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POSTGRADUATE IN TROPICAL MEDICINE AND INTERNATIONAL HEALTH

MODULE 2
CLINICAL & BIOMEDICAL SCIENCES OF TROPICAL DISEASES

Practical notes

HUMAN PARASITOLOGY IN TROPICAL SETTINGS

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Some preliminary remarks

About the taxonomy used in this syllabus:

Every species belongs to a Genus. This last one has a certain place in successively: a family, an order, a class and a phylum. The names of the classes and families, used in this syllabus, are not always those recommended by the International Zoological Nomenclature (Classification of the Committee of Nomenclature, Levine et al., 1980), they are however commonly encountered in parasitolgy. This simplified classification is used intentionally in an effort to avoid unnecessary complication.

Helminths of medical importance can be divided in two sub-groups:

- The phylum of Plathelminths (flat worms with rudimentary digestive tracts and a thin surface/skin through which a substantial part of the food is absorbed) comprises two classes that will be discussed in this syllabus:
  - The Trematodes: Non-segmented body and hermaphroditic (except for the schistosomes)
  - The Cestodes: Segmented body and hermaphroditic (every segment is bisexual)
- The phylum of Nemathelminths (round, cylindrical worms, non-segmented with complete digestive tract, separated sexes and with a tough, solid surface/skin) has only one important class:
  - The Nematodes

For the Protozoans (single-celled parasites), a simplified classification is suggested in this syllabus, mainly based on their type of movement, making it more useful in the laboratory diagnosis:

- The group of Amoebae: movement by pushing out the ectoplasm to form pseudopodia. Parasitic forms are always extra-cellular.
- The group of Flagellates: movement by means of one or more flagella, which are however not always present in the different evolutionary stages of the parasite.
- The group of Ciliata: movement and 'capturing' of food by means of co-operation of numerous cilia.
- The group of Sporozoa: no apparent way of moving. Parasitic forms are usually intracellular.

For some parasites no clear or unique classification is known at this time.

In this syllabus some bacteria and fungi are included, as their method of detection is the same as the ones already mentioned in these notes.

About the dimensions, given in this syllabus:

The dimensions of the parasites, given in this syllabus, are those, cited as the minimum and maximum dimensions by the World Health-Organisation (WHO) and/or the Centers for Disease Control and Prevention (CDC). In summarizing tables and schemes in this syllabus, the most commonly encountered dimensions will be given.

Idzi Potters
Philippe Gillet
Jan Jacobs

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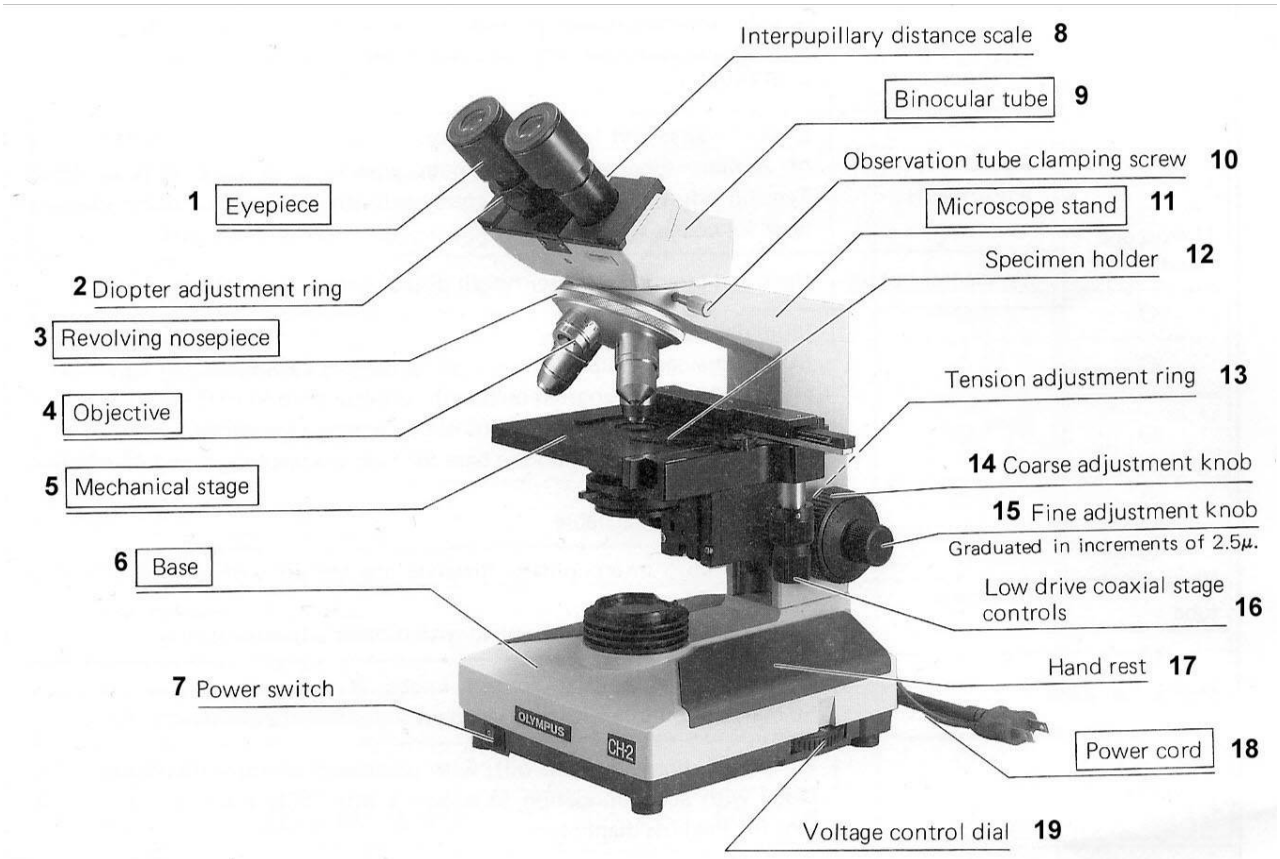
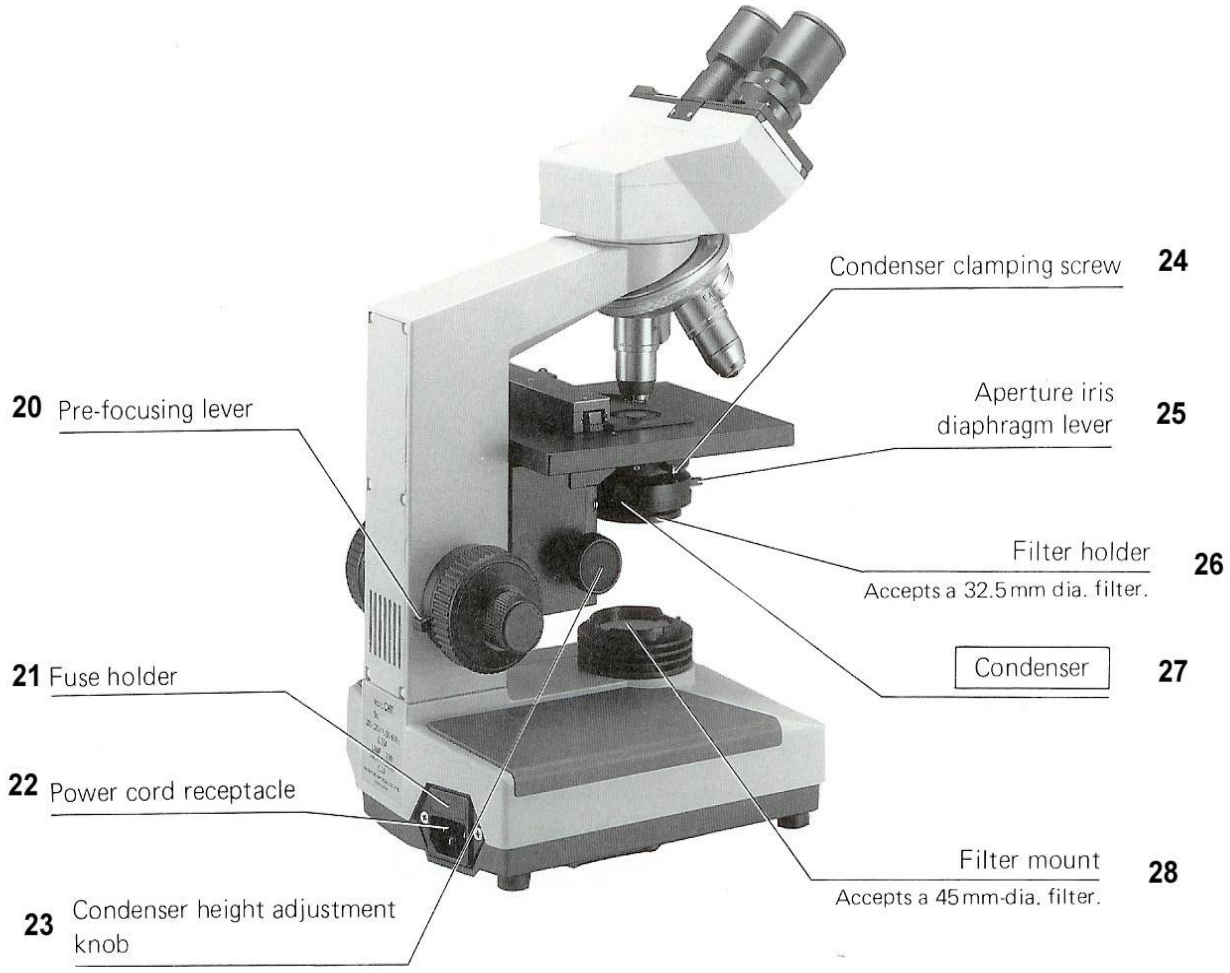
Used example: OLYMPUS, model CHK





Maintenance of the microscope

Already in the 17th century Antoni van Leeuwenhoek (1632-1723), a dutch biologist, developed the first, though rudimentary, microscope. Throughout the centuries different investigators have further expanded this device to the rather complicated tool that we know nowadays. The microscope consists of a series of extremely precise mechanical parts and extremely sensitive optics. For every microscope one should always check the enclosed manual for ideal transporting conditions and maintenance-techniques.
In isolated labs, the microscope is probably the most important tool one can find, for it can diagnose a whole set of bacterial infections (tuberculosis, meningitis,...) and parasitic infestations (trypanosomiasis, malaria,...). Even for other diagnostics, the microscope can give us useful surplus information (e.g. a count and/or identification of certain cell-types in biological samples).
Nevertheless the microscope remains a relatively expensive device which deserves, in other words, all our attention.

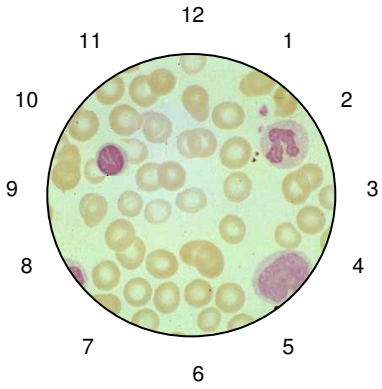
In a small laboratory, the microscope is an indispensable tool that should meet the highest standards. A poorly maintained microscope will drastically lower the quality and sensitivity of the tests. A microscope with fungal growth in the optical parts, which is not rare in moist and tropical conditions, is definitively out of order. For these reasons, the microscope should be handled and maintained with lots of care, so its lifespan can be prolonged for several years.

- Always avoid shocks. When transporting the microscope, a box or crate, especially designed for this purpose, should be used, in which the microscope is well immobilised.
- The microscope should be well-protected against dust. This is especially the case during the dry and warm season. Dust and sand-storms can seriously damage the mechanical parts of your microscope.
- In the warm and moist season fungal growth on lenses and prisms of the optical system poses the biggest problem.
- Certain precautions can be taken to avoid the problems mentioned above:
    - When using electrical microscopes, the intensity of the light-source should be set to the minimum intensity before switching off the microscope. This will substantially prolong the logevity of the light-bulb.
    - Always make sure that some spare lightbulbs for the microscope are in stock. Always keep the reference-numbers of all parts and accessories for replacing them if necessary.
    - One should remove the immersion-oil from the immersion-lenses on a daily basis by wiping it off with a non-fluffy, soft and clean tissue or with toilet-paper. Dried oil will cause the microscopic image to be foggy. When dried oil is sticking to the immersion-lenses, it can be removed, using a clean tissue, moistened with xylol or with a mix of ether and alcohol (1/1 v/v). This way of cleaning should be kept to a minimum and should never be used on internal lenses and prisms. After cleaning, the lenses should be dried immediately with another clean and dry tissue or toilet-paper. Also plastic lenses exist, which are not resistant to organic solvents. For this reason, one should always carefully check the manual of the microscope for suitable solvents.
    - Other lenses should be cleaned with another tissue, which is not used for the immersion-lenses, as any contact between oil and non-immersion-lenses should be avoided.
    - Never leave the tube of the microscope open (i.e without eyepieces) as this can cause dust and fungal spores to enter into the micrcoscope.
    - After every use, the microscope should be covered with a preferably cotton dust-sheet. Avoid plastic covers, as these will promote fungal growth, especially in moist circumstances.
    - The best thing to do is to place the microscope in an acclimatized room, but only when the acclimatization is never interrupted (danger of condensation within the optics of the microscope in case of fluctuating temperatures). At any rate, the acclimatization should never be set to a relatively low temperature. So, the temperature-shock in case of electrical break-down is not too big.
    - Local air-humidity can be diminished by placing an electrical dehumidifier, or even more simply, by placing dried silicagel-crystals (with saturation-indicator), near the microscope, in a closed cupboard.
    - After using the microscope one can also put it in a place, in which air-humidity is diminished by means of a low-power light bulb (± 20 Watt).

The microscopic field

The **microscopic field** is the circular image one sees at a certain magnification.

This microscopic field can be considered to be the plate of a clock.
Doing so, we can always locate any object in this microscopic field, starting from the centre:

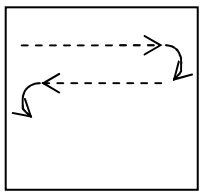


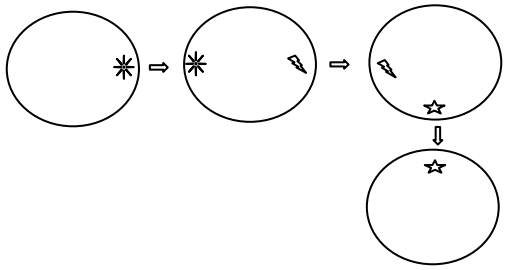
Examples:

- Between one and two o'clock we can see a trombocyte between 2 red blood cells.
- The object near two o'clock is a neutrophile.
- The object between four and five o'clock is a monocyte.
- Between the centre and ten o'clock, we can see a lymphocyte.
- At the edge, near eight o'clock, we can see part of a lymphocyte.

Systematic examination

The search for eggs and larvae of helminths and of Ciliates is performed with the 10x objective. **The entire preparation** is examined, leaving no parts missed out. To accomplish this, one should work systematically. Always start at a corner of the cover slip, e.g. the upper-left corner (in reality this will be the lower-right corner) and proceed by looking at the next microscopic field, with a small overlap. This means that each time when a field has been examined, an object in this field, e.g. a crystal, at three o'clock is chosen, and is brought towards nine o'clock. This second field is examined and so on. In this way, one should go in a straight line from the upper-left corner towards the upper-right corner of the cover slip. Once we arrive there, we choose an object at six o'clock and move it towards twelve o'clock. This results in arriving at the row below the one that has just been examined (again with a small overlap). This time we work from right to left. This way the complete preparation should be examined within the edges of the cover slip, untill arriving at the lower-right corner.





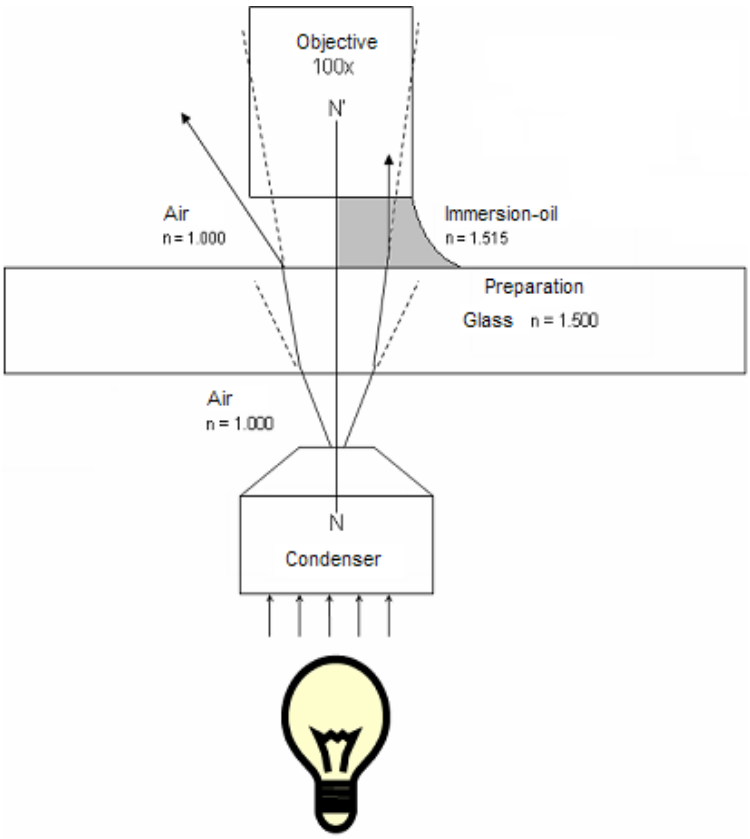
For searching some of the protozoans the 40x objective is used. In the same way as described above, a few overlapping rows (3 or 4) should be examined.
A partial systematic examination is used for permanently stained smears as well. Also here, the examined part should consist of overlapping rows.

Basic adjustments of the microscope

Objective	Condenser	Diaphragm	Immersion-oil	Mirror	Optical limit and purpose
10x	lowered	open/closed	no	flat	30 - 500 µm • Search for eggs and larvae • Search for Ciliates • (Entomology) • …
40x	halfway	open/closed	no	flat	5 - 30 µm • Details of eggs, larvae and ciliates • Search for certain protozoans by means of direct wet smear • (Counting of WBC and RBC) • …
100x	at the top	open	yes	concave	0,25 - 5 µm • Permanently stained smears • …

Immersion-objectives

Rays of light that pass the optical system of the microscope pass through different kinds of materials (glass, air, ...). Each one of these materials has a certain refracting capacity or refractional index "n". When light passes through these materials (with different refracting indices), the rays of light will be bent and sometimes lost (when they are bent so strongly they leave the optical system of the microscope). The level and way of bending depend on the refracting capacity of the environment and on the order of the transition.
When light passes from an environment with low refractional index to an environment with higher refractional index, it will be bent to the normal NN' of the optical system (right half of the scheme). When light passes from an environment with high refractional index to an environment with lower refractional index, it will be bent, away from the normal NN' of the optical system (left half of the scheme).



High-magnification objectives (50x, 100x) need large amounts of light. To avoid loss of light by refraction, immersion-oil with a sufficiently high refractional index is placed between the preparation and the objective.

Some examples of different refractional indices:

Air:	n = 1,000
Water:	n = 1,330
Normal glass:	n = 1,500
Immersion-oil:	n = 1,515
Canada balm:	n = ± 1,5

Calibration of the microscope

Principals :

For the correct identification of a series of parasites, the exact dimensions are of utmost importance, especially when we are talking about cysts of protozoans and eggs of helminths. For the approximate values of a certain objects dimensions, one can start by comparing it to other elements with known dimensions (e.g. eggs of *Schistosoma mansoni*, eggs of *Trichuris trichiura*, or with red blood cells etc). One can also make an estimation of any object's dimensions, by comparing it to the diameter of the microscopic field. To know this diameter exactly, the field-of-view-factor, mentioned on the eyepieces, is to be divided by the magnification of the used objective and by the tube-factor of the microscope. The result is expressed in millimeters. The field-of-view indicates the diameter (in mm) of the circular diaphragm, located halfway the eyepiece to limit the microscopic field. This field-of-view-factor can be found on the eyepiece, in most cases together with the magnification-factor of the eyepiece (e.g. 10x / **18**L). The tube-factor is a characteristic of the used microscope and can be found in its manual (in most cases it equals 1).

Example:
One uses eyepieces, indicating: 10x / 22.
When using a 10x objective on a microscope with tube-factor 1, this results in a diameter of the microscopic field of (22 : 10) : 1 = 2,2 mm or 2200 µm.

However, to know the exact dimensions of an object, one needs an eyepiece with a scale built inside (micrometer-eyepiece). This micrometer-eyepiece is basically a normal eyepiece, in which a lense is installed. This lense has a scale etched on it (usually divided in 100 equal parts). Since this scale is located in the eyepiece itself, it will represent different values (expressed in µm), depending on the objective being used.
This implicates however that for every different objective (and for every microscope) this micrometer-eyepiece will have to be calibrated. This means attaching a specific value (in µm) to one single division of the scale, and doing this for each objective that is used (and for each microscope). To determine this value, one needs a reference, which dimensions are known exactly. This reference usually consists of a slide on which a scale is etched (usually 1 to 2 mm), divided in 100 or 200 equal parts (each division being exactly 10 µm). Considering the high cost of these reference-slides and the limited use of them, one can replace them by a counting-chamber (without losing too much precision). Counting-chambers are for instance used in haematology to count different cell types and/or objects and can therefore be found in any routine-lab.

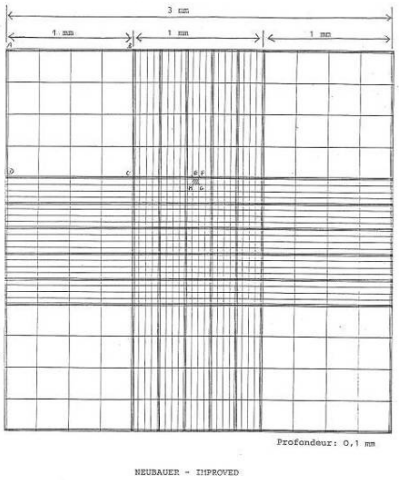
Materials and reagents :

Microscope, micrometer-eyepiece, (reference-slide or) counting-chamber, immersion-oil

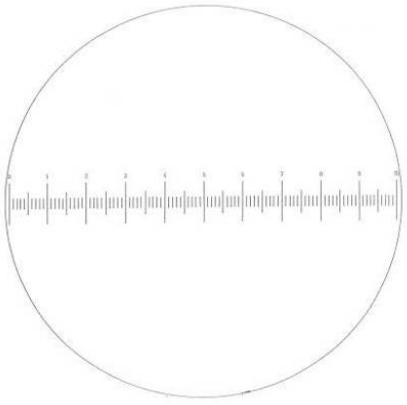
Calibration of an objective :

1. Place a counting-chamber (e.g. Neubauer Double Improved) on the mechanical stage of the microscope and manoeuvre the etched part of the counting-chamber to the centre of the microscopic field.
2. Remove the eyepiece, **of which the dioptrics are not adjustable,** and replace it by the micrometer-eyepiece.
3. Focus the microscopic image.
4. Turn the micrometer-eyepiece, placing the divisions of the scale parallel to the lines of the counting-chamber.
5. Move the counting-chamber (by moving the specimen holder) untill the 0-line of the micrometer-eyepiece corresponds exactly with a line of the counting-chamber. At high magnifications, the thickness of these lines will be such, that one should choose to make either the left or the right sides of these lines correspond.
6. Search, without moving the counting-chamber, a point at the opposite side of the microscopic field, where a division of the micrometer-eyepiece corresponds exactly with a line of the counting-chamber (as far away of the 0-line as possible).
7. Count the number of divisions of the micrometer-eyepiece between these two points of corresponding lines (in the scheme below 94 divisions of the micrometer-eyepiece can be counted).
8. Count the number of cells of the counting-chamber between the two points of corresponding lines and recalculate them to µm (in the scheme below we can see 6 cells of 250 µm + 5 cells of 200 µm, which results in a total of 2500 µm).
9. Then calculate the length of one division of the micrometer-eyepiece as follows :
   94 divisions of the micrometer-eyepiece = 2500 µm.
   1 division of the micrometer-eyepiece = 2500 / 94 = 26,6 µm.
10. Repeat all this for each objective that is to be calibrated.
11. In principle the calibration should be done only once for each microscope and for each objective that is being used. **Attention**: if the dioptrics of the micrometer-eyepiece are adjustable as well, the calibration will only be valid for de dioptrics used when calibrating!!! (The distance between eyepieces and tube influences the magnification)

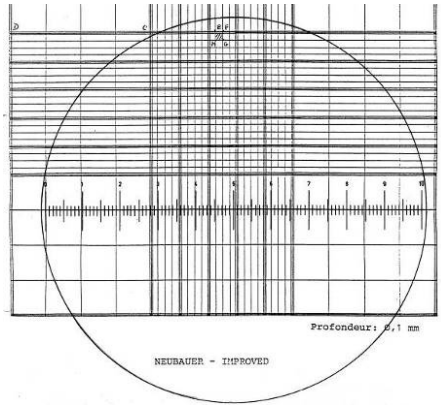
Neubauer counting-chamber (Double Improved)

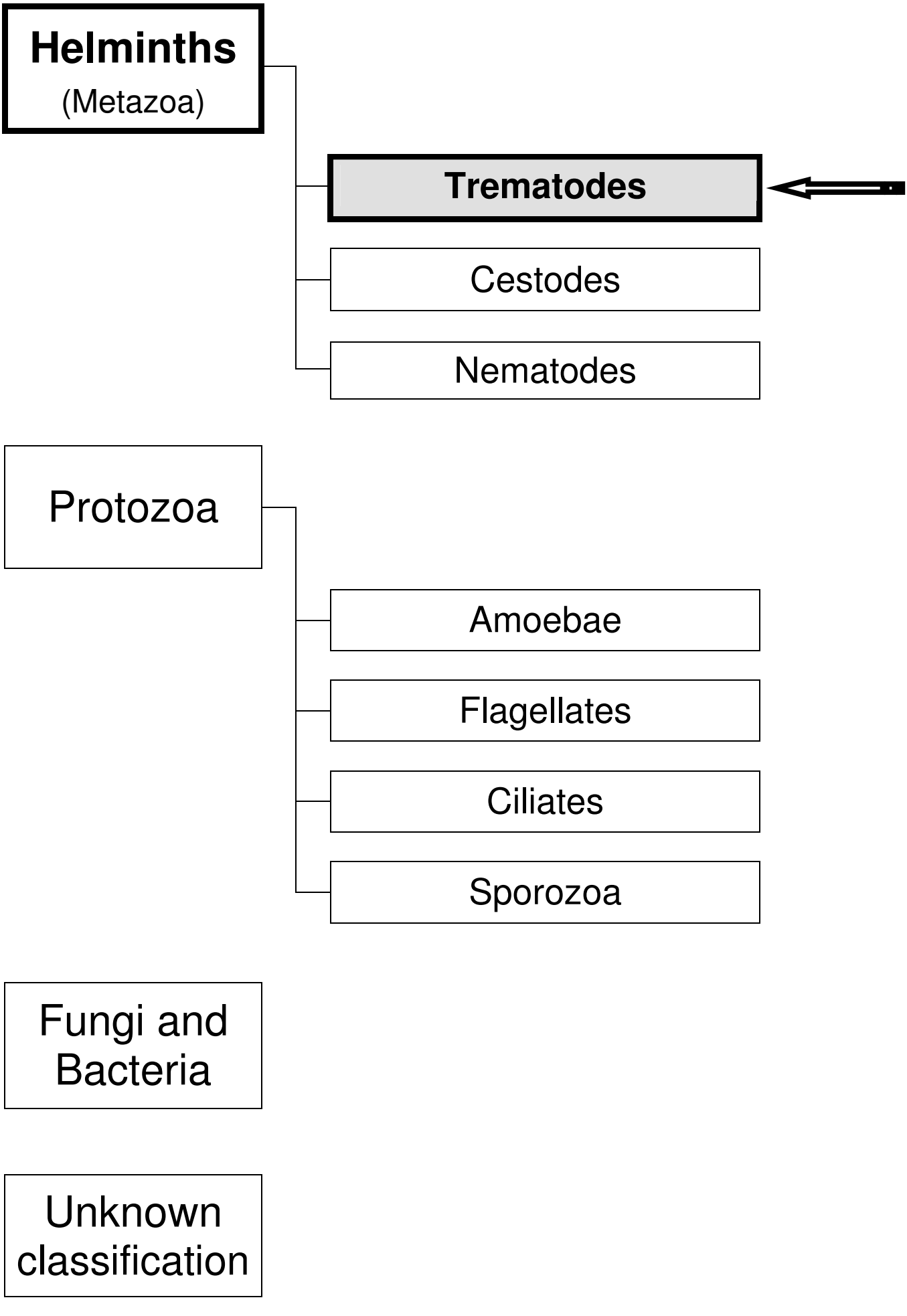


Micrometer-eyepiece

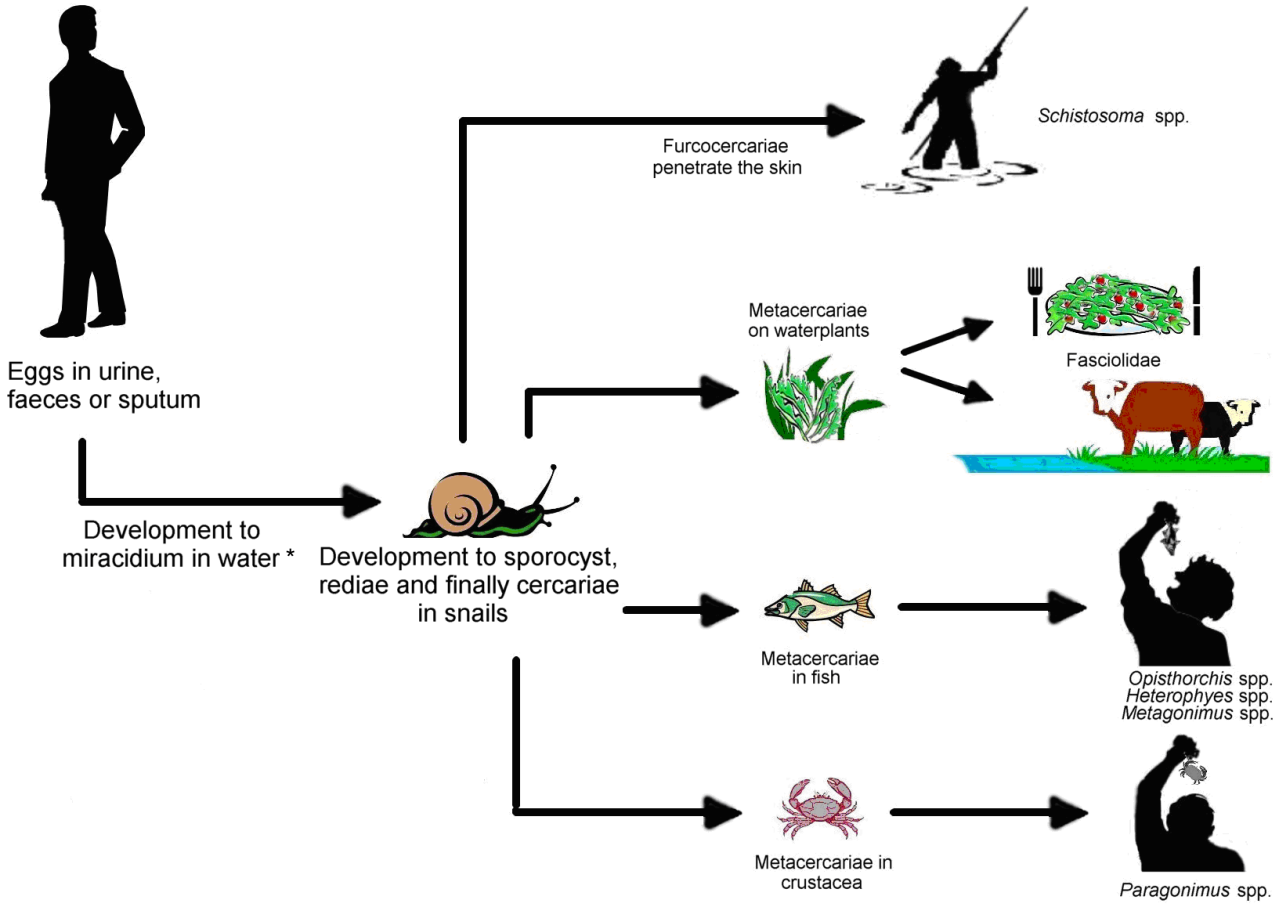


Calibration of the micrometer-eyepiece




Helminths
(Metazoa)
Trematodes
Cestodes
Nematodes
Protozoa
Amoebae
Flagellates
Ciliates
Sporozoa
Fungi and Bacteria
Unknown classification

Example of a life-cycle: *Trematodes*



* Except for *Opisthorchis* spp., *Heterophyes* spp. and *Metagonimus* spp., for which the eggs are to be taken up by the snail before the egg hatches and the miracidium comes out.

Schistosoma mansoni

Family : Schistosomatidae

Class : Trematodes

Geographic distribution :

- Tropical America
- Tropical Africa
- Middle East

Common name :

Intestinal bilharzia

Disease :

- NL : darm- of rectumbilharziose
- FR : bilharziose intestinale ou rectale
- EN : intestinal or rectal schistosomiasis
- ES : bilarciasis o esquistomiasis intestinal

Final host :

- Man
- Rodents (especially in Tropical America)
- Monkeys (especially in Africa)

Intermediate host :

- Freshwater snails (*Biomphalaria* spp.)

Transmission :

Transcutaneous : Furcocercariae from contamined water will penetrate the skin (or exceptionally the mucosa).

Localisation of the adult worm :

Lower mesenterial veins.

Diagnostic possibilities :

- Searching for eggs in faeces, using:
  - Direct examination
  - Concentration by sedimentation
  - Kato-Katz: used only for epidemiological purposes
- Searching the eggs by rectal biopsy: the biopsy is examined without staining or fixation
- Serology (mainly based on CCA (Circulating Cathodic Antigens) and CAA (Circulating Anodic Antigens))
  - searching antibodies in serum
  - detection of immune-complexes in blood
- (Antigen-detection in **urine**: based on CCA, in Lateral Flow Through Strip or in ELISA)

Morphology of the eggs :

Dimensions : 110-175 µm x 45-70 µm

Aspect : aspect of asymmetric bottle or oval

Shell : smooth

Contents : embryo (miracidium)

Colour : rather dark

Characteristics :
big lateral spine; acid-fast egg
(red when coloured with Ziehl-Neelsen)

Associated biological signs :

- Hypereosinophilia at invasion-phase (up to 30%), which normalizes later on
- Hyperleucocytosis at prepatent phase
- Liver-tests off chart and signs of portal hypertension

Possible confusion with :

- Other *Schistosoma* spp.
- Fasciolidae
- Spores of *Psorospermium haeckelii* (sporozoa which infests crayfish in eastern Europe)

Remarks :

- Sometimes the typical lateral spine will be invisible (pointing upward or downward). In these cases the egg can be turned/rolled by gently tapping the cover slip with a pen or a wooden spatula.
- As the eggs of *S. mansoni* do not detach easily from the inner intestinal wall, repeating the examination of faeces or rectal biopsy can prove useful.
- The eggs of *S. mansoni* sometimes are found in urine-samples.
- The adult's lifespan is estimated at 2 to 18 years. Eggs can be found in faeces approximately 25 to 60 days after initial infestation. Eggs produced per female: 100 to 300 eggs per day.
- Serological tests are group-specific. Cross-reaction with other trematodes is possible. The combination of two different techniques for the detection of antibodies (ELISA + IHA) yields a sensitivity of about 90%. The antibodies can remain in circulation for years after succesful treatment.

Schistosoma japonicum

Family : Schistosomatidae

Class : Trematodes

Geographic distribution :

Far East
(China, Filippines,…)

Common name :

Intestinal bilharzia

Disease :

- NL : darmbilharziose
- FR : bilharziose intestinale
- EN : intestinal schistosomiasis
  Katayama disease
- ES : enfermedad de Katayama

Final host :

- Man
- Lots of other animals

Intermediate host :

- Freshwater snails (*Oncomelania* spp.)

Transmission :

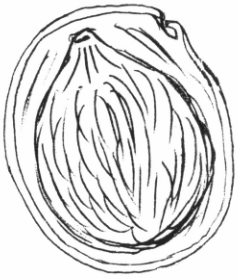
Transcutaneous : Furcocercariae from contamined water will penetrate the skin (or exceptionally the mucosa).

Localisation of the adult worm :

Upper mesenterial veins.

Diagnostic possibilities :

- Searching for eggs in faeces, using: - Direct examination
  - Concentration by sedimentation
- Searching for eggs, using rectal biopsy: the biopsy is examined without staining or fixation
- Serology (mainly based on CCA (Circulating Cathodic Antigens) and CAA (Circulating Anodic Antigens))
  - searching antibodies in serum
  - detection of immune-complexes in blood
- (Antigen-detection in **urine**: based on CCA, in Lateral Flow Through Strip or in ELISA)



Morphology of the eggs :

Dimensions : 68-100 µm x 45-80 µm

Aspect : oval to round

Shell : smooth

Contents : embryo (miracidium)

Colour : rather light/clear

Characteristics :
small lateral spine, more or less hidden in an indentation of the egg-shell; acid-fast egg
(red when coloured with Ziehl-Neelsen)

Associated biological signs :

- Hypereosinophilia at invasion-phase, which normalises later on
- Hyperleucocytosis at prepatent phase
- Liver-tests off chart and signs of portal hypertension

Possible confusion with :

- *Schistosoma mekongi*
- *Ascaris* spp.

Remarks :

- The name Katayama Fever, originally exclusively used for infestations with *Schistosoma japonicum*, is nowadays used to indicate de acute phase of schistosomiasis in general.
- The eggs of *S. japonicum* and *S.mekongi* are morphologically identical (only small differences in dimensions). Differentiation is only possible using geographic distribution.
- The Kato-Katz-technique is not usefull as these eggs have no typical shape and the miracidium clears up quite quickly.
- The eggs of *S. japonicum* sometimes are found in urine.
- The adult's lifespan is estimated at more than 25 years. Eggs can be found in faeces approximately 30 days after initial infestation. Eggs produced per female: 1.500 to 3.500 eggs per day.

Schistosoma mekongi

Family : Schistosomatidae

Class : Trematodes

Geographic distribution :

Along the river Mekong (Laos, Cambodja, Thailand) and in Malaisia.

Common name :

Intestinal bilharzia

Disease :

- NL : darmbilharziose
- FR : bilharziose intestinale
- EN : intestinal schistosomiasis
  Katayama disease
- ES : enfermedad de Katayama

Final host :

- Man
- Lots of other animals

Intermediate host :

- Freshwater snails (*Tricula aperta*)

Transmission :

Transcutaneous : Furcocercariae from contamined water will penetrate the skin (or exceptionally the mucosa).

Localisation of the adult worm :

Upper mesenterial veins.

Diagnostic possibilities :

- Searching for eggs in faeces, using: - Direct examination
  - Concentration by sedimentation
- Searching for eggs, using rectal biopsy: the biopsy is examined without staining or fixation
- Serology (mainly based on CCA (Circulating Cathodic Antigens) and CAA (Circulating Anodic Antigens))
  - searching antibodies in serum
  - detection of immune-complexes in blood
- (Antigen-detection in **urine**: based on CCA, in Lateral Flow Through Strip or in ELISA)

Morphology of the eggs :

Dimensions : 51-73 µm x 39-66 µm

Aspect : oval to round

Shell : smooth

Contents : embryo (miracidium)

Colour : rather light/clear

Characteristics :
small lateral spine, more or less hidden in an indentation of the egg-shell; acid-fast egg
(red when coloured with Ziehl-Neelsen)

Associated biological signs :

- Hypereosinophilia at invasion-phase, which normalises later on
- Hyperleucocytosis at prepatent phase
- Liver-tests off chart and signs of portal hypertension

Possible confusion with :

- *Schistosoma japonicum*
- *Ascaris* spp.

Remarks :

- The eggs of *S .japonicum* and *S. mekongi* are morphologically identical (only small differences in dimensions). Differentiation is only possible using geographic distribution.
- The Kato-Katz-technique is not usefull as these eggs have no typical shape and the miracidium clears up quite quickly.
- The eggs of *S. mekongi* sometimes are found in urine.
- The adult's lifespan is estimated at more than 25 years. Eggs can be found in faeces approximately 30 to 60 days after initial infestation. Eggs produced per female: 1.500 to 3.500 eggs per day.

Schistosoma intercalatum

Family : Schistosomatidae

Class : Trematodes

Geographic distribution :

Central Africa.
Infestations with this parasite are usually focussed in a few villages.

Common name :

Rectal bilharzia

Disease :

- NL : intestinale / rectale bilharziose
- FR : bilharziose intestinale / rectale
- EN : intestinal / rectal schistosomiasis
- ES : bilharziasis intestinal / rectal

Final host :

- Man
- Rodents?

Intermediate host :

- Freshwater snails (*Bulinus* spp.)

Transmission :

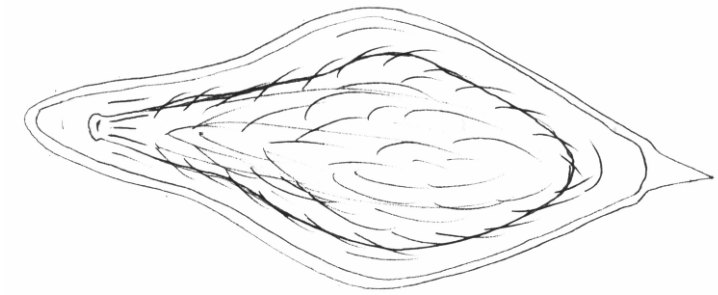
Transcutaneous : Furcocercariae from contamined water will penetrate the skin (or exceptionally the mucosa).

Localisation of the adult worm :

Rectal veins.

Diagnostic possibilities :

- Searching for eggs in faeces, using:
  - Direct examination
  - Concentration by sedimentation
  - Kato-Katz: used only for epidemiological purposes
- Searching for eggs, using rectal biopsy: the biopsy is examined without staining or fixation
- Serology (mainly based on CCA (Circulating Cathodic Antigens) and CAA (Circulating Anodic Antigens))
  - searching antibodies in serum
  - detection of immune-complexes in blood
- (Antigen-detection in **urine**: based on CCA, in Lateral Flow Through Strip or in ELISA)



Morphology of the eggs :

Dimensions : 140-240 µm x 50-85 µm

Aspect : usually symmetrical, stretched oval- to diamond-shaped

Shell : smooth

Contents : embryo (miracidium)

Colour : rather dark

Characteristics :
big terminal spine; acid-fast egg
(red when coloured with Ziehl-Neelsen)

Associated biological signs :

- Hypereosinophilia at invasion-phase, which normalises later on
- Hyperleucocytosis at prepatent phase
- liver-tests off chart and signs of portal hypertension

Possible confusion with :

- Other *Schistosoma* spp.
- Fasciolidae
- Spores van *Psorospermium haeckelii*

Remarks :

- Occasionally eggs of *S. intercalatum* are found in urine samples (differentiation with *S. haematobium* using Ziehl-Neelsen's staining).
- Natural hybrids exist between *S. haematobium* and *S. intercalatum*.
- The eggs of *S. intercalatum* sometimes have difficulties detaching from the inner intestinal wall. In these cases repeating the examination of faeces or rectal biopsy can prove useful.
- Eggs can be found in faeces approximately 50 to 60 days after initial infestation.
- Eggs produced per female: 150 to 400 eggs per day.

Schistosoma haematobium

Family : Schistosomatidae

Class : Trematodes

Geographic distribution :

- Africa
- Middle East
- India
- Portugal (very rare)

Common name :

Bilharzia of the bladder

Disease :

- NL : blaasbilharziose of urinaire bilharziose
- FR : bilharziose vésicale
- EN : urinary or vesical schistosomiasis
- ES : bilarciasis urinaria

Final host :

- Man
- (Monkeys)
- (Pigs)

Intermediate host :

- Freshwater snails (*Bulinus* spp.)

Transmission :

Transcutaneous : Furcocercariae from contamined water will penetrate the skin (or exceptionally the mucosa).

Localisation of the adult worm :

Veins of the bladder.

Diagnostic possibilities :

- Searching for eggs in urine, using: - sedimentation
  - filtration
- Serology (mainly based on CCA (Circulating Cathodic Antigens) and CAA (Circulating Anodic Antigens))
  - searching antibodies in serum
  - detection of immune-complexes in blood
- (Antigene-detection in **urine**: based on CCA, in Lateral Flow Through Strip or in ELISA)

Morphology of the eggs :

Dimensions : 112-170 µm x 40-70 µm

Aspect : symmetrically oval

Shell : smooth

Contents : embryo (miracidium)

Colour : yellow-grey

Characteristics :
small terminal spine
egg is NOT acid-fast !!!
(blue when coloured with Ziehl-Neelsen)

Associated biological signs :

- Hypereosinophilia at invasion-phase (up to 30%), which normalizes later on
- Hematuria and proteinuria
- Hyperleucocytosis at prepatent phase
- Kidney-tests off chart

Possible confusion with :

- *Schistosoma* spp. (especially *S. intercalatum*)
- Epithelial cells
- Spores of *Psorospermium haeckelii* (found in faeces !) (sporozoa which infests crayfish in eastern Europe)

Remarks :

- For specimen-collection, some points should be taken into consideration:
  - Collect the urine preferably between 10h00 and 14h00
  - Before urinating the patient should move/jump around a little bit
  - Before giving a urine-sample, the patient's bladder should be well-filled
  - As the eggs are particularly present in the last millilitres, maximum miction is advisable
- In the event of a negative result, the examination can be repeated a few days later.
- After some time the eggs of *S. haematobium* hatch and the extremely motile miracidia can be found in the urine.
- Occasionally eggs of *S. haematobium* are found in faecal samples (differentiation with *S. intercalatum* using Ziehl-Neelsen's staining).
- Natural hybrids exist between *S. haematobium* and *S. intercalatum*.
- Serological tests are group-specific. Cross-reaction with other trematodes is possible. The combination of two different techniques for the detection of antibodies (ELISA + IHA) yiels a sensitivity of about 90%. The antibodies can remain in circulation for years after succesful treatment.
- The adult's lifespan is estimated at 3 to 7 years. Eggs can be found in urine approximately 54 to 84 days after initial infestation. Eggs produced per female: 20 to 300 eggs per day.

Fasciola hepatica

Family : Fasciolidae

Class : Trematodes

Geographic distribution :

Worldwide

Common name :

Liverfluke

Disease :

- NL : leverdistomatose
- FR : distomatose du foie
- EN : fasciolasis, liver fluke infection
- ES : distomatosis hepatica

Final host :

Many vegetarians :
- Sheep
- Goat
- Cow
- Man
- Rodents
- …

Intermediate host :

- Amphibic freshwater snails (*Lymnea* spp.)

Transmission :

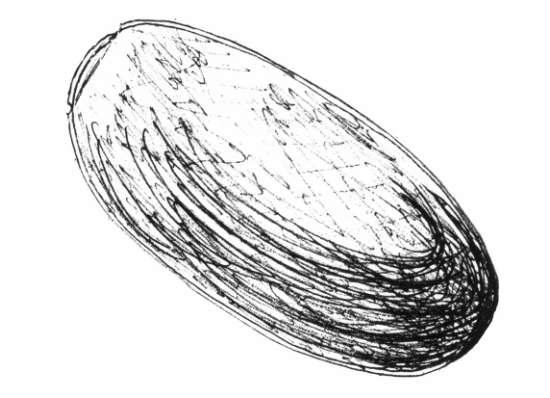
By eating waterplants, contaminated with metacercariae.

Localisation of the adult worm :

Biliary channels

Diagnostic possibilities :

- Searching for eggs in faeces, using: - Direct examination
  Usually the number of eggs in the faeces is rather low so a direct examination will not be sufficient.
  - **Concentration by sedimentation**
- Searching for eggs in duodenal aspirate
- Serology: searching for antibodies (against excretion and secretion Ag (ES Ag)) in serum
- (Antigene-detection in faeces (ES Ag))



Morphology of the eggs :

Dimensions : 120-150 µm x 63-90 µm

Aspect : symmetrically oval

Shell : smooth and thin wall

Contents : no embryo

Colour : yellow, brown-yellow or brown-grey

Characteristics :
operculum is present, but sometimes very difficult to observe

Associated biological signs :

- Hyperleucocytosis in invasion-phase
- Hypereosinophilia (up to 80% at invasion-phase)
- Liver-tests off chart

Possible confusion with :

- *Fasciola gigantica*
- *Fasciolopsis buski*
- *Schistosoma* spp.
- eggs of *Acarina* spp. (mites)

Remarks :

- The distinction between *Fasciola hepatica, Fasciola gigantica* and *Fasciolopsis buski* is microscopically almost impossible to make. There are natural hybrids as well between *F. hepatica* and *F. gigantica*, making it impossible to differentiate these two eggs. When one of these eggs is found in a faecal sample, a more correct answer would be *"Eggs of Fasciolidae found"*.
- Presence of eggs of *Fasciola hepatica* in faeces does not always indicate an infestation however. The possibility exists that these are passing eggs (eating of infected liver → "pseudo-fasciolasis").
- Soon after initial infestation (7 to 11 weeks) the larvae penetrating the parenchym of the liver will cause a pathology. At this point no eggs can be found in the faeces, but serology can be helpful: 2 to 4 weeks after infestation serology has a sensitivity of about 95% (there will be cross-reactivity with other trematodes). Approximately 1 year after the invasion-phase serology can become negative again (while the adult worms are still in the biliary channels).
- When the operculum is not visible, one can firmly press on the cover slip with a wooden spatula, trying to open the operculum in this manner.
- The adult's lifespan is estimated at more than 25 years. Eggs can be found in faeces approximately 4 to 10 weeks after infestation. The number of eggs produced daily per adult is rather high (± 25.000 eggs per adult). Probably, only a very limited number of these eggs can eventually be found in the patient's faeces.

Fasciola gigantica

Family : Fasciolidae

Class : Trematodes

Geographic distribution :

- Central- and South-Africa
- (West-Pacific)
- (Haïti)
- (Asia)

Common name :

Big liverfluke

Disease :

- NL : leverdistomatose
- FR : distomatose du foie
- EN : fasciolasis, giant liver fluke infection
- ES : distomatosis gigantica

Final host :

Different kinds of plant-eaters :

- Sheep
- Goats
- Cows
- Man
- Rodents
- …

Intermediate host :

- Aquatic freshwater snails (*Lymnea* spp.)

Transmission :

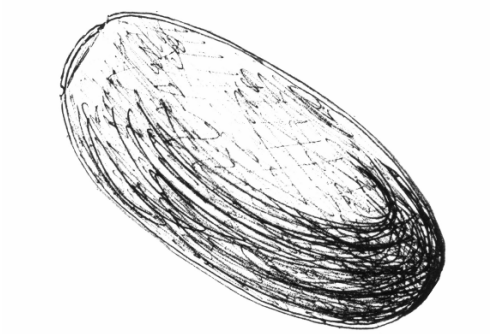
By eating waterplants, contaminated with metacercariae.

Localisation of the adult worm :

Biliary channels

Diagnostic possibilities :

- Searching for eggs in faeces, using:
  - Direct examination
    Usually the number of eggs in the faeces is rather low so a direct examination will not be sufficient.
  - Concentration by sedimentation
- Searching for eggs in duodenal aspirate
- **Serology: searching for antibodies (against excretion and secretion Ag (ES Ag)) in serum**
- (Antigene-detection in faeces (ES Ag))



Morphology of the eggs :

Dimensions : 135-190 µm x 68-94 µm

Aspect : symmetrically oval

Shell : smooth and thin wall

Contents : no embryo

Colour : yellow, brown-yellow or brown-grey

Characteristics : operculum is present, but sometimes very difficult to see

Associated biological signs :

- Hyperleucocytosis in invasion-phase
- Hypereosinophilia (up to 80% in invasion-phase)
- Liver-tests off chart

Possible confusion with :

- *Fasciola hepatica*
- *Fasciolopsis buski*
- *Schistosoma* spp.
- Eggs of *Acarina* spp. (mites)

Remarks :

- The distinction between *Fasciola hepatica, Fasciola gigantica* and *Fasciolopsis buski* is microscopically almost impossible to make. There are natural hybrids as well between *F. hepatica* and *F. gigantica*, making it impossible to differentiate these two eggs. When one of these eggs is found in a faecal sample, a more correct answer would be *"Eggs of Fasciolidae found"*.
- Presence of eggs of Fasciolidae in faeces does not always indicate an infestation however. The possibility exists that these are passing eggs (eating of infected liver → "pseudo-fasciolasis").
- Soon after initial infestation (7 to 11 weeks) the larvae penetrating the parenchym of the liver will cause a pathology. At this moment no eggs can be found in the faeces, but serology can be helpful: 2 to 4 weeks after infestation serology has a sensitivity of about 95% (there will be cross-reactivity with other trematodes). Approximately 1 year after the invasion-phase serology can become negative again (while the adult worms are still in the biliary channels).
- When the operculum is not visible, one can firmly press on the cover slip with a wooden spatula, trying to open the operculum in this manner.
- In human infestations, the adults of *Fasciola gigantica* are usually sterile. As no eggs can be found in this case, serology can prove useful. If the adults aren't sterile, eggs can be found in the patient's faeces approximately 12 to 15 weeks after infestation. Probably, only a limited number of eggs produced can eventually be found in the patient's faeces. The adult's lifespan is estimated at more than 11 years.

Fasciolopsis buski

Family : Fasciolidae

Class : Trematodes

Geographic distribution :

- China
- South-East Asia
- Far East
- Central Europe
- Indian continent

Common name :

Intestinal fluke

Disease :

- NL : darmdistomatose
- FR : distomatose intestinale
- EN : fasciolopsiasis
  Busk's fluke infection
- ES : distomatosis intestinal

Final host :

- Man
- Pig
- Dog (rare)
- Monkey
- Rabbit

Intermediate host :

- Freshwater snails (*Hippeutis* spp., *Segmentina* spp.)

Transmission :

By eating waterplants, contaminated with metacercariae.

Localisation of the adult worm :

Intestins (sucked to the wall of the ileum)

Diagnostic possibilities :

- Searching for eggs in faeces, using:
  - Direct examination
  - Concentration by sedimentation
    As there is no pathology with low wormloads and as egg-production is rather high, a concentration-technique should not be considered.

Morphology of the eggs :

Dimensions : 130-159 µm x 78-98 µm

Aspect : symmetrically oval

Shell : smooth and thin wall

Contents : no embryo

Colour : yellow, brown-yellow or brown-grey

Characteristics :
operculum is present, but sometimes very hard to see

Associated biological signs :

- Hypereosinophilia

Possible confusion with :

- *Fasciola hepatica*
- *Fasciola gigantica*
- *Schistosoma* spp.
- Eggs of *Acarina* spp. (mites)

Remarks :

- The distinction between *Fasciola hepatica, Fasciola gigantica* and *Fasciolopsis buski* is microscopically almost impossible to make. There are natural hybrids as well between *F. hepatica* and *F. gigantica*, making it impossible to differentiate these two eggs. When one of these eggs is found in a faecal sample, a more correct answer would be *"Eggs of Fasciolidae found"*.
- Rare cases of ectopic localisations (in myocard or brain) have been reported in literature.
- When the operculum is not visible, one can firmly press on the cover slip with a wooden spatula, trying to open the operculum in this manner.
- The adult's lifespan is estimated at about 6 months.
- Eggs can be found in faeces approximately 12 to 20 weeks after infestation.
- Eggs produced per adult: ±16.000 eggs per day.

Opisthorchis sinensis

Family : Opisthorchidae

Class : Trematodes

Geographic distribution :

- Far East (Japan, Korea, Hong-Kong, Taiwan, China, Kamchatka,…)

Common name :

Chinese liverfluke

Disease :

- NL : Chinese leverdistomatose
- FR : distomatose de Chine
- EN : Chinese liverfluke infection, clonorchiasis
- ES : distomatosis hepatica chinesco

Final host :

- Man
- Many fish-eating mammals

Intermediate host :

1st: Freshwater snails (*Bithynia* spp., *Alocinma* spp., *Parafossarulus* spp.)

2nd: Freshwater fish

Transmission :

By eating fish, contaminated with living metacercariae.

Localisation of the adult worm :

Biliary channels

Diagnostic possibilities :

- Searching for eggs in faeces, using: - Direct examination
  - Concentration by sedimentation
- Searching for eggs in duodenal aspirate
- Serology: searching for antibodies in serum (ELISA)

Morphology of the eggs :

Dimensions : 26-35 µm x 12-14 µm

Aspect : shape of asymmetrical amphora or stretched oval

Shell : smooth

Contents : embryo (miracidium)

Colour : brown-yellow

Characteristics : small knob (not always visible) on the pole, opposite to the well-visible operculum

Associated biological signs :

- Hypereosinophilia (up to 20%)

Possible confusion with :

- Other Opisthorchidae
- *Heterophyes* spp.
- *Metagonimus* spp.

Remarks :

- *Opisthorchis viverrini* (South-East Asia and Malaisia), *O. felineus* (*tenuicollis*) (South-East and Central Europe), etc. are liverflukes that have a biology and pathology similar to *O. sinensis*. Also their eggs (which can be found in faecal material) are practically indistinguishable.
- Serology is not very specific.
- The eggs of *Heterophyes heterophyes,* of *Metagonimus yokogawai* and of 13 other species of Heterophyidae that have been described (and which can also be found in faecal material) have a strong resemlance to these of the Opisthorchidae. The adult worms of *H. heterophyes* and of *M. yokogawai* live in the intestinal lumen however, causing only a very mild pathology (sometimes even asymptomatic). Their lifespan as adult worms is limited to about one year (while this is sometimes over 20 years for the Opisthorchidae).
- Smoking and/or pickling doesn't always kill the metacercariae.
- The adult's lifespan is estimated at more than 25 years.
- Eggs can be found in faeces approximately 1 to 4 weeks after infestation.
- Eggs produced per adult: 1.000 to 4.000 eggs per day.

Paragonimus spp.

Family : Troglotrematidae

Class : Trematodes

Geographic distribution :

- Sout-East Asia
- Africa
- America

Common name :

Lungfluke

Disease :

- NL : longdistomatose
- FR : distomatose pulmonaire
- EN : pulmonary distomatosis, oriental lungfluke disease
- ES : distomatosis pulmonar, duela pulmonar

Final host :

- Man
- Lots of different animals
  - Dog
  - Cat
  - Pig
  - Apes
  - …

Intermediate host :

1st: Freshwater snails (*Thiaridae* spp., *Oncomelania* spp.,…)

2nd: Freshwater crustaceans (lobster, crab,…)

Transmission :

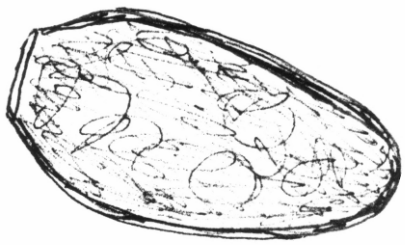
By eating crustaceans, infested with living metacercariae (raw, pickled, crab-meat as an aperitif,…)

Localisation of the adult worm :

Lungs (ectopic localisations are possible)

Diagnostic possibilities :

- Searching for eggs in sputum, using: concentration by sedimentation (after liquification)
  In case of high wormloads the sputum will often have brown to red dots in it, corresponding with important egg-masses.
- Searching for specific antibodies (against *Paragonimus westermani*) in blood



Morphology of the eggs :

Dimensions : 68-118 µm x 39-67 µm

Aspect : (sometimes irregularly) oval

Shell : thin shell with irregularities

Contents : no embryo

Colour : dork brown-yellow to greyish

Characteristics : operculum (sometimes hard to see)

Associated biological signs :

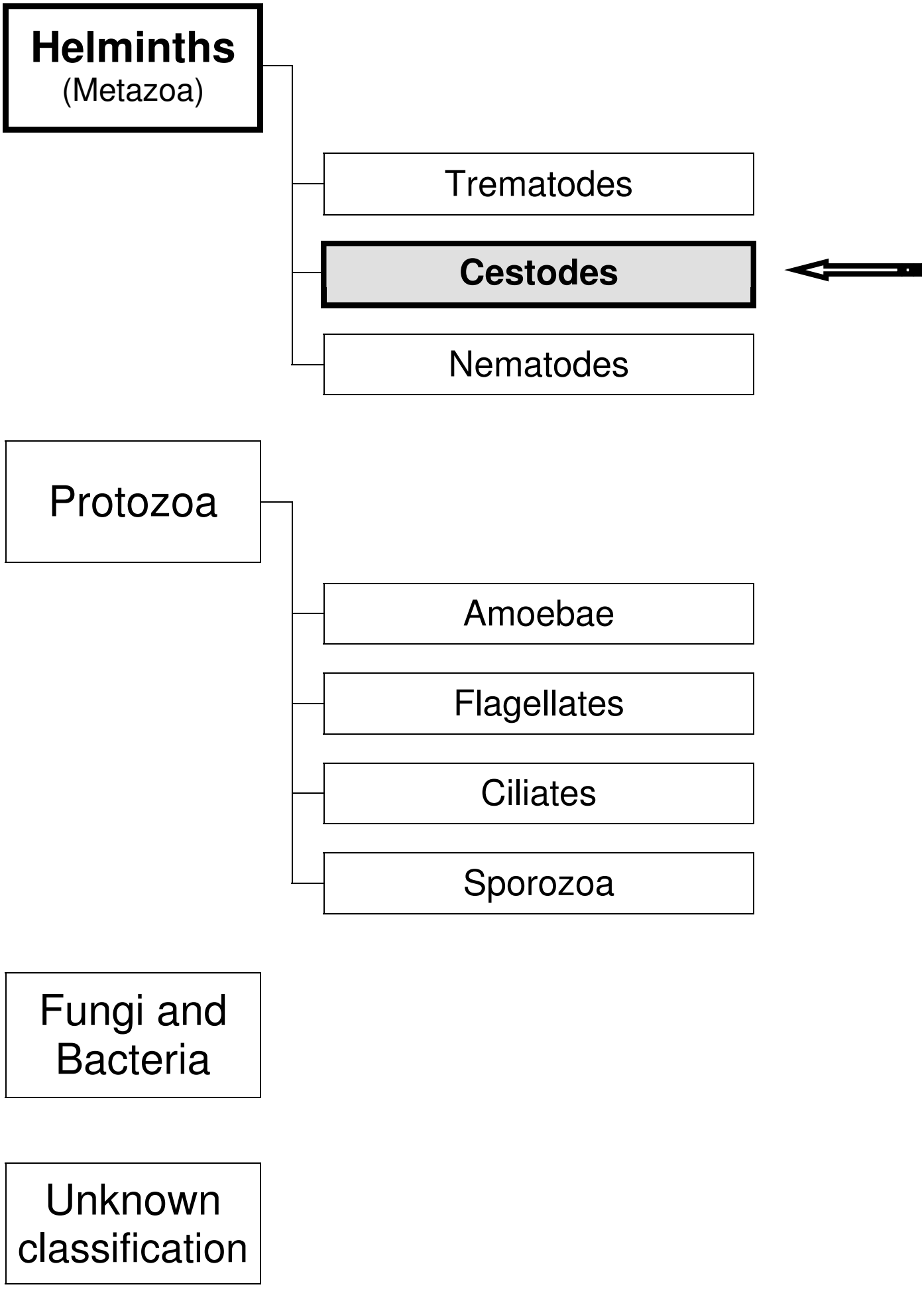
- Hypereosinophilia (in invasion-phase)
- Coughing up bloody sputum (chronic phase)

Possible confusion with :

- Eggs of *Diphyllobothrium latum* (in faeces)
- Eggs of *Ascaris* spp. (non fertile) (in faeces)
- Eggs of *Schistosoma mekongi* (in faeces)
- Eggs of *Schistosoma japonicum* (in faeces)
- Eggs of Fasciolidae (in faeces)

Remarks :

- One should never do a parasitological examination of (bloody) sputum before excluding a TB infection (risk of producing infectious aerosols).
- Eggs can be found in sputum only 2 to 3 months after infestation. The adult's lifespan is estimated at 10 to 20 years.
- Detection of eggs in sputum is not very easy, making serology particularly interesting, especially in case of low parasitaemias.
- When the operculum is not visible, one can firmly press on the cover slip with a wooden spatula, trying to open the operculum in this manner, thus making it visible and confirming the diagnosis.
- When examining sputum larvae can sometimes be found of worms, passing the lungs in their cycle of development (e.g. *Ascaris lumbricoides,* Ancylostomidae*, Strongyloides stercoralis,*...). Examination of sputum however is not the best way to search for these helminths!
- Examination of faeces is not indicated: the swallowed eggs will be passed in the faeces, but these will be hard to identify and very few in number. However, if these eggs are found in faeces, a sputum examination is indicated for confirmation.
- There is no morphological distinction possible between the different species of *Paragonimus* (*P. westermani, P.heterotremus, P. miyazakii, P. skyrjabini* (Asia, Africa?, South-America?), *P. africanus, P. uterobilateralis* (Africa), *P. kellicotti* (North-America), *P. mexicanus* (Latin America),…). The most correct answer to give when these eggs are found, is "eggs of *Paragonimus* spp. found".
- So far, the serological tests have no standardized protocol.


Helminths
(Metazoa)
Trematodes
Cestodes
Nematodes
Protozoa
Amoebae
Flagellates
Ciliates
Sporozoa
Fungi and Bacteria
Unknown classification

Diphyllobothrium latum

Family : Diphyllobothriidae

Class : Cestodes

Geographic distribution :

Cosmopolitic, more frequent in regions with temperate climate:
- North- and Central-Europe
- Japan, Taiwan, Filippines
- North- en South-America

Common name :

Fish tapeworm

Disease :
- NL : dibotriocefalose
- FR : diphyllobothriose humaine bothriocéphalose
- EN : broad fish tapeworm infection
- ES : botriocéfalosis

Final host :
- Man
- Many fish-eating mammals

Intermediate host :

1st: Small Copepoda (*Cyclops* spp., *Diatomus* spp.,…)

2nd: Freshwater fish (salmon, trout,…)

Transmission :

By eating fish, contaminated with living, plerocercoid larvae.

Localisation of the adult worm :

Attached to the inner wall of the small intestine

Diagnostic possibilities :
- Searching for eggs in faeces, using: - Direct examination
  As the number of eggs, produced by *D. latum* is quite high, a concentration-technique is usually not necessary.
- Identification of segments found in faeces (very rare)

Morphology of the eggs :

Dimensions : 58-76 µm x 40-51 µm
Aspect : symmetrically oval
Shell : thin
Contents : no embryo
Colour : rather dark
Characteristics : small knob (not always visible) on the opposite pole of the clearly visible operculum.

Associated biological signs :
- Megaloblastic macrocytic anaemia (Vitamine B12 deficiency)
- Leucopenia, thrombopenia, non-constant hypereosinophilia
- Elevated sedimentationspeed, hypoproteinemia, hypoalbuminemia, hypergammaglobulinemia

Possible confusion with :
- *Paragonimus* spp.

Remarks :
- Freezing of fish, contaminated with plerocercoid larvae, at -10°C during at least 24h, will kill these larvae.
- Sparganosis (or plerocercoidosis) is a disease in which *Diphyllobothrium* spp., using exclusively animals as a final host, are using humans as an unusual, paratenic (=no complete development in the host) intermediate host. In this case, only pro- or plerocercoid larvae are found in human host tissues. The localisation of these larvae will determine whether the course of the disease is rather mild and/or unnoticed (e.g. larvae in intestinal wall) or serious and/or dangerous evolution (e.g. larvae in ocular tissue). In cases of sparganosis no eggs are found in the faeces!
- Mature (non-motile) segments usually dissolve inside the intestins and hence will not be found in the faeces.
- Smoking or pickling of contaminated fish will not always kill the larvae!
- *D. latum, D. cordatum* (Northern-Europe, Northern-America,...), *D. pacificum* (South-Amerika),... are morphologically indistinguishable.
- The adult's lifespan is estimated at up to 30 years.
- Eggs can be found in faeces approximately 30 to 45 days after initial infestation.
- Eggs produced per adult: 35.000 to 100.000 eggs per day.

Taenia saginata

Family :	**Class :**
Taeniidae	Cestodes

Geographic distribution :

Cosmopolitic; rare in countries where no beef is eaten (hindu) or where systematic veterinary controls are conducted

Common name :

Unarmed tapeworm
Beef tapeworm

Disease :

- NL : teniasis
- FR : téniase
- EN : beef tapeworm infection
- ES : teniasis

Final host :

- Man

Intermediate host :

- Bovidae (cow, ox, buffalo, zebu,...)

Transmission :

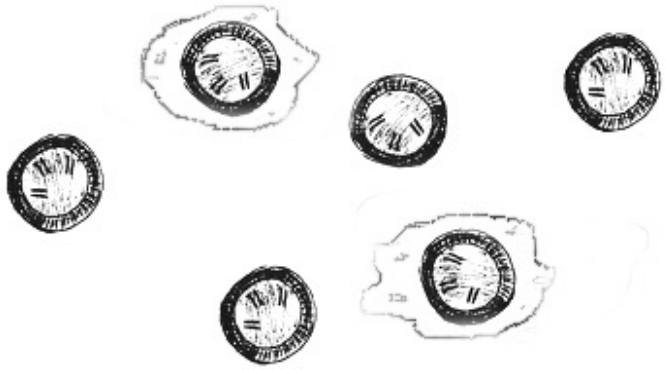
By eating beef, contaminated with living cysticerci (*Cysticercus bovis*).

Localisation of the adult worm :

Attached to the inner wall of the small intestine

Diagnostic possibilities :

- Macroscopically (finding the segments of the tapeworm)
- Microscopically by finding the eggs in the faeces: - Direct examination
  - Concentration by sedimentation
- The tape-test (for *Enterobius vermicularis*) can give an accidental diagnosis (eggs on peri-anal skin)
- (Searching copro-antigenes with dipsticks (good sensitivity, but no species-differentiation possible))
- (Serology: searching for specific antibodies)
- (Searching for specific DNA by PCR on faecal extractions (species-differentiation possible))
- **CAUTION:** When manipulating segments or adult tapeworms, always proceed with lots of caution (risk of cysticercosis in case of *T. solium*). Manipulation of segments or adult tapeworms is best done using tweezers. ALWAYS wear protective gloves when manipulating segments or adult tapeworms.



Morphology of the eggs :

Dimensions : 31-43 µm

Aspect : round or oval

Shell : thick wall with radial structure

Contents : hexacanth larva (3 pairs of hooks, all of which are not always visible)

Colour : dark-brown

Characteristics : sometimes some sort of mucus (periovular membrane) can be observed around the egg

Associated biological signs :

- At first: moderate hypereosinophilia (± 20%)

Possible confusion with :

- Air-bulbs
- *Taenia solium*
- Different kinds of pollen-grains

Remarks :

- In french the tapeworm is sometimes called “ver solitaire” (solitary worm), because it was thought that a person could be infected with only 1 tapeworm at a time. It has been proven several times already that a patient can bear more than one tapeworm at a time however.
- The eggs of *T. saginata* and those of *T. solium* are morphologically identical. When one of these is found in a examination of faeces the most correct answer should be “*eggs of Taenia spp. found*”.
- The difference between *T. saginata* and *T.* solium can be made by looking at mature segments or by looking at the scolex.
- Cysticerci in meat can remain alive for 8 months to one year, but can be killed by thorougly cooking the meat or by freezing it (at least 24h at –20°C).
- The microscopic examination (for eggs) of the faeces usually stays negative (because usually intact segments are excreted, even in between defecations).
- The first 10 to 12 weeks after infestation no segments or eggs will be found in the faeces (however, at this moment there will be a hypereosinophilia).
- Each mature segment contains about 100.000 eggs. One single tapeworm (counting as many as 2000 segments) can produce up to 600 million eggs each year (or about 2 million eggs per day), while the adult’s lifespan is estimated at more than 35 years.

Taenia solium

Family :	**Class :**
Taeniidae	Cestodes

Geographic distribution :

Cosmopolitic; rare in countries where no pork is eaten (moslims) or where systematic veterinary controls are conducted

Common name :

Armed tapeworm
Pork tapeworm

Disease :

- NL : teniasis, (neuro)cysticercose
- FR : téniase, (neuro)cysticercose
- EN : pork tapeworm infection, cysticercosis
- ES : teniasis, cisticercosis

Final host :

- Man

Intermediate host :

- Pig
- Occasionally man, with very serious pathology (cysticercosis)
- Dog, cat, ovidae and primates

Transmission :

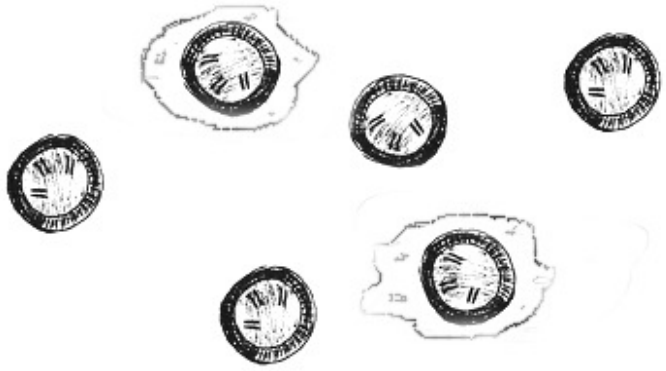
- By eating pork, contaminated with living cysticerci (*Cysticercus cellulosae*).
- By ingestion of the eggs (faeco-oral or by anti-peristaltism of mature segments) → cysticercosis

Localisation of the adult worm :

Attached to the inner wall of the small intestine

Diagnostic possibilities :

- Macroscopically (finding the segments of the tapeworm)
- Microscopically by finding the eggs in the faeces: - Direct examination
  - Concentration by sedimentation
- (The tape-test (for *Enterobius vermicularis*) can give an accidental diagnosis (eggs on peri-anal skin))
- (Searching copro-antigenes with dipsticks (good sensitivity, but no species-differentiation))
- (Serology: searching for specific antibodies by Western Blotting of serum or CSF (only in case of cysticercosis))
- (Searching for specific DNA by PCR on faecal extractions (with species-differentiation))
- Medical Imaging (only in case of cysticercosis)
- **CAUTION:** When manipulating segments or adult tapeworms, always proceed with lots of caution (risk of cysticercosis in case of *T. solium*). Manipulation of segments or adult tapeworms is best done using tweezers. ALWAYS wear protective gloves when manipulating segments or adult tapeworms.



Morphology of the eggs :

Dimensions : 31-43 µm

Aspect : round or oval

Shell : thick wall with radial structure

Contents : hexacanth larva (3 pairs of hooks, all of which are not always visible)

Colour : dark-brown

Characteristics : sometimes some sort of mucus (periovular membrane) can be observed around the egg

Associated biological signs :

- At first: moderate hypereosinophilia (± 20%)

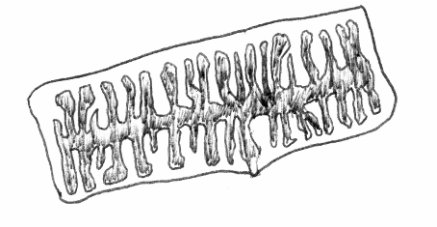
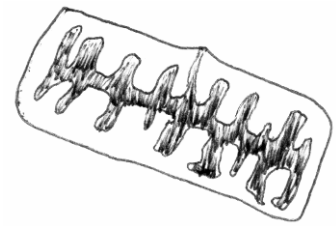
Possible confusion with :

- Air-bulbs
- *Taenia saginata*
- Different kinds of pollen-grains

Remarks :

- In french the tapeworm is sometimes called "ver solitaire" (solitary worm), because it was thought that a person could be infected with only 1 tapeworm at a time. It has been proven several times already that a patient can bear more than one tapeworm at a time however.
- The eggs of *T. saginata* and those of *T. solium* are morphologically identical. When one of these is found in a examination of faeces the most correct answer should be "*eggs of Taenia spp. found*".
- The difference between *T. saginata* and *T.* solium can be made by looking at mature segments or by looking at the scolex.
- Cysticerci in meat can remain alive for 8 months to one year, but can be killed by thorougly cooking the meat or by freezing it (at least 24h at –20°C). The eggs of *Taenia solium* can remain viable for at least 8 months.
- The first 5 to 12 weeks after infestation no segments or eggs will be found in the faeces (however, at this moment there will be a hypereosinophilia). The microscopic examination (for eggs) of the faeces usually stays negative (because usually intact segments are excreted with the faeces).
- In cases of cysticercosis cysticerci can be found in:
  - brain-tissue (cerebral) in 40% of the cases → hypereosinophilia
  - other cases are ocular (with moderate hypereosinophilia), muscular or sub-cutaneous (most of the time asymptomatic).
- Serological tests for cysticercosis can be negative, especially in cases of older infestations. Their sensitivity is about 90%, while having a specificity of about 97%.
- Each mature segment contains about 100.000 eggs. One single tapeworm (counting as many as 2000 segments) can produce up to 600 million eggs each year, while the adult's lifespan is estimated at more than 25 years.

Differential diagnosis of T. saginata / T. solium

Taenia saginata	***Taenia solium***
Scolex : Unarmed tapeworm  Diameter: 1-2 mm 4 suckers No rostrum No hooks	**Scolex :** Armed tapeworm  Diameter: 1 mm 4 suckers Rostrum with hooks
Length of the adult worm : Average of 5 to 10 m	**Length of the adult worm :** Average of 3 to 5 m
Mature segment :  Very motile at excretion. 15 to 32 fine, unilateral ramifications. Length of each segment: 12-15 mm	**Mature segment :**  Almost immobile at excretion. 7 to 16 larger, unilateral ramifications. Length of each segment: 10-12 mm
Morphology of the cysticercus : *Cysticercus bovis* (in cow) in muscular tissue. No hooks. Usually few cysticerci present. 	**Morphology of the cysticercus :** *Cysticercus cellulosae* (in pig and man) in muscular tissue or in the central nervous system. Hooks present. Usually lots of cysticerci present. 

Diagnostic possibilities :

- Macroscopically (finding the mature segments of the tapeworm):
  - Very motile segment, excretion in between defecations: *Taenia saginata*
  - Less motile segment / not excreted in between defecations:
    Differentiation is made by counting the ramifications of the central uterus-canal. To visualise these ramifications, the segment is pressed between two glass slides and looked at in front of a light-source, if necessary, after treating it with acetic acid or glycerine. When treating the segments, always treat more than one segment in regard of the possible overlap (15-16) of ramifications.

CAUTION: When manipulating segments or adult tapeworms, always proceed with lots of caution (risk of cysticercosis in case of *T. solium*). Manipulation of segments or adult tapeworms is best done using tweezers. ALWAYS wear protective gloves when manipulating segments or adult tapeworms.

Hymenolepis nana

Family : Hymenolepididae

Class : Cestodes

Geographic distribution :

Cosmopolitic, but more frequent in:
- Eastern and Southern Europe
- India
- America
- Northern Africa

Common name :

Dwarf tapeworm

Disease :
- NL : hymenolepiasis
- FR : hyménolépiase
- EN : dwarf tapeworm infection, hymenolepiasis
- ES : himenolepiasis

Final host :
- Man
- (Rodents)

Intermediate host :

H. nana is the only tapeworm infecting man, which has NO intermediate host.

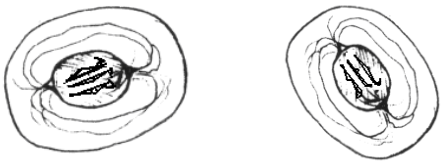
Vector: flour-worms ?

Transmission :
- Oral ingestion of the eggs.
- (Oral ingestion of flour-worms, infected with cysticercoid larvae)

Localisation of the adult worm :

Attached to the inner wall of the ileum

Diagnostic possibilities :
- Searching for eggs in faeces, using: - Direct examination
  - Concentration by sedimentation
- (Identification of the adult worm in faeces)



Morphology of the eggs :

Dimensions : 40-60 µm x 30-50 µm

Aspect : symmetrically oval or round

Shell : thin, smooth

Contents : embryophore (with 2 polar knobs and a few filaments) containing a hexacanth larva with well-visible hooks

Colour : transparent, colourless

Associated biological signs :
- Moderate hypereosinophilia

Possible confusion with :
- *Hymenolepis diminuta*
- *Hymenolepis fraterna*
- Pollen grains (barley)

Remarks :
- *Hymenolepis nana* is also known as *Vampirolepis nana*.
- The adult worm measures 1,5 to 4,0 cm, has 4 suckers and a scolex with numerous hooks.
- Mature segments already decompose inside the intestine and thus will not be found in the faeces.
- *Hymenolepis fraterna* is a similar cestode. Its final hosts are mainly rodents, but sometimes man can also be the final host. It needs an intermediate host however (fleas, cockroaches, flour-worms,...). The eggs of *H. fraterna* are morphologically identical to those of *H. nana.* When one of these eggs is found in a faeces sample the most correct answer should be "*eggs of Hymenolepis spp. found*".
- As only high levels of infestation will cause a pathology, concentration-techniques are of lesser importance.
- The possibility of auto-infestation (mainly with children) usually causes high levels of infestation, causing concentration-techniques to be less useful.
- The adult's lifespan is estimated at only a few months.
- Eggs appear in faeces about 20 days after initial infestation.

Hymenolepis diminuta

Family : Hymenolepididae

Class : Cestodes

Geographic distribution :

Cosmopolitic, but very rare (usually in regions with habitats in poor condition, where rodents have acces to food of man)

Common name :

Small tapeworm
Rat tapeworm

Disease :

- NL : hymenolepiasis
- FR : hyménolépiase
- EN : rat tapeworm infection hymenolepiasis
- ES : himenolepiasis

Final host :

- Rodents
- Man (zoonosis)

Intermediate host :

All kinds of arthropodes :

- Flea of rats
- Flour-worms
- Dung-beetles
- …

Transmission :

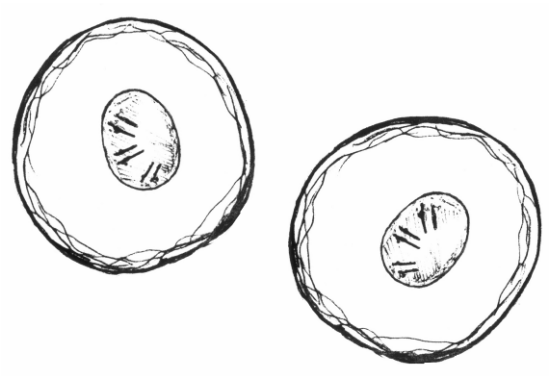
Accidental ingestion of insects, infested with cysticercoid larvae of *H. diminuta*.

Localisation of the adult worm :

Attached to the inner wall of the jejunum.

Diagnostic possibilities :

- Searching for eggs in faeces, using:
  - Direct examination
  - Concentration by sedimentation
  - (Concentration by flottation)
- (Identification of the adult worm in faeces)



Morphology of the eggs :

Dimensions : 70-86 µm x 60-80 µm

Aspect : usually round

Shell : rather thick and smooth

Contents : embryophore without polar knobs, containing a hexacanth larva with well-visible hooks; between the embyophore and the shell a filamentous mass can be observed, pressed against the outer wall.

Colour : brown to brown-yellow

Associated biological signs :

- Moderate hypereosinophilia

Possible confusion with :

- *Hymenolepis nana*
- *Hymenolepis fraterna*
- Spores of laurel and crocus

Remarks :

- Mature segments usually dissolve inside the intestine and hence will not be found in the faeces.
- The adult worm has a length of about 20 to 60 cm, has 4 suckers and does not possess hooks.
- Untill 2005, worldwide only less than 100 cases of infestation with *H. diminuta* were reported.
- Eggs appear in faeces about 20 days after initial infestation.
- The adult's lifespan is estimated at about 5 to 7 weeks.

Echinococcus granulosus

Family :	**Class :**
Taeniidae	Cestodes

Geographic distribution :

Cosmopolitic, especially in regions with lots of ovidae and bovidae.

Common name :

Hydatic cyst

Disease :

- NL : echinococcosis, hydatidose
- FR : échinococcose, hydatidose
- EN : echinococcosis, hydatidosis, hydatid disease
- ES : hidatidosis

Final host :

- Carnivores, **except man**

Intermediate host :

- Herbivores (especially sheep)
- Cow, horses,…
- **Man**

Transmission :

By ingestion of eggs (contact with carnivores (e.g. dog) or with contaminated food).

Localisation of the adult worm :

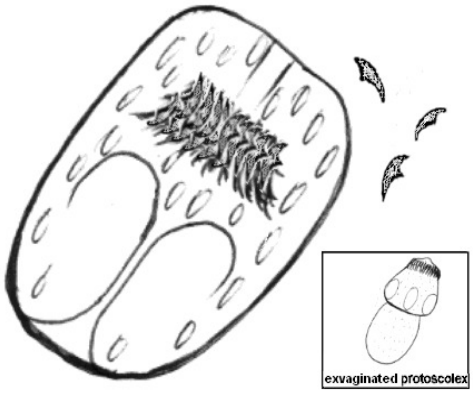
In the intestinal lumen of different kinds of carnivores (**except humans**).

Localisation of the hydatic cyst :

Usually hepatic (75%), sometimes pulmonary (10%), muscular (5%), splenic (3%), osseous (2%), renal (2%) or in other organs (3%).

Diagnostic possibilities :

- Clinical picture.
- Medical imaging.
- Immunological diagnosis (serology):
  - indirect immunofluorescence (IFAT) [screening]
  - Enzyme ImmunoAssay (EIA) [screening]
  - Indirect hemagglutination (IHA) [screening]
  - confirmation-test: immunoblot (arc 5 in double diffusion)
- (Identification of protoscolices and/or typical hooks in punction-fluid. The punction-fluid is clear; the hydatic sand can contain many protoscolices and/or liberated hooks.)



Morfologie van de scolices en haakjes :

Dimensions : protoscolex: ±170 µm x ±110 µm
hooks: ±20 µm

Aspect protoscolex: oval to round

Contents protoscolex: large number of hooks are clearly visible

Colour protoscolex: colourless, transparent

Associated biological signs :

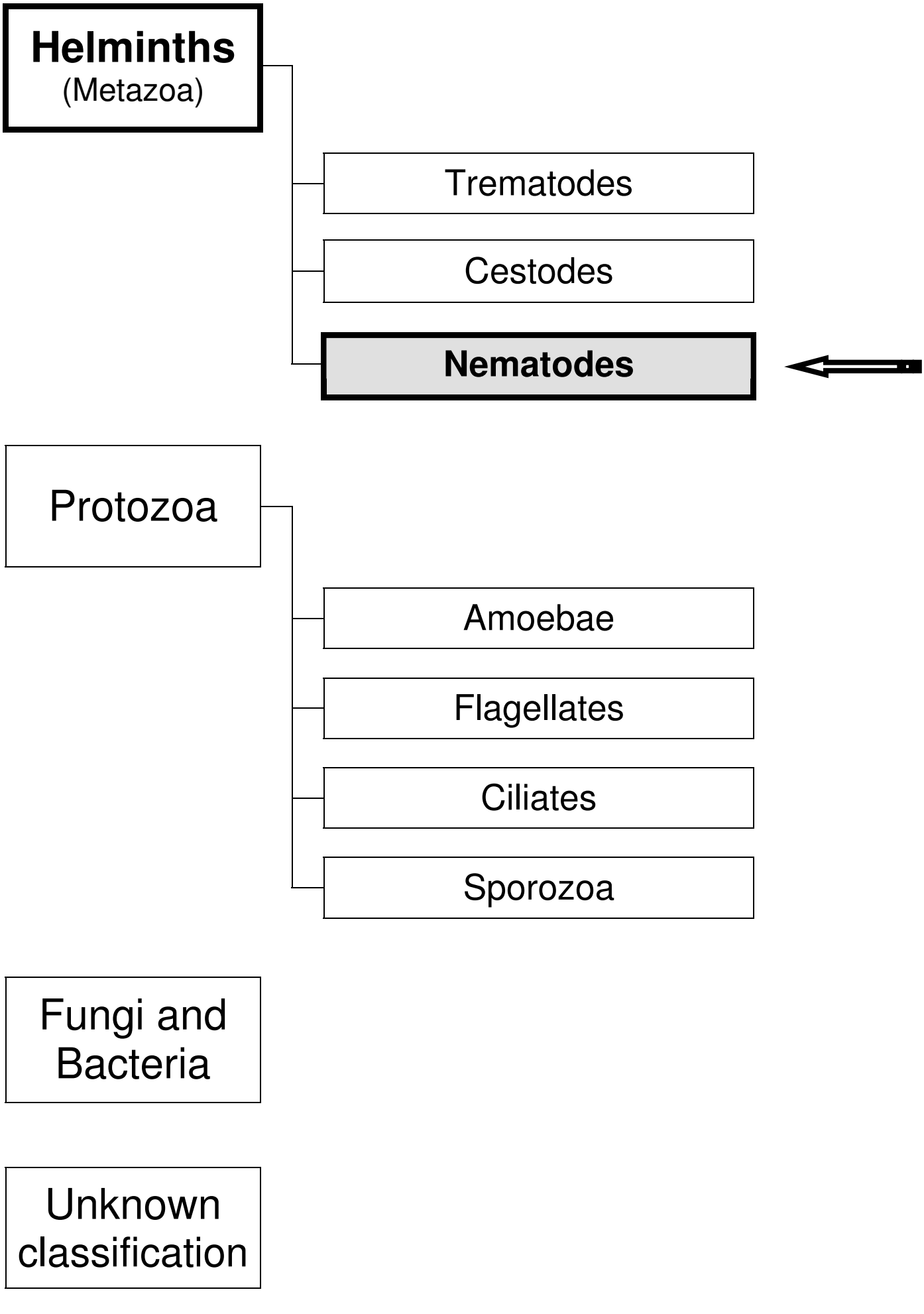
- Strong hypereosinophilia (especially in early stage)

Possible confusion with :

- other echinococcoses

Remarks :

- **Punction of hydatic cysts is NOT INDICATED.** By doing this, hydatic fluid with liberated protoscolices can disseminate and cause new hydatic cysts elsewhere in the body. Punction of hydatic cysts can also cause anaphylactic shock.
- Eggs of *Echinococcus* spp. are never found in human stools (eggs of *Echinococcus* spp. are morphologically identical to those of *Taenia* spp.).
- Hydatic cysts of *E. multilocularis* usually do not contain protoscolices (sterile cysts).
- Negative results in serology do not exclude echinococcosis (sensitivity of about 90%): indetectable antibodies, depending on the localisation, the integrity and the viability of the larvae. The sensitivity of serology is best in cases of hepatic or osseous localisations. Patients with calcified, dead or degenerating cysts are often seronegative.
- Serology for echinococcosis can be falsly positive in cases of infestations by other helminths, in cases of cancer and in cases of chronical immunological disorders. 5 to 25% of neurocysticercosis cases (*Taenia solium*) give positive serological reactions for echinococcosis. The same counts for cases of infestation with *Echinococcus multilocularis, Echinococcus vogeli* and *Echinococcus oligarthrus*.
- Other echinococcoses with almost identical life-cycles can (in rare cases) infest humans:
  - *E. multilocularis* (alveolar echinococcosis; northern hemisphere; fox tapeworm, usually with rodents as intermediate host (sometimes humans: 8 cases in Belgium between 1998 and 2005); serious pathology, usually hepatic localisation, more exceptionally pulmonary, renal or cerebral localisation).
  - *E. vogeli* (poly-cystic echinococcosis; Central- and South-America; tapeworm of wild dogs; intermediate host usually rodents (sometimes humans); usually hepatic or pulmonary localisations).
  - *E. oligarthrus* (extremely rare echinococcosis; Central- and South-America; cat tapeworm, usually rodents as intermediate host (sometimes humans); variable localisations possible).


Helminths
(Metazoa)
Trematodes
Cestodes
Nematodes
Protozoa
Amoebae
Flagellates
Ciliates
Sporozoa
Fungi and Bacteria
Unknown classification

Ascaris lumbricoides

Superfamily : Ascaridoidea

Class : Nematodes

Geographic distribution :
Cosmopolitic

Common name :
Roundworm

Disease :
- NL : spoelworminfectie
- FR : ascaridiase
- EN : giant roundworm infection, ascariasis
- ES : ascaridiasis

Final host :
- Man
- Pigs
- Other animals ?

Intermediate host:
~~Without intermediate host and without vector~~

Transmission :
Oral ingestion of ***embryonated eggs***

Localisation of the adult worm :
In lumen of small intestine

Diagnostic possibilities :
- Searching for eggs in faeces, using: - Direct examination
  As the number of produced eggs usually is very high, concentration-techniques usually are unnecessary.
- Macroscopic identification of adult worms in vomit or faeces.
- Radiological examination can reveal infestations with only male worms.
- Serology (detection of specific Ab) will be positive during the phase of larval migration or in cases of infestation with only male worms.

Morphology of the fertile eggs:

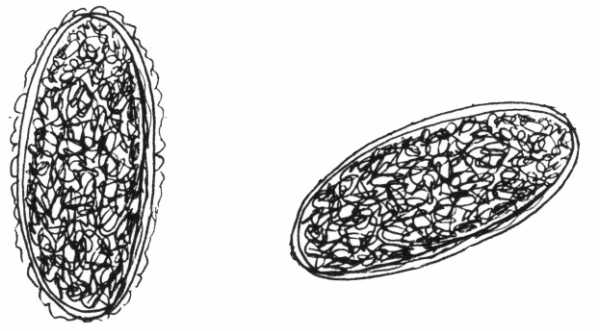
Dimensions : 45-84 µm x 35-58 µm

Aspect : oval or round

Shell : Thick, smooth wall, usually with bumpy aspect, caused by albumine-coating

Contents : usually one single round and granular cell (zygote)

Colour : brown-yellow (when the albumine-coating is absent, the colour will be lighter and the egg will be more transparent)



Morphology of the non-fertile eggs:

Dimensions : 78-105 µm x 38-55 µm

Aspect : oval, sometimes irregular shape

Shell : Thin, smooth wall, usually with bumpy aspect, caused by albumine-coating

Contents : homogenous, granular mass

Colour : brown-yellow (when the albumine-coating is absent, the colour will be lighter and the egg will be more transparent)

Associated biological signs :
- Strong hypereosinophilia during migration-phase, moderate hypereosinophilia later on (Lavier's curve)
- Hyperleucocytosis

Possible confusion with :
- *Ascaris suum* (pig's roundworm)
- Fasciolidae
- *Schistosoma japonicum/S. mekongi*
- Plant cells and spores
- Pollen grains of saffran (*Carthamus tinctorius*),…
- …

Remarks :
- When body temperature is fluctuating (fever, death) or in case of stress (e.g. medication) the adult worms (usually the male ones) leave the body of the host via anus, mouth or nose.
- *A. suum* can infest as well pig as man, but will only rarely become adult in man (usually causes visceral *larva migrans*).
- Considering its great morphological variability and its strong resemblance to a variety of vegetal structures, the egg of *Ascaris* spp. is considered to be the most difficult to identify.
- Sometimes the larvae of *Ascaris* spp. can be found in the sputum (migration of larvae). Examination of sputum however is not the best technique for diagnosis.
- The eggs of *A. lumbricoides* need a certain period of time for "*maturation*" in the external environment before becoming infectious. This period of time depends on climatological factors (temperature, humidity,...) but is at least 18 days.
- The adult's lifespan is estimated at about 1 year, sometimes up to 3 years. The production of eggs starts about 2 to 3 months after initial infestation. One single female can contain up to 27 million eggs and produces up to 200.000 eggs per day.

Toxocara canis

SuperFamily : Ascaridoidea

Class : Nematodes

Geographic distribution :

Cosmopolitic

Common name :

Dog roundworm

Disease :

- NL : viscerale of oculaire *larva migrans*, toxocarose
- FR : *larva migrans* viscérale ou oculaire, toxocarose
- EN : visceral or ocular *larva migrans,* toxocariasis
- ES : *larva migrans* visceral o ocular, toxocariasis

Final host :

- Dogs
- Man (no development to adult worms)
  → paratenic visceral *larva migrans*

Intermediate host:

Without intermediate host and without vector

Transmission :

Oral ingestion of ***embryonated eggs***

Localisation of the larvae :

Blood and tissues (no development to adult worms)

Diagnostic possibilities :

- Clinical picture
- Serology: searching for specific antibodies in blood
- (Finding larvae in the eye, using an ophthalmoscope → accidental diagnosis)

Morphology of the eggs :

Dimensions :

Aspect :

Shell :

Contents :

Colour :

Associated biological signs :

- Hypereosinophilia

Possible confusion with :

- Other migrating larvae (*Ancylostoma* spp., *Anisakis* spp.,...)
- *Larva currens* (*Strongyloides stercoralis)*

Remarks :

- **As the larvae do not develop to adult worms in human infestations, eggs are never found in human faeces.** If other eggs (e.g. those of *Ascaris lumbricoides, Trichuris trichiura,...*) are found in a patient's faeces, this indicates exposure to faecal material, increasing the probability of visceral *larva migrans* in case of clinical suspicion.
- *Toxocara cati* (cat roundworm) and *Baylisascaris procyonis* (racoon roundworm) also can cause *larva migrans* in humans, however more exceptional.
- Serology for *Toxocara* spp. is used only to confirm clinical suspicion (due to cross-reactivity). Serological tests, based on TES (Toxocara Excretory-Secretory antigens) offer high specificity, but cross-reactions with other nematodes are not rare. Evaluation of sensitivity and specificity of these serological tests continues to be hard due to the lack of parasitological methodes to confirm *Toxocara*-infestations (absence of a *Golden Standard*).

Anisakis spp.

SuperFamily : Ascaridoidea

Class : Nematodes

Geographic distribution :

Cosmopolitic
(consumption of raw fish)

Common name :

Herringworm

Disease :

- NL : anisakiase
- FR : anisakiase, anisakidose larvaire
- EN : anisakiasis, herringworm disease
- ES : anisakiasis

Final host :

- Aquatic mammals (seal,...)

Intermediate host :

1st: Crustaceans

2nd: Saltwaterfish (**Man** →paratenic)

Transmission :

By ingestion of fish, contaminated with ***living*** L3-larvae (macroscopically visible) (herring, sardine, mackerel,...)

Localisation of the larvae :

Parasitological deadlock (unless the larvae are excreted with the faeces).

Diagnostic possibilities :

- Clinical picture
- Serology: searching for specific antibodies in blood
- (Finding the larvae in tissues (endoscopy, gastroscopy, biopsy) → accidental diagnosis)

Morphology of the eggs :

Dimensions :

Aspect :

Shell :

Contents :

Colour :

Associated biological signs :

- Hypereosinophilia

Possible confusion with :

- Other migrating larvae (*Ancylostoma* spp., *Toxocara* spp.,...)
- *Larva currens* (*Strongyloides stercoralis)*

Remarks :

- **As the larvae do not develop to adult worms in human infestations, eggs are never found in human faeces (paratenic host).**
- Serology for anisakiasis is not very sensitive, however it is still the most important means of diagnosis.
- Larvae of *Anisakis* spp. can survive low temperatures (-5°C) also pickling and smoking (up to 50°C) does not kill the larvae!!!
- Freezing at -20°C during at least 24h does kill the larvae.
- *Anisakis simplex* and *Anisakis marina* are only sporadically found in the Netherlands, as they systematically and sufficiently freeze herring.
- *Contracoecum* spp., *Phocanema* spp. and *Pseudoterranova* spp., also causing anisakiasis, are still regularly found in Japan, but also in the United States of America, in Chili, in the United Kingdom, in Germany and in Belgium.

Enterobius vermicularis

SuperFamily : Oxyuroidea

Class : Nematodes

Geographic distribution :

Cosmopolitic, however more frequently in temperate climates

Common name :

Pinworm

Disease :

- NL : oxyuriase
- FR : oxyurose
- EN : pinworm infection, enterobiasis
- ES : enterobiasis, oxiuriasis humana

Final host :

- Man

Intermediate host:

Without intermediate host and without vector

Transmission :

Oral ingestion of ***embryonated eggs***

Localisation of the adult worm :

In lumen of caecum and adjacent parts.

Diagnostic possibilities :

- Searching for eggs, using the tape-test.
- Finding the adult females on the peri-anal skin (usually at night).
- (Finding the eggs in a faecal sample (in direct examination or after concentration by sedimentation). Examination of faeces, searching for eggs is not very efficient.)

Morphology of the eggs :

Dimensions : 50-60 µm x 20-32 µm

Aspect : asymmetrically oval

Shell : double and smooth

Contents : embryo present (granular mass or larva)

Colour : colourless and transparent

Associated biological signs :

- Moderate, slightly oscillating hypereosinophilia

Possible confusion with :

- Adult females can be confused with food-remnants (plant-fibers etc.), pieces of toilet-paper,…
- Air-bulbs in a tape-test can be confused with eggs

Remarks :

- Adult females of *E. vermicularis* have a length of about 8 to 15 mm.
- A tape-test can also reveal eggs of *Taenia* spp.
- Sometimes the eggs can be found in the urine-sediment of little girls (vaginal ectopical localisation).
- Auto-infestation is quite frequent (especially in children and psychiatric patients).
- As the eggs are placed on the peri-anal skin, they usually aren't found in the faeces.
- The tape-test sometimes is called Scotch-Test or Graham's Test.
- The tape-test is best performed in the morning before making one's toilet and before defecation.
- If the tape-test is performed 3 times in one week, one can reach a sensitivity of 95%.
- *E. grigorii* has been described in Europe, Asia and Africa, but its morphology, its life-cycle, the clinical aspect and the treatment of *E. grigorii* are identical to that of *E. vermicularis*. The difference between both can only be made by using molecular techniques (PCR).
- Development into adult takes about 3 weeks time and does not include any migration-phase.
- The adult's lifespan is estimated at less than 55 days.
- One single female can contain up to 10.000 eggs and produces about 500 eggs per day.

Trichuris trichiura

Superfamily : Trichuroidea

Class : Nematodes

Geographic distribution :

Cosmopolitic, more frequent in tropical and sub-tropical countries.

Common name :

Whipworm

Disease :

- NL : trichuriasis, zweepworm infectie
- FR : trichocéphalose, trichuriase
- EN : whipworm infection, trichuriasis
- ES : tricocefalosis

Final host :

- Man

Intermediate host:

Without intermediate host and without vector

Transmission :

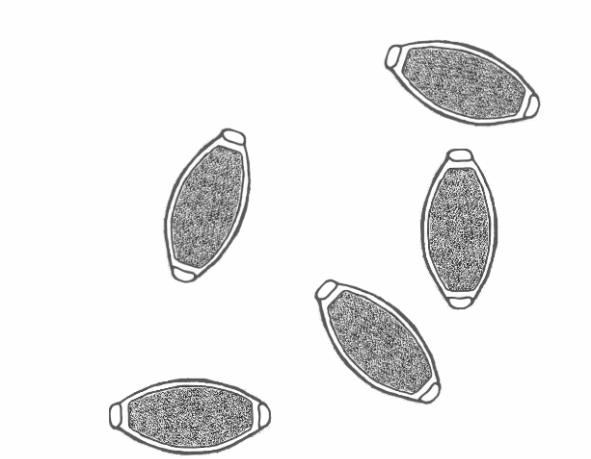
Oral ingestion of ***embryonated eggs***

Localisation of the adult worm :

Intestine
(in mucosa of caecum, appendix and large intestine)

Diagnostic possibilities :

- Searching for eggs in faeces, using: - Direct examination
  - Concentration by sedimentation
- Macroscopic: Sometimes the very typical adult worms can be found on/in the faeces.



Morphology of the eggs :

Dimensions : 49-65 µm x 20-29 µm

Aspect : lemon-shaped

Shell : thick, smooth, brown-yellow wall

Contents : homogenous, granular mass

Colour : orange-brown

Characteristics : two plugs; colourless, transparent and rounded polar knobs

Associated biological signs :

- Hypereosinophilia (± 15%)
- Especially in children, a high wormload can cause a ferriprivic, hypochromic anaemia

Possible confusion with :

- Eggs of *Capillaria* spp.
- *Trichuris vulpis*
- Pollen grains (Iris)

Remarks :

- The adult worms, measuring between 3 en 5 cm, are typically "whip"-shaped (the anterior part is thin and filamentous, while the posterior part is thick).
- Sometimes eggs can be found with abnormal aspect and/or abnormal thickness of the wall and/or abnormal colour etc. This phenomenon is usually caused by anti-helminthics or confusion with eggs of *T. vulpis* (*Trichuris* of dogs→ passing eggs).
- The eggs of *T. trichiura* need a certain period of time for "*maturation*" in the external environment before becoming infectious. This period of time depends on climatological factors (temperature, humidity,...) but lies between 10 days and several months.
- Counting the number of eggs (e.g. expressed in eggs per gram (EPG)) can be useful, because the pathology often depends on the degree of infestation.
- Some persons which are sensitive to this infection, already develop a pathology at low degrees of infestation.
- Development into adult entirely takes place in the intestinal lumen, not incuding any migration-phase.
- The adult's lifespan is estimated at 1 to 4 years (some sources mention lifespans up to 20 years).
- Eggs appear in faeces about 30 to 90 days after initial infestation.
- Eggs produced per female: 3.000 to 20.000 eggs per day.

Capillaria hepatica

SuperFamily : Trichuroidea

Class : Nematodes

Geographic distribution :

Cosmopolitic.

Common name :

Disease :

- NL : lever-capillariase
- FR : capillariose hépatique
- EN : hepatic capillariasis
- ES : capillariasis hepatica

Final host :

- Rodents
- Other animals
- Sometimes man (zoonosis)

Intermediate host :

None
(carnivore only serves to **diperse** the eggs)

Transmission :

Oral ingestion of ***embryonated eggs***

Localisation of the adult worm :

Parenchymal cells of the liver.

Diagnostic possibilities :

- Detection of eggs (or worms) in biopsy of the liver (in case of liver-infestation).

Morphology of the eggs :

Dimensions : 50-60 µm x 20-30 µm

Aspect : oval

Shell : Thick with usually a dense radial structure

Contents : homogenous, granular mass

Colour : dark brown-grey

Characteristics : two plugs; colourless, transparent polar knobs which stay **inside the shell**

Associated biological signs :

- hypereosinophilia
- Disturbed liver tests
- Anemia
- Hyperleukocytosis

Possible confusion with :

- Eggs of *Trichuris trichiura*
- Eggs of *Trichuris vulpis*
- Eggs of other *Capillaria* spp.

Remarks :

- The eggs of the different *Capillaria* spp. are morphologically identical: when one of these eggs are found, the most correct answer should be: *"Eggs of Capillaria spp. found"*.
- *Capillaria hepatica* is also known as *Hepaticola hepatica or Calodium hepaticum*.
- Eggs of *C. hepatica* which have been found in a faecal sample, are usually passing eggs (consumption of liver, infested with adult worms). After their passing, these eggs will develop outside the host's body into embryonated and infectious eggs. In this case, man serves as a carnivore to *disperse* the eggs.
- In cases of liver-infestation, the produced eggs will remain stuck in the host's liver. They will only get to the outer environment when the host dies (desintegration of the host's body) or when the host is consumed by a carnivore, causing the eggs to be passed in the external environment with the carnivore's faeces.
- The eggs of *C. hepatica* need a certain period of time for "*maturation*" in the external environment before becoming infectious. This period of time depends on climatological factors (temperature, humidity,...)
- Eggs appear in faeces about 2 to 3 weeks after initial infestation.
- The adult's lifespan is estimated at 1 to 4 months.
- Up to 2005, only about 100 cases have been reported in literature.

Capillaria philippinensis

SuperFamily : Trichuroidea

Class : Nematodes

Geographic distribution :

Endemic in the Philippines and Thaïland. Rare in Asia, Colombia and the Middle-East.

Common name :

Disease :

- NL : intestinale capillariase
- FR : capillariose intestinale
- EN : intestinal capillariasis
- ES : capillariasis intestinal

Final host :

- Fish-eating birds
- Sometimes man (zoonosis)

Intermediate host :

Freshwater-fish

Transmission :

Consumption of fish, infested with living larvae.

Localisation of the adult worm :

Lumen of small intestine.

Diagnostic possibilities :

- Detection of the eggs in faeces, using: - direct examination (zie p.2)
  - concentration by sedimentation (zie p.2)
- Macroscopically finding the adult worms in/on the faeces: ♂ : 2,3 – 3,2 mm
  ♀ : 2,5 – 4,3 mm

Morphology of the eggs :

Dimensions : 36-45 µm x 20-25 µm

Aspect : oval

Shell : Thick with usually a dense radial structure

Contents : homogenous, granular mass

Colour : dark brown-grey

Characteristics : two plugs; colourless, transparent polar knobs which stay **inside the shell**

Associated biological signs :

- Hypereosinophilia

Possible confusion with :

- Eggs of *Trichuris trichiura*
- Eggs of *Trichuris vulpis*
- Eggs of other *Capillaria* spp.

Remarks :

- *Capillaria philippinensis* is also known as *Paracapillaria philipinensis, Calodium philippinensis* or *Aonchotheca philippinensis*.
- The eggs of the different *Capillaria* spp. are morphologically identical: when one of these eggs are found, the most correct answer should be: *"Eggs of Capillaria spp. found"*.
- The adult females produce non-embryonated eggs in the intestinal lumen. Some of these eggs can however already embryonate inside the intestines, liberating infectious larvae. These larvae can cause an auto-infestation, resulting in very high parasitaemias.

Capillaria aerophila

SuperFamily : Trichuroidea

Class : Nematodes

Geographic distribution :

Cosmopolitic.

Common name :

Disease :

- NL : pulmonaire capillariase
- FR : capillariose pulmonaire
- EN : pulmonary capillariasis
- ES : capillariasis pulmonar

Final host :

- dogs, cat, fox, ...
- Sometimes man (zoonosis)

Intermediate host :

None

(earthworm???
→ only serves to **diperse** the eggs**?**)

Transmission :

Oral ingestion of ***embryonated eggs***

Localisation of the adult worm :

Lumen of the trachea and bronchia.

Diagnostic possibilities :

- Detection of the eggs in faeces, using:
  - direct examination (zie p.2)
  - concentration by sedimentation (zie p.2)
- Identification of adult worms in biopsy of the lungs.
- (Identification of the eggs in sputum.)

Morphology of the eggs :

Dimensions : 50-60 µm x 20-30 µm

Aspect : oval

Shell : Thick with usually a dense radial structure

Contents : homogenous, granular mass

Colour : dark brown-grey

Characteristics : two plugs; colourless, transparent polar knobs which stay **inside the shell**

Associated biological signs :

- Hypereosinophilia

Possible confusion with :

- Eggs of *Trichuris trichiura*
- Eggs of *Trichuris vulpis*
- Eggs of other *Capillaria* spp.

Remarks :

- *Capillaria aerophila* is also known as *Eocoleus aerophilus*.
- The eggs of the different *Capillaria* spp. are morphologically identical: when one of these eggs are found, the most correct answer should be: *"Eggs of Capillaria spp. found"*.
- The eggs of *C. aerophila* need a certain period of time for "*maturation*" in the external environment before becoming infectious. This period of time depends on climatological factors (temperature, humidity,...)
- The adult's lifespan is estimated at about 1 year.
- Up to 2005, less than 100 cases have been reported in literature.

Trichinella spp.

SuperFamily : Trichuroidea

Class : Nematodes

Geographic distribution :

Cosmopolitic
(except in Australia)

Common name :

Trichine

Disease :

- NL : trichinose
- FR : trichinose, trichinellose
- EN : trichinosis, trichinellosis
- ES : triquinosis

Final host :

- Mammals
- (Man)

Intermediate host:

Without intermediate host
and
without vector

Transmission :

Ingestion of meat, infested with living larvae.

Localisation of the adult worm :

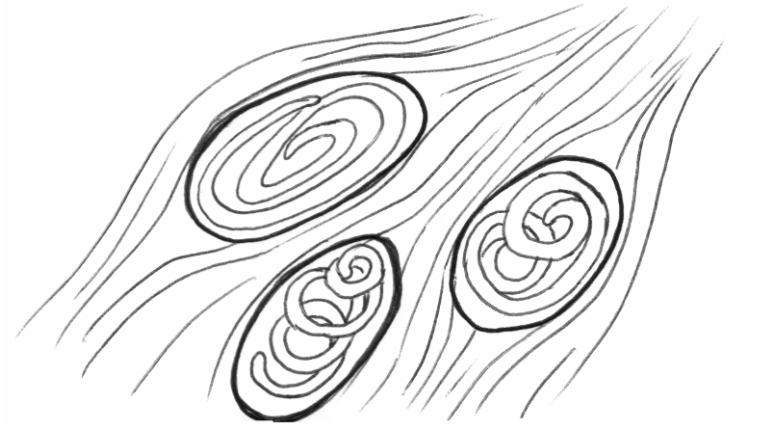
Mucosa of the small intestine

Localisatie larve :

Encapsulated in striated muscles

Diagnostic possibilities :

- (Suggestive) clinical picture.
- Serology (detection of specific Ab against ES-Ag)
- Detection of typical cysts in biosies of striated muscle-tissue.
  The biopsy is pressed between two glass slides and examined at low magnification (40x or 100x total magnification). Staining is not necessary.
  Concentration can be useful (see p.2).
- (Identification of adult worms or larvae in faeces)



Morphology of the cysts :

Dimensions : 400-500 µm x 250 µm

Aspect : oval to lemon-shaped, parallel with muscular fibres

Contents : rolled-up larva

Characteristics : calcification possible

Associated biological signs :

- Hypereosinophilia (especially in migrational phase)
- Elevated muscular enzymes in serum (CK, LDH, aldolase)

Possible confusion with :

- Other *Trichinella* spp.

Remarks :

- Muscular trichines are killed by:
  - pickling: during at least 6 weeks in 19% NaCl
  - heat: frying, coocking, etc. (during at least 10 minutes at 80°C)
  - cold: during at least 10 days at -18°C
- The adult worm (1-4 mm x 60-75 µm) or the larvae (80-120 µm) can exceptionally be found in the faeces. The adult's life span in the intestinal mucosa is limited (maximum 4 months). During this period the female worms produce 1000 to 2000 larvae which migrate to the striated muscles and encapsulate by "nurse cell"-forming, actively modifying the host cell to their proper needs and transforming the cellular membrane.
- *T. spiralis* (cosmopolitic, mammals and omnivores) is usually responsible for infestations of man. Other *Trichinella* spp. are however also found in man: *T. pseudospiralis* (cosmopolitic, mammals and birds), *T. nativa* (polar bears), *T. nelsoni* (african predators), *T. britovi* (carnivores in Europe and Western asia), ...
- The preferred serological tests are those based on TSL-1 ES-Ag as this Ag is found in all different *Trichinella* spp.
- Serology usually becomes positive 3 to 5 weekes after initial infestation.

Strongyloides stercoralis

SuperFamily : Rhabdiasoidea

Class : Nematodes

Geographic distribution :

Tropical and sub-tropical countries.
Also in temperate climates in certain circumstances (mining, tunnel-construction,…)

Common name :

Threadworm

Disease :

- NL : strongyloidosis
- FR : anguillulose
- EN : threadworm infection
  strongyloidiasis
- ES : estrongiloidiasis
  anguillulosis

Final host :

- Man
- Chimpansee
- Dog
- Cat

Intermediate host:

Without intermediate host
and
without vector

Transmission :

- In case of contact with ground or mud contaminated with infectious larvae, these larvae will penetrate the skin or the mucosa.
- Auto-infestation: trans-intestinal or trans-peri-anal

Localisation of the adult worm :

In mucosa of duodenum.

Diagnostic possibilities :

- Searching for eggs in faeces, using: - Direct examination
  - Concentration by sedimentation
- Sarching for rhabditiform (or filariform) larvae, using: - Direct examination
  - Concentration by sedimentation
  - **Baermann concentrationtechnique**
  - Agar-culture
- Serology (detection of specific Ab (ELISA) using Ag of filariform larvae)
- (Identification of the larvae in duodenal aspirate or in string-test (for *Giardia lamblia*))

Morphology :

The morphological aspects of the different stages have been brought together in a schematic overview.
In this overview, also the differential diagnosis with Ancylostomidae is given (see p.2).

Morphology of the rhabditiform larvae :

Dimensions : 200-300 µm x 16-20 µm

Mouth cavity : shallow (⅓ to ½ of the width of the larva)

Bulbus : present

Tail : pointed

Associated biological signs :

- Oscillating hypereosinophilia (going from 5% to 30%)
- Often presence of Charcot-Leyden crystals in faeces

Possible confusion with :

- Vegetal fibers and hairs
- Eggs and larvae of Ancylostomidae
- Eggs of *Trichostrongylus* spp.
- Eggs of *Ascaris* spp. (without albumine coating)
- Eggs of *Enterobius vermicularis*
- Eggs of *Acarina* spp. (mite)

Remarks :

- Strongyloidosis is a disease which can evolve quite seriously in case of immuno-suppression.
- The eggs of *S. stercoralis* usually already hatch inside the intestins, meaning that usually the **rhabditiform larvae** will be found in (fresh) faeces. Filariform larvae will only be found in faeces of at least several hours old or in case of auto-infestations.
- Eggs are only sporadically found in cases of serious diarrhoea and usually will contain an embryo.
- The adult's lifespan is rather limited, but the possibility of auto-infestation makes that an infestation with *S. stercoralis* can continue for as long as 30 years after leaving an endemic area. In cases of trans-peri-anal auto-infestation, the larvae, passing sub-cutaneously, will cause a typical *larva migrans*, called *larva currens.*
- Sometimes the larvae of *S. stercoralis* are found in a patient's sputum (migration of larvae). Examination of sputum however is not the best technique for diagnosis.
- Other *Strongyloides* spp. (*S. fuelleborni*) more common in chimpansees and baboons, can occur sporadically in humans, but with limited pathology. In these cases, usually eggs (with blastomeres) are found in (fresh) faeces.
- All other *Strongyloides* spp. (which usually occur in animals) can only develop to cutaneous *larva migrans* when infesting humans.
- Serology has a sensitivity of about 90%. When successfully treated, titers will drop significantly after 9 to 12 months.
- The larvae can be found in the patient's faeces about 2 to 3 weeks after initial infestation.

Ancylostoma duodenale

SuperFamily : Ancylostomatoidea

Class : Nematodes

Geographic distribution :

- Europe
- Northern-Africa
- Asia

Common name :

Hookworm

Disease :

- NL : mijnwormziekte
  ancylostomiasis
- FR : ankylostomiase
- EN : Old World hookworm infection
  ancylostomiasis
- ES : uncinariasis
  anaemia de los mineros

Final host :

- Man
- Pig
- Felidea, canidea
- Some monkey-species

Intermediate host:

Without intermediate host
and
without vector

Transmission :

In case of contact with ground or mud contaminated with infectious larvae, these larvae will penetrate the skin (or possibly the mucosa).

Localisation of the adult worm :

Attached to jejunum and duodenum.

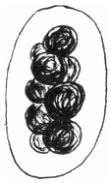
Diagnostic possibilities :

- Searching for eggs in faeces, using: - Direct examination
  - Concentration by sedimentation

  Sometimes (when the faeces cannot be examined immediately: >12h) the larvae (instead of the eggs) can be found in the faeces.

Morphology :

The morphological aspects of the different stages have been brought together in a schematic overview.
In this overview, also the differential diagnosis with *S. stercoralis* is given (see p.2).



Morphology of the eggs :

Dimensions :	60-80 µm x 35-40 µm
Aspect :	oval
Shell :	very thin and fine
Contents :	blastomeres (usually 4) in freshly produced faeces
Colour :	colourless and transparent with brown-greyish blastomeres

Associated biological signs :

- Hypereosinophilia (± 30%)
- Hypochromic, microcytic anaemia can occur in case of chronic infestation.
- Drop of iron in serum in case of chronic infestation

Possible confusion with :

- Vegetal fibers and hairs
- Eggs and larvae of *Strongyloides stercoralis*
- Eggs and larvae of *Necator americanus*
- Eggs of *Trichostrongylus* spp.
- Eggs of *Ascaris* spp. (without albumine coating)
- Eggs of *Acarina* spp. (mite)
- Eggs of *Heterodera* spp. (plant nematodes)

Remarks :

- The optimal temperature for the development of *A. duodenale* is about 21-27°C.
- As the eggs (and larvae) of *Ancylostoma duodenale, Ancylostoma ceylanicum* and *Necator americanus* are morphologically indistinguishable using an ordinary microscope, they are reffered to (in microscopic examinations as "eggs (or larvae) of Ancylostomidae".
- One should always be aware that in most cases the **eggs** (usually containing 4 blastomeres) are found in (fresh) faeces. Larvae are only found in faeces of more than 12h old.
- As a result of importation of parasites, geographic distribution is no longer a useful criterium for differentiation of *Ancylostoma duodenale* and *Necator americanus*.
- The consumption (and spill) of blood for *Ancylostoma duodenale* is estimated at about 0,2 ml per day per worm (0,02 ml for *Necator americanus*).
- Sometimes the larvae of *A. duodenale* are found in a patient's sputum (migrating larvae). Examination of sputum however is not the best technique for diagnosis.
- Other *Ancylostoma* spp. (which usually infest animals) can also infest humans. *A. ceylanicum* (hamsters) is morphologically and pathologically identical to *A. duodenale*. *A. caninum* (dog) and *A. braziliense* (cat) can only develop to cutaneous *larva migrans* when infesting humans.
- The eggs of *Oesophagostomum bifurcum* (a zoonotic parasite which can be found in Northern Togo and Ghana and which causes "multi-nodular disease") are morphologically identical to those of the Ancylostomidae.
- The adult's lifespan is estimated at 1 to 9 years.
- Eggs appear in faeces about 15 to 40 days after initial infestation.
- Eggs produced per female: 5.000 to 22.000 eggs per day.

Necator americanus

SuperFamily : Ancylostomatoidea

Class : Nematodes

Geographic distribution :

- America
- Africa
- China
- Indonesia
- Australia

Common name :

Hookworm

Disease :

- NL : mijnwormziekte
  ancylostomiasis
- FR : ankylostomiase
- EN : New World hookworm infection
  ancylostomiasis
- ES : uncinariasis
  anaemia de los mineros

Final host :

- Man

Intermediate host:

Without intermediate host
and
without vector

Transmission :

In case of contact with ground or mud contaminated with infectious larvae, these larvae will penetrate the skin.

Localisation of the adult worm :

Attached to jejunum and duodenum.

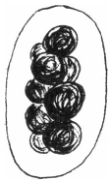
Diagnostic possibilities :

- Searching for eggs in faeces, using: - Direct examination
  - Concentration by sedimentation

  Sometimes (when the faeces cannot be examined immediately: >12h) the larvae (instead of the eggs) can be found in the faeces.

Morphology :

The morphological aspects of the different stages have been brought together in a schematic overview.
In this overview, also the differential diagnosis with *S.stercoralis* is given (see p.2).



Morphology of the eggs :

Dimensions :	60-80 µm x 35-40 µm
Aspect :	oval
Shell :	very thin and fine
Contents :	blastomeres (usually 8) in freshly produced faeces
Colour :	colourless and transparent with brown-greyish blastomeres

Associated biological signs :

- Hypereosinophilia (± 30%)
- (Hypochromic, microcytic anaemia can occur in case of chronic infestation)
- (Drop of iron in serum in case of chronic infestation)

Possible confusion with :

- Vegetal fibers and hairs
- Eggs and larvae of *Strongyloides stercoralis*
- Eggs and larvae of *Ancylostoma* spp.
- Eggs of *Trichostrongylus* spp.
- Eggs of *Ascaris* spp. (without albumine coating)
- Eggs of *Acarina* spp. (mite)
- Eggs of *Heterodera* spp. (plant nematodes)

Remarks :

- The optimal temperature for the development of *N. americanus* is about 25-35°C.
- As the eggs (and larvae) of *Ancylostoma duodenale, Ancylostoma ceylanicum* and *Necator americanus* are morphologically indistinguishable using an ordinary microscope, they are reffered to (in microscopic examinations as “eggs (or larvae) of Ancylostomidae”.
- One should always be aware that in most cases the **eggs** (usually containing 8 blastomeres) are found in (fresh) faeces. Larvae are only found in faeces of more than 12h old.
- As a result of importation of parasites, geographic distribution is no longer a useful criterium for differentiation of *Ancylostoma duodenale* and *Necator americanus*.
- The consumption (and spill) of blood for *Necator americanus* is estimated at about 0,02 ml per day per worm (0,2 ml for *Ancylostoma duodenale*).
- Sometimes the larvae of *N. americanus* are found in a patient’s sputum (migrating larvae). Examination of sputum however is not the best technique for diagnosis.
- The eggs of *Oesophagostomum bifurcum* (a zoonotic parasite which can be found in Northern Togo and Ghana and which causes “multi-nodular disease”) are morphologically identical to those of the Ancylostomidae.
- The adult’s lifespan is estimated at 4 to 20 years.
- Eggs appear in faeces about 15 to 40 days after initial infestation.
- Eggs produced per female: 3.000 to 6.000 eggs per day.

Strongyloides stercoralis / Ancylostomidae : overview

Strongyloides stercoralis

Egg containing larva :

50-76 µm
(usually 50-55 µm)

very rare

Rhabditiform larva :

< 1 hour

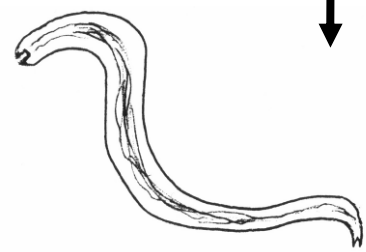
200-300 µm x 16-20 µm
(usually 225 x 18 µm)
Bulbus present
Shallow mouth-cavity
Pointed tail

Most common stage

Filariform larva :

several hours

500-550 µm x 20-24 µm
(usually 500 x 22 µm)
Bulbus absent
Shallow mouth-cavity
Bifurcated tail



Rare

Ancylostomidae

Egg containing blastomeres :

60-80 µm
(usually 60-65 µm)

most common stage

Rhabditiform larva :

± 12 hours

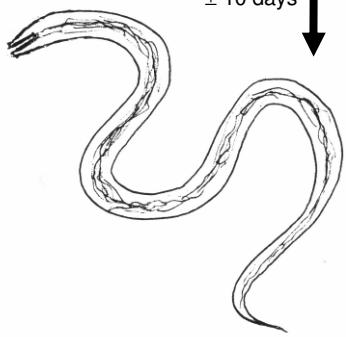
200-300 µm x 14-17 µm
(usually 250 x 16 µm)
Bulbus present
Deep mouth-cavity
Pointed tail

Rare

Filariform larva :

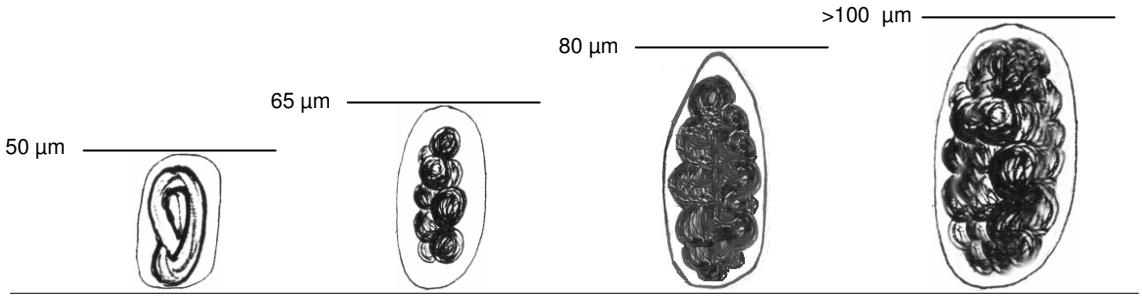
± 10 days

500-700 µm x 20-24 µm
(usually 700 x 22 µm)
Bulbus absent
Deep mouth-cavity
Pointed tail



very rare

Comparison :



Egg of *Strongyloides stercoralis* Egg of Ancylostomidae Egg of *Trichostrongylus* spp. Egg of *Acarina* spp. (mite)

Trichostrongylus spp.

SuperFamily : Trichostrongyloidea

Class : Nematodes

Geographic distribution :

Cosmopolitic

Common name :

Disease :

- NL : trichostrongylosis
- FR : trichostrongylose
- EN : trichostrongyliasis
- ES : trichostrongiliasis

Final host :

- Ovidae, goat
- Bovidae
- Camel-like animals, horse, pig
- Sometimes man (zoönose)

Intermediate host:

Without intermediate host and without vector

Transmission :

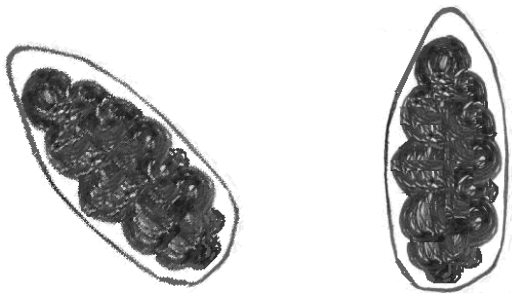
Oral ingestion of the filariform larvae.

Localisation of the adult worm :

Intestinal lumen

Diagnostic possibilities :

- Detection of the eggs in faeces, using : - Direct examination
  - Concentration by sedimentation



Morphology of the eggs :

Dimensions : 79-100 µm x 40-50 µm

Aspect : egg-shaped; one pole more stretched out than the other

Shell : thin and smooth

Contents : usually a lot of blastomeres; sometimes a morula

Colour : colourless and transparent with grey to brown-grey blastomeres

Associated biological signs :

- Hypereosinophilia
- (Hypochromic, microcytic anaemia can occur in case of chronic infestation)
- (Drop of iron in serum in case of chronic infestation)

Possible confusion with :

- Eggs of *Strongyloides stercoralis*
- Eggs of *Necator americanus*
- Eggs of *Ancylostoma* spp.
- Eggs of *Ascaris* spp. (without albumine coating)
- Eggs of *Acarina* spp. (mite)

Remarks :

- After (oral) infestation the larvae do not migrate to the lungs. The larvae develop to adult worms inside the intestinal lumen.
- All species of this superfamily, infecting man, are zoonoses. Some of them infest humans but sporadically, others are responsible for large scale infestations.

Onchocerca volvulus

SuperFamily : Filarioidea

Class : Nematodes

Geographic distribution :

- West- and Central Africa
- Yemen
- Central America
- North-East Brasil
- North-East Venezuela

Common name : (Filaria)

Disease :

- NL : onchocercose (rivierblindheid)
- FR : onchocercose (cécité des rivières)
- EN : river blindness, onchocerciasis
- ES : oncocercosis

Final host :

- Man

Intermediate host:

(Vector)

Simulium spp.

Transmission :

Active penetration of the skin (or via the sting-wound) by infectious larvae, placed on the skin during the blood-meal of an infested vector.

Localisation of the adult worm :

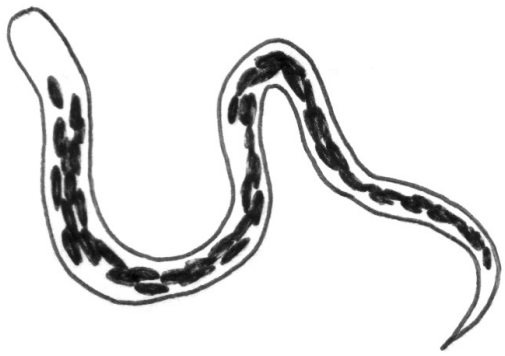
Sub-cutaneous tissues.
Free-living or sometimes multiple worms together, encapsulated in hard nodules (onchocercomes).

Localisation of the microfilaria :

Skin.

Diagnostic possibilities :

- Searching for microfilaria, using: - Skin-Snip (good screening-method)
  - Deep scarification
- Mazzotti's Test (this pathognomic test, using DEC, is not indicated for reasons of possible allergic reactions)
- Searching for adult worms in possibly present onchocercomes
  (Surgical intervention!!! Risk of allergic reaction!!!)
- Serology (searching for Ab (mostly IgE-type) in serum)
- Detection of specific DNA, using molecular techniques (PCR)
- Finding the microfilaria in the eye, using a split-lamp



Morphology of the microfilaria (Giemsa) :

Dimensions : 280-320 µm x 5-9 µm

Tail : Empty and pointed, usually curved

Head : long empty space (≈ 2x width of the larva)

Sheath : not present

Characteristics :
oval nuclei, usually not very clear

Associated biological signs :

- Persisting hypereosinophilia during infestation

Possible confusion with :

- Other microfilaria
- Zoonoses (*Dirofilaria* spp., *Microfilaria* spp.)
- Thread-like artefacts (cotton-threads etc.)
- Fungi (*Helicosporium*)
- Helminth-larvae during their migration-phase

Remarks :

- When performing a Mazzotti'sTest (using DEC, NOT using ivermectine) the microfilaria can sometimes be found in the blood and even in urine.
- *Onchocerca volvulus* is an aperiodical microfilaria.
- Deep scarifications always contain a small amount of blood, which explains the possibility of finding blood-microfilaria in such a preparation.
- Serological tests do not discriminate between the different species of microfilaria. Also cross-reactivity with other nematodes and with *E. granulosus* exists.
- It takes at least 6 months for the larvae to become adult worms. The adult's lifespan is estimated at more than 15 years. The microfilaria can survive up to 2 years in the skin.

Loa Loa

SuperFamily :	**Class :**
Filarioidea	Nematodes

Geographic distribution :

- Tropical rain-forests, mostly of Western- and Central Africa

Common name :

(Filaria)

Disease :

- NL : loasis
- FR : loase
- EN : eye worm disease, loiasis
- ES : loasis

Final host :

- Man
- Primates

Intermediate host:

(Vector)

Horse-flies
(*Chrysops* spp.)

Transmission :

Active penetration of the skin (or via the sting-wound) by infectious larvae, placed on the skin during the blood-meal of an infested vector.

Localisation of the adult worm :

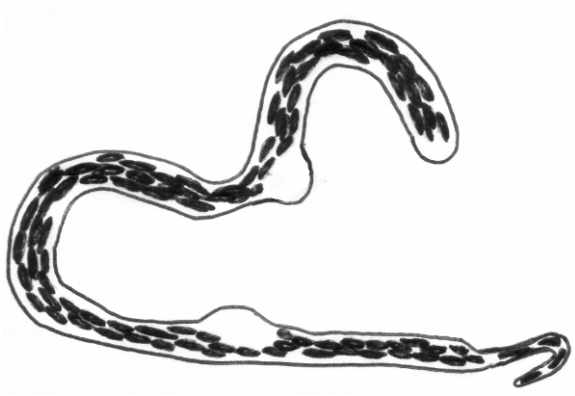
Sub-cutaneous tissues and conjunctiva.

Localisation of the microfilaria :

Blood.

Diagnostic possibilities :

- Examination of blood: Direct examination (not very efficient; no identification possible!)
  Thick blood smear: - Giemsa staining is a good technique, but the sheath usually doesn't take up the colour.
  - Carazzi staining is better, but more complex.
  Woo-technique: good concentration-technique (no identification possible!)
  KNOTT-method: very efficient concentration-method.
  After filtration onto a membrane, followed by staining (epidemiological purposes).
- Sometimes the adult worm (up to 7 cm long) passes under the conjunctiva of the eye ("accidental diagnosis").
- Serology (searching for Ab in serum)
- Detection of specific DNA, using molecular techniques (PCR)



Morphology of the microfilaria (Giemsa):

Dimensions : 230-250 µm x 5-7 µm

Tail : stretched, blunt tail, often folded

Head : small empty space

Sheath : present, but doesn't always stain with Giemsa.

Characteristics :
Oval shaped nuclei, filling the tail.
Two hernia-like saggings at about 1/3 and 2/3 of its body-length.

Associated biological signs :

- Persisting hypereosinophilia during infestation

Possible confusion with :

- Other microfilaria
- Zoonoses (*Dirofilaria* spp., *Microfilaria* spp.)
- Thread-like artefacts (cotton-threads etc.)
- Fungi (*Helicosporium*)
- Helminth-larvae during their migration-phase

Remarks :

- The microfilaria mainly circulate during the day in the periferal bloodstream (bloodsample best taken between 11 and 16 o'clock).
- The presence of microfilaria in the blood can fluctuate a lot.
- The searching for the microfilaria themselves often yields negative results during the first years of infestation.
- Very rarely the microfilaria of *Loa Loa* can be found in urine-samples.
- Serological tests do not discriminate between the different species of microfilaria. Also cross-reactivity with other nematodes and with *E. granulosus* exists.
- It takes 6 to 12 months for the larvae to become adult worms. The adult's lifespan is estimated at 4 to 7 years (up to 25 years according to certain authors).

Wuchereria bancrofti

SuperFamily : Filarioidea

Class : Nematodes

Geographic distribution :

- All tropical and subtropical regions (except very dry regions)
- Turkey (rare)

Common name :

Bancroft's filaria

Disease :

- NL : wuchereriasis
- FR : wuchereriase
- EN : bancroftian filariasis, lymphatic filariasis
- ES : wuchereriasis, filariasis linfática

Final host :

- Man

Intermediate host:

(Vector)

Different kinds of mosquitoes: Family of *Culicidae* (*Culex* spp., *Mansonia* spp., *Anopheles* spp., *Aedes* spp.)

Transmission :

Active penetration of the skin (or via the sting-wound) by infectious larvae, placed on the skin during the blood-meal of an infested vector.

Localisation of the adult worm :

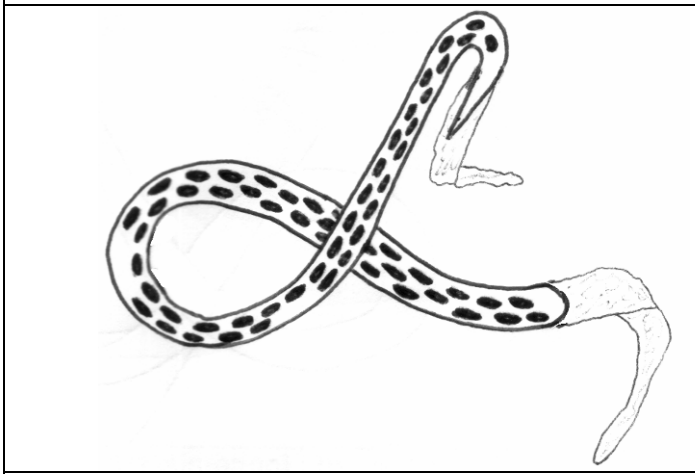
Lymphatic system.

Localisation of the microfilaria :

Blood.

Diagnostic possibilities :

- Examination of blood: Direct examination (not very efficient; no identification possible!)
  Thick blood smear: - Giemsa staining is a good technique, but the sheath usually doesn't take up the colour.
  - Carazzi staining is better, but more complex.
  Woo-technique: good concentration-technique (no identification possible!)
  KNOTT-method: very efficient concentration-method.
  After filtration onto a membrane, followed by staining (epidemiological purposes).
- Serology (searching for Ab (mostly IgG4-type) in serum)
- Searching for Ag in blood, using Dip-Stick (same sensitivity as KNOTT-method)
- Detection of specific DNA, using molecular techniques (PCR)
- Detection of adult worms and abnormal lymphatic dilatations, using medical imaging



Morphology of the microfilaria (Giemsa):

Dimensions : 240-300 µm x 7,5-10 µm

Tail : empty, pointed and often folded

Head : small empty space

Sheath : pink or greyish sheath, hardly stains with Giemsa or even not at all

Characteristics :
Small, separated nuclei;
first and last ones clearly oval-shaped

Associated biological signs :

- Persisting hypereosinophilia during infestation

Possible confusion with :

- Other microfilaria
- Zoonoses (*Dirofilaria* spp., *Microfilaria* spp.)
- Thread-like artefacts (cotton-threads etc.)
- Fungi (*Helicosporium*)
- Helminth-larvae during their migration-phase

Remarks :

- The microfilaria usually circulate at night in the periferal bloodstream (bloodsample best taken between 23 and 04 o'clock). Sometimes sub-periodically.
- For patients mostly working at night, the blood is best taken during the daytime.
- Sometimes the microfilaria of *Wuchereria bancrofti* can be found in urine (chyluria).
- In cases of elephantiasis the microfilaria usually cannot be found in the periferal bloodstream anymore!!!
- Serological tests do not discriminate between the different species of microfilaria. Also cross-reactivity with other nematodes and with *E. granulosus* exists.
- The microfilaria appear in the bloodstream about 8 to 12 months after initial infestation (maturation period). The adult's lifespan is estimated at more than 25 years. The microfilaria can survive up to 70 days.

Brugia malayi

SuperFamily : Filarioidea

Class : Nematodes

Geographic distribution :

- South- and East-Asia
- India

Common name : Malayan filaria

Disease :

- NL : Brugia filariosis
- FR : filariose Brugia
- EN : Malayan filariasis
- ES : filariasis de Malaia

Final host :

- Man
- Certain monkeys
- (Cats?)

Intermediate host:

(Vector)

Different kinds of mosquitoes: Family of *Culicidae* (*Mansonia* spp., *Anopheles* spp.)

Transmission :

Active penetration of the skin (or via the sting-wound) by infectious larvae, placed on the skin during the blood-meal of an infested vector.

Localisation of the adult worm :

Lymphatic system.

Localisation of the microfilaria :

Blood.

Diagnostic possibilities :

- Examination of blood: Direct examination (not very efficient; no identification possible!)
  Thick blood smear: - Giemsa staining
  - Carazzi staining is better, but more complex
  Woo-technique: good concentration-technique (no identification possible!)
  KNOTT-method: very efficient concentration-method.
  After filtration onto a membrane, followed by staining (epidemiological purposes).
- Serology (searching for Ab (mostly IgG4-type) in serum)
- Detection of Ag in blood
- Detection of specific DNA, using molecular techniques (PCR)
- Detection of adult worms and abnormal lymphatic dilatations, using medical imaging

Morphology of the microfilaria (Giemsa):

Dimensions : 175-230 µm x 5-6 µm

Tail : stretched, with two strongly isolated terminal nuclei

Head : long empty space (≈ 2x width of the larva)

Sheath : strongly coloured (but can be absent in sub-periodical microfilaria)

Characteristics : usually strongly rolled up in thick blood smear (not in KNOTT)

Associated biological signs :

- Persisting hypereosinophilia during infestation

Possible confusion with :

- Other microfilaria
- Zoonoses (*Dirofilaria* spp., *Microfilaria* spp.)
- Thread-like artefacts (cotton-threads etc.)
- Fungi (*Helicosporium*)
- Helminth-larvae during their migration-phase

Remarks :

- The microfilaria usually circulate at night in the periferal bloodstream (bloodsample best taken between 23 and 04 o'clock). *Brugia malayi* is sub-periodic in Indonesia, Maleisia, the Filippines and Thaïland (the microfilaria can always be found in the periferal bloodstream, but especially at night).
- In 50% of the sub-periodical microfilaria of *B. malayi* the sheath is absent. In the periodical microfilaria of *B. malayi*, the sheath usually is present, but prevents correct staining of the nuclei.
- For patients mostly working at night, the blood is best taken during the daytime.
- Sometimes the microfilaria of *Brugia malayi* can be found in urine.
- In cases of elephantiasis the microfilaria usually cannot be found in the periferal bloodstream anymore!!!
- Serological tests do not discriminate between the different species of microfilaria. Also cross-reactivity with other nematodes and with *E. granulosus* exists.
- It takes 67 to 98 days for the larvae to become adult worms. The adult's lifespan is estimated at more than 15 years.

Brugia timori

SuperFamily : Filarioidea

Class : Nematodes

Geographic distribution :

- Indonesian archipel: from Timor to Bali, including the satellite-islands

Common name :

Timorean filaria

Disease :

- NL : Timor filariosis
- FR : filariose du Timor
- EN : Timorean filariasis
- ES : filariasis de Timor

Final host :

- Man
- ?

Intermediate host:

(Vector)

Mosquitoes of the family of *Culicidae* (*Anopheles* spp.)

Transmission :

Active penetration of the skin (or via the sting-wound) by infectious larvae, placed on the skin during the blood-meal of an infested vector.

Localisation of the adult worm :

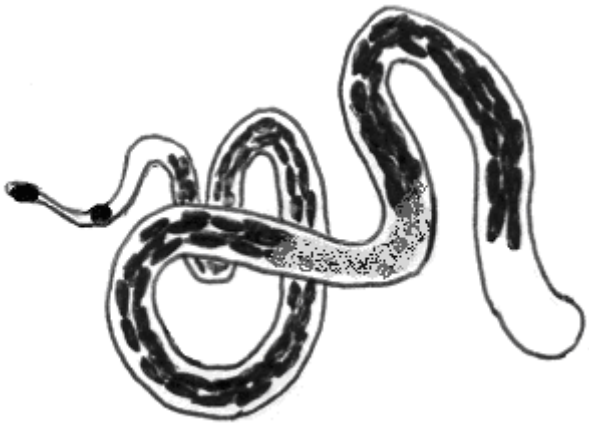
Lymphatic system.

Localisation of the microfilaria :

Blood.

Diagnostic possibilities :

- Examination of blood: Direct examination (not very efficient; no identification possible!)
  Thick blood smear: - Giemsa staining is a good technique, but the sheath usually doesn't take up the colour.
  - Carazzi staining is better, but more complex.
  Woo-technique: good concentration-technique (no identification possible!)
  KNOTT-method: very efficient concentration-method.
  After filtration onto a membrane, followed by staining (epidemiological purposes).
- Serology (searching for Ab (especially IgG4-type) in serum)
- Detection of Ag in blood
- Detection of specific DNA, using molecular techniques (PCR)
- Detection of adult worms and abnormal lymphatic dilatations, using medical imaging



Morphology of the microfilaria (Giemsa):

Dimensions : 265-325 µm x 4,4-6,8 µm

Tail : stretched, with two strongly isolated terminal nuclei kernen

Head : long empty space (≈ 2x to 3x width of the larva)

Sheath : present, but hardly stains with Giemsa or even not at all

Characteristics : usually strongly rolled up in thick blood smear (not in KNOTT); nuclei stain poorly at ⅓ of the microfilaria's length

Associated biological signs :

- Persisting hypereosinophilia during infestation

Possible confusion with :

- Other microfilaria
- Zoonoses (*Dirofilaria* spp., *Microfilaria* spp.)
- Thread-like artefacts (cotton-threads etc.)
- Fungi (*Helicosporium*)
- Helminth-larvae during their migration-phase

Remarks :

- The microfilaria of *Brugia timori* circulate strictly at night in the periferal bloodstream (bloodsample best taken between 23 and 04 o'clock). For patients mostly working at night, the blood is best taken during the daytime.
- Sometimes the microfilaria of *Brugia timori* can be found in urine.
- In cases of elephantiasis the microfilaria usually cannot be found in the periferal bloodstream anymore!!!
- Serological tests do not discriminate between the different species of microfilaria. Also cross-reactivity with other nematodes and with *E. granulosus* exists.

Mansonella streptocerca

SuperFamily : Filarioidea

Class : Nematodes

Geographic distribution :

- Rainforests of West- and Central Africa

Common name :

(Filaria)

Disease :

No specific name
(non or only slightly pathogenic)

Final host :

- Man
- Some monkey species

Intermediate host:

(Vector)

Mosquitoes
(*Culicoides* spp.)

Transmission :

Active penetration of the skin (or via the sting-wound) by infectious larvae, placed on the skin during the blood-meal of an infested vector.

Localisation of the adult worm :

Subcutaneous tissues.

Localisation of the microfilaria :

Skin.

Diagnostic possibilities :

- Identification of the microfilaria in skin-biopsies (skin-snip).
- Identification of the microfilaria in deep scarifications.

Morphology of the microfilaria (Giemsa):

Dimensions : 180-240 µm x 5-6 µm

Tail : blunt, usually curled with about 8 nuclei in line

Head : filled with nuclei

Sheath : absent

Characteristics :
microfilaria is crosier-shaped

Associated biological signs :

(non or only slightly pathogenic)

- Persisting hypereosinophilia during infestation

Possible confusion with :

- Other microfilaria
- Zoonoses (*Dirofilaria* spp., *Microfilaria* spp.)
- Thread-like artefacts (cotton-threads etc.)
- Fungi (*Helicosporium*)
- Helminth-larvae during their migration-phase

Remarks :

- *Dipetalonema* is an ancient name for the group containing (amongst others) *Mansonella* spp. (infesting man) and *Microfilaria* spp. (infesting animals).
- No specific name exists for this disease, as *M. streptocerca* is considered non or only slightly pathogenic. It should however be differentiated from pathogenic microfilaria.
- When both *Onchocerca volvulus* and *Mansonella streptocerca* infest a person, these two microfilaria are not always found in the same skin-regions.
- *Mansonella streptocerca* is an aperiodic microfilaria.
- *Mansonella streptocerca* is often found together with other microfilaria.
- Deep scarifications always contain a small amount of blood, which explains the possibility of finding blood-microfilaria in such a preparation.
- Serological tests do not discriminate between the different species of microfilaria. Also cross-reactivity with other nematodes and with *E. granulosus* exists.

Mansonella ozzardi

SuperFamily : Filarioidea

Class : Nematodes

Geographic distribution :

- Small regions in Central- and South-America
- Islands of the Pacific and the Caribees

Common name :

(Filaria)

Disease :

No specific name
(non or only slightly pathogenic)

Final host :

- Man
- Some monkey species

Intermediate host:

(Vector)

Mosquitoes
(*Culicoides* spp.
and *Simulium* spp.)

Transmission :

Active penetration of the skin (or via the sting-wound) by infectious larvae, placed on the skin during the blood-meal of an infested vector.

Localisation of the adult worm :

Abdominal cavity.

Localisation of the microfilaria :

Blood.

Diagnostic possibilities :

- Identification of the microfilaria in blood:
  Direct examination (not very efficient; no identification possible!)
  Thick blood smear: - Giemsa staining.
  - Carazzi staining is better, but more complex.
  Woo-technique: good concentration-technique (no identification possible!)
  KNOTT-method: very efficient concentration-method.
  After filtration onto a membrane, followed by staining (epidemiological purposes).

Morphology of the microfilaria (Giemsa):

Dimensions : 160-205 µm x 3-5 µm

Tail : empty and pointed tail (not always quite clear)

Head : filled with nuclei

Sheath : absent

Characteristics :
nuclei more or less mosaic-shaped

Associated biological signs :

(non or only slightly pathogenic)

- Persisting hypereosinophilia during infestation

Possible confusion with :

- Other microfilaria
- Zoonoses (*Dirofilaria* spp., *Microfilaria* spp.)
- Thread-like artefacts (cotton-threads etc.)
- Fungi (*Helicosporium*)
- Helminth-larvae during their migration-phase

Remarks :

- *Dipetalonema* is an ancient name for the group containing (among others) *Mansonella* spp. (infesting man) and *Microfilaria* spp. (infesting animals).
- No specific name exists for this disease, as *M. ozzardi* is considered non or only slightly pathogenic. It should however be differentiated from pathogenic microfilaria.
- *Mansonella ozzardi* is an aperiodic microfilaria.
- Serological tests do not discriminate between the different species of microfilaria. Also cross-reactivity with other nematodes and with *E. granulosus* exists.

Mansonella perstans

SuperFamily : Filarioidea

Class : Nematodes

Geographic distribution :

- Tropical and Northern-Africa
- Central- and South-America

Common name : (Filaria)

Disease : No specific name (non or only slightly pathogenic)

Final host :

- Man
- Certain monkeys

Intermediate host:

(Vector)

Mosquitoes (*Culicoides* spp.)

Transmission :

Active penetration of the skin (or via the sting-wound) by infectious larvae, placed on the skin during the blood-meal of an infested vector.

Localisation of the adult worm :

Abdominal cavity, pleural cavity, (pericard)

Localisation of the microfilaria :

Blood.

Diagnostic possibilities :

- Identification of the microfilaria in blood:
  Direct examination (not very efficient; no identification possible!)
  Thick blood smear: - Giemsa staining.
  - Carazzi staining is better, but more complex.
  Woo-technique: good concentration-technique (no identification possible!)
  KNOTT-method: very efficient concentration-method.
  After filtration onto a membrane, followed by staining (epidemiological purposes).

Morphology of the microfilaria (Giemsa):

Dimensions : 150-200 µm x 3-5 µm

Tail : blunt tail, last nucleus seems somewhat bigger, more intensely coloured (maximum 4 nuclei in line)

Head : filled with nuclei

Sheath : absent

Characteristics : nuclei are mosaic-shaped

Associated biological signs :

(non or only slightly pathogenic)

- Persisting hypereosinophilia during infestation

Possible confusion with :

- Other microfilaria
- Zoonoses (*Dirofilaria* spp., *Microfilaria* spp.)
- Thread-like artefacts (cotton-threads etc.)
- Fungi (*Helicosporium*)
- Helminth-larvae during their migration-phase

Remarks :

- *Dipetalonema* is an ancient name for the group containing (among others) *Mansonella* spp. (infesting man) and *Microfilaria* spp. (infesting animals).
- No specific name exists for this disease, as *M. perstans* is considered non or only slightly pathogenic. It should however be differentiated from pathogenic microfilaria.
- *M. perstans* is often found together with *Loa Loa* (or other microfilaria).
- *Mansonella perstans* is an aperiodic microfilaria.
- When a thick bloodsmear is prepared with blood containing **NO** anticoagulants, the microfilaria of *M. perstans* often are strongly rolled up. When using blood **WITH** anticoagulants, the microfilaria of *M. perstans* usually are nicely stretched out.
- Serological tests do not discriminate between the different species of microfilaria. Also cross-reactivity with other nematodes and with *E. granulosus* exists.
- It takes several months for the larvae to become adult worms.

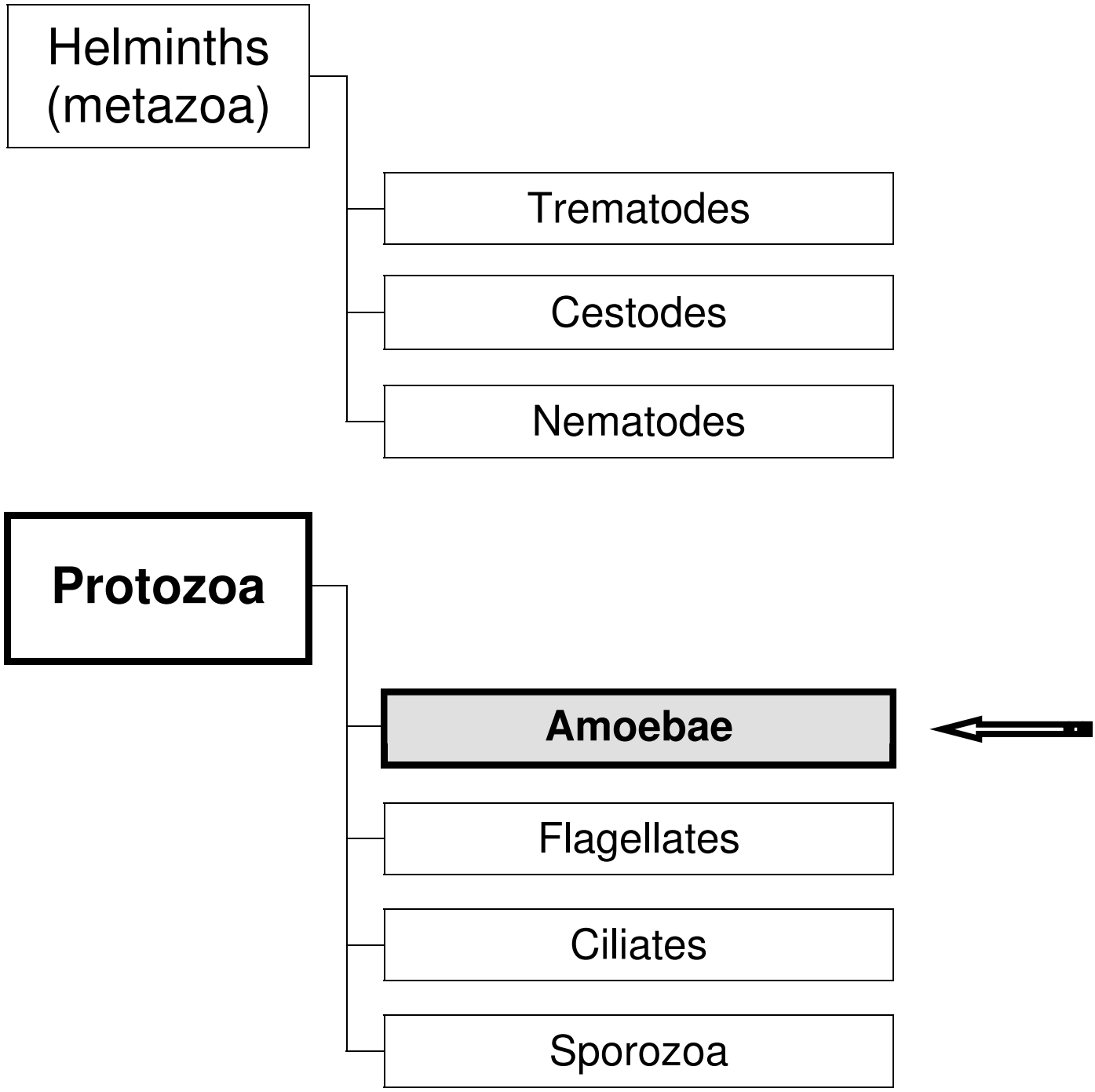
Characteristics of common human microfilaria species

Species Characteristic	*Wuchereria bancrofti*	*Brugia malayi*	*Brugia timori*	*Loa loa*	Mansonella *ozzardi*	Mansonella *perstans*	Mansonella *streptocerca*	Onchocerca *volvulus*
Geographical distribution	Tropical and Subtropical countries (except desert) Turkey (rare ?)	South and East, Indian subcontinent	Indonesian archipel from Timor to Bali and satellite-islands	Central and West Africa (rain forests)	Central and South America, Islands of the Pacific and Caribees	Central and West Africa, Central and South America	Central and West Africa (rain forests)	Central and West Africa, Central and South America
Vectors	Mosquitoes (family of culicidae)	Mosquitoes (family of culicidae)	Mosquitoes (family of culicidae)	Tabanid flies (*Chrysops* spp.)	Biting midges (*Culicoides* spp.) and blackflies (*Simulium* spp.)	Biting midges (*Culicoides* spp.)	Biting midges (*Culicoides* spp.)	Blackflies (*Simulium* spp.)
Habitat (adults)	Lymphatic system	Lymphatic system	Lymphatic system	Subcutaneous tissues, orbit	Subcutaneous tissues	Mesentery	Dermis	Subcutaneous tissues
Habitat (microfilariae)	Blood	Blood	Blood	Blood	Blood	Blood	**Skin**	**Skin**
Diagnostic	Blood examination	Blood examination	Blood examination	Blood examination	Blood examination	Blood examination	Deep scarification or skin biopsy	Deep scarification or skin biopsy
Periodicity of Microfilariae [1]	Nocturnal [2]	Nocturnal [3]	Nocturnal	Diurnal	Aperiodic	Aperiodic	Aperiodic	Aperiodic
Sheath	Present	Present	Present	Present	**Absent**	**Absent**	**Absent**	**Absent**
Width (µm)	7.5 -10	5 - 6	4.4 - 6.8	5 - 7	3 - 5	3 - 5	5 - 6	5 - 9
Length (µm)								
Smears	260 [240-300]	220 [175-230]	290 [265-325]	240 [230-250]	180 [160-205]	195 [150-200]	-	-
Formalin 2%	300 [275-320]	270 [240-300]	360 [330-385]	280 [270-300]	225 [200-255]	200 [180-225]	-	-
Biopsy	-	-	-	-	-	-	210 [180-240]	310 [280-320]
Tail	Tapered, anucleate	Tapered, subterminal and terminal nuclei widely separated	Tapered, subterminal and terminal nuclei widely separated	Tapered, Nuclei to end	Pointed, anucleate	Bluntly rounded, nuclei to end	Bluntly rounded, nuclei to end **crosier-shaped**	Pointed, anucleate
Cephalic space	Short	Long (twice as long as broad)	Long (twice [3] as long as broad)	Short	Very short	Very short	Very short	Long
Key features	Nuclei are well distinct, first and last ones oval shaped, Body in smooth curves, sheath unstained in Giemsa	Terminal and subterminal nuclei, sheath **stains** pink in Giemsa	Terminal and subterminal nuclei, sheath unstained in Giemsa	Two hernia, single row of nuclei to end of tail sheath unstained in Giemsa	Anucleated tail	Blunt tail filled with maximum 4 nuclei, mosaic shaped nuclei, last nucleus bigger or more intensely coloured	Blunt tail filled with nuclei (8 nuclei in line)	Empty tail

[1] The microfilariae of some species appear in the blood with a market nocturnal or diurnal periodicity. For nocturnal periodicity, a "night blood specimen" should be examined (maximum between 11 pm and 4 am). For diurnal periodicity, a "day blood specimen" should be examined (maximum between 11 am and 4 pm). Some microfilariae are present all the time in blood, but with an increase during the night (nocturnally subperiodic) or during the day (diurnally subperiodic).

[2] Nocturnally subperiodic in Thailand, diurnally subperiodic in New Caledonia and Polynesia.

[3] Nocturnally subperiodic in some parts of Indonesia, Malaysia, the Philippines and Thailand.


Helminths (metazoa)
Trematodes
Cestodes
Nematodes
Protozoa
Amoebae
Flagellates
Ciliates
Sporozoa
Fungi and Bacteria
Unknown classification

Entamoeba histolytica

Family : Entamoebidae

Class : Rhizopodea

Geographic distribution :

Worldwide

Common name :

Disease :

- NL : amoebiase
- FR : amibiase
- EN : amoebiasis
- ES : amoebiasis

Final host :

- Man
- Primates
- (Dogs, cats, ...)

Intermediate host:

Without intermediate host and without vector

Transmission :

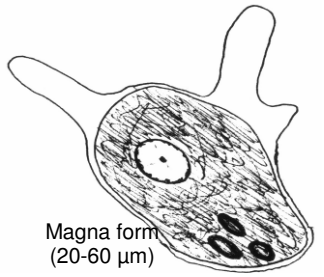
Faeco-oral, mature cysts ingestion.
(Sexual contact, cysts and trophozoites).

Localisation of the parasite :

Minuta form: Intestinal lumen, colon
Magna form: Intestinal mucosa and sub-mucosa (or liver, brain, lungs, …).

Diagnostic possibilities :

- Trophozoites detection in faeces (or in abscess pus, rectal biopsy or sputum) : direct examination.
- Cysts detection in (diarrhoeic) faeces : direct examination, iodine staining, concentration by sedimentation.
- Antigen detection in stool and species differentiation between *E. histolytica* and *E. dispar* in (ELISA, galactose adhesins).
- Detection of specific DNA, using PCR (various samples).
- Serology : antibody detection in serum (IFAT, ELISA, IHA, direct agglutination).
- Differentiation between *E. histolytica* and *E. dispar* (various samples : PCR or ELISA galactose adhesins) .



Magna form (20-60 µm)

Minuta form (10-60 µm)

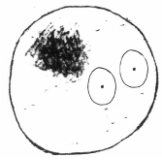
Morphology of the trophozoites :

Size : 10-60 µm (>20 for invasive forms)

Nucleus : 1 not so visible, central, central karyosome, fine and irregular granules of peripheral chromatin, evenly distributed.

Motility : quick, in one direction, finger-like pseudopods

Characteristics : Ingested RBC for the invasive form.



Morphology of the cysts :

Size : 10-20 µm

Morphology : Spherical or oval

Nucleus : up to 4, usually 1 or 2, central fine karyosome.

Characteristics : Immature cyst with 1 nucleus; sometimes chromatoid body, elongated with bluntly rounded ends; usually diffuse glycogen mass.

Associated biological signs :

- Leukocytosis For extra-intestinal amoebiasis: Leukocytosis, neutrophilia, ESR raised.

Possible confusion with :

- *Entamoeba dispar* (cysts and trophozoites)
- Others cysts of protozoa.
- Others trophozoites of amoebae.
- Leucocytes (cysts)
- Motile macrophages (trophozoites).

Remarks :

- Differentiation between the never pathogenic *E. dispar* and the sometimes pathogenic *E. histolytica*, based on morphologic characteristics of the cysts and trophozoïtes is not possible. Except in case of finding motile amoebae containing RBC which give the diagnosis of amibiasis. Otherwise, differentiation must be based on isoenzymatic or molecular analysis.
- Haematophageous trophozoites are found only in bloody diarrhoeic specimens. Specimens must be examined without delay, otherwise identification of the trophozoïtes becomes impossible because they lose their motility. Haematophageous trophozoïtes are also found in pus (viscous, yellow, brown or chocolate). Metastatic abscesses (liver, lung, brain,…) contain necrotic tissue, but few leucocytes and trophozoites. These trophozoites are most easily found at the periphery of the abscess. As the abscess is most oftenly punctured in its centre, the microscopic examination of the pus may fail to detect the trophozoites.
- *E. histolytica* (minuta form) remain confined to the intestinal lumen. In some cases, the trophozoïtes invade the intestinal mucosa, or through the bloodstream extra intestinal sites (magna form). Only *E. histolytica* (minuta form) and *E dispar* will produce cysts. Cysts are never present in stool during an intestinal amoebiasis.
- Serology is only useful for invasive amoebiasis (sensitivity of approximately 95% for extra intestinal infections, 70% for intestinal infections and 10% for asymptomatic persons who are passing *E. histolytica* cysts; specificity of approximately 95%). Detectable *E. histolytica* specific antibodies may persist for months after successful treatment.
- Antigen detection (galactose adhesions) may be useful to distinguish between *E. histolytica* and *E dispar* (sensitivity is about 70% and specificity about 97%)
- Cysts of *Entamoeba.moshkowskii* (a non pathogenic amoeba) look the same as cysts of *E. histolytica* and *E. dispar*. (Same species as *E. dispar* ?).
- *Entamoeba histolytica* cysts are resistant to the concentration of chlorine commonly used in the purification of domestic water supplies.

Entamoeba dispar

Family : Entamoebidae

Class : Rhizopodea

Geographic distribution :

Worldwide

Common name :

Disease :

Non pathogenic

Final host :

- Man
- Primates
- Other animals

Intermediate host:

Without intermediate host and without vector

Transmission :

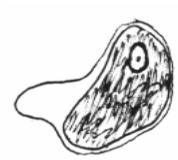
Faeco-oral, mature cysts ingestion.

Localisation of the parasite :

Intestinal lumen, colon

Diagnostic possibilities :

- Trophozoites identification in faeces : direct examination.
- Cysts identification in faeces : direct examination, iodine staining, concentration by sedimentation.
- Differentiation between *E. histolytica* and *E. dispar* : PCR or ELISA (galactose adhesins) .



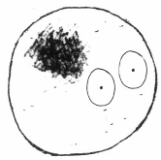
Morphology of the trophozoites :

Size : 10-20 µm.

Nucleus : 1 not so visible, central, central karyosome, fine and irregular granules of peripheral chromatin, evenly distributed.

Motility : quick, in one direction, finger-like pseudopods

Characteristics : Never contain ingested RBC



Morphology of the cysts :

Size : 10-20 µm

Morphology : spherical or oval

Nucleus : up to 4, usually 1 or 2, central fine karyosome.

Characteristics : Immature cyst with 1 nucleus; sometimes chromatoid body, elongated with bluntly rounded ends; usually diffuse glycogen mass.

Associated biological signs :

NON PATHOGENIC

Possible confusion with :

- *Entamoeba histolytica* (cysts and trophozoites)
- Others cysts of protozoa.
- Others trophozoites of amoebae.
- Motile macrophages (trophozoites)..

Remarks :

- Differentiation between the never pathogenic *E. dispar* and the sometimes pathogenic *E. histolitica*, based on morphologic characteristics of the cysts and trophozoïtes is not possible. Differentiation must be based on isoenzymatic or molecular analysis.
- The prevalence of *E. dispar* in man is higher than de prevalence of *E. histolytica* (at the ITM, more than 97% of patients who are passing *E. histolytica/dispar* cysts are infected by *E. dispar*).
- Cysts are found in more formed specimens, trophozoïtes are found mainly in diarrhoeic specimens.
- Specimens must be examined without delay, otherwise identification of the trophozoïtes becomes impossible because they lose their motility.
- Iodine stain will kill any trophozoïtes immediately, but stains the internal structure of the cysts.
- Antigen detection (galactose adhesions) may be useful to distinguish between *E. histolytica* and *E dispar* (sensitivity is around 70% and specificity is around 97%)
- *Entamoeba.moshkowskii* (another non pathogenic amoeba) looks perfectly alike (same species?).

Entamoeba hartmanni

Family : Entamoebidae

Class : Rhizopodea

Geographic distribution :

Worldwide

Common name :

Disease :

Non pathogenic

Final host :

- Man

Intermediate host:

Without intermediate host and without vector

Transmission :

Faeco-oral, mature cysts ingestion.

Localisation of the parasite :

Intestinal lumen.

Diagnostic possibilities :

- Trophozoites identification in faeces : direct examination.
- Cysts identification in faeces : direct examination, iodine staining, concentration by sedimentation.

Morphology of the trophozoites :

Size : 5-12 µm.

Nucleus : 1 not so visible, central, central karyosome, fine and irregular granules of peripheral chromatin, evenly distributed.

Motility : quick, in one direction, finger-like pseudopods

Characteristics : Never contain ingested RBC

Morphology of the cysts :

Size : 5-10 µm

Morphology : Spherical or oval

Nucleus : up to 4, usually 1 or 2, central fine karyosome.

Characteristics : Immature cyst with 1 nucleus; sometimes chromatoid body, elongated with bluntly rounded ends; usually diffuse glycogen mass.

"Small *histolytica*"

Associated biological signs :

NON PATHOGENIC

Possible confusion with :

- *Entamoeba histolytica/dispar* (cysts and trophozoites)
- Others cysts of protozoa.
- Others trophozoites of amoebae.
- Motile macrophages (trophozoites)..

Remarks :

- As non pathogenic parasites, *E. hartmanni* should not be looked for but should be distinguished from the (sometimes) pathogenic *E. histolytica*.
- Accurate measurement of cysts is essential for the correct distinction between *E. histolytica/dispar* and *E. hartmanni*.
- Iodine stain will kill immediately the trophozoïtes, but stains the internal structure of the cysts.

Entamoeba coli

Family : Entamoebidae

Class : Rhizopodea

Geographic distribution :

Worldwide

Common name :

Disease :

Non pathogenic

Final host :

- Man
- Primates
- Dogs
- Pigs

Intermediate host:

Without intermediate host and without vector

Transmission :

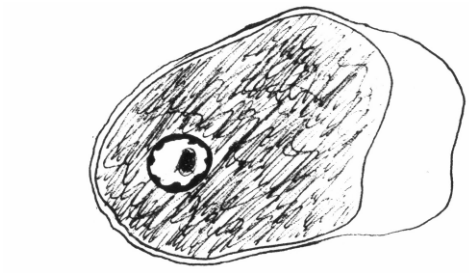
Faeco-oral, mature cysts ingestion.

Localisation of the parasite :

Intestinal lumen.

Diagnostic possibilities :

- Trophozoites identification in faeces : direct examination.
- Cysts identification in faeces : direct examination, iodine staining, concentration by sedimentation.



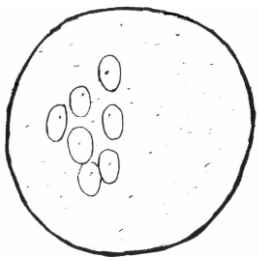
Morphology of the trophozoites :

Size : 15-50 µm.

Nucleus : 1, most of the time large and eccentric karyosome, fine and irregular granules of peripheral chromatin, evenly distributed.

Motility : Barely motile, large pseudopods

Characteristics : Never contain ingested RBC



Morphology of the cysts :

Size : 10-35 µm.

Morphology : Spherical or oval.

Nucleus : 1 to 8, well visible (out of the ordinary 8 to 16).

Characteristics : Tick shell; sometimes chromatoid body, with sharp or jagged ends; usually diffuse glycogen.

Associated biological signs :

NON PATHOGENIC

Possible confusion with :

- Others cysts of protozoa.
- Others trophozoites of amoebae.
- Motile macrophages (trophozoites).

Remarks :

- As non pathogenic parasites, *E. coli* should not be looked for but should be distinguished from the (sometimes) pathogenic *E. histolytica*.
- Iodine stain will kill any trophozoïtes immediately, but stains the internal structure of the cysts.

Endolimax nanus

Family : Entamoebidae

Class : Rhizopodea

Geographic distribution :

Worldwide

Common name :

Disease :

Non pathogenic

Final host :

- Man
- Primates
- Dogs
- Pigs

Intermediate host:

Without intermediate host and without vector

Transmission :

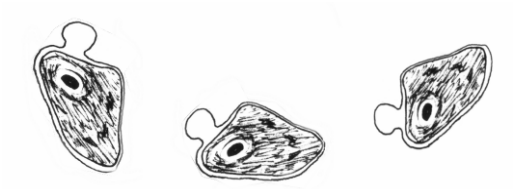
Faeco-oral, mature cysts ingestion.

Localisation of the parasite :

Intestinal lumen.

Diagnostic possibilities :

- Trophozoites identification in faeces : direct examination.
- Cysts identification in faeces : direct examination, iodine staining, concentration by sedimentation.



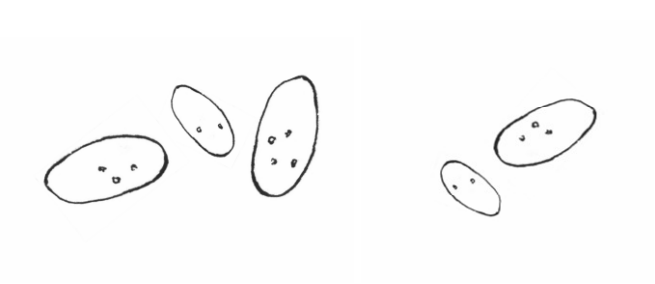
Morphology of the trophozoites :

Size : 6-12 µm.

Nucleus : 1, large and compact karyosome, no peripheral chromatin.

Motility : Slow, only 1 pseudopods.

Characteristics : Never contain ingested RBC



Morphology of the cysts :

Size : 5-10 µm.

Morphology : Oval, sometimes spherical.

Nucleus : 1 to 4, not well visible, (refringent dots).

Characteristics :

Associated biological signs :

NON PATHOGENIC

Possible confusion with :

- Others cysts of protozoa.
- Others trophozoites of amoebae.
- Motile macrophages (trophozoites).

Remarks :

- As non pathogenic parasites, *Endolimax nanus* should not be looked for but should be distinguished from the (sometimes) pathogenic *E. histolytica*.
- Iodine stain will kill any trophozoïtes immediately, but stains the internal structure of the cysts.
- *Endolimax nana*, is the old name for *Endolimax nanus.*

Iodamoeba butschlii

Family : Entamoebidae

Class : Rhizopodea

Geographic distribution :

Worldwide

Common name :

Disease :

Non pathogenic

Final host :

- Man
- Primates
- Pigs

Intermediate host:

Without intermediate host and without vector

Transmission :

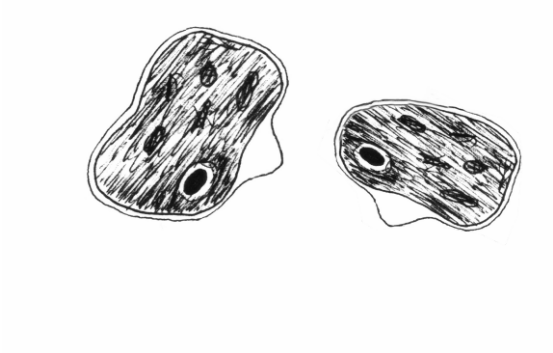
Faeco-oral, mature cysts ingestion.

Localisation of the parasite :

Intestinal lumen.

Diagnostic possibilities :

- Trophozoites identification in faeces : direct examination.
- Cysts identification in faeces : direct examination, iodine staining, concentration by sedimentation.



Morphology of the trophozoites :

Size : 8-20 µm.

Nucleus : One not so visible, large karyosome, no peripheral chromatin.

Motility : Slow, transparent pseudopods.

Characteristics : Never contain ingested RBC

Morphology of the cysts :

Size : 5-20 µm.

Morphology : Variable, oval, spherical.

Nucleus : One, compact.

Characteristics : Large glycogen vacuole (stained reddish-brown by iodine solution)

Associated biological signs :

NON PATHOGENIC

Possible confusion with :

- Others cysts of protozoa.
- Others trophozoites of amoebae.
- Motile macrophages (trophozoites).

Remarks :

- As non pathogenic parasites, *Iodamoeba butschlii* should not be looked for but should be distinguished from the (sometimes) pathogenic *E. histolytica*.
- Iodine stain will kill any trophozoïtes immediately, but stains the internal structure of the cysts.
- *Pseudolimax butschlii*, is the old name for *Iodamoeba butschlii.*

Entamoeba gingivalis

Family : Entamoebidae

Class : Rhizopodea

Geographic distribution :

Cosmopolitic

Common name :

Disease :

Non pathogenic

Final host :

- Man
- Dog
- Horse

Intermediate host:

Without intermediate host and without vector

Transmission :

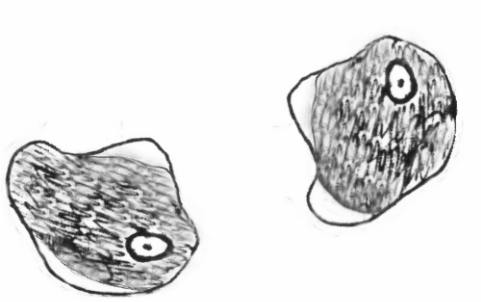
Direct oral transmission (kissing).

Localisation of the parasite :

Mouth cavity.

Diagnostic possibilities :

- Identification of trophozoïtes in saliva, using direct examination
- Identification of trophozoïtes in tooth-scrapings, using direct examination



Mophology of the trophozoïtes :

Dimensions : 10-25 µm

Nucleus : small karyosoma, surrounded by fine peripheral chromatin

Motion : directed movement, most of the time using different pseudopoda at the same time

Morphology of the cysts :

Associated biological signs :

Non pathogenic

Possible confusion with :

- *Entamoeba histolytica* (pulmonary amoebiasis)

Remarks :

- The presence of *Entamoeba gingivalis* usually indicates poor mouth hygiene.
- ATTENTION: *Entamoeba gingivalis* is to be differentiated from *Entamoeba histolytica* coming from the lungs (pulmonary amoebiasis), the latter containing RBCs!!!

Naegleria fowleri

Family : Vahlkampfiidae

Class : Rhizopodea

Geographic distribution :

Cosmopolitic

Common name :

Disease :

Exceptional !

- NL : primaire amoeben-meningo-encefalitis
- FR : méningo-encéphalite amibienne primitive
- EN : primary amebic meningoencephalitis
- ES : meningoencefalitis amibiana primaria

Final host :

Freeliving organism

Accidentally : Man

Intermediate host:

Without intermediate host and without vector

Transmission :

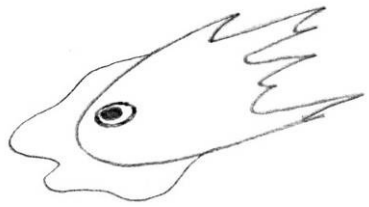
Uptake of infested water through the nose.

Localisation of the amoeboïd trophozoïte:

Central Nervous System.

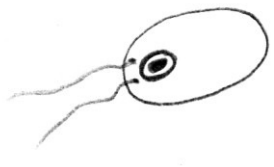
Diagnostic possibilities :

- Detection of the amoeboïd trophozoïtes in CSF using direct examination, after Giemsa-staining, using cultures.
- Identification of the amoeba using immunofluorescence (marked specific Ab).



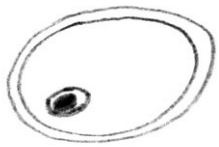
Mophology of the amoeboïed trophozoïtes: in CSF

Dimensions :	10 – 20 µm.
Movement :	Very actif ; rounded «exploding» pseudopodes
Nucleus :	large with a big karyosoma
Characteristics :	multiple vacuoles in the cytoplasm



Mophology of the flagellated trophozoïtes: in water

Dimensions :	10 – 20 µm.
Nucleus :	1 nucleus.
Characteristics:	2 to 4 flagella



Mophology of the cysts : in water

Dimensions :	10 – 20 µm.
Shape :	Oval
Shell :	Double, thick and smooth shell
Nucleus :	1 single nucleus

Associated biological signs:

- purulent CSF
- hyperalbuminorachia
- normal or lowered glycorachia

Possible confusion with :

- *Acanthamoeba* spp.
- *Balamuthia mandrillaris*
- Motile macrofages
- Cysts and trophozoïtes of other amoeba en flagellates (contaminants)

Remarks :

- *Naegleria* spp. and amoeba of *Acanthamoeba* spp. are free-living organisms, usually found in water (lakes, swimming-pools, water-distribution, water from heating installations and aircoes,…). Their optimal multiplying temperature is ±35°C. *Naegleria fowleri,* the only *Naegleria* sp. that can occasionally be pathogenic for man, is found only in warm water (20-25°C), A*canthamoeba* species can also be found in colder water. *Balamuthia mandrillaris* was never isolated from the external environment up till now.
- Primary amoebic-meningo-encefalitis is usually encountered as small epidemics (persons that came simultanuously in contact with infested water (swimming-pools,…)).
- *Naegleria fowleri* is a strange parasiet, as it has 3 different stages in its evolutionary cycle: the amoeboïd trophozoïte (parasitic form), the flagellated trophozoïte (free-living and multiplying form) and the cyst (protected form in the external environment). The cysts are well-protected against dryness, but are sensitive to chlorine (4 ppm).
- Primary amoebic-meningo-encefalitis causes purulent CSF, containing especially poly-nuclear WBCs (500-20.000 cells/mm^3), hyperalbuminorachia, normal or lowered glycorachia, a negative bacteriological examination of CSF with presence of numerous motile amoeba.

Acanthamoeba spp.

Familie : Hartmanellidea

Klasse : Rhizopodea

Geographic distribution :

Cosmopolitic

Common name :

Disease:
Very exceptional encephalitis !

- NL : amoeben keratitis
  [granuleuze amoeben-encefalitis]
- FR : ulcère cornéen amibien
  [encéphalite granuleuse amibienne]
- EN : keratitis and corneal amebic ulcer
  [chronic granulomatous amebic encephalitis]
- ES : ulceras corneales amibiana
  [encefalitis amibiana granulosa]

Final host :

Freeliving organism

Accidentally : Man

Intermediate host:

Without intermediate host and without vector

Transmission :

Direct eye contact

Localisation of the amoeboïd trophozoïte and cyst:

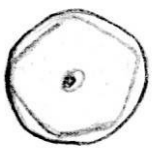
Skin, eye, [central nervous system].

Diagnostic possibilities :

- Detection of amoeboïd trophozoïtes and cysts in cornea-scrapings:
  - direct examination, after Giemsa-staining,…
- Detection of amoeboïd trophozoïtes and cysts in biopsies (skin, cornea, brain-tissue) :
  - direct examination, after Giemsa-staining,…
- Identification of the amoeba using immunofluorescence (marked specific Ab).
- [Culture].
- [Detection of amoeboïd trophozoïtes and cysts in CSF:
  - direct examination, after Giemsa-staining,…]

Mophology of the amoeboïed trophozoïtes:

Dimensions :	10 – 40 µm.
Movement:	slow, granular pseudopodes
Nucleus :	large with a big karyosoma
Characteristics:	efilated or filamentous pseudopodes; multiple vacuoles



Mophology of the cysts :

Dimensions :	10 – 20 µm.
Form :	oval
Shell :	double shell with polygonal outer structure
Nucleus :	1 single nucleus, usually hardly visible.
Characteristics:	polygonal aspect

Associated biological signs:

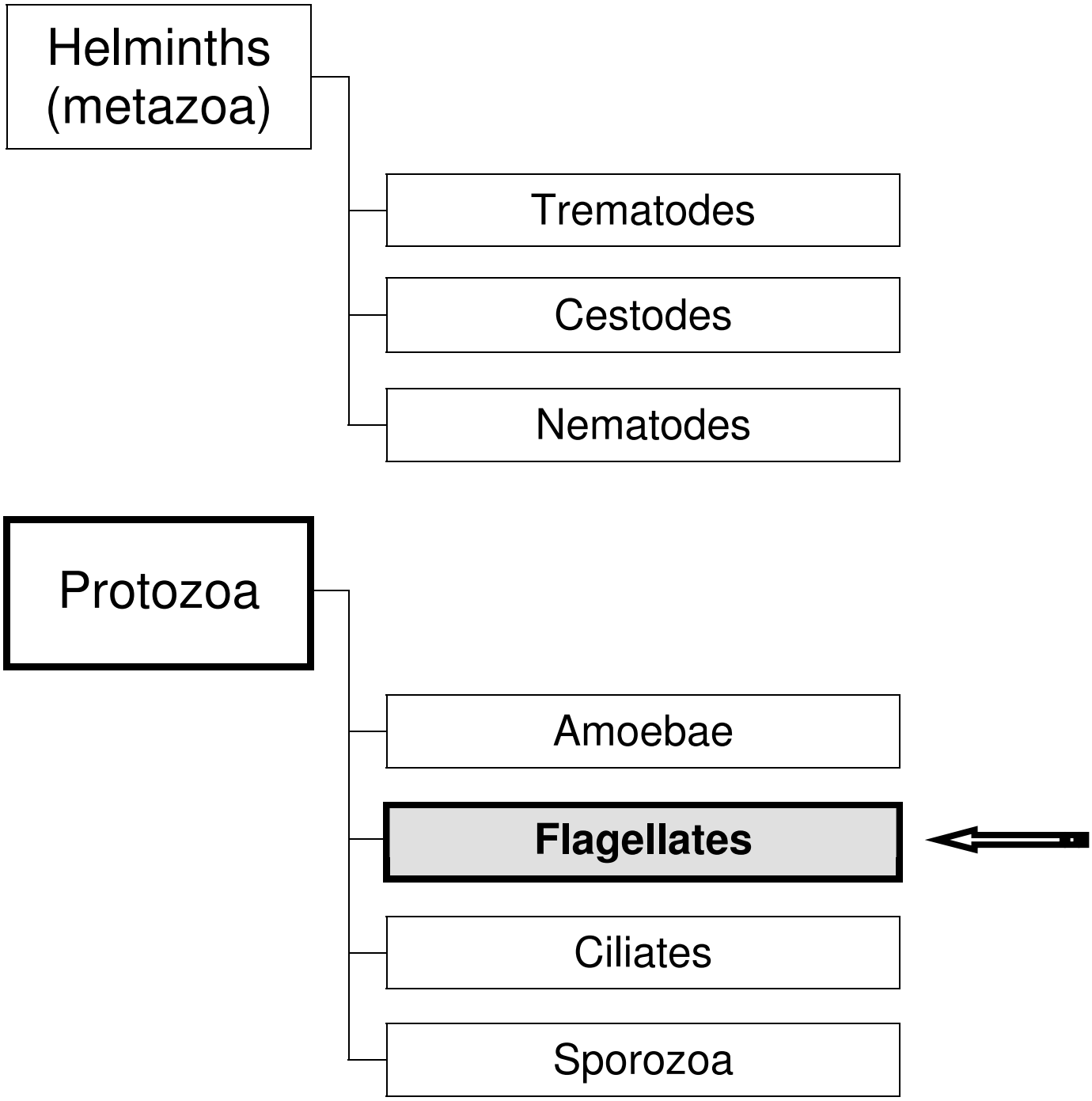
- hyperalbuminorachia
- normal or lowered glycorachia

Possible confusion with :

- *Naegleria* spp.
- *Balamuthia mandrillaris*
- plantspores
- Motile macrofages
- Cysts and trophozoïtes of other amoeba (contaminants)

Remarks :

- *Naegleria* spp. and amoeba of *Acanthamoeba* spp. are free-living organisms, usually found in water (lakes, swimming-pools, water-distribution, water from heating installations and aircoes,…). Their optimal multiplying temperature is ±35°C. *Naegleria fowleri,* the only *Naegleria* sp. that can occasionally be pathogenic for man, is found only in warm water (20-25°C), A*canthamoeba* species can also be found in colder water. *Balamuthia mandrillaris* was never isolated from the external environment up till now.
- Several *Acanthamoeba* spp. can evolve pathogenically (opportunistic): *A. polyphaga, A. castellani, A. culbertsoni, A. astronyxis, A. hatchetti, A. rhysodes*,…
- In contrast to *Naegleria fowleri*, *B. mandrillaris* and the amoeba of *Acanthamoeba* spp. have only two evolutionary stages: an amoeboïd and a cyst-stage.
- The amoeba of *Acanthamoeba* spp. and of *Balamuthia mandrillaris* are morphologically identical. Both of these amoeba can cause keratitis after damage to the cornea (scratchings, trauma, contactlenses,…) or encephalitis (opportunistic, in case of immuno-deficiëncy).
- Granulary amoebic-encefalitis is found in combination with clear CSF, containing especially mono-nuclear WBCs, hyperalbuminorachia, normal or lowered glycorachia, a negative bacteriological examination of CSF with presence of motile amoeba (hard to find however) and/or polygonal-shaped cysts.


Helminths (metazoa)
Trematodes
Cestodes
Nematodes
Protozoa
Amoebae
Flagellates
Ciliates
Sporozoa
Fungi and bacteria
Unknown classification

Dientamoeba fragilis

Superfamily : Monocercomonadidea

Class : Rhizopodea

Geographic distribution :

Cosmopolitic

Common name :

Disease :

No specific name exists for this (still hypothetical) disease.

Final host :

- Man

Intermediate host:

Without intermediate host and without vector ?

Transmission :

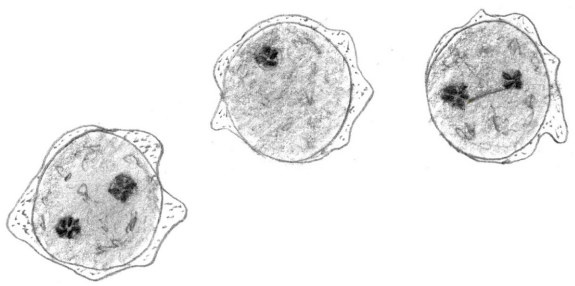
- Faeco-oral transmission of the trophozoïtes?
- The hypothesis that transmission occurs, using helminth eggs (such as those of *E. vermicularis*) is yet to be proven!

Localisation of the trophozoïte :

Lumen of the colon

Diagnostic possibilities :

- Detection of the trophozoïtes in permanently stained slides of faeces (e.g. iron-hematoxylin-staining; see p.2).
- Identification of the trophozoïtes in permanently stained slides after detection in direct wet smears
- in vitro cultivation on xenic media
- Detection of specific DNA in faeces, using PCR
- Serology: detection of specific Ab



Mophology of the trophozoïtes: in permanently stained slides

Dimensions : 4-20 µm

Nucleus : 1 to 2 nuclei, clearly fragmented (4 to 8 fragments), sometimes connected by a centrodesmose; no peripheral chromatin

Movement : very actif amoeboïd movement, using pseudopodes (in direct wet smears); immobile after fixation and/or staining

Characteristics: About 60% of the trophozoïtes has two typically fragmented nuclei

Morfologie van de cysten :

Associated biological signs:

- Pathogenic capacity not yet proven!

Possible confusion with :

- amoebic trophozoïtes
- Motile macrofages (in direct wet smears)
- Cysts and trophozoïtes of *Endolimax nanus* (in permanently stained slides)

Remarks :

- The pathogenic capacity of this flagellate has not yet been proven.
- As *D. fragilis* does not form cysts and as the trophozoïtes are quite fragile, the stoolsample should be fixed **immediately** after production (e.g. in SAF-fixative; see p.2).
- To identify *D. fragilis* the typically fragmented nuclei should be visualized, by using permanently stained slides (e.g. iron-hematoxylin staining; see p.2).
- The staining of faecal specimens is very time-consuming and the reading of the slides demands a high level of experience.
- While having a typical amoeboïd aspect and lacking any flagella, this parasite is classified amongst the flagellates, based on its genetic characteristics

.

Giardia lamblia

Family : Hexamitidae

Class : Zoomastigophorea

Geographic distribution :

Worldwide

Common name :

Disease :

- NL : giardiase / lambliase
- FR : giardiase
- EN : giardiosis
- ES : giardiasis

Final host :

- Man (reservoir)
- Primates
- Pigs
- Rodents (reservoir ?)

Intermediate host :

Without intermediate host and Without vector

Transmission :

Faeco-oral, mature cysts ingestion.

Localisation of the parasite :

Duodenum and first part of the jejunum

Diagnostic possibilities :

- Trophozoites detection in faeces : direct examination.
- Cysts detection in faeces : direct examination, iodine staining, concentration by sedimentation.
- Trophozoites detection in duodenal aspirate (or using a string-test (Enterotest®)): direct examination.
- Antigen detection in faeces (ELISA).
- Serology : antibody detection in serum (IFAT).
- Detection of specific DNA, using PCR.

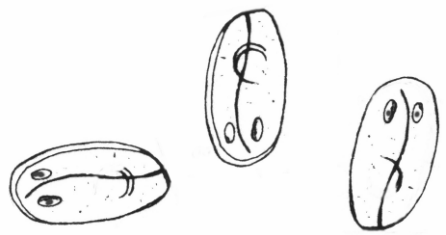
Morphology of the trophozoites :

Size : 10-20 µm x 6-8 µm x 1-4 µm

Nucleus : 2 anteriorly placed

Motility : In a definite direction, as a falling dead leaf

Characteristics : pear-shaped (frontal view), spoon–shaped (lateral view), 8 flagella.



Morphology of the cysts :

Size : 8 -19 µm x 7-10 µm

Morphology : oval

Nucleus : 2 to 4 anteriorly placed

Characteristics : Axostyle in S-shape, parabasal bodies in comma-shape. Cytoplasm's retraction is common (shell appears to be double walled).

Associated biological signs :

- Usually without any symptoms
- Watery diarrhoea without blood or mucus
- Hypovitaminosis, especially in children (malabsoption of fatty acids)

Possible confusion with :

- *Chilomastix mesnili* (trophozoites)
- Other trophozoites of non pathogenic flagellates
- Other cysts of protozoa

Remarks :

- Cysts are found in more formed specimens, trophozoïtes are found mainly in diarrhoeic specimens.
- Fresh specimens must be examined without delay, otherwise identification of the trophozoïtes becomes impossible because they lose their motility.
- Iodine stain will kill any trophozoïtes immediately, but stains the internal structure of the cysts.
- The cysts are excreted irregularly. The sensitivity for microscopic examination of only one specimen is about 75%. Several specimens collected at different times may need to be examined.
- As *E. histolytica, Giardia* cysts are resistant to the concentration of chlorine commonly used for the purification of domestic water supplies.
- *Giardia intestinalis, Giardia duodenalis* and *Lamblia intestinalis* are old names for *Giardia lamblia.*

Chilomastix mesnili

Family: Retortamonadidea

Class : Retortamonadea

Geographic distribution :

Worldwide

Common name :

Disease :

Non pathogenic

Final host :

- Man
- Monkeys
- Pigs

Intermediate host:

Without intermediate host and without vector

Transmission :

Faeco-oral, mature cysts ingestion.

Localisation of the parasite :

Intestinal lumen.

Diagnostic possibilities :

- Trophozoites identification in faeces : direct examination.
- Cysts identification in faeces : direct examination, iodine staining, concentration by sedimentation.

Morphology of the trophozoites :

Size : 6-24 µm x 4-7 µm

Nucleus : 1 anteriorly placed,

Motility : In one definite direction, in a spiral

Characteristics :
Pear-shaped with a sharp pointed end , 4 flagella : 3 anterior and 1 posterior.

Morphology of the cysts :

Size : 4-10 µm

Morphology : Pear-shaped

Nucleus : One, central

Characteristics :
Cytoplasm with fibrils (atrophic flagella).

Associated biological signs :

NON PATHOGENIC

Possible confusion with :

- *Giardia lamblia* (trophozoites).
- Other non pathogenic flagellates (trophozoites)
- Other cysts of protozoa.

Remarks :

- As non pathogenic parasites, *Chilomastix mesnili* should not be looked for but should be distinguished from pathogenic amoebae or flagellates.
- Iodine stain will kill immediately the trophozoïtes, but stains the internal structure of the cysts.
- *Retortamonas hominis* (non pathogenic parasite for man and monkey's) and *Enteromonas hominis* (non pathogenic parasite for man and animals) are similar but smaller (4 -10 µm).

Trichomonas vaginalis

Family : Trichomonadidae

Class : Parabasalea

Geographic distribution :

Worldwide

Common name :

Disease :

- NL : trichomoniase (vaginitis, uretritis)
- FR : trichomoniase
- EN : trichomonosis
- ES : tricomoniasis urogenital

Final host :

- Man

Intermediate host:

Without intermediate host and without vector

Transmission :

Sexual transmission

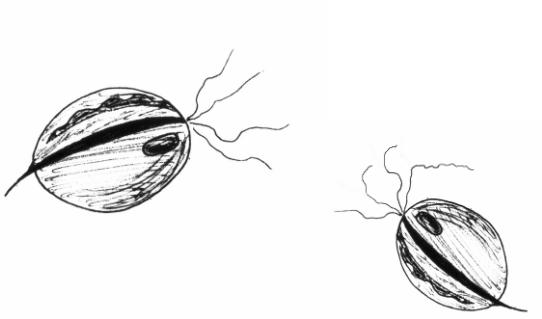
Rare mechanical transmission (wet objects : towels, gynaecological instruments, …)

Localisation of the parasite :

Urogenital tract.

Diagnostic possibilities :

- Trophozoites detection in vaginal discharges or in urethral pus : direct examination.
- Trophozoites detection in urine : Direct examination, concentration by sedimentation.
- [Antigen detection on urine or on vaginal discharge : ELISA, IFAT, …].
- [Culture].
- Detection of specific DNA, using PCR.



Morphology of the trophozoites :

Size : 5-19 µm x 3-12 µm

Nucleus : 1 anteriorly placed, hardly visible.

Motility : Often slight (on the spot) and not progressional.

Characteristics : 4 anteriorly placed flagella, axostyle, ondulating membrane.

NO CYST STAGE

Morphology of the cysts :

NO CYST STAGE

Associated biological signs :

- Purulent vaginal discharge, prurit.
- Usually asymptomatic (men).

Possible confusion with :

- Other *Trichomonas* spp. (contamination).
- Other flagellates (faecal or water contaminants).

Remarks :

- Specimens must be examined without delay, otherwise identification of the trophozoïtes becomes impossible (loss of motility).
- As the parasites live in the urethra, the first drops of urines (after a period of 4 to 5 hours in which the patient did not urinate) have to be examined for trophozoites.

Pentatrichomonas hominis

Family : Trichomonadidae

Class : Parabasalea

Geographic distribution :

Worldwide

Common name :

Disease :

Non pathogenic

Final host :

- Man
- Monkeys
- Pigs
- Dogs
- Rodents
- …

Intermediate host:

Without intermediate host and without vector

Transmission :

Faeco-oral, **Trophozoites** ingestion.

Localisation of the parasite :

Intestinal lumen.

Diagnostic possibilities :

- Trophozoites identification in faeces : direct examination.

Morphology of the trophozoites :

Size : 8-15 µm x 4-6µm

Nucleus : 1 anteriorly placed, hardly visible.

Motility : Often slight (on the spot) and not progressional.

Characteristics : 4 anteriorly placed flagella, 1 posteriorly placed flagellum, axostyle, ondulating membrane.

NO CYST STAGE

Morphology of the cysts :

NO CYST STAGE

Associated biological signs :

NON PATHOGENIC

Possible confusion with :

- *Giardia lamblia.*
- Other (non pathogenic) flagellates.

Remarks :

- As non pathogenic parasites, *Pentatrichomonas hominis* should not be looked for but should be distinguished from pathogenic flagellates.
- Pentatrichomonas hominis stays motile quite long in faeces (no cyst stage).
- Another non pathogenic parasite, with a similar morphology (*Trichomonas tenax*) lives in the oral cavity. *Trichomonas elongata* and *Trichomonas buccalis* are old names for this parasite.
- *Trichomonas hominis* and *Trichomonas intestinalis* are old names for *Pentatrichomonas hominis.*

Trypanosoma brucei gambiense

Family : Trypanosomatidae

Class : Mastigophorea

Geographic distribution :

West and Central Sub-Saharian Africa
(between 15°N and 25°S)
Dense vegetation and **riverbanks**

Common name :

West African sleeping sickness

Disease:

- NL : West-Afrikaanse trypanosomiase.
- FR : Trypanosomiase chronique de l'Afrique de l'Ouest.
- EN : West African Trypanosomiasis
- ES : Enfermedad del sueño forma Gambiana.

Final host:

- Man
- (Domestic and semi-domestic animals)
- (Wild animals: buffaloes, etc.)

Intermediate host:

(Vector)

Tsetse fly

(*Glossina palpalis* Group)

Transmission:

- Trypomastigotes injected with saliva through the skin when an infested tsetse fly takes a blood meal.
- Blood transfusion (fresh blood).
- (Congenital transmission: rare).
- (Mechanical transmission by an insect bite: exceptional)

Localisation of the parasite :

- Stage I: lymph and blood.
- Stage II: (lymph and blood), central nervous system

Diagnostic possibilities:

- Serology (detection of Ab in serum or blood): CATT (screening method), latex (especially for Nigeria), ELISA, IF, …
- Examination of lymph node aspirate for trypomastigotes (cervical lymph glands: Winterbottom's sign).
- Examination of blood for trypomastigotes :
  - in a wet smear (low sensitivity).
  - in a (thin or) thick blood film stained with Giemsa.
  - Woo (micro-haematocrite centrifugation).
  - QBC.
  - miniature anion exchange centrifugation technique (mAECT).
- Examination of cerebrospinal fluid for trypomastigotes (diagnostic and stage determination) :
  - in a wet smear (low sensitivity).
  - after single or double centrifugation.
- Detection of specific DNA using PCR

Morphology of the trypomastigotes (Giemsa):

Dimensions : 15-25 µm

Cytoplasm : Pale blue, with a flagellum and an undulating membrane.

Contents : A large central (purple) nucleus and a small kinetoplast (red).

Characteristics : Trypanosomes may show a variety of forms (pleomorphic).

Associated biological signs :

- Stage I: (erythrocyte sedimentation rate ↑ [IgM↑], moderate leukocytosis, thrombocytopenia, anaemia, …)
- Stage II: (idem + CSF perturbation)

Possible confusion with:

- *Trypanosoma brucei rhodesiense*
- Animal's trypanosomiases (*T. brucei brucei*,...)
- Motile bacteria (*Borrelia* spp. or water contaminants) or exflagellation of *Plasmodium* spp. for wet smear.
- *Trypanosoma cruzi* (South America)
- *Trypanosoma rangeli* (South America)

Remarks :

- Microscopically, the trypanosomes of *T. b. gambiense* and *T. b. rhodesiense* look alike. Clinical status and geographical location will give the species diagnosis.
- Because of the risks associated with the drug treatment, particularly in stage II, it is essential to confirm the diagnosis parasitologically (microscopically) before commencing treatment. A number of serologic assays are available to aid in the diagnosis of trypanosomiasis, but their variable sensitivity and specificity mandate that decisions about treatment be based on demonstration of the parasite. The parasitological confirmation by a second technician may be useful.
- Because of the risks associated with the drug treatment, at least 4 of the 5 characteristics must be present for a sure diagnosis in Giemsa stained films: dimension, nucleus, kinetoplast, cytoplasm, flagellum (and undulating membrane).
- Because of the host immunity response, several specimens may need to be examined before detecting the trypanosomes (successive populations with different surface antigens).
- Blood samples should be taken on heparin for motile detection. Do not use EDTA or citrate as anticoagulant.
- The examination for motile trypanosomes must be done as soon as possible: Trypanosomes are unable to survive for more than 15-20 minutes outside of the host's body (glucose consumption). Trypanosomes are also immobilised by sunlight.
- The stage determination is based on the central nervous system invasion. Following the WHO recommendations for stage determination, the CSF has to be examined at least on white blood cell number, total protein concentration, presence of trypanosomes. Since there is no close relationship between these 3 parameters, all of them should be examined. Other parameters may be useful to use : Measurement of IgM concentration, detection of specific antibodies, …
- *T. b. gambiense* provokes a chronic illness which, without treatment, always ends in the patient's death. The *T. b. gambiense* type II strain (found at the Ivory Coast) however causes an illness with a more acute evolution.

Trypanosoma brucei rhodesiense

Family : Trypanosomatidae

Class : Mastigophorea

Geographic distribution :

East-Africa
(between 15°N and 25°S)
(savanna)

Common name :

East African sleeping sickness

Disease:

- NL : Oost-Afrikaanse trypanosomiase
- FR : trypanosomiase aigue de l'Afrique de l'Est
- EN : East African Trypanosomiases
- ES : enfermedad del sueño forma Rodesiana

Final host:

- Wild animals
- (Domestic animals)
- (Man)

Intermediate host:

(Vector)

Tsetse fly

(*Glossina morsitans* Group)

Transmission:

- Trypomastigotes injected with saliva through the skin when an infested tsetse fly takes a blood meal.
- (Blood transfusion).
- (Mechanical transmission by an insect bite: exceptional).

Localisation of the parasite :

- Stage I: lymph and blood.
- Stage II: (lymph and blood), central nervous system

Diagnostic possibilities:

- Examination of lymph node aspirate for trypomastigotes (less useful for *T. b. rhodesiense*).
- Examination of blood for trypomastigotes :
  - in a wet smear (low sensitivity).
  - in a (thin or) thick blood film stained with Giemsa.
  - Woo (micro-haematocrite centrifugation).
  - QBC.
  - Miniature anion exchange centrifugation technique (mAECT).
- Examination of cerebrospinal fluid for trypomastigotes (diagnostic and stage determination) :
  - in a wet smear (low sensitivity).
  - after single or double centrifugation.
- Detection of specific DNA using PCR
- Serology : not very useful because of the fast disease's evolution.

Morphology of the trypomastigotes (Giemsa):

Dimensions : 15-25 µm.

Cytoplasm : Pale blue, with a flagellum and an undulating membrane.

Contents : A large central nucleus and a small kinetoplast (red).

Characteristics : Trypanosomes may show a variety of forms (pleomorphic).

Associated biological signs :

- Stage I: (erythrocyte sedimentation rate ↑ [IgM↑], moderate leukocytosis, thrombocytopenia, anaemia, …)
- Stage II: (idem + CSF perturbation)

Possible confusion with:

- *Trypanosoma brucei gambiense*
- Animal's trypanosomiases (*T. brucei brucei*,...)
- Motile bacteria (*Borrelia* spp. or water contaminants) or exflagellation of *Plasmodium* spp. for wet smear.
- *Trypanosoma cruzi* (South America)
- *Trypanosoma rangeli* (South America)

Remarks :

- Microscopically, the trypanosomes of *T. b. gambiense* and *T. b. rhodesiense* look the same. Clinical status and geographical location will give the species diagnosis.
- Because of the risks associated with the drug treatment, particularly in stage II, it is essential to confirm the diagnosis parasitologically before starting treatment. A number of serological assays are available to aid in the diagnosis of trypanosomiasis, but their variable sensitivity and specificity mandate that decisions about treatment be based on demonstration of the parasite. The parasitological confirmation by a second technician may be useful.
- Because of the risks associated with the drug treatment, at least 4 of the 5 characteristics must be present for a sure diagnosis in Giemsa stained films : dimension, nucleus, kinetoplast, cytoplasm, flagellum and an undulating membrane.
- Because of the host immunity response, several specimens may need to be examined before detecting the trypanosomes (successive populations with different surface antigens).
- Blood sample should be taken on heparin for motile detection. Do not use EDTA or citrate as anticoagulant.
- The examination for motile trypanosomes must be done as soon as possible: Trypanosomes are unable to survive for more than 15-20 minutes outside of the host body (glucose consumption). Sunlight also immobilizes the trypanosomes.
- The stage determination is based on the central nervous system invasion. Following the WHO recommendations for stage determination, the CSF has to be examined at least on white blood cell number, total protein concentration, presence of trypanosomes. Since there is no close relationship between these 3 parameters, all of them should be examined. Other parameters may be useful : Measurement of IgM concentration, detection of specific antibodies, …
- *T. b. rhodesiense* provokes an acute illness which, without treatment, always ends in the patient's death. The *T. b. rhodesiense* type Zambia strain (found at Zambia and Malawi) however causes an illness with a more chronic evolution.

Trypanosoma cruzi

Family : Trypanosomatidae

Class : Mastigophorea

Geographic distribution :

South-America
(From South of USA down to Argentina)

Common name :

American Trypanosomiases

Disease:

- NL : ziekte van Chagas
- FR : maladie de Chagas
- EN : Chagas disease
- ES : enfermedad de Chagas

Final host:

- Man.
- Wild animals.
- Domestic animals.

Intermediate host:

(Vector)

Blood sucking bug

"Kissing Bugs"

(Triatominae)

Transmission:

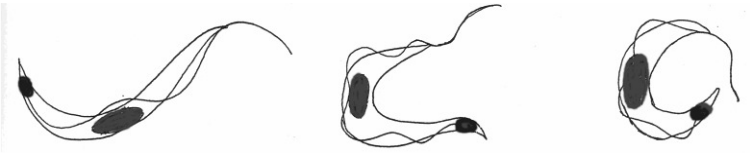
- Metacyclic trypomastigotes from bug's faeces will penetrate the bite wound or the conjunctiva.
- Blood transfusion (fresh blood).
- Congenital transmission.
- (Food or drinks, soiled with the faeces of infected bugs or containing dead bugs?)

Localisation of the parasite :

- In blood (trypomastigote form)
- Intracellular in tissue (amastigote form)

Diagnostic possibilities:

- Serology (CFT, IFAT, ELISA, agglutination, Dot Blot, …)
- (Examination of lymph node aspirate [or biopsy] for amastigotes).
- Examination of blood for trypomastigotes (acute or congenital phase):
  - in a wet smear (low sensitivity).
  - in a (thin or) thick blood film stained with Giemsa.
  - Woo (micro-haematocrite centrifugation).
  - QBC.
  - Strout concentration technique.
- Xenodiagnosis: searching parasites in feaces of *T. infestans* nyphs (takes up to 60 days for the result).
- Blood culture (KIVI : kit for *in vitro* isolation, NNN-medium [Novy-Nicolle-McNeal agar]).
- Detection of specific DNA using PCR



Morphology of the trypomastigotes (blood after Giemsa staining):

Dimensions : 15-25 µm x 2-6 µm.
Cytoplasm : Pale blue, with a flagellum and an undulating membrane.
Contents : A large central nucleus and a **big** kinetoplast (red).
Characteristics : Usually C, U or S shaped.

Morphology of the amastigotes (biopsy after Giemsa staining):

Dimensions : 4-6 µm.
Cytoplasm : Round, pale blue.
Contents : A central nucleus and a small kinetoplast (red).
Characteristics : Intra or extra- cellular.

Associated biological signs :

- Romaña's sign or chagoma for the acute phase.
- Perturbation in hepatical tests for the chronic phase.

Possible confusion with:

- *Leishmania* spp. (amastigotes).
- *Trypanosoma rangeli*.
- Motile bacteria (*Borrelia* spp. or water contaminants) and exflagellation of *Plasmodium* spp. for wet smears.
- *Trypanosoma brucei rhodesiense* (Africa).
- *Trypanosoma brucei gambiense* (Africa).
- Animal's trypanosomiases (*T. brucei brucei*,...)

Remarks :

- In areas where *T. rangeli* (non-pathogenic species transmitted by *Rhodnius* bugs) is found together with *T. cruzi*, these parasites must be differentiated by the size of their kinetoplast.
- The examination for motile trypanosomes must be done as soon as possible: Trypanosomes are unable to survive for more than 15-20 minutes outside of the host's body (glucose consumption). Trypanosomes are also immobilized by sunlight.
- The blood sample should be taken on heparin for motile detection. Do not use EDTA or citrate as anticoagulant.
- In chronical Chagas disease, the numbers of circulating trypanosomes are too few to be detected using easy techniques. Culture, xenodiagnosis or serology must be used.
- The amastigotes develop into trypomastigotes which are released into the blood. No multiplication of the parasite occurs in its trypomastigote stage in the blood.
- In a biopsy, it is possible to find amastigotes, but also epimastigotes (intermediary forms between amastigotes and trypomastigotes).

Trypanosoma rangeli

Family : Trypanosomatidae

Class : Mastigophorea

Geographic distribution :

South-America
(Brazil, Venezuela, Colombia, Panama, El Salvador, Ecuador and Guatemala)

Common name :

Disease:

Non pathogenic

Final host:

- Man.
- Wild animals.
- Domestic animals.

Intermediate host:

(Vector)

Blood sucking bug

Rhodnius Bugs

(Triatominae)

Transmission:

- Metacyclic trypomastigotes injected with saliva through the skin when an infested vector takes a blood meal.
- [Metacyclic trypomastigotes, from bug's faeces will penetrate the skin or conjunctiva.]
- Blood transfusion (fresh blood).
- Congenital transmission.
- (Food or drinks, soiled with the faeces of infected bugs or containing dead bugs ?)

Localisation of the trypomastigotes :

In blood (trypomastigote form)

Diagnostic possibilities:

- Identification of trypomastigotes in (Giemsa) stained blood-preparations: species differentiation possible
- Detection of specific DNA using PCR

Morphology of the trypomastigotes (blood after Giemsa staining):

Dimensions : 30 - 35 µm x 2 – 6 µm.

Cytoplasm : Pale blue, with a flagellum and an undulating membrane.

Contents : A large central nucleus and a small kinetoplast (red).

Characteristics : Trypanosomes may show a variety of forms (pleomorphic).

Associated biological signs :

Non pathogenic

Possible confusion with:

- *Trypanosoma cruzi*.
- Motile bacteria (*Borrelia* spp. or water contaminants) and exflagellation of *Plasmodium* spp. for wet smears.
- *Trypanosoma brucei rhodesiense* (Africa).
- *Trypanosoma brucei gambiense* (Africa).
- Animal's trypanosomiases (*T. brucei brucei*,...)

Remarks :

- In areas where *T. rangeli* is found together with *T. cruzi*, these parasites must be differentiated by the size of their kinetoplast.
- Microscopically, the trypanosomes of *T. b. gambiense*, *T. b. rhodesiense* and *T. rangeli* look the same. Clinical status and geographical location will give the species diagnosis.
- In man, *T. rangeli* will only be found in blood (and not in tissue).
- There is no cross immunity between *T. rangeli* and *T. cruzi*.

Leishmania spp.

Family : Trypanosomatidae

Class : Mastigophorea

Geographic distribution :

Various disease forms in southern Europe, tropical and sub-tropical countries.

Common name :

Disease:

- NL : (viscerale, cutane en muco-cutane) leishmaniose
- FR : leishmaniose (viscérale, cutanée et muco-cutanée)
- EN : leishmaniasis (visceral, cutaneous and mucocutaneous)
- ES : leishmaniasis (visceral, cutaneas y mucocutaneas)

Final host:

- Man.
- Various animals (depending on *Leishmania* spp.).

Intermediate host:

(Vector)

Sand flies

Phlebotomus spp. (Old World)

Lutzomya spp. and *Psychodogylus* spp. (New World)

Transmission:

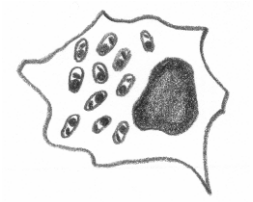
- Metacyclic forms injected with saliva through the skin when an infested vector takes a blood meal.
- Direct contact with leishmaniasis lesions.
- Blood transfusion.
- Crushing an infested vector on (damaged) skin or on the mucosa.
- [Contact with infested vector's faeces: exceptional]

Localisation of the parasite :

Macrophages and other phagocytic cells (amastigotes)

Diagnostic possibilities :

- Microscopic detection of amastigotes in Giemsa stained slides of relevant tissue [lesion's exsudate or biopsy (cutaneous or mucocutaneous leishmaniasis), aspirations from the spleen, liver, bone marrow, lymph gland (visceral leishmaniasis)].
- Isolation of the parasite in culture (Nogushi Wenyon, Tobie's Rabbit Blood, …)
- Montenegro's test (leishmanin test) (for epidemiological studies).
- Serology : antibody detection in serum (IFAT, ELISA, DATS)
- Detection of specific DNA using PCR (diagnosis and (sub-)species differentiation).



Morphology of the amastigotes (Giemsa):

Size : 1,5 - 3 µm x 2,5 – 6,5 µm.

Cytoplasm : Round or oval, pale blue.

Contents : A large central nucleus and a small rod-shaped kinetoplast (red).

Characteristics : the amastigotes may be found extra- or intra-cellularly

Associated biological signs :

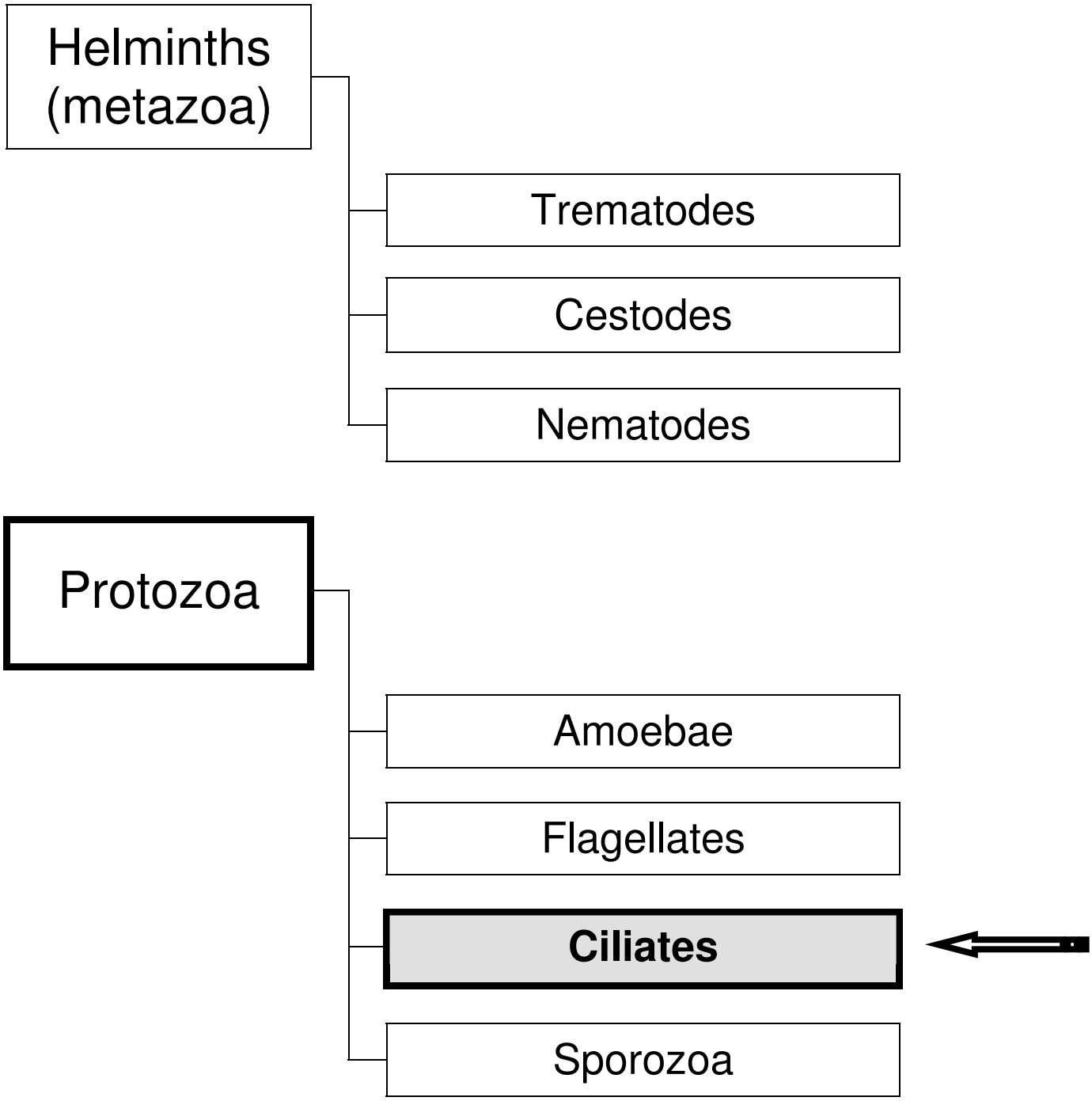
- Visceral leishmaniasis : leucopenia, thrombocytopenia and anaemia
- Perturbation in coagulation and hepatic tests, increases of globulins in blood [IgG] (ESR ↑, positive formol gel test), low serological albumin levels

Possible confusion with:

- Bacteria (diplococci)
- *Trypanosoma cruzi* (Intracellular forms)
- *Toxoplasma gondii*

Remarks :

- Microscopically, the different species of *Leishmania* look the same. Differentiation must be based on monoclonal antibodies or isoenzymatic or molecular analysis. Geographical location may be indicative.
- In case of visceral leishmaniasis, the sensitivity for the parasites detection in Giemsa stained slides depends on the tissue collected: approximately 95% for aspirates from the spleen, 50-85% for bone marrow, 70% for liver biopsies and 65% for lymph gland aspiration. Splenic or hepatic aspirations should be avoided with regard to possible complications during collection.
- Serology is mainly useful for visceral leishmaniasis. The antibodies are even detectable before clinical signs. Sensitivity is good (except for immunocompromised patients). Cross reactions occur with other Trypanosomatidae. Because of the poor antibody response in cutaneous and mucocutaneous leishmaniasis, serological tests are of little value in diagnosis. The formol gel test can prove very useful in cases of visceral leishmaniasis. It shows increases of immunoglobulins (IgG) in the blood (and therefore however is a non-specific test).
- Untreated visceral leishmaniasis always ends in death of the patient.


Helminths (metazoa)
Trematodes
Cestodes
Nematodes
Protozoa
Amoebae
Flagellates
Ciliates
Sporozoa
Fungi and Bacteria
Unknown classification

Balantidium coli

Family : Balantidiae

Class : Listomatea

Geographic distribution :

Rare and Worldwide

Contact with pigs

Common name :

Disease:

- NL : balantidiose
- FR : balantidiase
- EN : balantidiasis
- ES : balantidiasis

Final host :

- Pigs
- Man
- Monkeys, dogs rats

Intermediate host:

Without intermediate host and without vector

Transmission :

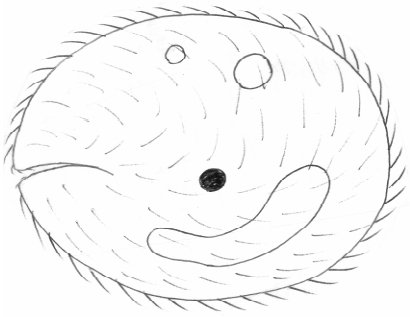
Faeco-oral, mature cysts ingestion.

Localisation of the parasite :

Intestinal lumen.

Diagnostic possibilities :

- Trophozoites detection in faeces : direct examination.
- Cysts detection in faeces : direct examination, iodine staining, concentration by sedimentation.
- Trophozoites detection in tissue collected during endoscopy.



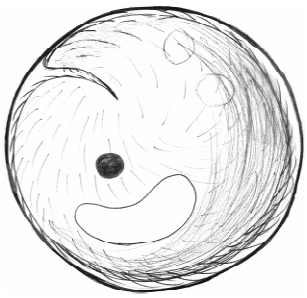
Morphology of the trophozoites :

Size : 50-200 µm x 40-70 µm

Nucleus : 2 : one macronucleus (kidney shaped) and one micronucleus.

Motility : In one definite direction. Rapid revolving motility.

Characteristics :
Body surface covered by longitudinal rows of cilia. Cytostome most of the times visible, beating cilia can be seen around the cytostome.



Morphology of the cysts :

Size : 40-65 µm

Morphology : Spherical or oval.

Nucleus : 2 : one macronucleus (kidney shaped) and one micronucleus.

Characteristics :
Thick-walled, cytostome and cilia may be seen in younger cyst.

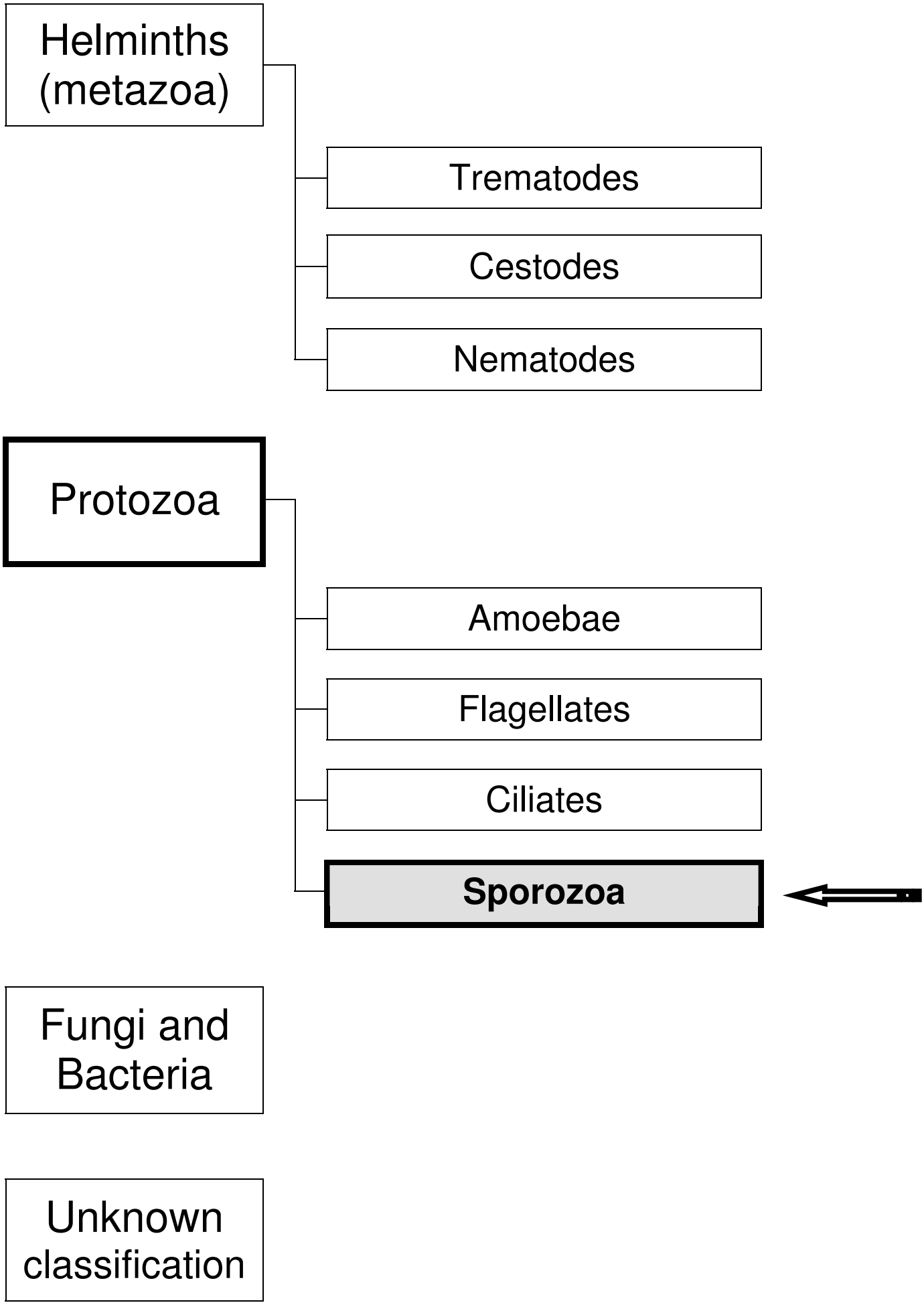
Associated biological signs :

Possible confusion with:

- Plant cells.
- Other non pathogenic ciliates: infusiora type (contamination from the atmosphere).

Remarks :

- It is the only ciliate which can parasitize humans. There are a lot of different ciliates in the soil or in the water. Always consider contamination.
- Cysts are found in more formed specimens, trophozoïtes are found mainly in diarrhoeic specimens.
- Trophozoïtes and cysts are excreted irregularly. Several specimens collected at different times may need to be examined.
- Specimens must be examined without delay, otherwise identification of the trophozoïtes becomes impossible because they lose their motility.
- Iodine stain will immediately kill any trophozoïtes.
- *Balantidium coli* usually remains confined to the intestinal lumen. In some rare cases, the trophozoites invade the intestinal mucosa, or (exceptionally) through the bloodstream extra intestinal sites (liver, lungs,…).
- This parasite is more frequently found in patients working at pig-farms.


Helminths (metazoa)
Trematodes
Cestodes
Nematodes
Protozoa
Amoebae
Flagellates
Ciliates
Sporozoa
Fungi and Bacteria
Unknown classification

Plasmodium falciparum

Family : Plasmodiidae

Class : Haemosporidea

Geographic distribution :

Tropical and subtropical regions
- America
- Africa
- Asia (airports!!!)

Common name :
- Malaria
- Paludism

Disease:
- NL : kwaardaardige tertiana, pernicieuze koorts
- FR : fièvre tierce maligne, fièvre pernicieuse
- EN : falciparum malaria
- ES : tertiana maligna, fiebre perniciosa

Final host:
- Man

Intermediate host:

(Vector)

Female mosquitoes

Anopheles spp.

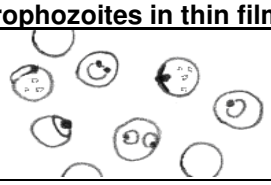
Transmission:
- Sporozoites inoculation during a blood meal by a malaria infested vector.
- Blood transfusion.
- Congenital transmission.

Localisation of the parasite :
- Intracellular in parenchymal cells of the liver.
- Intracellular in red blood cells.

Diagnostic possibilities:
- Blood parasites detection :
  - In a thin blood film (species differentiation and quantification).
  - In a thick blood film (pre-differentiation of species and quantification).
  - In QBC (pre-differentiation of species and quantification).
- Serology
  - *Plasmodium*'s specific Ag detection : HRP-II, Aldolase, pLDH (rapid tests)
  - *Plasmodium*'s specific Ab detection : IFA, ELISA, … (epidemiology)
- Detection of specific DNA using PCR
- (Malaria pigment and/or RBC alterations detection with an haematological analyser)

Trophozoites in thick film :

Trophozoites in thin film:



Schizonts in thick film :

Schizonts in thin film:

Gametocytes in thick film:

Gametocytes in thin film:

Main characteristics in thick film:
- Usually only trophozoites and gametocytes seen (except for severe infections).
- Small ring or comma forms, often 2 chromatin dots.
- Banana shaped gametocytes (if present).
- Uniform image.

Main characteristics in thin film :
- Infected erythrocyte has normal size and shape.
- Usually only trophozoites and gametocytes seen (except for severe infections)
- Small ring forms, trophozoites may lie on RBC membrane (accolé forms or marginal forms)
- Polyparasitism common.
- Banana shaped gametocytes (if present).
- Maurer's clefts possible (few large pink granules in older infected RBC; only at pH=8).

Main associated biological signs :
- Fever.
- Anaemia (haemolytic).
- Trombocytopenia.
- Hypoglycaemia.
- High (direct) bilirubin and LDH level.
- Low haptoglobin level.

Possible confusion with:
- Blood platelets
- Other *Plasmodium* spp.
- Howell-Jolly bodies
- *Babesia* spp. and *Theileria* spp.
- *Toxoplasma gondii* (~ gametocytes)

Remarks :
- In a thick blood film, the uniform aspect of *Plasmodium falciparum* may help to make the differential diagnosis.
- Exceptionally, in heavy infection, no parasites will be found (*Plasmodium falciparum* infested RBCs adhere to endothelium in organ capillaries = sequestration)
- The presence of schizonts of *P. falciparum* in the periferal bloodstream and/or the presence of hemozoïne in WBCs indicate severe infestations.
- Gametocytes are not seen early in the infection (about 10 days after initial infection), so their absence from blood films should not exclude a diagnosis. Gametocytes can exceptionally be round or exhibit exflagellation!
- Parasitemia up to 50% of RBCs infested and more (2.500.000 parasites/µl of blood or more).

Plasmodium vivax

Family : Plasmodiidae

Class : Haemosporidea

Geographic distribution :

(at least 16°C isotherm during the hot season)

- America
- Asia
- Africa
- …

Common name :

- Malaria
- Paludism

Disease:

- NL : goedaardige tertiana
- FR : fièvre tierce bénigne
- EN : vivax malaria
- ES : tertiana benigna

Final host:

- Man

Intermediate host:

(Vector)

Female mosquitoes

Anopheles spp.

Transmission:

- Sporozoites inoculation during a blood meal by a malaria infested vector.
- Blood transfusion.
- Congenital transmission.

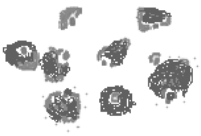
Localisation of the parasite :

- Intracellular in parenchymal cells of the liver (hypnozoïtes possible).
- Intracellular in red blood cells.

Diagnostic possibilities:

- Blood parasites detection :
  - in thin blood film (species differentiation and quantification).
  - in thick blood film (pre-differentiation of species and quantification).
  - in QBC (pre-differentiation of species and quantification).
- Serology
  - *Plasmodium*'s specific Ag detection : Aldolase, pLDH (rapid tests)
  - *Plasmodium*'s specific Ab detection : IFA, ELISA, … (epidemiology)
- Detection of specific DNA using PCR
- (Malaria pigment and/or RBC alterations detection with an haematological analyser)

Trophozoites in thick film :



Trophozoites in thin film:

Schizonts in thick film :

Schizonts in thin film:

Gametocytes in thick film:

Gametocytes in thin film:

Main characteristics in thick film:

- All stages may be found in the same film.
- Typical amoeboid old trophozoites.
- Mature schizonts with 16 merozoites (or more).
- Small pink dots (Schüffner's stippling) in "ghost" of RBC (only for old forms).

Main characteristics in thin film :

- Large and/or irregular host cells.
- All stages may be found in the same film.
- Typical amoeboid old trophozoites.
- Mature schizonts with 16 merozoites (or more).
- Fine Schüffner's stippling (only for old forms).

Main associated biological signs :

- Fever.
- Thrombocytopenia.

Possible confusion with:

- Platelets
- Other *Plasmodium* spp.
- Howell-Jolly bodies
- *Babesia* spp. and *Theileria* spp.

Remarks :

- Relapses are a feature of *P. vivax* malaria, due to the delayed release of merozoites from liver cells (hypnozoites).
- In a thick blood film, the cloud-like aspect of *Plasmodium vivax* may help to make the differential diagnosis.
- Rarely found in Duffy negative population (Central Africa). Duffy = blood-group Ag which *P. vivax* needs to attach to and invade RBC.
- All stages (trophozoites, schizonts and gametocytes) may be found at the same time in the peripheral blood.
- Parasitemia up to 4% of RBCs infested (or 200.000 parasites/µl of blood).

Plasmodium ovale

Family : Plasmodiidae

Class : Haemosporidea

Geographic distribution :

- Tropical and sub-tropical Africa
- West Pacific
- South America (small focusses)

Common name :

- Malaria
- Paludism

Disease:

- NL : goedaardige tertiana
- FR : fièvre tierce bénigne
- EN : ovale malaria
- ES : fiebre tertiana

Final host:

- Man

Intermediate host:

(Vector)

Female mosquitoes

Anopheles spp.

Transmission:

- Sporozoites inoculation during a blood meal by a malaria infested vector.
- Blood transfusion.
- Congenital transmission.

Localisation of the parasite :

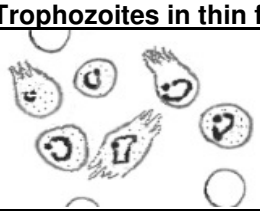
- Intracellular in parenchymal cells of the liver (hypnozoïtes possible).
- Intracellular in red blood cells.

Diagnostic possibilities:

- Blood parasites detection :
  - In thin blood film (species differentiation and quantification).
  - In thick blood film (pre-differentiation of species and quantification).
  - In QBC (pre-differentiation of species and quantification).
- Serology
  - *Plasmodium*'s specific Ag detection : Aldolase, pLDH (rapid tests)
  - *Plasmodium*'s specific Ab detection : IFA, ELISA, … (epidemiology)
- Detection of specific DNA using PCR
- (Malaria pigment and/or RBC alterations detection with an haematological analyser)

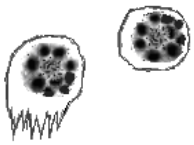
Trophozoites in thick film :

Trophozoites in thin film:



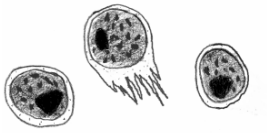
Schizonts in thick film :

Schizonts in thin film:



Gametocytes in thick film:

Gametocytes in thin film:



Main characteristics in thick film:

- All stages may be found in the same film.
- Parasites quite small and compact.
- Mature schizonts usually contain 8 merozoites.
- Large pink/red dots (Schüffner's stippling) in "ghost" of RBC.

Main characteristics in thin film :

- Large host cells, often oval, with torn jagged edges.
- All stages may be found in the same film.
- Parasites quite small and compact.
- Mature schizonts usually contain 8 merozoites.
- Prominent pink/red dots (Schüffner's stippling) in the infected erythrocyte.

Main associated biological signs :

- Fever.

Possible confusion with:

- Platelets
- Other *Plasmodium* spp.
- Howell-Jolly bodies
- *Babesia* spp. and *Theileria* spp.

Remarks :

- Relapses are a feature of *P. ovale* malaria, due to the delayed release of merozoïtes from liver cells (hypnozoïtes).
- All stages (trophozoites, schizonts and gametocytes) may be found at the same time in the peripheral blood.
- Parasitemia up to 4% of RBCs infested (or 200.000 parasites/µl of blood).

Plasmodium malariae

Family : Plasmodiidae

Class : Haemosporidea

Geographic distribution :

More or less cosmopolite

- Common in Africa and Asia
- Less common in America

Common name :

- Malaria
- Paludism

Disease:

- NL : goedaardige quartana
- FR : fièvre quarte bénigne
- EN : malariae malaria
- ES : la cuartana

Final host:

- Man

Intermediate host:

(Vector)

Female mosquitoes

Anopheles spp.

Transmission:

- Sporozoites inoculation during a blood meal by a malaria infested vector.
- Blood transfusion.
- Congenital transmission.

Localisation of the parasite :

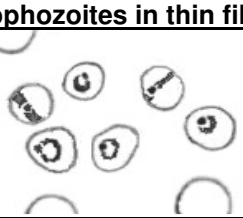
- Intracellular in parenchymal cells of the liver.
- Intracellular in red blood cells.

Diagnostic possibilities:

- Blood parasites detection :
  - In thin blood film (species differentiation and quantification).
  - In thick blood film (pre-differentiation of species and quantification).
  - In QBC (pre-differentiation of species and quantification).
- Serology
  - *Plasmodium*'s specific Ag detection : Aldolase, pLDH (rapid tests)
  - *Plasmodium*'s specific Ab detection : IFA, ELISA, … (epidemiology)
- Detection of specific DNA using PCR
- (Malaria pigment and/or RBC alterations detection with an haematological analyser)

Trophozoites in thick film ;

Trophozoites in thin film:

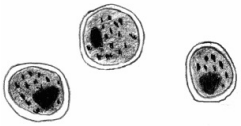


Schizonts in thick film :

Schizonts in thin film:

Gametocytes in thick film:

Gametocytes in thin film:



Main characteristics in thick film:

- All stages may be found in the same film.
- Mature schizonts contain 6-12 merozoites, arranged in rosette formation around a clump of pigment.
- Parasites small, compact, densely stained.
- Pigment already present in early stages.

Main characteristics in thin film :

- Infected RBCs are normal or smaller.
- All stages may be found in the same film.
- Parasites small, compact, densely stained.
- Mature schizonts contain 6-12 merozoites, arranged in rosette formation around a clump of pigment.
- Pigment already present in early stages.
- Trophozoïtes in characteristic band form (rare).

Main associated biological signs :

- Fever

Possible confusion with:

- Platelets
- Other *Plasmodium* spp.
- Howell-Jolly bodies
- *Babesia* spp. and *Theileria* spp.

Remarks :

- Parasitaemia is usually low (less than 1% of RBCs infested or 50.000 parasites/µl of blood).
- All stages (trophozoites, schizonts and gametocytes) may be found at the same time in the peripheral blood.
- In a thick blood film, the "dirty" aspect of *Plasmodium malariae* may help to make the differential diagnosis.
- *Plasmodium knowlesi*, a malaria parasite infesting monkeys and sporadically humans in the area of Malaysia and Borneo, has the same morphology as *P. malariae*, but with a significantly higher parasitaemia. Because *P. knowlesi* replicates every 24h, prompt effective treatment is essential. **All patients coming from Southeast Asia presenting "*P. malariae*" hyperparasitaemia should receive intensive management as appropriate for severe falciparum malaria.**

Characteristics of *Plasmodium* species in thin blood films

Characteristics	*Plasmodium falciparum*	*Plasmodium vivax*	*Plasmodium malariae*	*Plasmodium ovale*
Incubation period	7 - 21 days Mean = 12	8 - 31 days Mean = 14	19 - 37 days Mean = 28	11 – 16 days
Hypnozoites	No	Yes	No	Yes
Relapse	No, but sometimes recrudescence up to 1 year	Yes, ± 6 months to 4 years	Possible after a long period of time (recrudescence up to 52 years)	Possible, but most of the time natural suppression
Asexual cycle in blood	24-48 hours	48 hours	72 hours	48 hours
Size of infected erythrocyte	Normal (infects all RBCs)	Large and often pale blue staining (infects young RBCs)	Normal or smaller (infects old RBCs)	Large, normal or smaller; oval with torn jagged edges
Dots in the infected erythrocyte	Maurer's clefts (pH=8): in some infected RBCs with mature trophozoites	Schüffner's dots : small pink dots found in some infected RBCs with mature trophozoites	No	Schüffner's dots : large red dots practically always present
Stages found	Most of the time, only trophozoites and/or gametocytes. Exceptionally, schizonts and young gametocytes (severe infections)	All stages may be found	All stages may be found	All stages may be found
Parasite density	Up to 2.500.000/µl and more	Maximum 200.000/µl (4%)	Maximum 50.000/µl (1%)	Maximum 200.000/µl (4%)
Young trophozoite :				
Cytoplasm	Small, fine pale ring Occasional "accolé" forms	Quite big ring	Compact ring	Quite compact
Chromatin	1 to 2 red dots	Quite big, sometimes 2 dots	Generally 1dot	1 dark dot
Vacuole	Visible	Big	Not or barely visible	Not or barely visible
Pigment	No	No	Sometimes	No
Polyparasitism	Often	Sometimes	Usually not	Usually not
Mature trophozoite :				
Cytoplasm	Little amoeboïd, most of the time lying on the red cell membrane	Amoeboid, fragmented, may almost fill the RBC	Compact, sometimes amoeboïd ; Typical band form	Little amoeboïd, do not fill the RBC
Chromatin	1 to 2 medium size red dots	1 or 2 quite big dots	1 to 2 big dots	1 tot 2 dark black dots
Vacuole	1 or more, variable size	1 or more big vacuoles	Not visible	Small, not all the times visible
Pigment	Exceptionally, 1 black dot	Small, brown, scattered, sometimes 1 compact mass	Yes (big, black mass)	Very rare
Young schizont :				
Cytoplasm	Irregular, few	Large, irregular	Compact	Compact
Chromatin	2 – 4	2 or more, variable size	2 – 4	2 – 4
Vacuole	No	Sometimes up to 4 small vacuoles	NO	NO
Pigment	Clumped in one mass	Dark, scattered	Dark, scattered on the parasite	Dark, scattered on the parasite

	Plasmodium falciparum	***Plasmodium vivax***	***Plasmodium malariae***	***Plasmodium ovale***
Mature schizont :				
Cytoplasm	2/3 of the RBC	May almost fill the RBC	May almost fill the RBC	Do not fill the RBC
Chromatin	8 – 24 or more	12 – 24, most of the time 16	6 -12, most of the time 8, clustered around	4 – 16
Pigment	Single dark mass	Concentrated in 1 or 2 masses	Compact mass, sometimes central	Concentrated in 1 or 2 masses
Macrogametocyte (female gametocyte) :				
Cytoplasm	Pale blue, banana shaped, exceptionally round, RBC most of the time not visible	Pale blue, round of oval, may almost fill the RBC	Pale blue, round, may almost fill the RBC	Pale blue, round, do not fill the RBC
Chromatin	1 well defined mass	1 well defined mass	1 well defined mass	1 well defined mass
Pigment	Dark, scattered rice-grain like, lying on the nucleus	Brown granualtions, scattered on the parasite	Dark granulations, scattered on the parasite	Dark granulations, scattered on the parasite
Microgametocyte (male gametocyte) :				
Cytoplasm	Pink-blue, banana shaped, exceptionally round, RBC most of the time not visible	Pink-blue or colorless, round or oval; may almost fill the RBC	Pink-blue or colorless, round or oval; may almost fill the RBC	Pink-blue or colorless, round or oval; fillnot the RBC
Chromatin	1 big diffuse mass	1 big, diffuse chromatin mass	1 big, diffuse mass	1 big, diffuse mass
Pigment	Dark, scattered rice-grain like, scatered on the nucleus	Brown-black, scaterred on the parasite	Dark, brown-black, scaterred on the parasite	Dark, scaterred on the parasite
Main characteristics :				
	➢ Infected erythrocytes have normal size. ➢ Only trophozoites and gametocytes usually seen (except in severe infections) ➢ Small ring forms, trophozoites may lie on red cell membrane (marginal forms) ➢ Polyparasitism common. ➢ Banana shaped gametocytes. ➢ Maurer's clefts, few large pink granules can be found in some infected red cells (pH=8). ➢ Often very high parasite density : can be over 50%	➢ Enlarged or irregular host cell. ➢ All stages usually seen in the same film. ➢ Typical amoeboid old trophozoites. ➢ Mature schizonts with 16 merozoites (or more). ➢ Fine Schüffner's stippling (only for old forms). ➢ Medium parasite density : up to 4%.	➢ Infected cells are normal or smaller. ➢ All stages usually seen in the same film. ➢ Parasites small, compact, densely stained. ➢ Mature schizonts contain 8 merozoites, arranged in rosette formation around a clump of pigment. ➢ Pigment early present. ➢ Trophozoites in the characteristic band form (rare) ➢ Low parasite density: up to 1%	➢ Infected cells enlarged, oval, with torn jagged edges. ➢ All stages usually seen in the same film. ➢ Parasites quite small and compact. ➢ Mature schizonts contain 8 merozoites. ➢ Prominent pink/red dots (Schüffner's stippling) in the infected erythrocyte. ➢ Medium parasite density: up to 4%

Characteristics of *Plasmodium* species in thick blood films

Characteristics	*Plasmodium falciparum*	*Plasmodium vivax*	*Plasmodium malariae*	*Plasmodium ovale*
Dots surrounding parasite	No	Schüffner dots: fine stipplings in "ghost" of host red cells	No	Schüffner dots: big stipplings in "ghost" of host red cells
Stages found	Most of the time, only trophozoites and/or gametocytes. Exceptionally schizonts (severe infections)	All stages may be found	All stages may be found	All stages may be found
Trophozoites :				
Size	Small to medium	Small to big	Small	Small to medium
Form	Ring and comma forms common	Broken ring or irregular form	Ring or round, compact	Ring or round, compact
Cytoplasm	Regular, fine to fleshy	Irregular or fragmented	regular, dense	Fairly regular
Chromatin	1 to 2 dots	Quite big, occasionally 2 dots	Single large dot	1 dense dot
Pigment	Exceptionally compact mass	Scattered, fine	Scattered, coarse	Scattered, big
Schizonts :				
Size	Small, compact	Big	Small, compact	Medium
Chromatin	8 to 24 or more	12 to 24, most of the times 16	6 to 12, most of the times 8	4 to 16, most of the times 8
Pigment	Single dark mass	Diffuse mass	Concentrated mass, occasionally in the center	Concentreted
Gametocyte :				
Form	Banana shaped, exceptionally round	Round, big	Round, compact	Round, medium size
Chromatin	1 well defined mass	1 well defined mass	1 well defined mass	1 well defined mass
Pigment	Scattered, coarse rice grain like	Scattered, fine	Scattered, big mass	Scattered big dots
Main characteristics :				
	➢ Only trophozoites and gametocytes usually seen (except in severe infections). ➢ Small ring or comma forms, often 2 chromatin dots. ➢ Banana shaped gametocytes. ➢ Uniform image.	➢ All stages may be seen in the same film. ➢ Typical amoeboid old trophozoites. ➢ Mature schizonts with 16 merozoites (or more). ➢ Small pink dots (Schüffner's stippling) in "ghost" of red cells (only for old forms).	➢ All stages may be seen in the same film. ➢ Mature schizonts contain 6-12 merozoites, arranged in rosette formation around a clump of pigment. ➢ Parasites small, compact, densely stained. ➢ Pigment early present.	➢ All stages may be seen in the same film. ➢ Parasites quite small and compact. ➢ Mature schizonts contain 8 merozoites. ➢ Large pink/red dots (Schüffner's stippling) in "ghost" of red cells.

Toxoplasma gondii

Family : Sarcocystiidae

Class : Coccidea

Geographic distribution :

Worldwide

Common name :

Disease:

- NL : toxoplasmose
- FR : toxoplasmose
- EN : toxoplasmosis
- ES : toxoplasmosis

Final host:

- Felidae

Intermediate host:

- Various animals
- Man

Transmission:

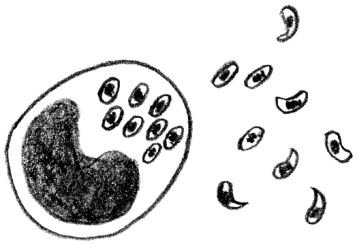
- Transparenteral (blood transfusion and organ transplantation).
- Ingestion of sporulated oocysts [by way of cats].
- Ingestion of meat contamined by bradyzoites (tachyzoites) [by way of sheep].
- Ingestion of bradyzoites after meat manipulation.

Localisation of the parasite :

Intracellular, in internal organs

Diagnostic possibilities:

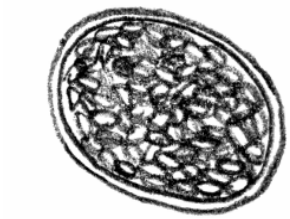
- Serology (IgM, IG G, Ig A, [Ig E] . Especially in detecting congenital infections in utero.
- Tachyzoites detection in Giemsa stained preparations (various specimens : lymph nodes or bone marrow aspirates, CSF, placenta, bronchoalveolar lavage,…).
- Bradyzoites or tachyzoites detection in histological preparation.
- (Parasites detection after culture or animal inoculation)
- Detection of specific DNA using PCR.
- X-ray, scanner, …



Morphology of the trophozoites (man):

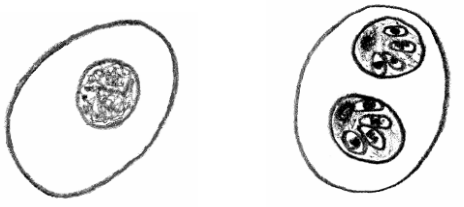
(Giemsa)

5 - 12 µm x 2 – 4 µm, intracellular (Round or ovale) or extracellular (water-drop shaped). Pale blue cytoplasm and central red nucleus.



Morphology of the cysts (man-animals) :

(Mainly in brain or muscular tissues). Up to 200 µm, spherical or oval, with a thick shell. The cyst contains 50 to 3.000 **bradyzoites**.



Morphology of the oocysts (cat faeces) :

10-15 µm, spherical or oval, non sporulated oocyst contains a granular masse. Sporulated oocyst contains 2 **sporocysts** containing 4 **sporozoites**

Main associated biological signs :

- Various
- (immunocompromission)

Possible confusion with :

- *Leishmania* spp., *Pneumocystis* spp. and *Sarcocystis* spp.
- *Plasmodium falciparum* (gametocytes).

Remarks :

- Parasitaemia is usually low.
- For immunocompromised patients, it is possible to find tachyzoites in peripheral blood monocytes.
- Serological tests may help for immunocompromised patients, but the interpretation of tests results is often difficult.

Sarcocystis spp.

Family : Sarcocystiidae

Class : Coccidea

Geographic distribution :

Worldwide

Common name :

Disease:

- NL : (Intestinale of musculaire) sarcocystose
- FR : Sarcocystose (intestinale ou musculaire)
- EN : (Intestinal or muscular) Sarcocystosis
- ES : Sarcocystosis intestinal or muscular

Final host:

- Carnivorous
- Omnivorous
- (Man)

Intermediate host:

- Pigs
- Herbivorous
- (Man)

Transmission:

- Intestinal form : Ingestion of meat contamined by cysts [intermediate host].
- Muscular form : faeco-oral : ingestion of sporocysts [final host].

Localisation of the parasite :

- Intestinal form : Schizogony in epithelial cells of the intestine. Oocysts excreted in the faeces.
- Muscular form : Schizogony in epithelial cells of the blood vessel, muscular cysts formation.

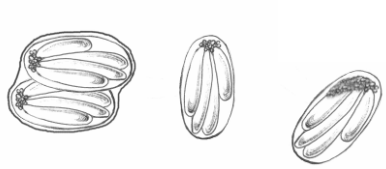
Diagnostic possibilities:

Intestinal form :

- Oocysts detection in faeces : Direct examination, concentration by sedimentation or by flotation.
- Detection of specific DNA using PCR.

Muscular form :

- Cysts (sarcocysts) identification in muscular biopsy.



Morphology of the oocysts :

Faeces, direct examination

Size : 11-17 µm x 10-16 µm

Morphology : Oval or variable.

Contents : 2 sporocysts with 4 banana shaped sporozoites inside

Characteristics : Sporocysts in faeces are all the time sporulated and contain a refringent mass. Oocysts are fragile. Most of the times, the sporocysts are free in faeces.

Main associated biological signs :

- Watery diarrhoea without blood
- Often hypereosinophilia.
- Often presence of Charcot-Leyden crystals in the faeces

Possible confusion with :

- *Giardia lamblia* (cysts).
- Animal coccidians (oocysts in transit).

Remarks :

- The number of parasites found is usually very low, rendering concentration-techniques quite useful. For the intestinal form, oocysts can be detected in the faeces starting from about two weeks after infestation
- Muscular or intestinal infestation are rarely serious and often asymptomatic. This parasite is more an opportunist.
- Humans may serve as either final (Intestinal infection with *Sarcocystis bovi-hominis* (herbivorous as intermediate host) or *Sarcocystis sui-hominis* (pigs as intermediate host)) or intermediate host (Muscular infection with different species of S*arcocystis* with a variety of different final hosts).
- Oocysts present in human faeces are always sporulated and are not infectious to humans.
- During the short blood part of their life cycle (muscular form only), rare sporozoites may be found in blood (to be differentiated from *Toxoplasma* spp. or gametocytes of *Plasmodium falciparum.*
- *Isospora hominis*, is the old name for the human intestinal form of *Sarcocystis* spp.
- When stained with the rapid technique of Heine, the oocysts (and sporocysts) show as non-stained, strongly refractile structures on a pink to purple background.
- As the oocysts (and sporocysts) are acid-fast structures, they will stain pink on a blue background, when using the (modified) Ziehl-Neelsen technique. Some of the oocysts (and/or sporocysts) will not stain however.
- The oocysts (and sporocysts) can also be detected by their autofluorescence. They light up violet at a wavelenghth of 365 nm, green at 405 nm and blue to violet at 436 nm. This technique can only be used on thin preparations of untreated faeces or faeces treated with SAF or 10% formaline solution (if desired after concentration using sedimentation).

Isospora belli

Family : Eimeriidae

Class : Coccidea

Geographic distribution :

Worldwide

Common name :

Disease:

- NL : isosporose
- FR : isosporose
- EN : isosporosis
- ES : isosporosis

Final host:

- Man

Intermediate host:

Without intermediate host and without vector

Transmission:

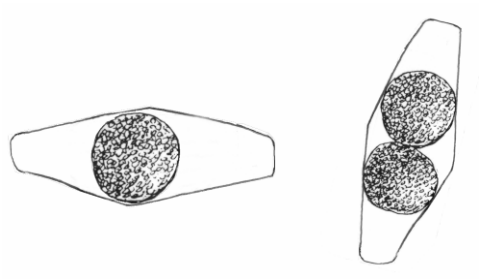
Faeco-oral : ingestion of sporulated oocysts.

Localisation of the parasite :

Intracellular, schizogony in epithelial cells of the intestine.

Diagnostic possibilities :

- Oocysts detection in faeces : direct examination, concentration by sedimentation.
- Oocysts detection in duodenal aspirate (or using a Stringtest (Enterotest®) or in duodenal biopsy : direct examination.
- Detection of specific DNA using PCR.



Morphology of the oocysts :

Faeces, direct examination

Size : 25-33 µm x 12-16 µm

Morphology : Ellipsoidal.

Contents : 1 then 2 non sporulated sporoblasts (granular mass)

Characteristics : Fine and transparent shell.

Main associated biological signs :

- Watery diarrhoea without blood.
- Often eosinophilia.
- Often presence of Charcot-Leyden crystals in the faeces.

Possible confusion with :

- Animal coccidians (oocysts in transit).

Remarks :

- Human infection with *Isospora belli* is rarely serious and often asymptomatic. This parasite is more an opportunist.
- Oocysts of *Isospora belli* are difficult to distinguish with a 10x objective (they are too transparent). The examination should be done with a 40x objective.
- Because the oocysts may be passed in small amounts and intermittently, repeated stool examinations and concentration procedures are recommended.
- Oocysts of *Isospora belli* are unsporulated at the time of excretion and do not become infective until sporulation is completed. They need a certain period of time for "maturation" in the environment before becoming infectious. This maturation depends on climatological factors (temperature, humidity) but is at least some days. Once sporulated, the oocyst contains two sporocysts, each containing four sporozoïtes.
- *Isospora belli* oocysts are quite resistant to disinfectants (cresol, iodine, chlorine,…). They are killed by ammonium or formalin.
- When stained with the rapid technique of Heine, the oocysts show as non-stained, strongly refractile structures on a pink to purple background.
- Most of *Isospora belli* oocysts are acid fast (pink to red when coloured with Ziehl-Neelsen). Some oocysts may stain palely or will be colourless but the typical ellipsoidal shape will still be visible.
- The oocysts can also be detected by their autofluorescence. They light up violet at a wavelenghth of 365 nm, green at 405 nm and blue to violet at 436 nm. This technique can only be used on thin preparations of untreated faeces or faeces treated with SAF or 10% formaline solution (if desired after concentration using sedimentation).

Cyclospora cayetanensis

Family : Eimeriidae

Class : Coccidea

Geographic distribution :

Worldwide

Common name :

Disease:

- NL : cyclosporose
- FR : cyclosporose
- EN : cyclosporosis
- ES : cyclosporosis

Final host:

- Man
- Other animals ?

Intermediate host:

Without intermediate host and without vector

Transmission:

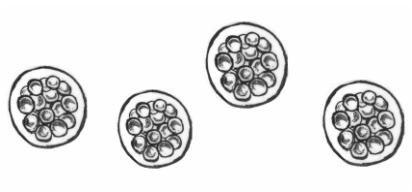
Faeco-oral : ingestion of sporulated oocysts.

Localisation of the parasite :

Intracellular, schizogony in epithelial cells of the intestine.

Diagnostic possibilities :

- Oocysts detection in faeces : direct examination, concentration by sedimentation.
- Oocysts detection in duodenal aspirate (or using a Stringtest (Enterotest®) or in duodenal biopsy : direct examination.
- Detection of specific DNA using PCR.



Morphology of the oocysts :

Faeces, direct examination

Size : 8 -10 µm.

Morphology : Round.

Contents : Numerous small spherical elements (“morula”)

Characteristics :
Thin shell but well visible.
Transparent, “lens” aspect.

Main associated biological signs :

- Watery diarrhoea without blood.

Possible confusion with :

- Animal coccidians (oocysts in transit).
- *Cryptosporidium* spp. (Ziehl-Neelsen).
- Yeast (Ziehl-Neelsen).

Remarks :

- Human infection with *Cyclospora cayetanensis* is rarely serious and often asymptomatic. This parasite is more an opportunist.
- Oocysts of *Cyclospora cayetanensis* are not stained by iodine.
- Because the oocysts may be passed in small amounts and intermittently, repeated stool examinations and concentration procedures are recommended.
- When freshly passed in stools, the oocyst is not infective (direct faeco-oral transmission cannot occur). In the environment, sporulation occurs after days or weeks (depending on temperature, humidity,…), resulting in an oocyst, containing 2 sporocysts, each containing 2 sporozoites.
- Most oocysts of *Cyclospora cayetanensis* are acid fast. They are variably stained by Ziehl-Neelsen, with different oocysts ranging from colourless to deep purple.
- When stained with the rapid technique of Heine, the oocysts show as non-stained, strongly refractile structures on a pink to purple background.
- The oocysts can also be detected by their autofluorescence. They light up violet at a wavelenghth of 365 nm, green at 405 nm and blue to violet at 436 nm. This technique can only be used on thin preparations of untreated faeces or faeces treated with SAF or 10% formaline solution (if desired after concentration using sedimentation).

Cryptosporidium spp.

Family : Cryptosporiidae

Class : Coccidea

Geographic distribution :

Worldwide

Common name :

Disease:

- NL : cryptosporidiose
- FR : cryptosporidiose
- EN : cryptosporidiosis
- ES : cryptosporidiosis

Final host:

- Man
- Various animals

Intermediate host:

Without intermediate host and without vector

Transmission:

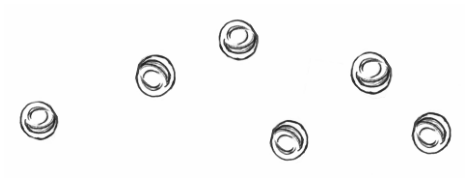
Faeco-oral : ingestion of sporulated oocysts.
(Air borne ?)

Localisation of the parasite :

Intracellular, schizogony in epithelial cells of the intestine.
(intracellular, in the lungs, the liver or pancreas: exceptional)

Diagnostic possibilities :

- Oocysts detection in faeces : Heine staining, Ziehl-Neelsen staining (direct or after concentration by sedimentation).
- [Oocysts detection in Bronchoalveolar lavage or in biopsy.]
- Antigen detection in faeces (ELISA).
- [Serology : antibody detection in serum (IFAT, ELISA, DATS).]
- Detection of specific DNA using PCR.



Morphology of the oocysts :
(in faeces, after specific staining)

Size : 3 -8 µm.

Morphology : Round.

Contents : Small moon-shaped elements (sporozoites)

Characteristics :
Oocysts are acid fast (staining pink to red with Ziehl-Neelsen).

Main associated biological signs :

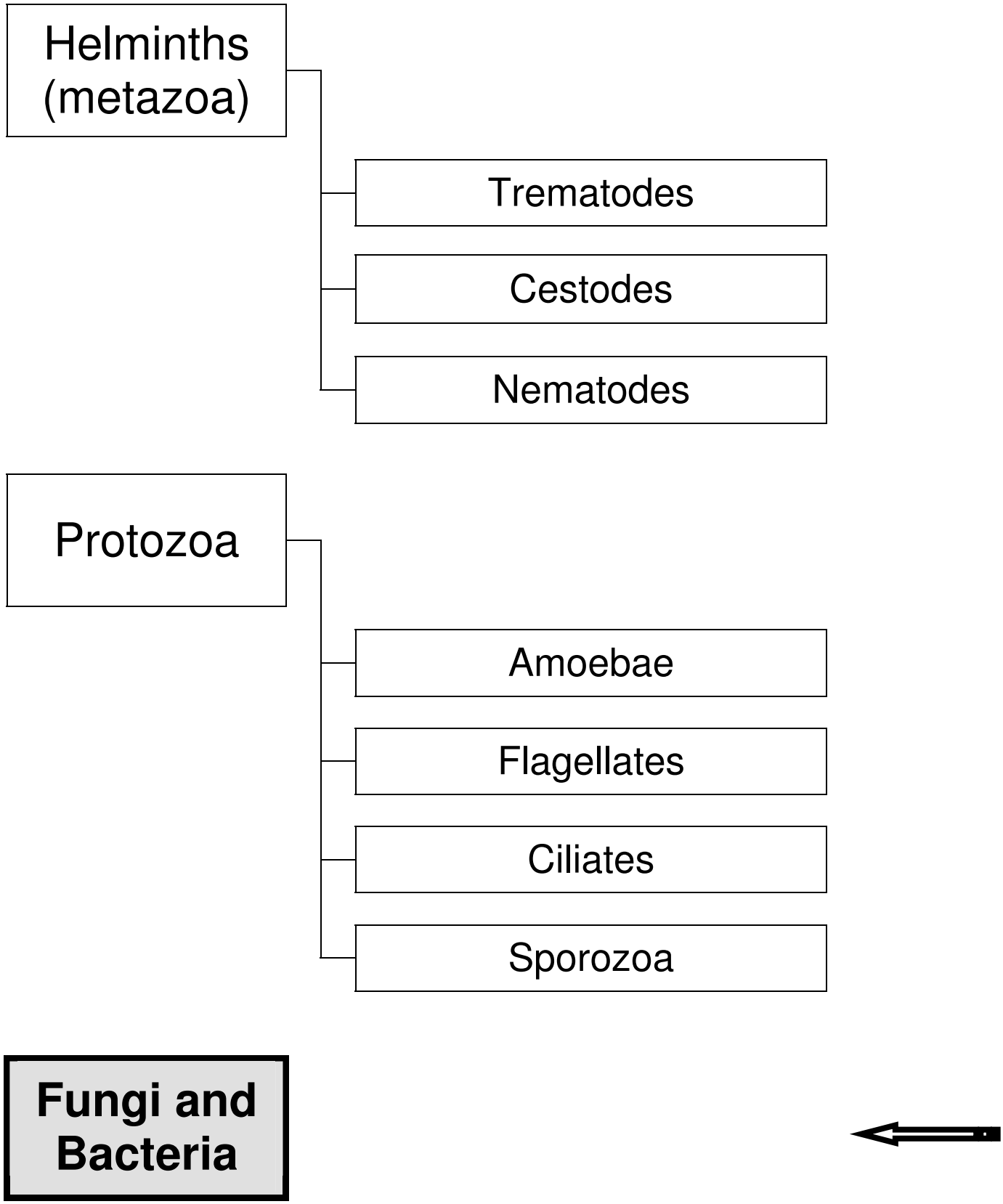
- Watery diarrhoea without blood.
- Often presence of Charcot-Leyden crystals in the faeces.

Possible confusion with :

- *Cyclospora* spp. (Ziehl-Neelsen).
- Yeast (Ziehl-Neelsen).

Remarks :

- Human infection with *Cryptosporidium* spp. is rarely serious. In immunocompetent persons, symptoms are usually short lived (1 to 2 weeks); they can be chronic and more severe in immunocompromised patients. This parasite is more an opportunist.
- The oocyst is directly infective (direct faeco-oral transmission can occur) and remains infective for extended periods. Auto-infection can occur. Great care should be taken in places where immunocompromised patients reside.
- The identification of *Crysptosporidium* oocysts in direct preparation or with iodine staining is impossible. Specific staining is needed.
- *Cryptosporidium* spp. is mainly found in water (drinking or recreational water). Oocysts are resistant to the concentration of chlorine normally used for swimming pools or for the treatment of domestic water supplies.
- *Cryptosporidium parvum* and *C. hominis* (together known as *C. parvum* genotype 1) are the main species infesting man. Infestations caused by *C. felis* (cats), *C. meleagridis* (birds), *C. canis* (dogs), *C. nasorum* (fish), *C. muris* (rodents), … have been reported however. Host specificity does not appear to be strict.
- There is no established specific therapy for human cryptosporidiosis.
- Most oocysts of *Cryptosporidium* spp. are acid fast. They stain pink to red by Ziehl-Neelsen, some oocysts will not stain at all.
- When stained with the rapid technique of Heine, the oocysts show as non-stained, strongly refractile structures on a pink to purple background.
- The oocysts can also be detected by their autofluorescence. They light up violet at a wavelenghth of 365 nm, green at 405 nm and blue to violet at 436 nm. This technique can only be used on thin preparations of untreated faeces or faeces treated with SAF or 10% formaline solution (if desired after concentration using sedimentation).


Helminths (metazoa)
Trematodes
Cestodes
Nematodes
Protozoa
Amoebae
Flagellates
Ciliates
Sporozoa
Fungi and Bacteria
Unknown classification

Pneumocystis jiroveci

Family : Pneumocystidaceae

Fungus

Class : archiascomycetes

Geographic distribution :

Worldwide

Common name :

Disease:

- NL : pneumonie door *Pneumocystis carinii*
- FR : pneumonie à *Pneumocystis carinii*
- EN : *Pneumocystis carinii* pneumonia
- ES : neumonia *Pneumocystis carinii*

Final host:

- Man

Intermediate host:

?

Vector ?

Transmission:

airborne ?

Most healthy children have a positive serology
→ reservoir ?
→ latent infections ?

Localisation of the parasite :

- Lungs
- (Extra-pulmonar dissemination)

Diagnostic possibilities :

- X-ray of the lungs.
- Parasite detection in bronchoalveolar lavage, induced sputum or biopsy : Toluidine blue O, Giemsa staining, RAL 555, …
- Detection of specific DNA using PCR.

Morphology of the parasite :

"Trophozoites" : Or small oval with one nucleus
2 – 4 µm (immature)
Or amoeboid with one nucleus
4 – 10 µm (mature)

"Pre-cysts" : 2 to 6 nucleus, tick shell,
3 – 6 µm

"Cysts" : contain 8 parasites, tick shell,
4 – 8 µm

"empty cysts" : Tick shell,
4 – 8 µm

Main associated biological signs :

- immunodepression.

Possible confusion with :

- *Histoplama capsulatum*.

Remarks :

- Toluidine blue O: The walls of cysts or pre-cyst are stained in blue.
- Giemsa: Trophozoites and internal structures of the cysts are stained in blue with a red nucleus. Often only the nuclei will stain. The cyst walls always remain unstained.
- *Pneumocystis carinii* is the old name for *Pneumocystis jiroveci.*

Borrelia spp.

(Not considered: *B. burgdorferi*)

Family : Spirochataceae

Bacteria

Class : Spirochaetes

Geographic distribution :

Worldwide and epidemic
(louse borne borreliosis)

Regional and endemic
(tick borne borreliosis)

Common name :

Disease:

- NL : Recurrente koorts, Borreliose (teken~ of luizen~)
- FR : Fièvres récurrentes à poux ou à tiques, Borreliose.
- EN : Relapsing fever, louse or tick borne Borreliosis.
- ES : Borreliosis, fiebre recurrente por piojos o por garrapatas.

Final host:

- Man
  (louse borne borreliosis)
- Man and animals
  (Tick borne borreliosis)

Intermediate host:

(Vector)
(louse borne borreliosis)

Pediculus humanus corporis

(Vector and reservoir)
(Tick borne borreliosis)

Ornithodorus spp.

Transmission:

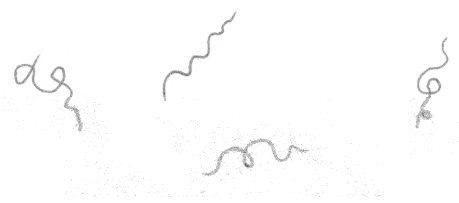
- Crushing an infested vector on the skin of the mucosa. **(louse borne borreliosis)**
- Bacteria injected with saliva through the skin when an infested vector takes a blood meal. **(tick borne borreliosis)**
- (Blood transfusion : rare).
- (Congenital : exceptional).

Localisation of the bacteria :

- Blood (CSF).

Diagnostic possibilities :

- Bacteria detection in blood : direct examination, Giemsa stained tick (or thin) blood film, Woo
- (Bacteria detection in CSF or urine : direct examination, Giemsa staining.)
- [Serology : antibody detection in serum]



Morphology of the bacteria :

Fine and long bacteria,

Helical bacteria

10-20 µm x 0,5µm

Stain mauve-blue (Giemsa stain)

actively motile (direct examination)

Main associated biological signs :

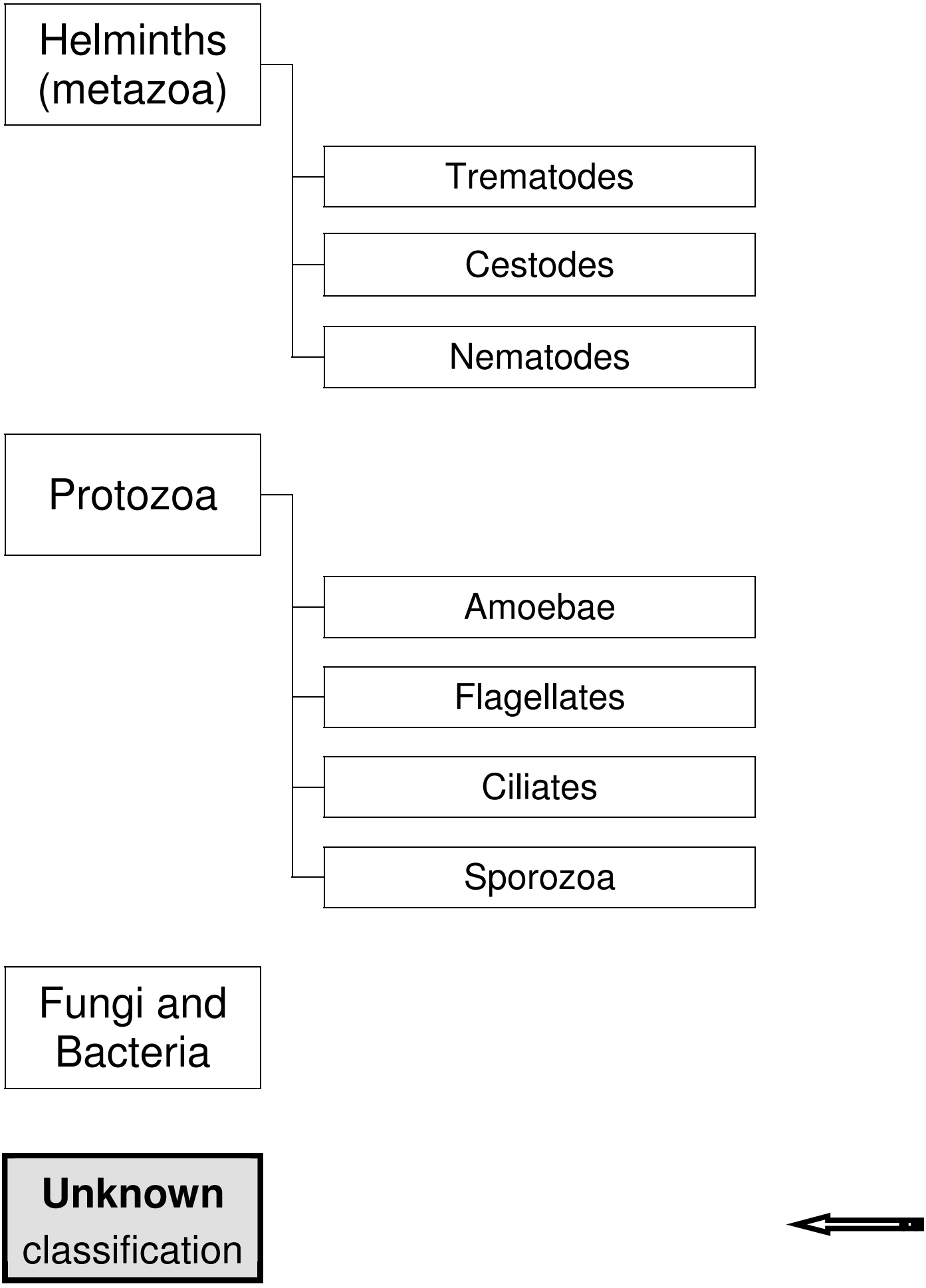
- Fever
- Thrombocytopenia, often perturbation of coagulation tests.
- Often anaemia.
- Leukocytosis (in 30% of the cases) or leukopenia

Possible confusion with :

- *Treponema* spp.
- *Leptospira* spp.
- *(*exflagellation of *Plasmodium* spp. gametocytes)

Remarks :

- The *Borrelia* species which cause relapsing fever cannot be distinguished morphologically. In tick borne borreliosis, fewer bacteria are found in the blood than in louse borne borreliosis.
- The blood should be collected during periods of fever because this is when the bacteria are at the highest concentration.
- Several serological tests have been developed for diagnosing relapsing fever, but antigens for the tests are not generally available and show high cross-reactivity with *Treponema* spp.
- For Lyme disease, caused by *Borrelia burgdorferi* (transmitted by hard ticks, but not causing relapsing fever), blood examination is not recommended (too few bacteria present in blood). Good serological tests are available.
- **Louse borne borreliosis :** *Borrelia recurrentis* : still epidemic in the highlands of Central and East Africa and less frequent in North Africa and the South American Andes.
- **Tick borne borreliosis :** Occur sporadically worldwide **:** *Borrelia duttoni* (Madagascar, Central Africa,…); *Borrelia hispanica* (Central and North Africa, Spain, …); *B. persica* (Egypt, Iran, …); *B. turicatae, B. parkeri* and *B. hermsii* (USA); *B. venezuelensis* (South America);… For tick borne borreliosis, transovarian transmission occurs in the vector.


Helminths (metazoa)
Trematodes
Cestodes
Nematodes
Protozoa
Amoebae
Flagellates
Ciliates
Sporozoa
Fungi and Bacteria
Unknown classification

Blastocystis hominis

Family : Opalinidae

"incertae sedis"

Class : Blastocystea

Geographic distribution :

Worldwide

Common name :

Disease:

NON PATHOGENIC ?

Final host:

- Man
- ?

Intermediate host:

Without intermediate host

Vector ?

Transmission :

Faeco-oral, cysts-like ingestion ?

Localisation of the parasite :

Intestinal lumen.

Diagnostic possibilities :

- Cyst-like detection in faeces : **Direct examination**, concentration by sedimentation.

Morphology of the cysts-like :

Size : 5 -30 µm.

Morphology : Round or oval.

Contents : Large central vacuole surrounded by small, multiple nuclei**.**

Main associated biological signs :

NON PATHOGENIC ?

Possible confusion with :

-

Remarks :

- Concentration techniques will lyse the organisms, except if the specimen has been fixed by formalin.
- Knowledge of the life cycle of *Blastocystis hominis* and transmission is still under investigation. The taxonomic classification is mired controversy (yeasts, fungi, protozoa, …?).
- Whether *Blastocystis hominis* can cause symptomatic infection in humans is a point of active debate. This because of common occurrence of the organism in both asymptomatic and symptomatic persons.

Diagnostic techniques in parasitology

Parasitological examination of faeces

The examination of faeces (or coprological examination) aims to detect those parasites that leave their host by the digestive tract and are thus found in the stools.
When taking a stool sample great care should be taken not to contaminate the sample with urine (this can cause distortion of vegetative forms of protozoans). Also avoid tests that can interfere in the coprological examination (e.g. after a Ba-transit or enema the patient should wait at least one week before delivering a faecal sample to the lab). The sample should be given to the lab in a proper packing. The faeces should never be packed in absorbing materials (paper, cardboard, matchboxes, etc.) Ideal are plastic or glass air-tight containers. These are however not always accessible in tropical regions. In this case, one can also use a fresh banana-leaf.
When the sample arrives in the laboratory, it should first be checked for its consistency, presence of blood or mucus and presence of worms or parts of worms. The microscopic examination should be done as soon as possible, giving priority to liquid and/or bloody samples, because in these samples one can possibly find living protozoans which will decompose (or at least immobilize) relatively quickly after production of the sample. This will complicate (or even make impossible) their identification. Samples that cannot be examined immediately should be stored at +4°C. When samples are stored for a long period of time, or when a sample should be sent by mail, it is advisable to add a product to the stools that prevents fermentation without altering the aspect of possible parasites (formaldehyde, merthiolate, SAF,…)
Some limitations of the parasitological examination of stools:

- The secretion of parasites or parasitic elements can sometimes be intermittent. When clinically suspecting someone of a parasitic infestation, a second examination of stools, produced a few days later, can prove very useful.
- In the rare case of infestation with only male helminths, no eggs or larvae will be secreted.
- Nor is there any secretion of parasitic elements during latent periods, e.g. maturation of the parasite in the body of its host, periods of internal migration, ...
- Some parasites of the intestinal tract cannot be detected by looking for parasitic elements in the stools of the host (e.g. eggs of *Enterobius vermicularis* which are placed on the peri-anal skin, eggs of certain tapeworms which are secreted in intact segments,…). This compels us to carefully study the biology of these parasites.
- Sometimes, the eggs found in a stool sample do not indicate an actual infestation, but are merely "passed" in the stools by eating contaminated foods (e.g. eggs of Fasciolidae in liver).
- …

Fixation of a stool-sample

This technique (especially intended for detection of trophozoïtes) avoids degeneration of possibly present parasites in a stool-sample, while preserving the parasite's morphology. This allows identification of the parasites after permanent staining (e.g. Iron-Hematoxylin Staining of Kinyoun (see p. 2)).
Mix thoroughly at least 3 volumes of SAF (see p. 2) with 1 volume of stools (as soon after production as possible; max 15-30 minutes). The stools and SAF should be mixed well by shaking for at least 20 seconds. The recipient containing SAFshould have a label indicating the dangers of SAF and the final level of liquid (after the stools have been added).

Direct examination

Direct examination consists of a microscopic examination of a faecal sample, before any other products are added. This examination should be made as soon as possible after receiving the sample at the lab.
When the faecal sample is liquid, one can simply take a drop of faeces and place it on a slide. Cover this drop with a cover slip (20mm x 20mm). This preparation should be examined completely and systematically with the 10x objective (total magnification of 100x), looking for eggs, larvae and ciliata. Details are observed using the 20x or 40x objective. The searching itself is done in any case with the 10x objective. After that about 100 microscopic fields are examined using the 40x objective (total magnification of 400x). At this moment one should look only for cysts and/or vegetative stages of protozoans (possible cysts and/or trophozoites of *Balantidium coli* are found already with the 10x objective).
When the stool sample has a (more or less) normal consistency, a drop of saline is first put on a slide. Then, a small amount of the faecal sample is put into suspension by means of a wooden spatula. The whole is covered with a cover slip (20mm x 20mm). Examine as described above.
The quantity of faeces examined this way is about 2 mg.

Modified Kato-Katz technique

This method uses a rather large amount of faeces (±0,05 g). The faeces is placed on a glass slide. In order to render this thick layer of faeces sufficiently transparent for microscopic examination, it is covered with a piece of cellophane (30mm x 30mm) that has been soaking for at least 24h in glycerinewater (30/70 v/v). The whole is slightly flattened and examined microscopically after ½ to 24h. The clarification-time needed, depends on the egg that is looked for and on the glycerine-concentration used. This technique is only useful in helminthology.
The disadvantage of this technique is that, after some time, all the eggs will become transparent and thus invisible. Thin-shelled eggs will clarify a lot sooner than thick-shelled ones, rendering this technique inappropriate for routine-analysis. However, this technique is very useful when looking for only one kind of egg (e.g. *Schistosoma mansoni* eggs), and when all other eggs that might be present are of no interest. In this case, the incubation-period can be adapted to the egg one is looking for. This technique is widely used for epidemiological purposes. When desired, the result can be expressed in a quantitative way (using a constant amount of faeces).

Lugol staining of wet smears for cysts of protozoa

This technique facilitates identification of cysts of protozoa. The iodine in the Lugol's staining solution will colour the cysts yellow to yellow-brown, colouring the chromatine of the nuclei more intensely than the cytoplasm. This will render the morphological aspects of the nuclei more visible.
A small amount of stools (fresh stools or concentrated material) is put in suspension in a drop of Lugol's staining solution, using a wooden spatula. The Lugol's staining solution (d'Antoni's Lugol-solution) used for parasitology is more concentrated than the one used for bacteriology. When using a staining solution which is not concentrated enough, the cysts won't take up enough colour and their identification will become even harder. The preparation should not be made too thick, as this will cause the cysts' internal structures to become practically invisible. Preparations are examined with a 40x objective.

Heine-staining

This staining-technique is used especially to look for oocysts of *Cryptosporidium* spp. A small drop or amount of faeces (if desired concentrated material can be used) is placed on a glass slide. Add 1 to 3 drops of carbol-fuchsin solution. Mix well and prepare 1 or 2 thin smears from this suspension. Let the preparations dry on the bench (max 30 min), place a drop of immersion-oil on the preparation and examine with the 100x objective.
The *Cryptosporidium*-oocysts will show as small, round, non-stained and highly refractile structures of about 3 to 5 µm in diameter. The background will be pink to purplish.
As the use of the 100x objective drastically lowers the sensitivity, the preparation can be looked at with the 50x immersion-objective (very expensive objective), or the drop of immersion-oil can be covered with a cover slip and the preparation can be examined with the 40x objective.

Modified Ziehl-Neelsen staining

Prepare a thin smear of the faeces-sample (if necessary diluted in saline). Let the preparation dry on the bench and fixate it by placing it in methanol during 2 to 3 minutes. Cover the complete preparation with carbol-fuchsin solution during 10 minutes. Heating isn't necessary when searching *Cryptosporidium* spp.! Rinse in water and destain with acid alcohol untill no colour comes off anymore. Rinse again with water. Counterstain with methyleneblue-solution during 30 seconds. Rinse once more with water and let the preparation dry. Examine the slide with the 50x or 100x objective (oil-immersion).
Cryptosporidium-oocysts will show as small, round, red to violet structures of about 3 to 5 µm in diameter. Internally, some more intensely coloured spots can be observed.
Take care not to confuse the oocysts with yeast-cells!

Iron-Hematoxylin Staining of Kinyoun

This staining technique is very useful to detect infestations with protozoa (and especially trophozoïtes). The picric acid will differentiate the hematoxyline by removing more colour from fecal debris than from protozoa. It also removes more colour from the protozoa's cytoplasm than from their nuclei, resulting in a moderately grey-blue stained protozoa, containing dark-blue to black nuclei, on a rather clear background. Cysts usually stain darker than trophozoïtes. Chromatoïd bodies and phagocytized RBCs stain dark-grey to black. Helminth eggs will stain too intensely and will not be easily identified in the stained preparation. Acid-fast structures will stain clearly pink to deep-purple.
Identify 1 clean glass slide per patient. Mix 1 drop of Mayer's Albumin (see p. 2) with 1 drop of feces, fixed with SAF (see p. 2). Make a thin smear, using a wooden stick (e.g. cotton swab) and let it dry at room-temperature for 20 minutes to 2 hours.

Staining procedure: (place the slides on a paper tissue between each staining step to minimize carry-over!!!)

- fix in ethanol 70% (see p. 2) during 10 minutes
- wash in tap-water (not running) during 2 minutes
- stain in carbol-fuchsin solution (see p. 2) during 20 minutes
- wash in running tap-water during 1 minute (constant stream of water in container)
- destain in hydrochloric alcohol (see p. 2) during 2 minutes
- wash in running tap-water during 1 minute (constant stream of water in container)
- stain in iron-hematoxylin working-solution (see p. 2) during 8 minutes
- wash in demineralised water (not running) during 1 minute
- destain in picric acid working-solutiong (see p. 2) during 4 minutes
- wash in running tap-water during 10 minutes (constant stream of water in container)
- dehydrate during 3 minutes in alcohol-ammonia solution (see p. 2)
- dehydrate during 5 minutes in ethanol 100%
- dehydrate during 5 minutes in a second container with ethanol 100%
- dehydrate during 5 minutes in xylol
- dehydrate during at least 5 minutes in a second container with xylol

Place a few drops of Entellan® onto the wet preparation and cover with a coverslip. Let the preparation dry overnight at room-temperature and examine with a 50x (or 100x) oil-immersion objective during 5 to 10 minutes. Morphological details should be looked at with a 100x oil-immersion objective.

- The iron-hematoxylin working-solution should be checked before each run by adding a few drops of working-solution to a recipient with (alkaline) tap-water. A blue colour should develop.
- The staining containers should be covered after each run to avoid evaporation of reagents.
- The iron-hematoxylin working-solution, the picric acid working-solution, the 70% ethanol fixation-step, the destaining-step with hydrochloric alcohol and the dehydration-step with ammonia-alcohol solution should be replaced each week.
- The washing-steps with tap-water and with demineralised water should be freshly prepared each run.
- The carbol-fuchsin solution should be replaced each month.
- The steps with absolute ethanol and with xylol should be replaced when becoming "foggy" or when visible accumulation of water at the bottom of the container occurs.

Concentration-techniques

The goal of these techniques is to concentrate any possible parasites, present in small numbers, to a smaller volume of faeces. This smaller volume of faeces, having a higher concentration of parasites in it, can then be examined microscopically. These concentration-techniques however will kill any trophozoites of protozoa, present in a sample. This means that concentration-techniques can never replace a direct examination, but can only be used as supplementary techniques.
Many variations exist in this group of techniques, but most of them are based on differences in specific gravity. Some examples of specific gravities:

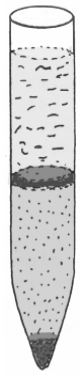
	specific gravity
Zinc-sulphate 33 % solution in water	1,180
Formol-solution 10 % in saline	1,019
Ether	0,714
Egg of *Ancylostoma duodenale*	1,055
Cyst of *Giardia lamblia*	1,060
Cyst of *Entamoeba histolytica*	1,065 - 1,070
Cyst of *Entamoeba coli*	1,065 - 1,070
Cyst of *Endolimax nana*	1,065 - 1.070
Egg of *Ascaris lumbricoides* (fertilized)	1,110
Egg of *Trichuris trichiura*	1,150
Cyst of *Chilomastix mesnili*	1,180
Egg of *Ascaris lumbricoides* (unfertilized)	1,200

The most simple and widely used techniques will be discussed here.

Sedimentation-technique: modified technique according to Ritchie

This technique suspends faeces in a low-density fluid (saline). Faecal residues are dissolved in ether (defatting agens). After centrifugation, the parasites can be found in the sediment. This technique concentrates all helminth eggs and larvae, as well as all cysts of protozoa.

- suspend about 3g of faeces in about 42ml of saline (formolated if desired) and homogenize well
- transfer about 1,5ml of this suspension to a conical centrifugation-tube
- add about 3,5ml of saline (formolated if desired)
- add 5ml of ether (or gasoline if no ether is available)
- if desired, 2 drops of xylol can be added
- close the tube firmly with a rubber stopper and shake vigorously during about 1 min.
- remove stopper and centrifuge at 2000 rpm (± 650 *x g*) during 3 to 5 minutes



- after centrifugation, 4 layers can be observed
- if necessary, the coagulum is loosened from the inner wall of the centrifugation-tube, using a spatula
- the centrifugation-tube is poored out with one supple movement
- transfer the sediment to a glass slide, using a pipette
- cover with a cover slip and perform a systematic, microscopic examination

CAUTION:
Ether is an extremely flammable and/or explosive substance when brought in contact with an open flame or spark. Recipients, once opened, are to be kept in a high (dispersion of fumes) and cool place in the lab. As ether is very volatile, recipients should be closed well and hermetically. A recipient with ether should NEVER be kept in the refrigerator: ether-fumes will build up and the whole may explode when opening the fridge (spark of refrigerator-light). Recipients in use should never be placed together in a cupboard. Avoid having large amounts of ether in the lab.

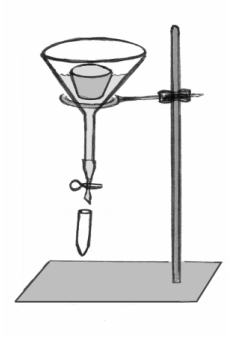
Flottation-technique: modified technique according to Willis

This technique will cause low-density parasitic elements to float on the surface of the flotation-fluid. This method can be used to concentrate cysts and oocysts of protozoa, as well as for eggs of cestodes and nematodes. This concentration-technique however can not be used to concentrate trematode-eggs, because of their high density. Saturated solutions of NaCl or $ZnS0_4$ can be used as flottation-fluids.

- suspend about 5g of faeces in about 100ml of flotation-fluid (homogenize well)
- sieve if desired
- poor the (sieved) suspension in a narrow tube (untill the surface becomes convex)
- gently place a cover slip (20mm x 20mm) on the liquid-surface
- let stand for about ½ hour (parasites will float up against the cover slip)
- transfer the cover slip (in horizontal position) to a glass slide and completely examine microscopically

Baermann-concentration for larvae

This (very sensitive) technique is meant to demonstrate larvae of *Strongyloides stercoralis*. The test is based on hygro- en thermotropism of the larvae. The test can only be done however on well-formed, freshly produced faeces (soft and liquid stools and faecessamples of more than 6h old cannot be used).



About 20g of FRESHLY PRODUCED and WELL-FORMED faeces is placed in a sieve (meshes ±1 mm) (if desired the stools can be placed in the sieve on a piece of gauze, making it easier to remove the faeces afterwards). In turn, the sieve is placed in a glass funnel, to which a piece of rubber tubing is attached. The rubber tubing is closed firmly with a clamp. Carefully fill the funnel with luke-warm water (30-35°C) until the faeces are practically submerged. Let the whole stand for at least 6h (e.g. overnight). Next, collect 10ml of the water in a conical centrifugation-tube by carefully opening the clamp. Centrifuge at 2000 rpm (± 650 *x g*) during 10 minutes. Carefully poor out the tube (immediately after centrifugation) and examine the sediment (WITHOUT adding Logol's solution) with the 10x objective (presence of (probably living) larvae).

Parasitological examination of urine

Some limitations of the parasitological examination of urine:

- The secretion of parasites or parasitic elements can sometimes be intermittent. When clinically suspecting someone to have a parasitic infestation, a second examination of urine, produced a few days later, can prove very useful.
- In the rare case of infestation with only male helminths, no eggs or larvae will be secreted.
- Nor is there any secretion of parasitic elements during latent periods, e.g. maturation of the parasite in the body of its host, periods of internal migration, ...
- …

For the detection of *S. haematobium* eggs, a sedimentation or a filtration technique can be used. The sedimentation technique is less sensitive than the filtration technique, but is cheaper and is easier to implement in a small laboratory.
The filtration technique is mostly used for epidemiological purposes, when a quantitave result is demanded.

Sedimentation

The urine should be collected in a conical recipient. The sample should be left on the table for about 1 hour, giving it the opportunity to form a sediment. Carefully decant the urine, leaving about 20 ml of the sediment in the recipient. The remaining urine is transferred into two conical centrifugation tubes. Centrifuge at 2500 rpm (± 1000 x *g*) during 5 minutes.
After centrifugation, the tubes should be emptied with one supple movement. Mix the sediment and transfer it to a glass slide. Cover with a cover slip and systematically examine it with the 10x objective (for eggs of *Schistosoma haematobium*). Next, about 200 fields should be examined with the 40x objective (for trophozoites of *Trichomonas vaginalis*).

IMPORTANT:

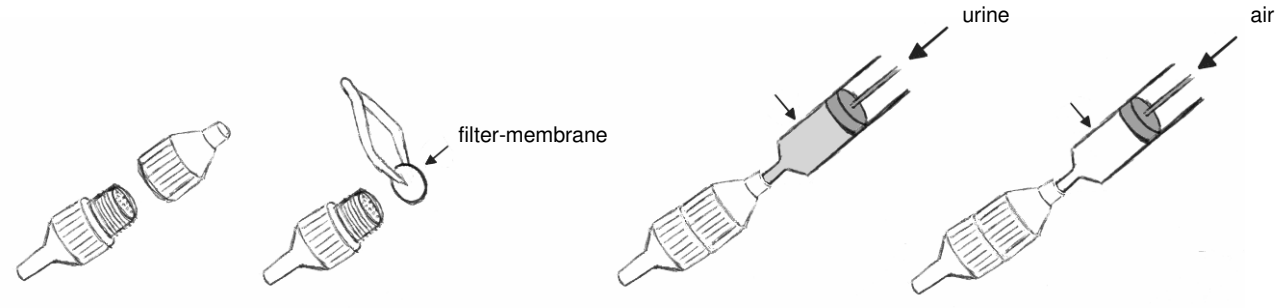
When collecting a specimen for searching eggs of *S. haematobium*, some points should be taken into consideration:

- Collect the urine preferably between 10h00 and 14h00
- Before urinating the patient should move/jump around a little bit
- Before giving a urine-sample, the patient's bladder should be well-filled
- As the eggs are particularly present in the last millilitres, maximum miction is advisable

When searching for *Trichomonas vaginalis* the first fraction of urine should be collected!

Filtration

This is a useful technique to look for eggs of *Schistosoma haematobium* in urine.
The urine sample should be well-mixed. Then 10 ml of urine is pressed through a small filter (meshes of 20 µm). Next, air should be pressed through the filter to clear out the filter.
The eggs will not pass through and thus stay on top of the filter. The filter is transferred to a glass slide and examined with the 10x objective.
Different kinds of filters exist (paper, nylon, polycarbonate). The filter-holders can be opened to place or take out the filters. Except for the paper filters, all filters can be used several times. For this, they should be placed in sodium-hypochlorite-solution (10%) during the night, then rinsed, next placed a few hours in a soap-solution, rinse very thoroughly and dry. The cleaned filters should be checked with a microscope (10x objective) before using them again.
This filtration method allows determination of the number of eggs per ml of urine.



Parasitological examination of vaginal secretions

Using a steril cotton swab, a sample is taken, preferrably from the cervix-mouth. The sample is pressed out immediately on a clean glass slide. The fluid is covered with a cover slip and examined immediately with the 10x and the 40x objectives. If the preparation is too thick, a drop of saline can be added.
This technique can be used to demonstrate infections with *Trichomonas vaginalis.* The parasite will draw the attention by its typical shaky movements, causing any neighbouring cells to move with it. Immobilised parasites will be hard or impossible to identify. This is the reason why samples should be examined immediately (before immobilisation of the parasites).

Parasitological examination of sputum

This test is useful when searching eggs of *Paragonimus* spp. in sputum. When examining sputum larvae can sometimes be found of worms, passing the lungs in their cycle of development (e.g. *Ascaris lumbricoides,* Ancylostomidae*, Strongyloides stercoralis,*...). Examination of sputum however is not the best way to search for these helminths!

IMPORTANT: Hemoptesis can be an indication for infestations with *Paragonimus* spp. However, it can also indicate tuberculosis. For this reason, bloody sputum-samples should be checked for acid-fast rods, prior to parasitological examination.

Instructions should be given to each patient on how to produce a good sputum sample. A good procedure on how to collect a sputum sample will improve the final results and lower the risks for health personnel.

The infection risk for health personnel (and other surrounding people) is very real when a patient, suspected of TB, starts coughing. For this reason, the collection of the sputum sample should be done outside, far away from other people, taking into account the direction of the wind. If this is not possible for any reason, a separate, well ventilated room in the building, should be used (verify the position of the patient according to the winds and air-streams coming from opened windows and doors).
Each recipient for the collection of the sputum sample should be labeled in advance (date, name, number, etc.). To avoid sample inversion, these data should be on the recipient itself, rather than on the lid of the recipient. The recipients should be single-use, made of hard plastic, having a lid that screws on and having an opening of about 2 to 3 cm. If this is not possible, recipients of glass (which can be re-used), having the same characteristics, can be used (decontaminate, clean and sterilize before each use of course). The recipients should be made of transparent material to allow visual evaluation of the quality of the sputum sample, without having to open the recipient.

The sputum itself has to be coughed up from as deep in the lungs as possible. This is best achieved by inhaling deeply several times and then exhaling strongly. A volume of sputum of about 3 to 5 ml is preferred and should contain solid or purulent particles (saliva is not a good sample because of the low number of pulmonary bacilla in it and because of the possible presence of commensal mycobacteria. This also counts for nasal secretions). When a patient is being treated for TB, it is possible that the sputum sample is not purulent.
It is best to collect 2 to 3 sputum samples: the first should be collected on the spot (at the first consultation), the second at home (when the patient wakes up in the morning) and if possible a third on the following consultation. The entire processus thus takes two days. Usually the best sample is the early morning sputum. For this reason, some TB programs use two early morning sputums and one sputum on the spot.

A good sputum sample should not look like water. Its colour should be white-yellowish, sometimes with a little bit of blood in it (purely blood is not a good sample). When a sample looks like saliva, it should be refused and the patient should be encouraged to produce a new sample of good quality. If no better sample is obtained (e.g. during treatment), a watery sample can be analyzed however.
The quality of a sputum sample can also be evaluated after preparation onto a slide and after staining: a saliva sample will be very thin, usually containing "bubbles". Under a microscope, a real sputum will contain mucus filaments and WBCs, while a saliva sample will pricipally contain epithelial cells (a sputum sample has about 20 times more WBCs than epithelial cells)
A sputum sample can be kept for more than one week at room temperature before bacteriological analysis (for acid-fast rods) is done. This however is not the case for cultures and for parasitological analysis.

Direct parasitological examination

After excluding TB, a sputum sample is transferred to a conical centrifugation-tube. Add at least the same amount of 1% NaOH or 1% KOH solution. Close the tube firmly and shake vigourously during about 1 minute. Let it stand for ½ to 1 hour, shake again very vigourously and centrifuge at 2500 rpm (± 1000 *x g*) during 5 minutes.
After centrifugation, the tube should be poured out with one supple movement. Mix the sediment and transfer it to a glass slide. Cover with a cover slip and examine systematically with the 10x objective. The NaOH's or KOH's function is to clear up and liquify the sputum.

Remark: Although several larvae (*Ascaris* spp., Ancylostomidae, etc...) will pass the lungs during their evolution, examination of sputum is not the best technique for diagnosis of these infestations.

RAL 555 staining

This technique is used for the detection of *Pneumocystis jiroveci* in bronchoalveolar lavage or in a lung biopsy or –punction. This technique stains the **inner structures of the cysts**. This technique can be replaced by Giemsa staining (of thin films). Centrifuge the sample at 2000 rpm (± 650 *x g*) during 10 minutes and spread out the sediment on a glass slide, making a thin film (or make prints of the biopsy on a glass slide). Let the preparation dry at room temperature, then fix the slide using methanol. Place the slide during 25 seconds in the RAL-reagent 2 (watery eosin-solution, code RAL 361643). Without rinsing the slide, place the slide for 40 seconds in RAL-reagent 3 (watery methylene-blue-solution, code RAL 361653). After rinsing the slide using running tap water, let it dry at room temperature. Examine the preparations microscopically using 10x eye-pieces and a 100x objective. Information concerning RAL reagents and their distributors can be found at http://www.reactifs-ral.fr (french).

Toluidine blue O staining

(Chalvardjian et al., 1963, modified by Marty et al. 1981)
This technique is used for the detection of *Pneumocystis jiroveci* in bronchoalveolar lavage or in a lung biopsy or –punction. This technique stains the **cyst walls**. Centrifuge the sample at 2000 rpm (± 650 *x g*) during 10 minutes and spread out the sediment on a glass slide, making a thin film (or make prints of the biopsy on a glass slide). Let the preparation dry at room temperature, then fix the slide using methanol. Place the slide during 5 minutes in sulfatation reagent. Rinse the slide using running tap water. Place the slide during 5 minutes in Toluidine blue O solution. Pass the slide trough 3 different recipient with isopropyl alcohol (1 minute in each recipient). Let the preparation dry at room temperature and examine microscopically using 10x eye-pieces and a 100x objective.

Parasitological examination of duodenal fluid

This technique is useful for searching eggs of Fasciolidae, Opisthorchidae and *Heterophyes heterophyes* and for searching trophozoites of *Giardia lamblia*.

Duodenal fluid is obtained by tubation. The obtained fluid is centrifuged in a conical test-tube at 2500 rpm (± 1000 *x g*) during 5 minutes. After centrifugation, the tube is poured out. Mix the sediment and examine it with the 10x objective. For *Giardia lamblia* the diagnosis should be confirmed with the 40x objective, if desired, Lugol's staining solution can be added.

Attention: staining with Lugol's solution will kill the trophozoites and identification will have to be done based on morphology!

Tape test

Used for searching eggs of *Enterobius vermicularis*, this test reveals the eggs that are placed on the peri-anal skin by the female adult worms (especially during the evening and night). The procedure is hence best performed early in the morning, before making one's toilet and before defecation.

A trasparent adhesive tape is placed, sticky side out, over the bottom of a round-bottomed test-tube. The tape is then pressed against the peri-anal skin. The eggs, if present, will stick to the tape. Next, the tape is placed on a glass slide and examined with the 10x objective. The tape itself serves as a cover slip. If desired, a drop of saline can be placed between the tape and the slide, to obtain a better image. The ends of the tape should be kept dry (for adhesion).

Remark: Accidentally, eggs of *Taenia* spp. can be found in a tape test (usually it will be the eggs of *T. saginata*).

Examination of segments of *Taenia* spp.

For the differentiation of both *Taenia* spp. the segments can be examined for the number of uterus-ramifications. To render the segments more transparent, they can be cleared up in a 50% glycerine solution for 30 minutes or heated to 60°C in a 5% solution of acetic acid (also ordinary vinegar (as found in any kitchen) can be used without diluting it) for about ½ to 1 hour. Next, the treated segments are pressed between two glass slides and examined in front of a light source. As the segments have become more transparent, the uterus-ramifications can easily be observed and counted.

CAUTION: When manipulating segments or adult tapeworms, always proceed with lots of caution (risk of cysticercosis in case of *T. solium*). Manipulation of segments or adult tapeworms is best done using tweezers. ALWAYS wear protective gloves when manipulating segments or adult tapeworms.

Parasitological blood examination

These techniques are applicable on the following infectious agents: blood microfilaria, *Plasmodium* spp., *Trypanosoma* spp., *Babesia* spp. and some *Borrelia* spp.. Yet each of the following techniques is not always useful for all of these parasites.

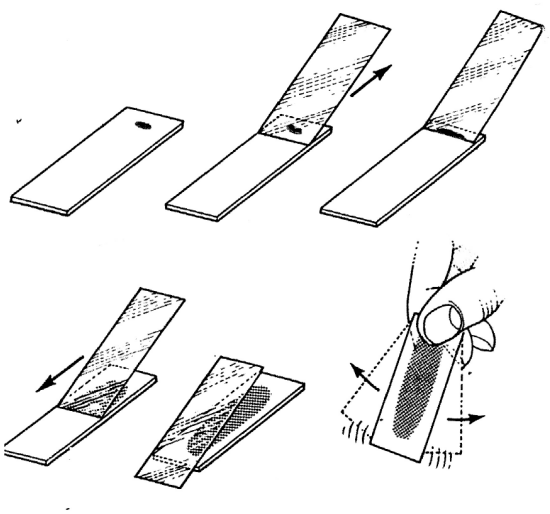
Direct examination

This is the easiest method, but also the least sensible to detect rather big and motile parasites in the blood (trypanosomes and microfilariae). The microscopical examination demands a certain time of attentive observation. An important inconvenience is that the slides must be examined immediately after their preparation (max 10-15 minutes after the bloodsample is taken), especially for searching trypanosomes, since these parasites are recognised by their motility. Nevertheless this test can be of interest, especially on the parasitical peak of *Trypanosoma rhodesiense.* At this moment rather important numbers of trypanosome parasites can be found in the periferal blood, making more complicated and expensive techniques unnecessary. For microfilarae, species identification is not possible. The detection threshold for direct examination is about 15 to 40 microfilaria and 10.000 trypanosomes per ml of blood.

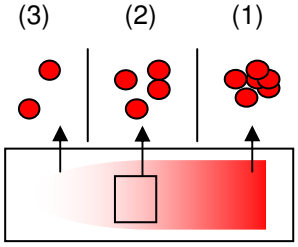
A drop of blood (capillary or venous blood sample taken on anticoagulant, such as EDTA for searching microfilaria or heparin for trypanosomes) is deposed on a slide and covered with a cover slip. The preparation is immediately microscopically examined with objective 10x (or 20x or 40x).

Thin blood film

This low sensitive method in parasitology is only useful for the precise identification of *Plasmodium* species.



A small drop of blood (capillary blood sample or venous blood taken on anticoagulant, such as EDTA for searching microfilaria or heparin for trypanosomes) is placed at one side of a glass slide. Then the small side of another slide (preferably with a polished border) is placed on the first, close to the drop of blood, forming an angle of 45°. Contact of the slide is made with the blood in order to make it spread between the two slides. Then move the upper slide towards the other side. The hand is moved in a steady and uninterrupted way, taking along and spreading the complete drop of blood into a thin and homogeneous layer. Immediately afterwards the slide is shaken between two fingers to dry it instantly in order to avoid deformation of the red blood cells.
A good thin blood film should be thick enough at the starting point (1) becoming less thick in the middle (2) to end in a "flame"-like shape at the end (3). The middle zone is best examined microscopically as the different cells will be lying next to one another here.



The preparation is fixed by covering it with concentrated methanol for 3 minutes.
After evaporation of the methanol, the preparation can be stained using **Giemsa**:
Cover the complete preparation with a 3.5 % diluted Giemsa buffered solution, pH 7.2 (or pH 8.0). For each slide 5 drops of concentrated Giemsa (VWR nr. 1.09204) are added to 4 ml of buffered water in a cylinder. Cover the slides with this solution and stain for 20 to 30 minutes.
Rinse by gently dripping tap water on the preparation. Let dry and examine under the microscope with an oil-immersion objective 100x (or oil-immersion objective 50x).
To obtain a good coloration it is necessary that the diluted water has the correspondent pH for its purpose (see below). Water that is too acid will result in too reddish coloration, while a too alkaline buffer will show a dominating blue coloration.

pH	6.6	6.8	7.0	7.2	7.4	7.6	7.8	8.0
Use	**Hematology**	**Hematology and Leishmaniosis**	**Paludism**	**Paludism**		**Trypanosomiasis**		**Paludism* and Microfilaria**

* pH 8.0 will show possible Maurer's clefts of *P. falciparum*

In the context of a small laboratory it is however preferable to standardise the pH of the dilution buffer to pH 7.2 for all the examinations, including haematological preparations.

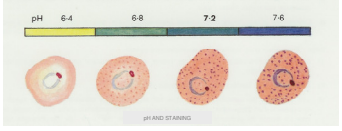
To avoid degradation of the Giemsa stocksolution (acid-alkaline reaction), the staining solution should be prepared just before use. The Giemsa stocksolution should also be filtered before use (e.g. when part of it is put in a dripping flask for daily use).

GIEMSA
(hemolysis and staining)

Eosin (acid) ➔ stains the alkaline elements.
Methylene blue (alkaline) ➔ stains the acid elements.
Azur (alkaline) ➔ stains the acid elements.

➔ Fresh dilution of the stain.

➔ Influence of the pH.



The **Pappenheim panoptical staining** (May-Grünwald-Giemsa) can be used instead of the Giemsa staining, but it won't necessarily give a remarkable quality gain:
Once the thin film is dry, it should be covered completely with May-Grünwald solution (VWR nr. 1.01424). Let the solution act for 3 minutes. For this staining technique, fixation of the preparation using methanol is not necessary: the first staining step (using May-Grünwald) causes fixation at the same time as the staining itself. Without removing the May-Grünwald solution, add (drop by drop) 4 ml of buffered water (pH adapted to the intended use: see table above). Let the preparation stand for 1 minute, then remove the staining solution by holding the slide inclined. Without rinsing the slide, cover the complete preparation with a 3.5 % diluted Giemsa buffered solution (pH adapted to the intended use: see table above). For each slide 5 drops of concentrated Giemsa (VWR nr. 1.09204) are added to 4 ml of buffered water in a cylinder. Let stain for 20 to 30 minutes. Rinse by gently dripping tap water on the preparation. Let dry and examine under the microscope with an oil-immersion objective 100x (or oil-immersion objective 50x).

Note : Counting parasites in thin blood films (Malaria and *Babesia spp.)* :

Select an area of the thin blood film where the total number of red cells is approximately 200 per field. Count the number of infected red blood cells in at least 25 fields using the 100x objective and 10x ocular. If red blood cells count in blood is available and accurate (this needs an electronic cell analyser), calculate the number of parasites per µl of blood as follows:

$$\frac{\text{number of parasitized RBC}}{\text{number of examined RBC}} \times \text{RBC-count} = \text{number of parasites / µl of blood}$$

Otherwise, calculate the percentage of parasitized red blood cells as follows :

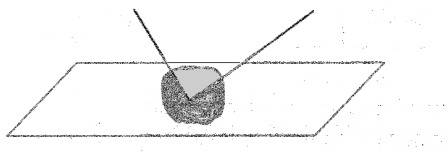
$$\frac{\text{number of parasitized RBC}}{\text{number of examined RBC}} \times 100 = \% \text{ of infected RBC}$$

The percentage of infected red blood cells can help to monitor patients with heavy parasitaemia.

If the number of RBC/µl of blood is not known, it is estimated that 1% of RBC parasitized corresponds to about 50.000 parasites/µl of blood.

Thick blood film

This method is a concentration technique. It takes about 4 times the blood quantity (20 µl) of the thin blood film (5 µl). Since the blood is less spread out, the eventual parasites are more frequent per surface unit.
It is mainly used for the search for plasmodia, trypanosomes, microfilaria and *Borrelia* spp. but also for the detection of Toxoplasmosis, *Leishmania* spp. and others. The detection threshold is in the range of 5 to 10 (100) plasmodia per µl of blood, 1000 trypanosomes per ml of blood and 15 to 40 microfilariae per ml of blood.
A blood drop is placed on a glass slide and immediately defibrinated by mixing with the corner of another slide in circular movements to obtain a blood layer of the correct thickness on a surface of about 1.5 cm diameter. (Defibrination is better than the use of an anticoagulant. If an anticoagulant should be used, EDTA is the best one, except for searching trypanosomes where heparin is preferred) The thickness of the preparation can be taken as correct if a normal print (such as this) can still be seen, but not read, through the film when it is held above the text.



Let it dry without warming up to avoid fixation of the red blood cells, since this fixation will avoid their destruction. The tick blood film may only be stained after complete drying.
Cover the complete preparation with a 3.5 % diluted Giemsa buffered solution, pH 7.2 (or pH 8.0). For each slide 5 drops of concentrated Giemsa (VWR nr. 1.09204) are added to 4 ml of buffered water in a cylinder. Cover the slides with this solution and let stain for 20 to 30 minutes.
[For the detection and identification of microfilaria, the staining time is best doubled to 40 minutes, the staining solution should be two times more concentrated (10 drops of concentrated Giemsa per 4 ml of buffered water) and the staining is best performed at pH 8.0]
The watery Giemsa solution has a double action: haemolysis and staining.
Rinse by ***gently*** dripping tap water on the preparation. Let dry and examine under the microscope with an oil-immersion objective 100x (or oil-immersion objective 50x). The red blood cells will be destroyed by haemolysis, leaving only the white blood cells and eventual parasites behind.
To obtain stainings of good quality the same measurements for the pH of the staining solutions are taken into account (see thin blood film).

Note: Parasite count in a thick blood film (only applicable in the context of malaria):

The search for parasites is considered negative if no parasites have been found during examination of at least 200 microscopical fields with objective 100x (or objective 50x). This takes about 5 to 10 minutes for a well trained technician.

For the parasite count in a thick blood film, the number of asexual parasites forms is expressed per 200 leucocytes (Count as much as necessary parasites in the microscopical fields as to obtain 200 leucocytes). For *Plasmodium falciparum* the gametocytes must be reported separately. Starting from the leukocyte concentration, the number of parasites can be calculated per µl of blood:

$$\frac{\text{Number of asexual parasites counted}}{\text{Number of leukocytes counted}} \times \text{Leukocytes concentration} = \text{Number of Parasites / µl blood}$$

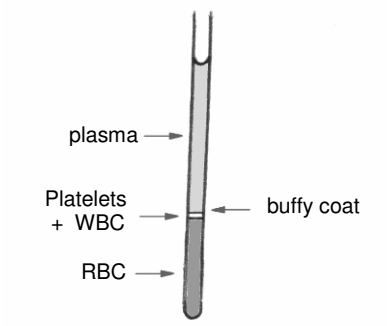
If the leukocyte count is not done, the WHO recommends applying 8.000 leukocytes per µl as a value for all the patients.

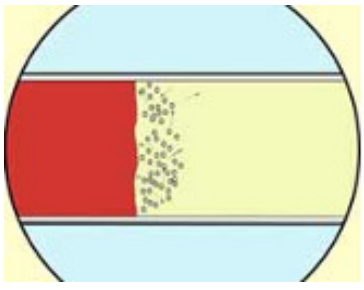
In non-immune persons even low parasitaemia can cause pathological phenomena. Especially in non-immune subjects, there is a remarkable correlation between the *P. falciparum* parasite concentration and the seriousness of the symptoms.
(Please note: *P. falciparum* infections examination of series of preparations made each 6 to 12 hours show important fluctuations of parasite concentration. This is caused by the restraint of the parasitised erythrocytes at the moment of schizogony. In infections with synchronical development of parasites, they can disappear temporary from the samples.)

For the routine of a small tropical laboratory, an estimation of the parasitical load in the thick blood film is sufficient for *Plasmodium* spp. This system indicates the relative majority of the asexual parasite forms with a code from 1 to 4 plus, as follows:

1 - 9	**asexual parasites**	**/ 100 fields**	**= +**
1 - 9	**asexual parasites**	**/ 10 fields**	**= ++**
1 - 9	**asexual parasites**	**/ 1 field**	**= +++**
> 10	**asexual parasites**	**/ 1 field**	**= ++++**

Buffy-coat (WOO)





This method is a rapid concentration technique for detecting motile parasites in blood (trypanosomes or microfilaria). It's easy to perform, but needs an electrical micro-haematocrite centrifuge. This technique is quite sensitive (parasite concentration from 50-60 µl of blood, detection threshold in the range of 250 trypanosomes or 25 microfilaria per ml of blood).

A haematocrite tube filled with 50-60 µl of blood is centrifuged at 12.000 g for 5 minutes. The components of blood separate according to their densities. Trypanosomes and microfilariae will be concentrated above the buffy coat. After centrifugation, the haematocrite tube is placed on a tubeholder and the plasma, just above the buffy coat layer is microscopically examined (10x or 20x objective; 10x or 15x eyepiece). The trypanosomes are very small but can be detected by careful focusing and providing not too intensive light. The preparation must be examined within a few minutes after blood taking and centrifuged, otherwise the trypanosomes will become less active and therefore more difficult to detect. The trypanosomes will also migrate into the supernatant plasma and be missed.
Virtually, all the parasites found in the 60 µl can be visualised by rotating the tube under the microscope.
For microfilariae, the species identification is not possible with this technique. Motile microfilariae will make it impossible to see whether there are also trypanosomes. Breaking the capillaries to obtain a buffy coat for staining with Giemsa is not recommended for safety reasons, but can be used in this case for parasites identification and/or species differentiation.

QBC

The QBC (Qualitative Buffy Coat) test developed by Becton and Dickenson is an expensive concentration method for detecting malaria parasites, trypanosomes or microfilariae in the peripheral blood. The detection threshold is in the range of 100 plasmodia per µl of blood, 250 trypanosomes or 25 microfilaria per ml of blood.

In the QBC system, parasites are concentrated by centrifuging blood in a special capillary tube. This special tube is coated with acridine orange and an anticoagulant. DNA and RNA take up acridine orange stain. The combination DNA-acridine orange fluoresces green after UV excitation and the combination RNA-acridine orange fluoresces green-yellow. Expansion of the centrifugally separated cell layers is achieved with a plastic float. Following centrifugation, the white cells, platelets, and the upper layer of the RBC can be found in the space between the float and inside the wall of the capillary. Examination under a fluorescence microscope (or a special Paralens objective, 50 x), of the interface region between the light RBC and granulocytes, permits detection of malaria parasites (RBC containing plasmodia are less dense than normal ones and concentrate just below the leukocytes).

Malaria parasites fluoresce green-yellow against a dark red background with the nucleus of trophozoites or merozoites of schizontes fluorescing bright green. Trypanosomes and microfilariae will be concentrated above the buffy coat. Motile microfilariae will make it impossible to see whether there are also trypanosomes. Breaking the capillaries to obtain a “buffy coat” for staining with Giemsa is not recommended for safety reasons, but can be used in this case for parasites identification and/or species differentiation.

The equipment, required for the QBC system and the special disposable capillary, are very expensive. Field evaluations of the QBC have shown, to be less sensitive than thick blood films in detecting low parasitaemias (< 100 malaria parasites/µl). Malaria species differentiation is difficult (or impossible) and considerable skill and experience are required to process and examine the tubes correctly and confidently. The use of this technique is not recommended for microfilariae detection, but it’s a good technique for trypanosomes detection.

Knott

Good (concentration-)technique for searching microfilaria at low parasitaemias.
2 ml of blood on anticoagulans is taken by venous punction. The blood is added to 30 ml of 2% formaline solution. This mixture is transferred to 4 conical centrifugation tubes and centrifuged at 1000 rpm (± 160 *x g*) during 15 minutes (or let stand on the bench overnight). The supernatans is decanted and the sediment can either be examined immediately (without the possibility of differentiation of species) or 4 thin smears can be made, left to dry, fixed with methanol during 3 minutes and microscopically examined after Giemsa-staining.

Strout

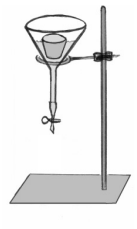
This technique is used for diagnosis of *Trypanosoma cruzi* infestations.
20 ml of blood (without any anticoagulans) is used. After formation of the blood-clot, the clot is removed and the remaining serum is centrifuged at 2500 rpm (± 1000 *x g*) during 5 minutes. The sediment is examined as is (the parasites will have their motility reduced or even lost, moreover it will be impossible to differentiate between *T. cruzi* and *T. rangeli*) or after Giemsa-staining.

mAECT

This specific technique for trypanosomes detection can be used in research programs or in big screening programs. mAECT (mini Anion Exchange Centrifugation Technique) consists of separating the trypanosomes from venous blood by anion exchange chromatography and concentrating them in the bottom of a sealed glass tube by low speed centrifugation (3000 RPM).
After centrifugation, the tip of the glass tube is examined under the microscope (objective 10x), for the presence of motile trypanosomes. The mAECT is available from : Prof. J.-J. Muyembe, Institut National de Recherche Biomédicale (INRB), Avenue de la Démocratie. Kinshasa, R.D. Congo. E-mail : inrb.rdc@ic.cd.
The large blood volume used (300 µl) allows detection of less than 100 trypanosomes/ml.

Parasitological examination of tissues

Concentration technique for the detection of Trichinella larvae



The muscular biopsy is placed into a sieve (meshes of ±1mm), which in turn is placed into a glass funnel, to which a piece of rubber tubing is attached. The rubber tubing is closed firmly with a clamp. Carefully fill the funnel with a digestion-solution of 2% pepsine and 0,5% HCl (see p. 2). Let the whole stand for about 2 hours at 37°C (digestion of the biopsy). Release the clamp carefully to collect the first few drops on a glass slide, cover with a coverslip and examine at low magnification (10x objective – details studied with 40x objective).

Skin tissues

Skin-snip

Good technique for *Onchocerca volvulus* (and *Mansonella streptocerca*).
The skin-snip should be performed close to any onchocercomes, if present, except when the onchocercomes are located in a troublesome area (e.g. the face). Desinfect the target skin-area. The skin is punctured superficially with a sterile needle and pulled upwards a little bit. Next, a thin slice is taken of with a bistouri and placed on a glass slide in a drop of saline. Cover the whole with a cover slip. Examine the preparation with a 10x objective, looking for microfilaria swarming out (½ to 1h after sampling).
This method is useful for screening a large population. Every biopsy is put seperately in a small conical centrifugation-tube, together with a little bit of saline. After about ½ to 1h the biopsy is taken out and the remaining fluid is centrifuged at 2500 rpm (± 1000 *x g*) during 5 minutes. The tubes are gently poured out and the sediment is examined on a slide with cover slip, without coloration (without possibility of differentiation of species) or after Giemsa-staining.

Deep scarification

Good technique for *Onchocerca volvulus* (and *Mansonella streptocerca*).
The scarification should be performed on the same skin-areas as the skin-snip (see above). After thoroughly desinfecting the skin, it is etched with the tip of a bistouri or small needle over a length of about 1 to 1½ cm. The skin should be torn more or less, liberating the lymphatic fluid. While doing this, enough pressure should be applied around the incision, causing a small amount of blood to appear. Three incisions should be made in this manner, with a few mm distance each. Next, enough pressure should be applied around the incisions with index and thumb, causing some blood and lymphatic fluid to surface. Collect a mixture of lymphatic fluid and blood by applying a glass slide to the incision-area. Apply as much material on the glass slide as possible. Let dry and stain with Giemsa.
Using this method, not only skin- but also blood-microfilaria, *Trypanosoma* spp., *Plasmodium* spp. and *Borrelia* spp. may be found. It is evident that scarification is not the appropriate technique for diagnosing these parasites.

Skin-biopsy for Leishmania spp.

The biopsy is to be taken at the outer edge of the leasion. Using sterile tweezers, prints of the biopsy are made onto clean glass slides. Cell-clusters will remain on the glass slide (="tissue-clash"). Let the preparation dry, fix with methanol and stain as if it were a thin film (see p. 2).

Exsudate

The exsudate on superficial skin- or mucosal leasions can be used to search for *Leishmania* spp. (cutaneous and mucocutaneous leishmaniasis).
Using a pipet or sterile needle a drop of the exsudate is transferred to a glass slide and spread out a bit. Let the preparation dry, fix with methanol and stain as if it were a thin film (see p. 2).

Bone-marrow

Can be spread out as a thin film (see p. 2) or as soft tissue (zie below: "Splenic and hepatic biopsy"). Let the preparation dry, fix with methanol and stain as if it were a thin film (see p. 2).

Splenic and hepatic biopsy

Soft tissue can carefully be spread out onto a glass slide (= "tissue-film"). Let the preparation dry, fix with methanol and stain as if it were a thin film (see p. 2).
For more resistant tissues, prints can be made onto a glass slide, using sterile tweezers. Cell-clusters will remain on the glass slide (="tissue-clash"). Let the preparation dry, fix with methanol and stain as if it were a thin film (see p. 2).

Rectal biopsy

The biopsy is flattened between two glass slides, without staining or fixation, and examined with the 10x objective (for eggs of *Schistosoma* spp. or trophozoites of *Balantidium coli*) or with a 40x objective (for trophozoites of *Entamoeba histolytica*).

Parasitological examination of lymph node aspirate

The standard method of diagnosis of African trypanosomiasis (especially in gambiense infections) in the early stage is to search for trypanosomes in aspirates from enlarged cervical lymph nodes (adenopathy). Trypanosomes will first invade the lymph nodes (possibly even before antibodies are formed). The swollen lymph nodes usually are those at the base of the patient's neck. They usually will be round, elastic and can be moved around under the skin. Most of the time these lymph nodes will not be hard, not resistant to pressure and will not be painful. The presence of such swollen lymph nodes is a very suspicious sign in endemic regions, but will however be absent in 20 to 40% of positive cases. Also the contrary can be true: in several cases the lymphatic fluid will be positive for Trypanosomes, while the blood examination remains negative. For this reason, it is absolutely necessary to do a systematic palpation of the cervical lymph nodes during a survey and to aspirate all suspect lymph nodes.

Immobilize the lymph node between thumb and index and carefully insert a needle in the centre of the lymph node. Turn the needle a bit, while massaging the lymph node. remove the needle from the lymph node and carefully place it onto a syringe with the piston pulled to the end. Place a drop of aspiration onto a glass slide by slowly pressing the piston inward. Examined immediately as a wet preparation under a microscope (40x objective). The wet preparation must be examined as soon as possible after the fluid has been collected. This because trypanosomes are unable to survive for more than 15-20 minutes in an aspirate once it has been taken. The organisms become rapidely inactive and will be lysed.

The contents of the needle may also be used to prepare a thin film. After fixation in methanol and staining by the Giemsa method, examine the preparation under the microscope with an immersion objective 100x (or objective 50x).
This procedure is only recommended for Toxoplasma detection or amastigotes forms of *Trypanosoma cruzi* or *Leishmania* spp.

Parasitological examination of cerebrospinal fluid

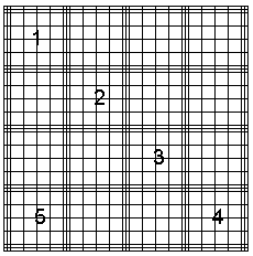
This technique is used for stage determination, follow-up, and sometimes also (in case of clinical suspicion) for diagnosis of *Trypanosoma gambiense or rhodesiense* infestations. Of course, the same technique may be used for *Toxoplasma*, *Cryptococcus*, *Naegleria*, *Acanthamoeba* detection in CSF and also for eosinophlic meningitis (*Angiostrongylus*) or bacterial meningitis.

After collecting the CSF sample, the analyse should be carried out as soon as possible since cells and parasites start to lyse rapidly (10 minutes for trypanosomes for example).
For trypanosomiasis, stage determination is necessary to choose an appropriate treatment with minimal risk for the patient. The second meningo-encephalitic stage is caused by trypanosome invasion in the central nervous system. The only way to determine whether the trypanosomes have invaded the central nervous system is by examination of the CSF, assuming that the changes observed in the CSF reflect the events going on in the central nervous system. Different parameters may be used : white blood cells number, total protein or globulin concentration, presence of trypanosomes, …).
The definition of normal CSF is based on a cell number ≤ 5 cells/µl, the absence of parasites, and a low level of protein concentration. Since there is no close relationship between these parameters, the WHO recommends the use all of them together.

Leukocyte count

The same technique is also used in haematology and in bacteriology (cf. haematology and bacteriology notes). Due to the low number of cells in normal CSF, cell counting chambers used for CSF counting should be medium or big volume counting chambers (Fuchs Rosenthal, Malassez, Neubauer, …). It may be good to use disposable KOVA counting chambers (less volume error, but for about 0,1 € per test). Carefully clean and mount the cell counting chamber (absence of dust or oil, use of special cover slip, presence of Newton rings). Gently mix the fresh and un centrifuged CSF before filling the chamber. If the liquid overflows into channel, the procedure should be restarted. Leave the counting chamber for 5 minutes on the bench to allow the cells to settle. Count the cells with the 10 x objective as explained below (depending on the type of counting chamber). If necessary, use the 40 x objective to make sure that the cells you count are leukocytes and not red blood cells. It is a good habit to do a second counting when the result is less than 20 leukocytes/µl.

Fuchs Rosenthal counting chamber



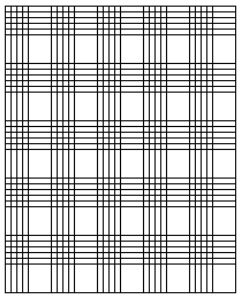
Area : 4 mm x 4 mm

Depth : 0.2 mm

Total volume: 3,2 mm³ or µl

For an undiluted CSF, count the cells in squares 1, 2, 3, 4 and 5. The number of cells counted in these 5 squares equals the number of cells per µl of CSF.

Mallassez counting chamber



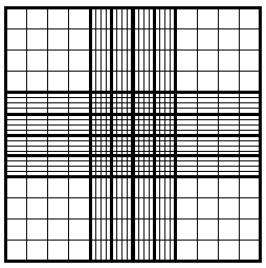
Area : 2 mm x 2,5 mm

Depth : 0.2 mm

Total volume : 1 mm³ or µl

For an undiluted CSF, the number of cells counted in the entire counting chamber equals the number of cells per µl of CSF.

Neubauer (Double Improved) counting chamber



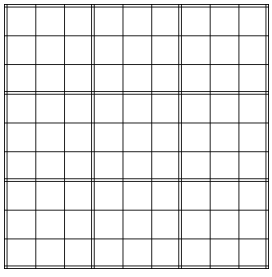
Area : 3 mm x 3 mm

Depth : 0.1 mm

Total volume : 0.9 mm³ or µl

For an undiluted CSF, the number of cells counted in the entire counting chamber multiplied by 10/9 equals the number of cells per µl of CSF.

Kova disposable counting chamber



1 plastic slide contains 10 counting chambers. 1 chamber consists of 9 big squares divided in 81 small squares.

Total Volume : 1µl

Volume of 1 small square : 0,0123 µl

For an undiluted CSF, the number of cells counted in the entire counting chamber equals the number of cells per µl of CSF.

Direct examination

Sometimes, during the cell counting procedure, motile parasites may be detected. It can be trypanosomes, indicating a trypanosomiasis stage II, or amoeboid trophozoites (*Naegleria* or *Acanthamoeba* spp., confusion possible with motile macrophage). Exceptionally, cysts of *Acanthamoeba* spp. may be detected.

Examination after single centrifugation

Several ml of CSF are centrifuged in a conical tube at low speed (10 minutes, 3.000 rpm or 1.000 *x g*) and the supernatant is carefully removed without touching the bottom of the tube. The supernatant may be used for biochemical investigations (protein, glucose, …). The remaining drop or sediment (invisible at low cell counts) is resuspended and a small drop of the remaining fluid is transferred on a clean microscopic slide and covered with a cover slip. The preparation is immediately microscopically examined with a 10 x objective (oculars of 10 x or 15 times) to confirm presence of trypanosomes or other pathogens. The preparation should be scanned completely and systematically. From the sediment, additional smears for detection of other pathogens can be prepared, which can be coloured with Gram, methylene blue, Giemsa or india ink (cf. bacteriology).

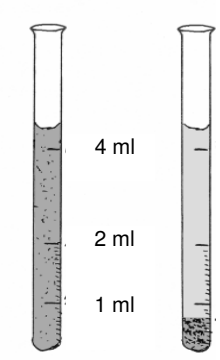
Examination after double centrifugation

The sensitivity of trypanosomes detection in CSF can be improved further by double centrifugation. Several ml of CSF are centrifuged in a conical tube at low speed (10 minutes, 3.000 rpm or 1.000 *x g*) and the supernatant is carefully removed without touching the bottom of the tube. The supernatant may be used for biochemical investigations (protein, glucose, …). The remaining drop or sediment is taken up in one or two micro-haematocrite tubes which are flame-sealed and centrifuged for 5 minutes at high speed (12.000 rpm or 15.000 *x g*). Capillaries are put on a microscopic slide and the cover slip is placed on the sealed end. Clean water is added between the slide and the cover slip to avoid diffraction during microscopic examination (10 x objective, ocular 10 x or 15 x). Proceed as soon as possible : chances of detecting trypanosomes decrease strongly in function of time. [cf. Woo].

CSF total protein

A wide variety in protein quantification methods for CSF is available. These techniques are not well standardised : Different proteins produce different absorbances and the protein concentration values are relative rather than absolute. The different reaction of CSF albumin and IgG with the different reagents used for protein determinations is no exception to this rule [the protein in CSF is mostly constituted of albumin (in normal conditions 70 %) and IgG (30%), which are both derived from the plasma]. Different concentrations may be obtained, depending on the protein used as calibrator (generally pure albumin or IgG). Therefore, various normal reference values for total protein concentration in CSF can be found.
For each method and for each calibrator, the cut-off value has to be established on the spot. Reference ranges vary a lot. The table below is given as example.

Method	Cut off (in g/l)
TCA 5 % precipitation	0,25
Sulfosalicylic acid 3 % precipitation	0,45
Coomassie brillant blue Colorimetry	0,37
BCA method	No data available for CSF



The **Sicard and Cantaloube** method is an easy technique, based on protein coagulation and sedimentation by acid and heat : The Sicard and Cantaloube tube is filled up to 4 ml with centrifuged CSF. The tube is slightly heated to a temperature of 50°C. 12 drop of 30 % (w/v) TCA are added. Close the tube and turn it 3 times to mix the contents (**never shake**). Place the tube perfectly vertically and leave it for at least 5 hours and maximum 24 hours. The height of the sediment indicates the protein concentration. Normal CSF total protein concentrations obtained with this method are below 0,4 g/l

Pandy's globulin test

☠ **CAUTION :** Phenol is a highly corrosive and harmfull chemical. This product must be manipulated with extreme care.

This easy technique may be used to screen for increases in CSF globulin when it is not possible to measure CSF total protein.

Because of it cut off value, this test, based on globulin precipitation by phenol, should be used only on CSF. Pipette about 1ml of saturated phenol solution into a small tube. Place the tube in front of a black background. Using another pipette, add (drop by drop) 3 drops of CSF. Examine the solution after the addition of each drop. If globulins are present, a white cloud forms as the drop of CSF mixes with the reagent (cigarette smoke like). The test is negative if no white cloud forms as the drops of CSF mix with the reagent, or when there is a slight cloudiness that redissolves. A positive Pandy's test occurs in all forms of meningitis, in amoebic and trypanosomiasis meningoencephalitis (stage II), but also in cerebral malaria, brain tumours, cerebral injury, spinal cord compression,…

Formol gel test

This cheap test is a good non specific indicator for the increased serum levels of gammaglobulin. Because of its simplicity, this test may be quite useful to assist in the diagnosis of visceral leishmaniasis (or as screening test for blood-donation?). It is a non specific screening test which if positive requires further investigation. The test is positive in about 90 % of patients with visceral leihsmaniasis. It becomes positive about 3 months after infection and negative about 6 months after a sucessful treatment. Results may be less reliable in case of HIV co-infection. Increased gamma globulin concentrations in serum are also seen following hepatitis B infection, certain malignant diseases (myeloma, Waldnström macroglobulinaemia,…). Increased gamma globulin levels which occur in case of malaria, trypanosomiasis, leprosis,… are not high enough to provided a positive result.

Collect 2-5 ml of blood and allow it to clot. When the clot begins to retract (30 to 60 minutes after collection), centrifuge (10 minutes at 4000 *x g*) the blood to obtain clear serum. Transfer 1 ml of RBC-free serum to a small glass tube, add 2 drops of concentrated formalin solution (37% w/v), mix and allow to stand for up to 2 hours.

A positive result is shown by gelling or/and whitening of the serum. Usually after 5 minutes, sometimes after 20 minutes. In the beginning of the infection, this may take up to 2 hours. A negative result is recorded when there is no gelling or whitening of the serum after 2 hours.

Reagents and staining solutions

Acetic acid solution 5%

☠ Caution: Acetic acid is flammable, toxic and extremely corrosive.
Acetic acid is also irritating for the eyes.
This product is always to be manipulated in a fume-cupboard, away from open flames and with extreme caution.

- distilled water: 70 ml
- concentrated acetic acid: 5 ml

Never add water to a recipient containing a concentrated acid!!! Adding even the smallest amount of water to a concentrated acid will produce an extremely violent reaction, causing the recipient to EXPLODE.
Measure 70ml of distilled water and put in a brown, glass bottle of 250 ml. Measure 30ml of concentrated acetic acid in a dry cilinder and add slowly and carefully to the water. During this process the solution will heat up (exothermal reaction)!
This solution can be kept at least 1 year at room temperature in a brown, tightly closed, glass bottle.

Acid alcohol 3%

☠ Caution: Ethanol is extremely flammable!
Concentrated hydrochloric acid is extremely corrosive. Hydrochloric acid fumes are toxic.
This product is always to be manipulated in a fume-cupboard and with extreme caution.

- ethanol 96%: 970 ml
- concentrated HCl: 30 ml

Put 970ml of ethanol 96% in a brown, glass bottle. Slowly and carefully add 30ml of hydrochloric acid. Mix well.
This solution can be kept indefinitely at room temperature in a brown, tightly closed, glass bottle.

Alcohol-ammonia solution

☠ Caution: Ethanol is extremely flammable!
Ammonia is a corrosive product. Ammonia fumes are irritating.
These products are to be manipulated in a fume-cupboard and with extreme caution.

- ethanol 70%: 250 ml
- ammonia 25%: 3 ml

Mix the ethanol 70% and the ammonia 25%.
This solution can be kept 1 week at room temperature.

Alcohol 70% solution

☠ Caution: Ethanol is extremely flammable!

- ethanol 95%: 730 ml
- distilled water: 270 ml

Mix the ethanol 95% and the water.
This solution can be kept at least 1 year at room temperature in a brown, tightly closed, glass bottle.

Carbol-fuchsin

☠ Caution: Phenol is extremely corrosive and toxic!
Ethanol is flammable!

Preparation of fuchsin-stock solution:

- alkaline fuchsin: 25 g
- ethanol 96%: 250 ml

The alkaline fuchsin (25g is one small bottle) is transferred to a brown glass bottle of 250ml. Next 250ml of ethanol 96% is added. Tightly close the bottle and shake vigorously three times on the same day. Let the solution rest during the night. If there is still a red precipitation present, some more ethanol should be added.
If desired, the alkaline fuchsin can be dissolved in methanol instead of in ethanol. The bottle with the saturated stock solution doesn't have to be rinsed or washed out each time a new batch is made. From time to time a small amount of fuchsin and alcohol can be added. As long as there is a small amount of powder on the bottom of the solution, the solution is saturated.
This stock solution can be kept a few years at room temperature in a brown, tightly closed, glass bottle.

Preparation of phenol-stock solution (5% v/v):

- penol (carbol) molten cristals: 50 ml
- distilled water: 950 ml

Normally, phenol is colourless. If the phenol has a pinkish colour, it has expired and should not be used anymore.
The phenol cristals should be heated up to 45°C in a warm-water bath. Measure 50ml phenol in a cilinder (also preheated at 45°C to avoid recristallisation in the cilinder). Alternatively, use a flask of 50g phenol (Fluka 77610). The phenol is transferred into 950ml of distilled water.
This solution can be kept for some months at room temperature in a tightly closed, glass bottle.

Preparation of the workingsolution:
Mix 100 ml of filtered fuchsin-stock solution and
900 ml of phenol-stock solution 5%
This solution can be kept for at least 2 years at room temperature in a brown, tightly closed, glass bottle. Filter again before use.

Digestion-solution for biopsies

☠ Caution: Concentrated hydrochloric acid is extremely corrosive. Hydrochloric acid fumes are toxic. Manipulate this product with extreme caution in a fume cupboard.

- distilled water: 1000 ml
- pepsine: 20 g
- NaCl: 8,5 g
- concentrated HCl: 5 ml

Pour 1000 ml of distilled water into a brown glass bottle. Carefully and slowly add 5 ml of concentrated hydrochloric acid. Mix well. Add the pepsine and the NaCl. Mix until all is in solution.
The final solution can be kept at least 1 year at room temperature in a brown, well closed, glass bottle.

Formolated saline 10%

☠ Caution: Formol is corrosive and toxic. Formol-fumes are irritating for eyes and mucosa. Formol is to be manipulated in a fume-cupboard (or with open windows).

- commercialy available formol (formaldehyde 37%): 100 ml
- physiological saline: 900 ml

Mix the formol and the physiological saline well.
This solution can be kept for at least 2 years at room temperature in a brown, tightly closed, glass bottle.

Formolated water 2%

☠ Caution: Formol is corrosive and toxic. Formol-fumes are irritating for eyes and mucosa. Formol is to be manipulated in a fume-cupboard (or with open windows).

- commercialy available formol (formaldehyde 37%): 2 ml
- distilled water: 98 ml

Mix formol and distilled water well.
This solution can be kept at least 2 years at room temperature in a brown, tightly closed, glass bottle.

Glycerinewater

- glycerine: 300 ml
- distilled water: 700 ml

For faster clarification, use a more concentrated solution:

- glycerine: 500 ml
- distilled water: 500 ml

If desired, 10ml of malachitegreen or methyleneblue (3% solution in water) can be added per liter glycerinewater in order to boost contrast in the microscopic preparation.
This solution can be kept for at least 1 year at room temperature in a brown, tightly closed, glass bottle.

Iron-hematoxylin solution

☠ Caution: Ethanol is extremely flammable!

Hematoxylin stock solution :

- hematoxylin (VWR 1.15938): 10 g
- ethanol 100%: 1000 ml

Dissolve the hematoxylin powder in the ethanol in a clear glass bottle! Let the solution ripen for at least 2 weeks in sunlight before use.
This solution can be kept one year at room temperature in a clear, tightly closed, glass bottle.

☠ Caution: Concentrated hydrochloric acid is extremely corrosive. Hydrochloric acid fumes are toxic. Manipulate this product with extreme caution in a fume cupboard.

Mordant stock solution :

- Ammonium-iron(II) sulphate ($(NH_4)_2Fe(SO_4)_2.6H_2O$): 10 g
- Ammonium-iron(III) sulphate ($(NH_4)Fe(SO_4)_2.12H_2O$): 10 g
- concentrated hydrochloric acid (HCl 37%): 10 ml
- distilled water: 1000 ml

Dissolve the ammonium-iron(II) sulphate and the ammonium-iron(III) sulphate in about 800 ml of distilled water. Add carefully and slowly 10 ml of concentrated hydrochloric acid. Add distilled water up to 1000 ml.
This solution can be kept one year at room temperature in a, tightly closed, glass bottle.

Working-solution :

- hematoxylin stock solution: 80 ml
- mordant stock solution: 80 ml

Mix the two solutions well. The mixture will become slightly warm. Let the mixture cool down for at least 2 hours before use.
This solution can be kept one week at room temperature or for staining 200 preparations.

KOH-solution 1%

☠ Caution: Potassiumhydroxyde is extremely corrosive!

- potassiumhydroxyde pellets (KOH): 1 g
- distilled water: 100 ml

Dissolve the potassiumhydroxyde in the distilled water.
During this process the solution will heat up (exothermal reaction)!
This solution can be kept for 2 years at room temperature in a brown, tightly closed, glass bottle.

Lugol's staining solution according to d'Antoni

☠ Caution: Lugol's solution is a product containing iodine, which is very volatile and can interact with metals and other products in its close environement!
Sublimated iodine is toxic when inhaled (vapors) and in contact with the skin.

- potassium-iodine (KI): 7,5 g
- distilled water: 500 ml
- sublimated iodine (I_2): 5 g

First, the potassium-iodine is added to the distilled water. Next, the sublimated iodine is ground and the potassium-iodine solution is added little by little.
The solution can be kept unfiltered for one year at 4°C in a brown, tightly closed, glass bottle. For use, a small amount of this solution should be filtered and can be kept from this moment on for 1 month at room temperature.

Mayer's albumin

- fresh egg-white: 20 ml
- glycerin 87%: 20 ml

Mix and shake the egg-white and the glycerin vigorously by hand during at least 1 minute. Let the mixture stand for 30 minutes. A layer of foam will apear on top of the solution. Eliminate the layer of foam.
This solution can be kept 3 months at 4°C.

Methyleneblue solution

☠ Caution: Ethanol is extremely flammable!

- methyleneblue (powder): 25 g
- ethanol 96% : 250 ml

The methyleneblue (25g is one flask) is transferred to a brown, glass bottle of 250ml. 250 ml of ethanol 96% is added. Tightly close the bottle and shake vigorously three times on the same day. Let the solution rest during the night. If there is still a blue precipitation present, some more ethanol should be added.
If desired, the methyleneblue can be dissolved in methanol instead of in ethanol. The bottle with the saturated solution doesn't have to be rinsed or washed out each time a new batch is made. From time to time a small amount of methyleneblue and alcohol can be added. As long as there is a small amount of powder on the bottom of the solution, the solution is saturated.
This stock solution can be kept a few years at room temperature in a brown, tightly closed, glass bottle.

NaOH-solution 1%

☠ Caution: Sodiumhydroxyde is extremely corrosive!

- sodiumhydroxyde pellets (NaOH): 1 g
- distilled water: 100 ml

Dissolve the sodiumhydroxyde in the distilled water.
During this process the solution will heat up (exothermal reaction)!
This solution can be kept for 2 years at room temperature in a brown, tightly closed, glass bottle.

Pandy's reagent

☠ **Caution:** Phenol is a highly corrosive and harmful chemical. This product must be manipulated with extreme care. Phenol is also hydroscopic.

- phenol (C_6H_6O): 50 g
- distilled water: 500 ml

Crystallised phenol is normally colourless. When the colour is pink violet, it has expired and should no longer be used. Packages of 50 g are available [e.g. : Fluka 77610]
Pandy's reagent is a saturated phenol solution. Bring the phenol in a brown flask of 1000 ml. Add 500 ml of distilled water. Agitate intensely. Let rest for 1 day. Verify that enough non dissolved phenol remains. If so, filter and store in a brown flask. If all the phenol is dissolved, add 10 g phenol and wait one more day before filtering.
The solution can be kept some years in a brown, hermetically closed bottle**.**

Physiological saline

- NaCl: 8,5 g
- distilled water: 1000 ml

Dissolve the NaCl in the distilled water.
This solution can be kept a few weeks at room temperature. Check regularly for growth of bacteria or fungi: the solution should be replaced when a cloudy aspect is observed after shaking.

Picric acid solution

☠ **Caution:** Picric acid is flammable and explosive when dry.
Avoid shocks, frottations and dispersion of small particles.
This product is to be manipulated with extreme caution and far away from ignition sources.

Stock solution :

- picric acid ($C_6H_3N_3O_7$): 13 g
- distilled water: 1000 ml

Dissolve the picric acid in the distilled water, using a water bath at 60°C.
This solution can be kept one year at room temperature in a brown, tightly closed, glass bottle.

Working-solution :

- picric acid stock solution: 80 ml
- distilled water: 80 ml

Mix the two solutions well. This solution can be kept one week at room temperature or for staining 200 preparations.

SAF-fixative

☠ **Opgepast:** Acetic acid is flammable, toxic and extremely corrosive.
Acetic acid is also irritating for the eyes.
This product is always to be manipulated in a fume-cupboard, away from open flames and with extreme caution.
Formol is corrosive and toxic. Formol-fumes are irritating for eyes and mucosa.
Formol is to be manipulated in a fume-cupboard (or with open windows).

- sodium-acetate ($C_2H_3NaO_2$): 1,5 g
- distilled water: 92,0 ml
- acetic acid ($C_2H_4O_2$): 2,0 ml
- commercialy available formol (formaldehyde 37%): 4,0 ml

Mix these products in exactly the same order as described above in a dark brown flask and shake well.
NEVER add water to a recipient containing concentrated acetic acid!!! Addition of even the smallest amount of water to an acid, will produce such a strong reaction, causing the recipient to explode.
This solution can be kept at least 1 year at room temperature in a brown, tightly closed, glass bottle.

Saturated NaCl-solution

- sodiumchloride (NaCl): 250 g
- distilled water: 1000 ml

Add the sodiumchloride to the water and heat up the whole to dissolve as much of the salt as possible. The solution can be used once it has reached room temperature again.
This solution can be kept a few months at room temperature.

Saturated $ZnS0_4$-solution

- zinc-sulphate ($ZnS0_4$): 330 g
- distilled water: 1000 ml

Add the zinc-sulphate to the water and heat up the whole to dissolve as much of the salt as possible. The solution can be used once it has reached room temperature again.
This solution can be kept a few months at room temperature.

Sörensen buffer solution

Stock solution :

KH_2PO_4 : 9,078 g/l in distilled water (solution A)
$Na_2HPO_4.2H_2O$: 11,876 g/l in distilled water (solution B)

Both stock solutions can be kept several weeks at 4°C in dark, glass bottles. Check regularly for growth of bacteria or fungi: the solution should be replaced when a cloudy aspect is observed after shaking.

Working-solution :

Use	pH	Volume solution A (in ml)	Volume solution B (in ml)	Volume distilled water (in ml)
Haematology	**6,6**	64,0	36,0	900
Haematology	**6,8**	50,0	50,0	900
Malaria	**7,0**	39,0	61,0	900
Malaria	**7,2**	28,0	72,0	900
	7,4	19,2	80,8	900
Trypanosomes	**7,6**	13,0	87,0	900
	7,8	8,5	91,5	900
Malaria / Microfilaria	**8,0**	5,5	94,5	900

The Sörensen buffering-solution can be replaced by buffering-pills of pH 7,2 (available with VWR (formerly known as *Merck*) nr.9468).
According to instructions: 1 pill per liter water (distilled or filtered).
According to our experience: depending on the quality of the used water, one pill can buffer up to 10 liters of water (lowering the price in a spectacular way!). This should however always be checked by staining a few smears and evaluating the results.
This buffering-solution can be kept for one month at room temperature.

Sulfatation reagent

☠ Caution: Diethylic ether is extremely flammable and will ignite and explode quickly when in contact with a flame or spark. Manipulate this product far away from ignition sources. Recipients, once opened, are to be kept in a high (dispersion of fumes) and cool place in the lab. As diethylic ether is very volatile, recipients should be closed well and hermetically. A recipient with diethylic ether should NEVER be kept in the refrigerator: diethylic ether-fumes will build up and the whole may explode when opening the fridge (spark of refrigerator-light).
Recipients in use should never be placed together in a cupboard. Avoid having large amounts of diethylic ether in the lab.
Sulphuric acid is extremely coorosive. Sulphuric acid fumes are toxic. This product is to be manipulated with extreme caution in a fume cupboard (or with open windows).

- diethylic ether: ±140 ml
- concentrated sulphuric acid : 100 ml
- distilled water: 40 ml

Mix thoroughly 140 ml of diethylic ether and 40 ml of ditilled water in a decantation ampoule. Wait until two layers are formed. Eliminate the lower layer (water) as well as some ml of the upper layer (ether-hydrate) to clean the tubing. Transfer the ether hydrate into an Erlenmayer-flask, which has been placed **in ice**. Add carefully (drop by drop) the sulphuric acid, while shaking the Erlenmayer-flask.
This solution can be kept one month in a brown, tightly closed, glass bottle. The solution should be discarted when two layers are observed.

Toluidine blue O solution

☠ Caution: Ethanol is extremely flammable!
Toluidine blue O is toxic when inhaled, in contact with skin or when ingested.
Toluidine blue O fumes are irritating for the eyes and mucosa.
Manipulate this product in a fume cupboard (or with open windows), using gloves.
Concentrated hydrochloric acid is extremely corrosive. Hydrochloric acid fumes are toxic. Manipulate this product with extreme caution in a fume cupboard.

- toluidine blue O: 320 g
- distilled water: 60 ml
- concentrated hydrochloric acid (HCl 37%): 2 ml
- absolute ethanol: 140 ml

Dissolve the toluidine blue O powder (e.g. Sigma T0394) in the distilled water, using a magnetic stirrer. Very carefully add the 2 ml of hydrochloric acid (while stirring). Then **slowly** add the ethanol.
This solution can be kept several months in a brown, tightly closed, glass bottle.

Trichloroacetic acid 30% (w/v)

☠ Caution: Trichloroacetic acid is a strongly corrosive and deliquescent chemical with an irritating vapour. Handle with care in well ventilated area (or in a fume-cupboard)

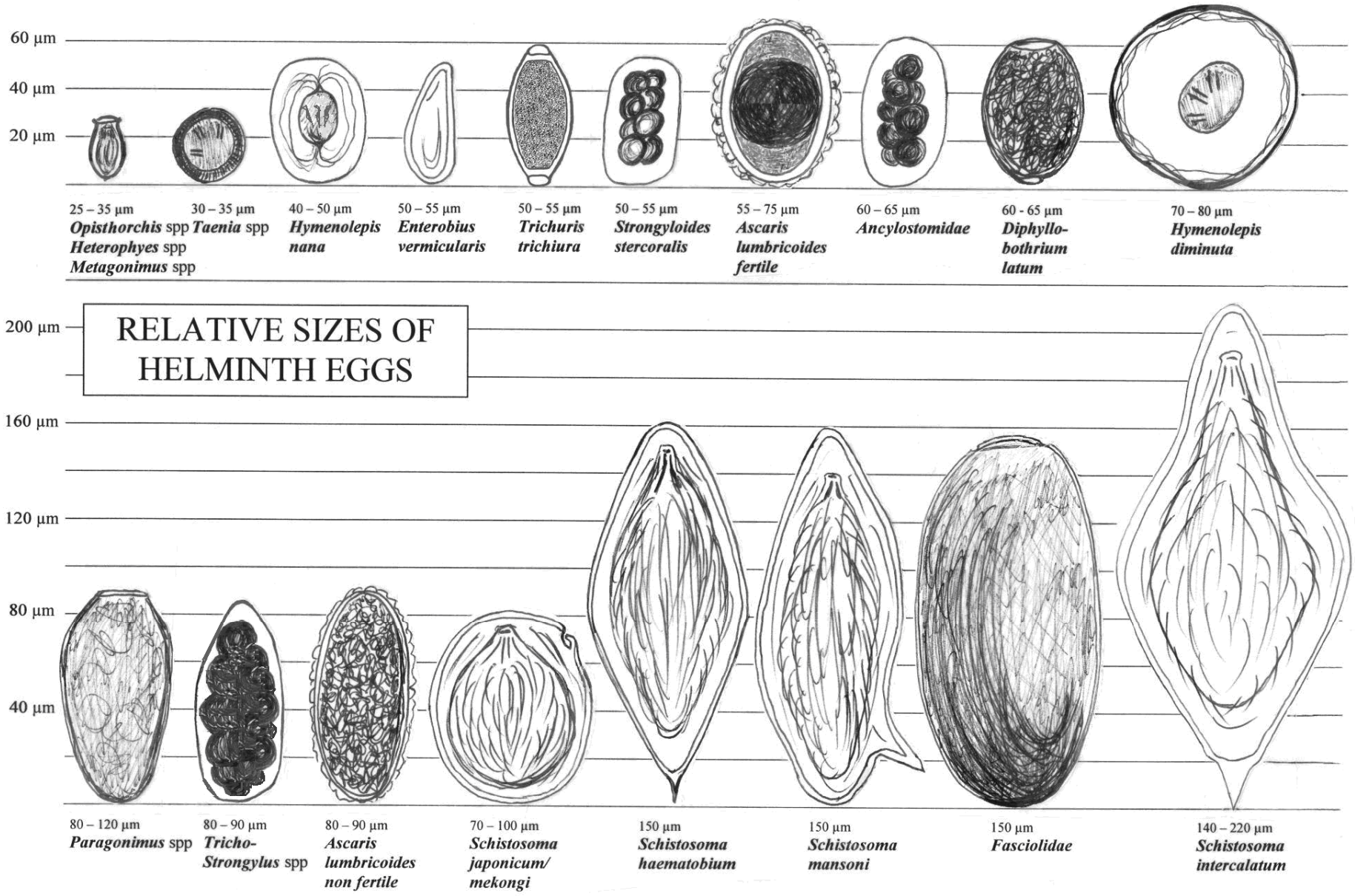
- trichloroacetic acid ($C_2HCl_3O_2$): 30 g
- distilled water: 100 ml

Dissolve the trichloroacetic acid in the distilled water.
The solution can be kept some weeks in the refrigerator (4°C).

Abbreviations

µl	microliter(s)
µm	micrometer(s)
Ag	Antigen(s)
Ab	Antibody (~ies)
e.g.	*ex grata*, for instance
i.e.	*it est*, this is
CAA	Circulating Anodic Antigen
CATT	Card Agglutination Test for Trypanosomiasis (*Trypanosoma b. gambiense*)
CCA	Circulating Cathodic Antigen
CDC	Centers for Disease Control and Prevention
CSF	CerebroSpinal Fluid
DEC	DiEthylcarbamazine Citrate
EDTA	Ethylene-Diamine Tetra-Acetic acid (anticoagulentium)
EIA	Enzyme ImmunoAssay
ELISA	Enzyme Linked ImmunoSorbent Assay
EN	English
ES	Spanish
ES-Ag	Excretor/Secretor-Antigen
ESR	Erythrocyte Sedimentation Rate
FR	French
g	centrifugal force
g	gram(s)
GGT	Gamma-Glutamyl-Transpeptidase
HCl	Hydrochloric acid
HIV	Human Immunodeficiency Virus
Ht	Haematocrit
IFAT	Immuno-Fluorescent Antibody Test
IgE	Immunoglobulin E
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IHA	Indirect HemAgglutination
KI	Potassium-iodine
KIVI	Kit for In Vitro Isolation (*Trypanosoma cruzi*)
KOH	PotassiumHydroxide
LDH	LactateDehydroGenase
mg	milligram(s)
min	minute(s)
ml	milliliter(s)
NaCl	SodiumChloride
NaOH	SodiumHydroxide
NL	Dutch
PCR	Polymerase Chain Reaction
QBC	Quantitative Buffy Coat
RBC	Red Blood Cell(s)
rpm	revolutions per minute
SAF	Sodium acetate – Acetic acid – Formalin (fixative)
sp.	species (only the mentioned one)
spp.	species (all the ones, belonging to a certain Genus)
TBC	Tuberculosis
h	Hour(s)
WBC	White Blood Cell(s)
WHO	World Health Organization
ZnSO4	Zinc-sulphate

Schematic overview of the helminth eggs



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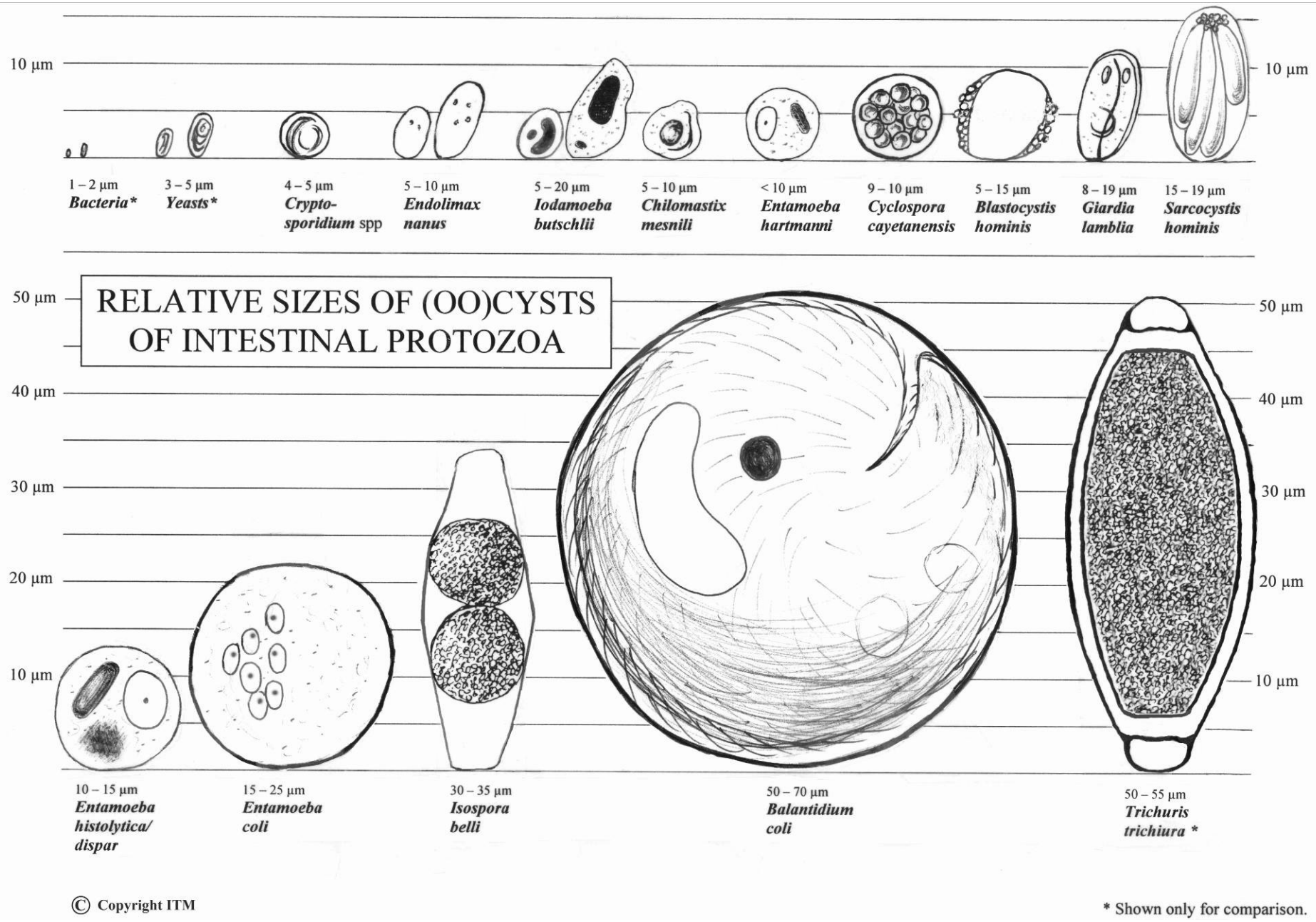
Some useful parameters concerning parasitic helminths (filaria not included)

Organism	Time between initial infestation and appearance of fist eggs/larvae (maturation)	Estimation of daily egg-production (per adult/female)	Estimated lifespan
Ancylostoma duodenale	15 - 40 days	5.000 - 22.000	1 - 9 years
***Anisakis* spp.**			
Ascaris lumbricoides	2 - 3 months	200.000	1 - 3 years
Capillaria aerophila			+/- 1 year
Capillaria hepatica	2 - 3 weeks		1 - 4 months
Capillaria philippinensis			
Clonorchis sinensis	1 - 4 weeks	1.000 - 4.000	up to more than 25 years
Diphyllobothrium latum	30 - 45 days	35.000 - 100.000	up to 30 years
Echinococcus granulosus			
Enterobius vermicularis	+/- 3 weeks	+/- 500	up tot 55 days (but auto-infestation)
Fasciola gigantica	12 - 15 weeks	worms usually sterile	up to more than 11 years
Fasciola hepatica	4 - 10 weeks	25.000 (only few arrive in faeces)	up to more than 25 years
Fasciolopsis buski	12 - 20 weeks	+/- 16.000	+/- 6 months
Hymenolepis diminuta	+/- 20 days		5 - 7 weeks
Necator americanus	15 - 40 days	3.000 - 6.000	4 - 20 years
***Paragonimus* spp.**	2 - 3 months		10 - 20 years
Schistosoma haematobium	54 - 84 days	20 - 300	3 - 7 years
Schistosoma intercalatum	50 - 60 days	150 - 400	
Schistosoma japonicum	30 days	1.500 - 3.500	up to more than 25 years
Schistosoma mansoni	25 - 60 days	100 - 300	2 - 18 years
Schistosoma mekongi	30 - 60 days	1.500 - 3.500	up to more than 25 years
Strongyloides stercoralis	2 - 3 weeks		short (but auto-infestation)
Taenia saginata	10 - 12 weeks	up to 2.000.000	up to more than 35 years
Taenia solium	5 - 12 weeks		up to more than 25 years
Toxocara canis			
***Trichinella* spp.**			up to 4 months
***Trichostrongylus* spp.**			
Trichuris trichiura	30 - 90 days	3.000 - 20.000	1 - 4 years (20 years ?)
Hymenolepis nana	+/- 20 days		some months

Schematic overview of (oo)cysts of intestinal protozoa



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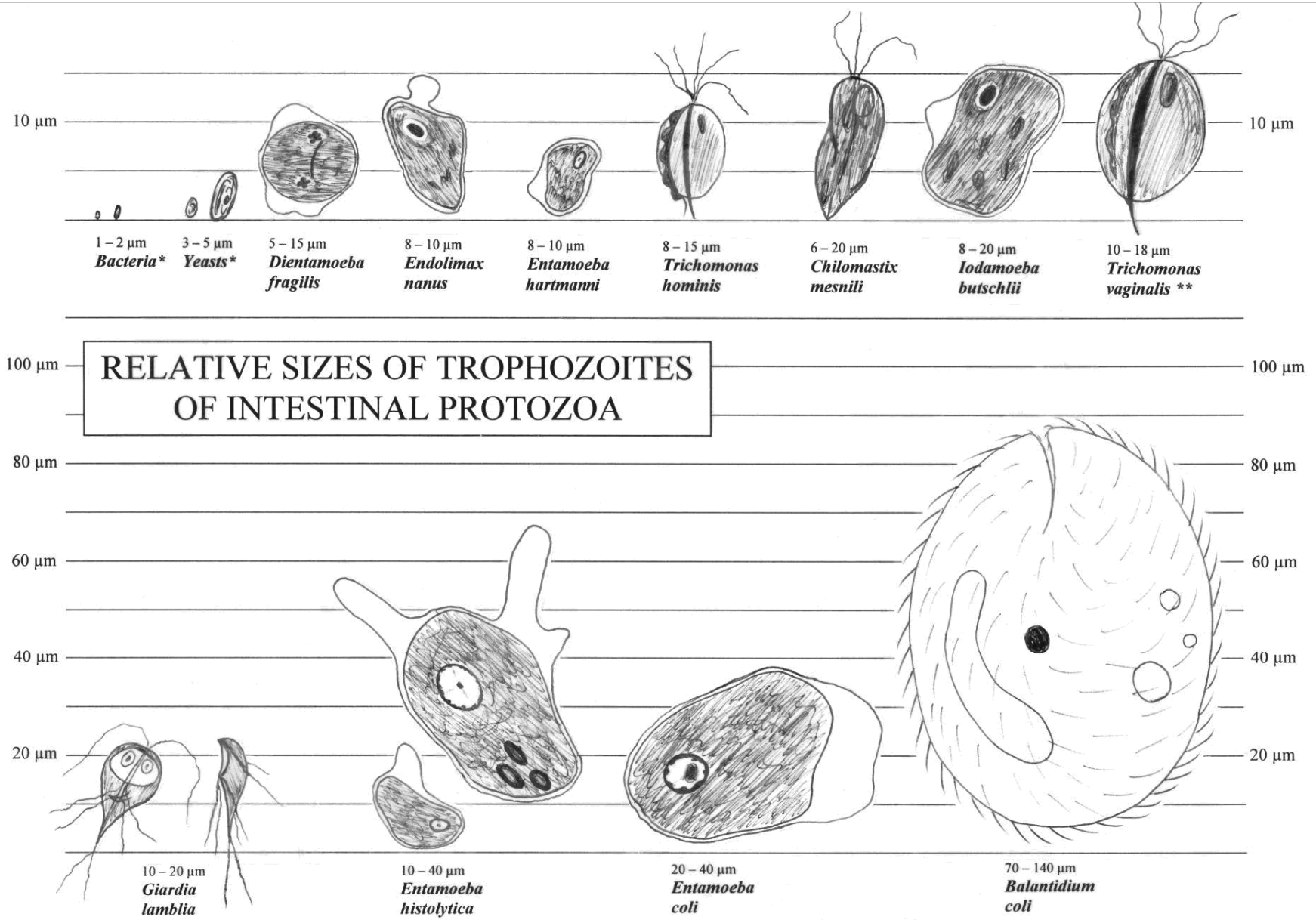
* Shown only for comparison.

Schematic overview of trophozoites of intestinal protozoa



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* Shown only for comparison.

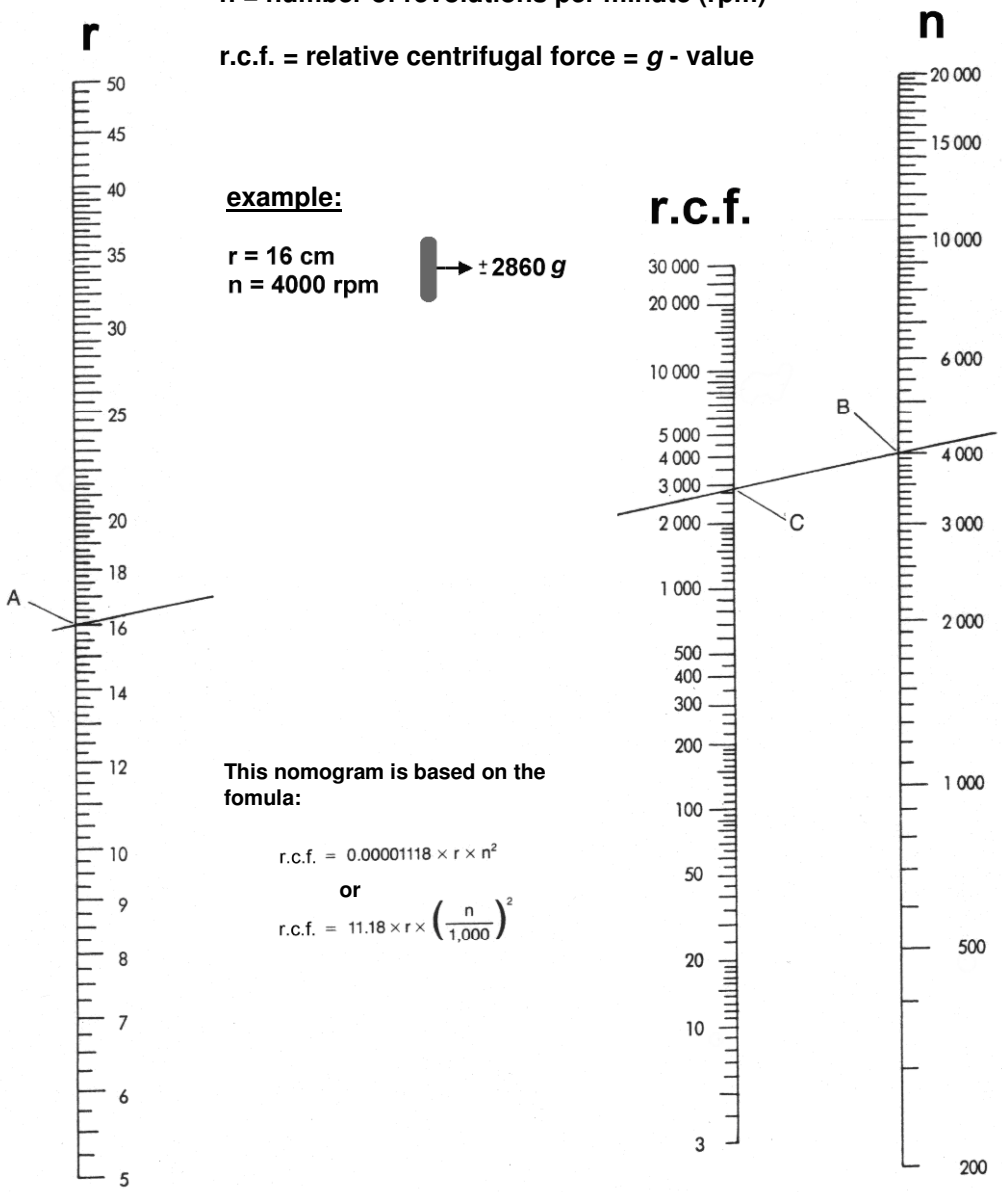
** Found in vaginal discharge or urine.

Nomogram

r = radius of the rotor (in cm)

n = number of revolutions per minute (rpm)

r.c.f. = relative centrifugal force = *g* - value



example:

r = 16 cm
n = 4000 rpm

→ **± 2860 *g***

This nomogram is based on the fomula:

$$\text{r.c.f.} = 0.00001118 \times r \times n^2$$

or

$$\text{r.c.f.} = 11.18 \times r \times \left(\frac{n}{1{,}000}\right)^2$$

MORPHOLOGY OF BLOOD CELLS IN A MAY-GRÜNWALD-GIEMSA STAINED BLOOD FILM

CELL TYPE	SIZE	NUCLEUS			CYTOPLASM		
GRANULOCYTES	µm	FORM	COLOR	CHROMATIN STRUCTURE	QUANTITY	COLOR	GRANULES
LEUKOCYTES : POLYMORPHONUCLEAR GRANULOCYTES							
IMMATURE NON SEGMENTED NEUTROPHILS "Band forms" or "S" shaped	12 – 15	Horseshoe, Central curvature is maximum a third part of the width of the lobes [1]	Clear blue purple	Strands of fine chromatin	abundant +++	Dusty rose (=very small granules)	small granules, light purple or violet Not always present
SEGMENTED NEUTROPHILS	12 – 15	2 to 5 lobes [2]	Deep blue purple	Rather thick and coarse	+++	Rose	Small granules, Pink or pink mauve
EOSINOPHILS	12– 15	Usually a bi-lobed nucleus	Blue purple	Rather thick and coarse	+++	Rose	Many large, uniform granules, red orange
BASOPHILS	11 – 13	Hardly visible lobes, not well separated (polymorph)	Blue purple	Rather thick and coarse, covered by granules	+++	Light rose	Very large, well separated, variable granules Deep purple Small in number

[1] Left deviation of the Arneth formula: an increase over 16 % of non segmented neutrophils, yet immature forms, occurring in inflammations, but also in stress conditions,… 2 to 5 segmented neutrophils are the major fraction of the neutrophils in a normal leukocyte type.

[2] Right deviation of the Arneth formula: in contrast with the left deviation, where segmented cells are rarely seen, this image shows hyper-segmented neutrophils, with 5 or more lobes. A hyper-segmentation is characteristic for megaloblastic anaemia. In the early phase, more than one neutrophil with 6 lobes per 100 granulocytes is found.

CELL TYPE	SIZE	NUCLEUS			CYTOPLASM		
AGRANULOCYTES	µm	FORM	COLOR	CHROMATIN STRUCTURE	QUANTITY	COLOR	GRANULES
LEUKOCYTES : MONOMORPHONUCLEAR AGRANULOCYTES							
SMALL LYMPHOCYTES	7 -10	Round or oval Or slightly indented	Deep purple	Big clumps of intensely stained chromatin	(-) or +	Sky blue (Often absent)	
LARGE LYMPHOCYTES	10 – 15	Round or oval Or slightly indented	Red, purple	Clumps of deep stained chromatin and other clumps which are less intensely stained	++	Sky blue	Absent or a few granules azurophils (rose violet)
MONOCYTES	15 – 25	Round, oval, indented or bean form	Blue to slightly violet	Fine, spongy like	+++ Vacuoles often demonstrable.	Grey or blue grey	Very fine granules (dusty like), azurophils (rose violet)
ERYTHROCYTES	6,7 – 7,7				Biconcave discus shape	rose	none
TROMBOCYTES	1,5 – 2 (5)				Slightly blue		Reddish

Some useful internet-sites

- Site for human parasitology (CDC):

  http://www.dpd.cdc.gov/dpdx

- Site for veterinary parasitology (Merck):

  http://www.merckvetmanual.com/mvm/index.jsp?cfile=htm/bc/22600.htm

- Site for human parasitology (medical chemical corporation):

  http://www.med-chem.com/Para/Index.htm

- Site for mycology:

  http://www.doctorfungus.org/index.htm

- Site for common laboratory-techniques – links to parasitology, haematology etc. (Australia):

  http://www.hoslink.com/malaria.htm

- Site for malaria-information (Australian Government):

  http://www.rph.wa.gov.au/labs/haem/malaria

- Site for new lab-tests:

  http://www.finddiagnostics.org

- Site for human parasitology (in dutch):

  http://www.medische parasitologie.nl

- Site for veterinary parasitology:

  http://www.wormers-direct.co.uk