

Diagnosis of the Parasitic infections

General Microbiology

2nd year student

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The Reality of Parasites

- 1.3 billion persons infected with *Ascaris* (1: 4 persons on earth).
- 300 million with Schistosomiasis.
- 100 million new malaria cases/ year.

Diagnosis of Parasitic Infections

1. Clinical
2. Laboratory

Purpose of laboratory diagnosis :

- Confirmation of clinical suspicion.
- Identification of unsuspected infection.

Collect the Information of the Patient

1- Provisional diagnosis

- a. History (Age, occupation, residency, previous infection).
- b. Complaint .
- c. Clinical examination.

2- Confirmed diagnosis:

- a. Laboratory investigations .
- b. Radiology .
- c. Surgical intervention (Exploratory)

Specimens

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

DIAGNOSIS

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graph TD; A[DIAGNOSIS] --> B[DIRECT]; A --> C[INDIRECT]; A --> D[MOLECULAR]; B --> B1[Urine]; B --> B2[Stool]; B --> B3[Sputum]; B --> B4[Biopsy]; B --> B5[Blood]; B --> B6[Aspirates]; C --> C1[IHAT]; C --> C2[LAT]; C --> C3[IFAT]; C --> C4[ELISA]; C --> C5[CFT]; D --> D1[DNA probes]; D --> D2[PCR];
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The diagram is a hierarchical flowchart. At the top is a box labeled 'DIAGNOSIS' with a red border. A horizontal line extends from this box, with three vertical lines branching downwards to three boxes: 'DIRECT', 'INDIRECT', and 'MOLECULAR'. Each of these three boxes has a vertical line extending downwards to a larger box containing a list of specific diagnostic methods. The 'DIRECT' box lists: Urine, Stool, Sputum, Biopsy, Blood, Aspirates. The 'INDIRECT' box lists: IHAT, LAT, IFAT, ELISA, CFT. The 'MOLECULAR' box lists: DNA probes, PCR.

DIRECT

**Urine
Stool
Sputum
Biopsy
Blood
Aspirates**

INDIRECT

**IHAT
LAT
IFAT
ELISA
CFT**

MOLECULAR

**DNA probes
PCR**

Urine examination

Parasites detected in urine :

Helminths:

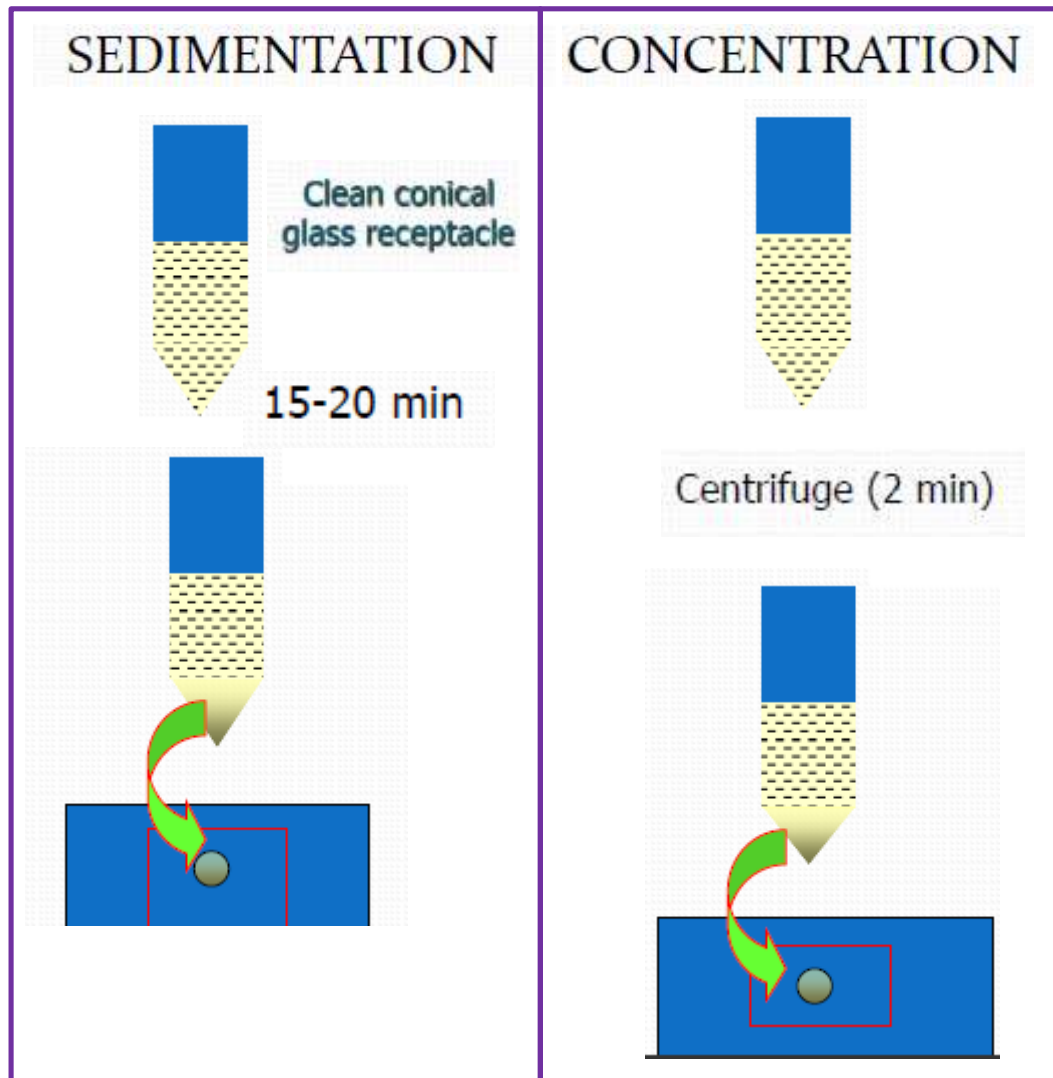
- Schistosoma haematobium eggs.
- Enterobius vermicularis eggs in female patients.
- Microfilaria of Wuchereria bancrofti.

Protozoa:

- Trichomonas vaginalis trophozoite in female patients.
- Temporary stains, such as methylene blue is helpful to see *T. Vaginalis*.

Note: Urine specimen should be centrifuged at $400 \times g$, the sediment mixed with a drop or two of saline, and examined by wet mount.

Urine examination

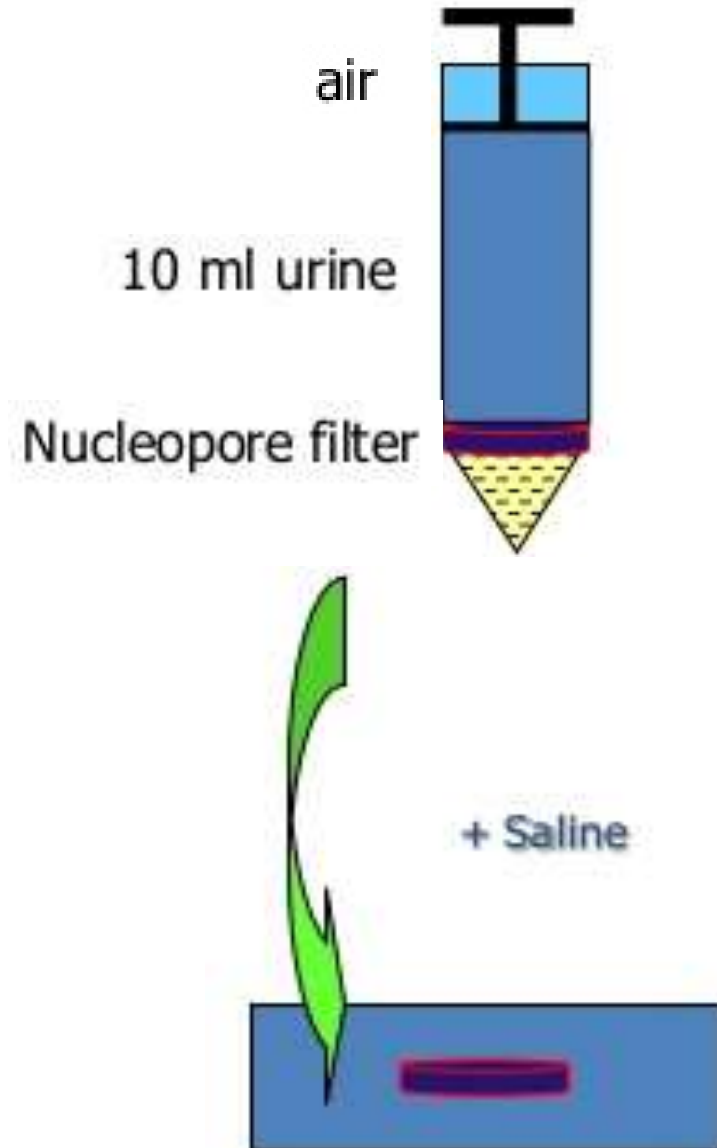


Urine examination

Urine examination

Other techniques:

- **Membrane Filter technique:**
 - For concentration of *Schistosoma* eggs.
 - Fill a syringe with urine, pass the urine through a filter.
 - Remove the filter and place it on the slide, and examine it microscopically.



Stool examination

Sample collection:

- Sample is collected in clean, dry container
- Handled carefully.
- Collect it into wide mouth, clean, sterile, leak proof container
- Samples in some cases fresh (amoeba, ciliates).
- Do not refrigerate stool.
- Liquid and soft stool examined within 15 min.
- Not mixed with urine or disinfectant (as they will kill trophozoites).

Preservation of stool specimens:

Aim:

- To preserve protozoan morphology.
- To prevent the continued development of some helminthic eggs and larvae.
- The most common preservative used is 10% formalin.

Stool examination

Microscopic Examination of Faecal Specimens:

- 1- Direct Smears.
- 2- Direct wet mount
- 3- Concentration methods.



Stool examination

Direct Smears.

Principle

- To assess the worm burden of a patient
- To provide a quick diagnosis of a heavily infected specimen
- To check organism motility



Stool examination

Direct wet mount:

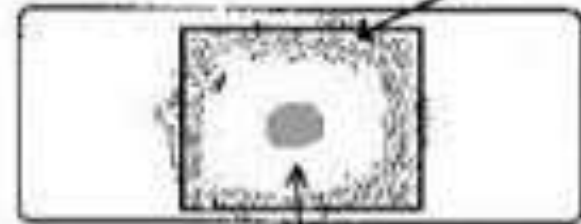
- To detect motile protozoan trophozoites.
- Small amount of faeces
- Few drops of saline
- Sometimes add lugol's iodine (nuclear details, glycogen vacuole in cyst).
- Protozoa (trophozoite), cyst, eggs and larva of helminths.

Drop of stool



cover slip

petroleum jelly



cover slip

depression slide

Stool examination

Concentration methods

- Used if parasites are scanty in the sample.
- Two types:

1- Floatation (eggs and cyst float , solution of high specific gravity)

- i. Saturated sodium chloride
- ii. Zinc sulphate centrifugation floatation (cyst, nematodes).

2- Sedimentation (solution of low specific gravity):

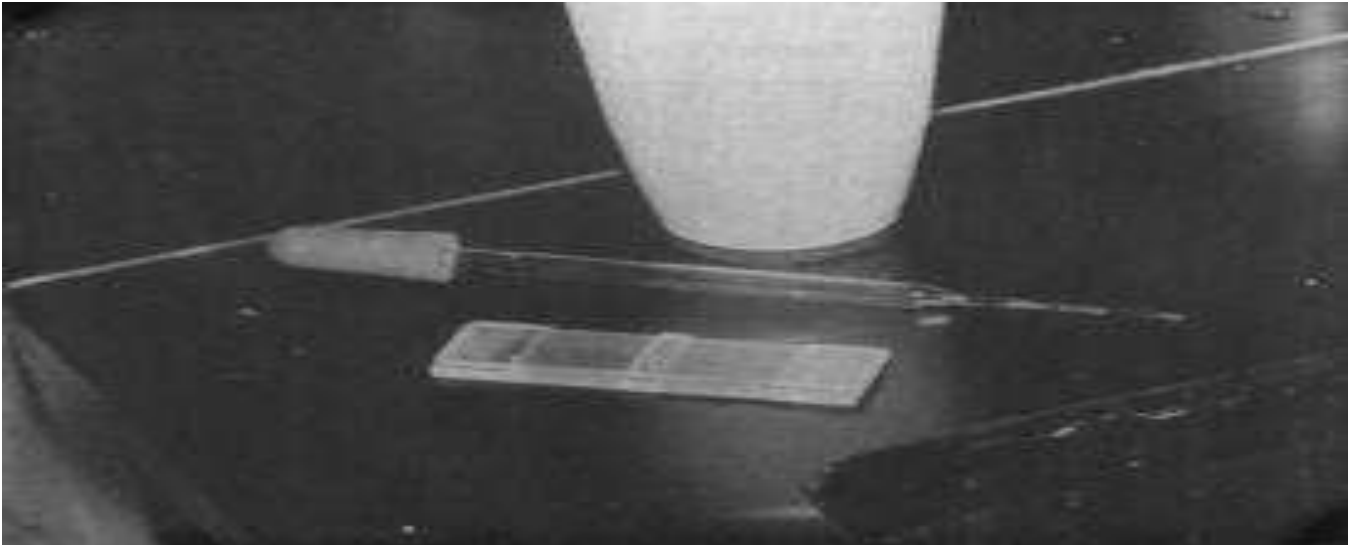
formol ether

Egg count in 1 gram

Stool examination

Concentration methods

Stoll's technique for counting helminth egg



3 gm stool and 42 ml water

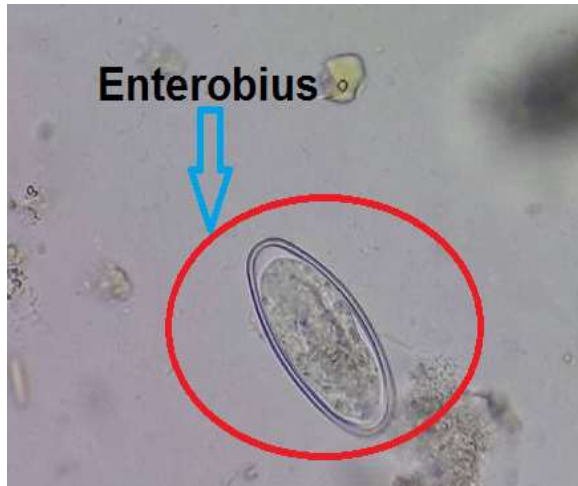
0.15 ml on slide

Multiply result in 100

Number in 1 gm

Stool examination

Microscopic Examination of Faecal Specimens: Direct Smears



Sputum examination

Sputum examination

- ✓ Abnormally, it is purulent, bloody, contains rusty brown particles (Paragonimus).

Technique for examination:

- ✓ Add on a sputum sample equal volume of NaOH to dissolve the mucus.

- ✓ Leave this combination for a while, then centrifuge at 200xg for 5 minutes, then examine the sediment.

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

Sputum examination

Parasitology

Macroscopic

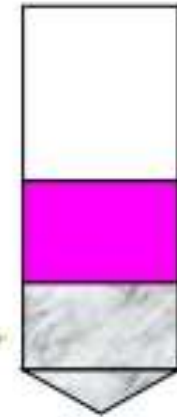
Microscopic

Appearance

Concentration

Bloody (Parag)

Rusty brown
(Parag)



NaOH
Sputum

Centrifuge



Take the sediment



Sputum examination

Parasites that could be detected in sputum:

1. The inhabitant in the lung:

- ✓ Paragonimus

2. Migratory larvae:

- ✓ Ascaris
- ✓ Hook worm (Ancylostoma)
- ✓ Strongyloides.

3. Parasites causing pathology in the lung:

- ✓ Trophozoites of Entamoeba histolytica.
- ✓ Hydatid sand due to rupture of hydatid cyst that could be present in the lung.

Blood examination

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

Blood sample will be used for :

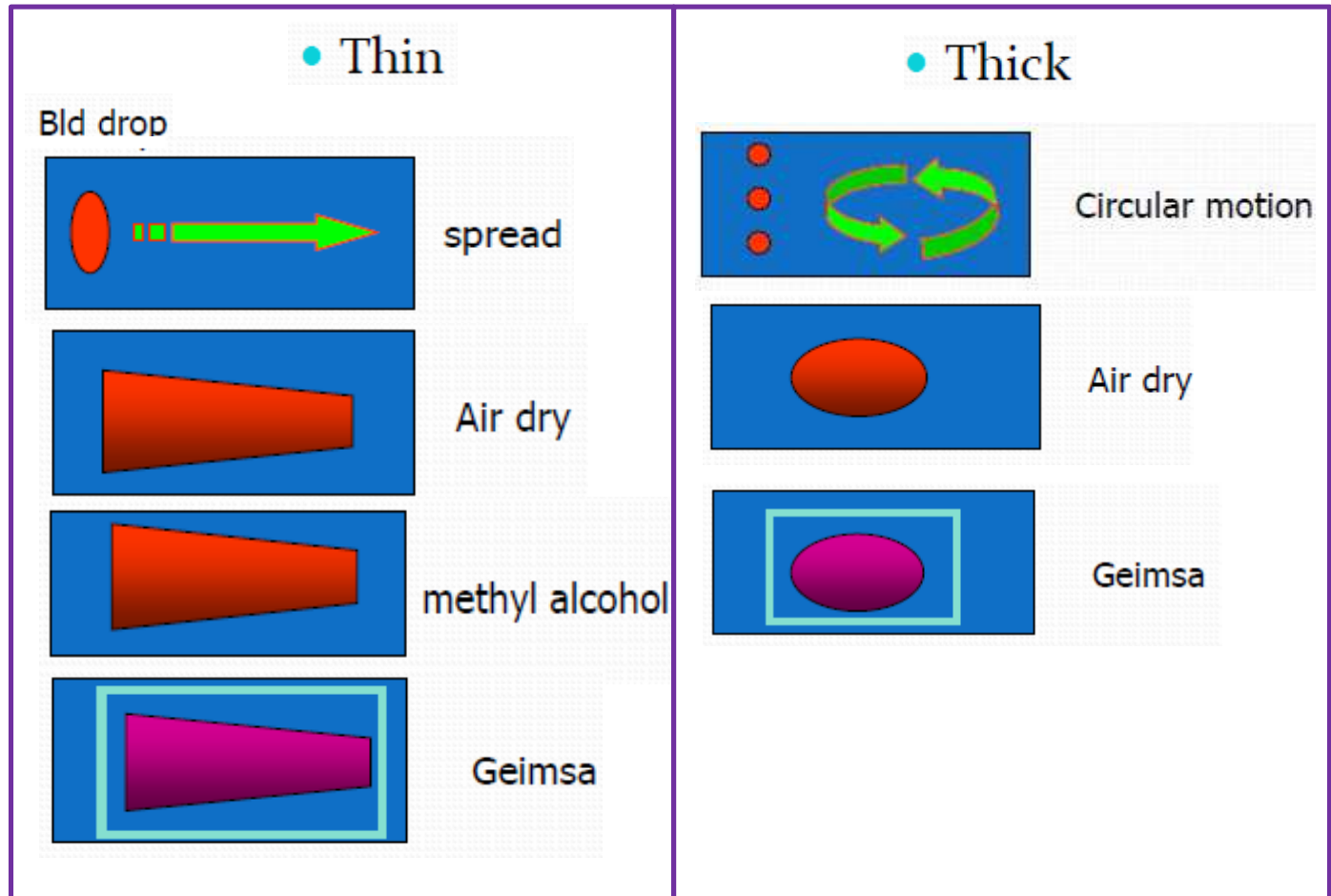
– Microscopic examination (Thin Smear, Thick smear, Wet mount for microfilaria).

- Molecular diagnosis
- Detection of parasite antigen
- Isolation of organisms
- Special tests

Blood examination

Two types of blood films can be made, thin and thick blood films:

- ✓ Thick films.
- ✓ Thin films.



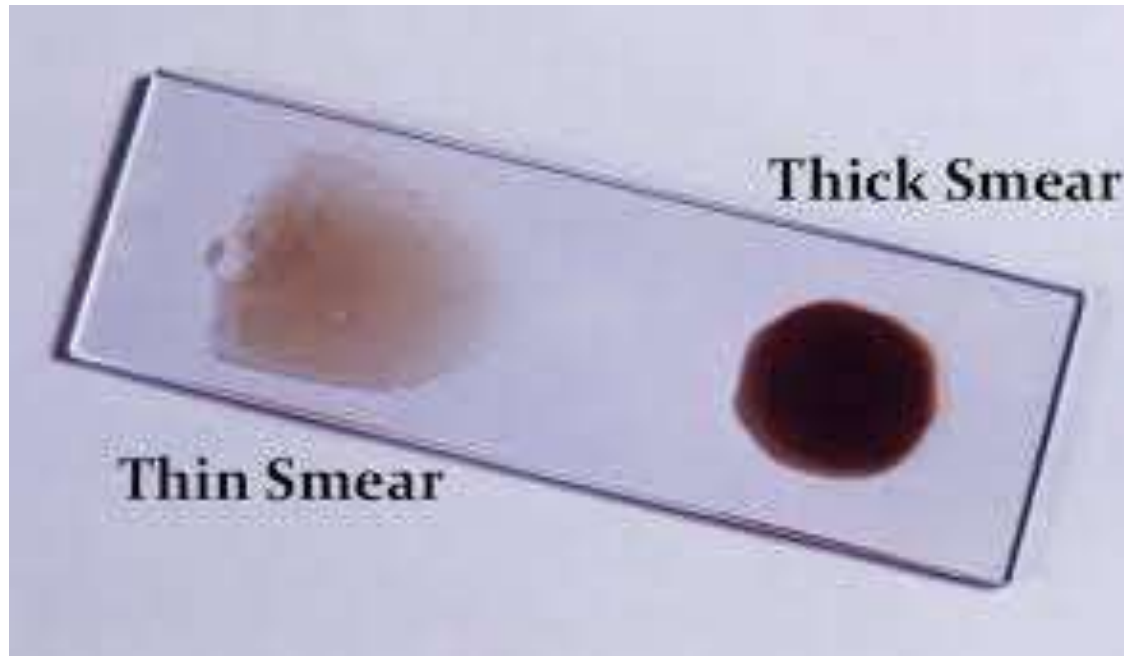
Blood examination

Thick blood film

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

Thin blood film

In malaria Parasitized red blood cells



Blood examination

Parasites that could be detected in blood film:

- Malaria
- Trypanosoma (African and American).
- Microfilaria of all types Filaria except Onchocerca volvulus.
- Indian type of Leishmania donovani.

Examination of other Specimens

1. Lung and Liver

- Aspiration from lung and liver could be examined for:
 - ✓ Pneumocytosis
 - ✓ Amoebiasis

Technique: The use of proteolytic enzymes is recommended to free the organisms from the aspirate material

- ✓ Hydatid Disease

2. Lymph nodes, Spleen, Liver, Bone Marrow and Spinal Fluid: Aspirated material may be examined for presence of trypanosomes, leishmanial forms and amoebae.

3. Cutaneous Ulcers : Leishmaniasis

Harmful effects of the parasite on the host

- Many parasites cause harmful effects to their host, Such effects comprise:
 - **Wasting (cachexia)**
African trypanosomiasis and leishmaniasis may lead to severe loss of weight in both animals and man.
 - **Superinfections**
In the case of (muco)cutaneous leishmaniasis ulcerations may lead to superinfections with bacteria

Harmful effects of the parasite on the host

- **Immunodepression**

Malaria, bilharziosis, etc., lead to a certain degree of immune suppression which renders the infected host more susceptible to other diseases.

- **Allergic reactions**

- **Anaphylactic shock** : may be induced by the sudden release of large amounts of parasite internal antigens into the bloodstream.

Harmful effects of the parasite on the host

- **Mechanical damage**

- In the case of malaria the lysis of erythrocytes does lead to haemolysis and anaemia.
- In the case of ascaris infection the presence of the worms in the small intestine may lead to intestinal occlusions

• **Reflexes** (intestinal contractions-ascaris)

• **Irritation of skin and tissues** by ecto- and endoparasites