Medical Laboratory

NTQF Level III

Learning Guide #43

Unit of Competence: **Perform Parasitological Examination**

Module Title**:** **Perform Parasitological Examination**

LG Code: HLT MLT3 07 0919

TTLM Code: HLT MLT3 07 0919V1

LO1: **Identify concept of human parasitology**

Instruction Sheet 1	Learning Guide #1

This learning guide is developed to provide you the necessary information regarding the following content coverage and topics –

1. **Basic Concepts of Medical Parasitology**
    - Definition of terminologies
    - Host parasite interactions
    - Techniques in Medical Parasitology
        - Wet mount
        - Concentration techniques
        - Staining
        - Immunodiagnostic techniques
    - Life cycle, morphological stage and classification of parasites
        - Medical Helminthological
            - ✓ Nematodes
            - ✓ Intestinal nematodes
            - ✓ Tissue and blood nematodes
        - Cestodes
        - Trematodes
        - Medical Protozoology
            - ✓ Intestinal protozoa
            - ✓ Protozoa of urogenital tract
            - ✓ Hemo-flagellates
            - ✓ Malaria Parasites

This guide will also assist you to attain the learning outcome stated in the cover page. Specifically, upon completion of this Learning Guide, you will be able to –

- Identify concepts of human parasitology.
- Identify principles of host and parasite interaction.
- Identify methods of parasitological examinations.
- Differentiate lifecycles and diagnostic stage of parasites.

Learning Instructions:

1. Read the specific objectives of this Learning Guide.
2. Follow the instructions described in number 3 to 16.
3. Read the information written in the “Information Sheets 1”. Try to understand what are being discussed. Ask your trainer for assistance if you have hard time understanding them.
4. Accomplish the “Self-check 1” in page 7.
5. Ask from your trainer the key to correction (key answers) or you can request your trainer to correct your work. (You are to get the key answer only after you finished answering the Self-check 1).
6. If you earned a satisfactory evaluation proceed to “Information Sheet 2”. However, if your rating is unsatisfactory, see your trainer for further instructions or go back to Information sheet 1.
7. Submit your accomplished Self-check. This will form part of your training portfolio.
8. Read the information written in the “Information Sheet 2”. Try to understand what are being discussed. Ask your trainer for assistance if you have hard time understanding them.
9. Accomplish the “Self-check 2” in page 11.
10. Ask from your trainer the key to correction (key answers) or you can request your trainer to correct your work. (You are to get the key answer only after you finished answering the Self-check 2).
11. Read the information written in the “Information Sheets 3”. Try to understand what are being discussed. Ask your trainer for assistance if you have hard time understanding them.
12. Accomplish the “Self-check 3” in page 19.

13. Ask your trainer the key to correction (key answers) or you can request your trainer to correct your work. (You are to get the key answer only after you finished answering the Self-check 3).
14. If you earned a satisfactory evaluation proceed to “Operation Sheet 1” in page 21; However, if your rating is unsatisfactory, see your trainer for further instructions or go back to Information sheet 3.
15. Read the “Operation Sheet 1 and try to understand the procedures discussed.
16. Do the “LAP test” in page 22 (if you are ready). Request your trainer to evaluate your performance and outputs. Your trainer will give you feedback and the evaluation will be either satisfactory or unsatisfactory. If unsatisfactory, your trainer shall advice you on additional work. But if satisfactory you can proceed to Learning Guide #2.

Information Sheet-1	Identify concepts of human parasitological

Identify concepts of human parasitological

- Introduction to parasitology**:**

  Medical Parasitology: Parasitology is the most fascinating branch of biology that studies parasites and their relationship with their hosts. Medical parasitology, on the other hand, deals with parasites that cause diseases to human beings or Medical parasitology is the science that studies or deals with parasites infecting humans. The most accepted definition in medicine is, Medical parasitology is a subject that also deals with the biological features of medically important parasites, the relationship between the human being and the parasites, the diagnosis, treatment, prevention and control of the parasitic diseases. In general, while it is important to classify bacteria and fungi and viruses as parasites, parasitology has traditionally been limited to parasitic protozoa, helminthes, and arthropods. It follows, then, that parasitology encompasses elements of Protozology, helminthology, and medical arthropodology. Human parasitology, an important part of parasitology, studies the medical parasites including their morphology, life cycle, the relationship with host and environment.

- **Definition of terms**:

  - ✓ Parasitology: is a science that deals with parasites
  - ✓ Medical parasitology: is that study of parasites that causes disease in man

- ✓ Parasite: is an organism living temporarily or permanently in or on another organisms (host) from which is physically or physiologically dependent up on other
- ✓ A host: is an organism which harbors a parasite and provides food. A host is much bigger than a parasite and has lower reproductive capabilities compared to its parasite.
- ✓ A vector: is an organism an arthropod which transfers infective forms of a parasite from one host to the other.
- ✓ Infective Stage: it is a stage when a parasite can invade human body and continue to live there. The infective stage of ascaris is the embryonated egg.
- ✓ Route of infection: is the specific entrance through which the parasite invades the human body. Hookworms invade human body by skin. Man gets infection with Ascaris by mouth.
- ✓ Modes of infection: means how the parasite invades human body, such as the cercariae of the blood fluke actively penetrate the skin of a swimming man and the infective Ascaris eggs are swallowed by human.
- ✓ Helminthes: are parasitic worms that feed on a living host to gain nourishment and protection, while causing poor nutrient absorption, weakness and disease in the host. The following groups of worms are classed as **helminths**: Nematodes or roundworms. Trematodes, which includes flukes or flatworms. Cestodes or tapeworms.
- ✓ Protozoa: are single celled organisms. They come in many different shapes and sizes ranging from an Amoeba which can change its shape to Paramecium with its fixed shape and complex structure. They live in a wide variety of moist habitats including fresh water, marine environments and the soil.
- ✓ Geohelminthes or Soil Transmitted Helminthes: refers to the helminthes which complete their life cycles not requiring the processes of the

development in intermediate hosts. They have only one host and a simple life cycle, such as Ascaris, Hookworm, Pinworm and etc.

- ✓ Biohelminthes: refers to the helminths which have to undergo the development in intermediate hosts to complete their life cycles, such as filarial, liver fluke, pork tapeworm and so on.

Self-check 1	Written test

Write True if the statement is correct and False if it is incorrect

1. Medical Parasitology deals with parasites that cause disease. (2 points)
2. A parasite is an organism that can survive without help of other organism.(2 points)
3. A vector can transmit an infective stage of a parasite.(2 points)

Note: satisfactory rating is 4 points, unsatisfactory 2 points. You can ask your instructor for copy of correct answer.

Answer Sheet

1._____

2. _____

3. _____

Score = _____

Rating: _____

Name: _____ Date: _____

Information Sheet-2	**Host parasite interactions**

✓ Host parasite interactions

Any organism that spends a portion or all of its life closely associated with another living organism of a different species is known as a symbionts (or symbiotic), and the relationship is known as symbiosis (living together of organisms of two different species). There are four categories of symbiosis are commonly recognized: commensalism, phoresis, parasitism, and mutualism. The scope of this part is limited to relationships of medical importance, and, since parasitism is the major type of symbiosis meeting this criterion, definitions of the other forms is included for clarification only. **A. Commensalism:** Commensalism does not involve physiologic interaction or dependency between the two partners, the host and the commensal. Literally, the term means “eating at the same table.” In other words, commensalism is a type of symbiosis in which spatial proximity allows the commensal to feed on substances captured or ingested by the host. The two partners can survive independently. Although at times certain nonpathogenic organisms (e.g., protozoa) are referred to as commensals, this interpretation is incorrect since

they are physiologically dependent on the host and are, therefore, parasites. An example of commensalism is the association of hermit crabs and the sea anemones they carry on their borrowed shells.

B. Phoresis: The term phoresis is derived from the Greek word meaning "to carry." In this type of symbiotic relationship, the phoront, usually the smaller organism mechanically carried by the others usually larger organism or the host. Unlike commensalism, there is no dependency in the procurement of food by either partner. Phoresis is a form of symbiosis in which no physiologic interaction or dependency is involved. Both commensalism and phoresis can be considered spatial, rather than physiologic, relationships. Examples of phoresis are the numerous sedentary protozoans, helminthes eggs/larvae stages, algae, and fungi that attach to the bodies of aquatic arthropods, turtles, cockroaches (in case of human infections), etc.

C. Parasitism: Parasitism is another type of symbiotic relationship between two organisms: a parasite usually the smaller of the two, and a host upon which the parasite is physiologically dependent. The relationship may be:

Permanent, as in the case of tapeworms found in the vertebrate intestine,

Temporary, as with female mosquitoes, some leeches, and ticks, which feed intermittently on host blood. Such parasites are considered obligatory parasites because they are physiologically dependent upon their hosts and usually cannot survive if kept isolated from them.

Facultative parasites, on the other hand, are essentially free-living organisms that are capable of becoming parasitic if placed in a situation conducive to such a mode. An example of a facultative parasite is the amoeba Naegleria. Unlike commensals, parasites derive essential nutrients directly from the host, usually from such nutritive substances as blood, lymph, cytoplasm, tissue fluids, and host-digested food.

D. Mutualism: is an association in which the mutualistic and the host depend on each other physiologically. Simply the association is obligatory and living apart is impossible. That is, they must live together and one cannot survive in the absence of the other. A classic example of this type of relationship occurs

between certain species of flagellated protozoans and the termites in whose gut they live.

Note: there is less overlap between phoresis/commensalism and parasitism as well as between phoresis/commensalism and mutualism than between parasitism and mutualism.

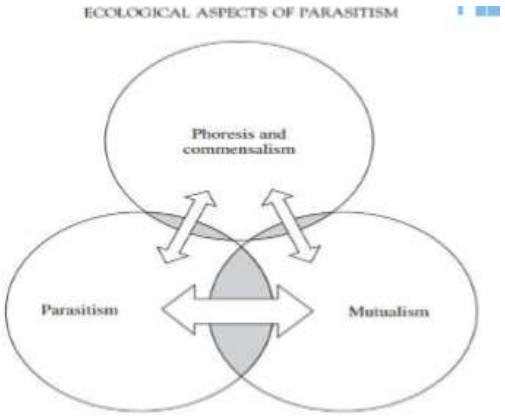

Figure 1.1Overlap between the major categories of symbiosis.

Self-check 2	Written test

Answer the following questions (2 points for each questions)

1. Relationship between two different species is:
   A. Commensalism
   B. Phoresis
   C. Symbiosis
   D. Parasitism
2. Eating at the same table is;
   A. Commensalism
   B. Phoresis
   C. Symbiosis
   D. Parasitism
3. The dependence of one organism on other for food and shelter:
   A. Commensalism
   B. Phoresis
   C. Symbiosis

D. Parasitism

4. An association of two different species for common benefit is:
   A. Mutualism
   B. Commensalism
   C. Parasitism
5. A form of symbiosis in which no physiologic interaction or dependency is involved is;
   A. Commensalism
   B. Phoresis
   C. Symbiosis
   D. Parasitism

Note: satisfactory rating is 6 points, unsatisfactory 4 points. You can ask your instructor for copy of correct answer.

Answer Sheet

1._____

2. _____

3. _____

4._____

5. _____

Score = _____

Rating: _____

Name: _____ Date: _____

Information Sheet-3	Techniques in Medical Parasitology

✓ Techniques in Medical Parasitology

Basic diagnostic techniques for parasitic infection includes

♣ Morphological diagnosis (Macroscopic /Microscopic diagnosis)

By morphological diagnosis the stages of the parasites to be detected are eggs, larva and adult stage for **Helminths** and cyst, trophozoite stages for protozoans.

- Macroscopic: using the naked eye for the presence of some adult worms. *E.g. Ascaris, E. vermicularis and gravid Taenia* spp.

- Microscopic: The majority of intestinal, blood, urinary and skin parasites are usually detected microscopically. Stained or unstained preparation of different specimens (such as blood, stool, urine, CSF, etc…) are examined either:

- **Directly**: Wet mount can be prepared directly from fecal material or from concentrated specimens.

  Basic types of wet mount are:

  - Saline: is initial microscopic examination of stool by direct stool smear. It primarily used to demonstrate adult worms, eggs, larvae of helminths and cysts and trophozoites of protozoan parasites. Can also reveal the presence of Red blood cells and White blood cells or pus cells.
  - Iodine: used mainly to stain glycogen and the nuclei of cysts of protozoa.
  - Materials and reagents used for wet mount preparation are the following.
    - Slides, cover slides, applicator sticks, and marker for labelling, microscope.
    - Reagents: normal saline 85%, Lugos iodine.

♣ **Concentration techniques:** The direct examination of faeces is essential to detect motile parasites and is usually adequate to detect significant helminth infections. Important exceptions are Schistosoma species because only a few eggs are usually produced even in moderate and severe infections, therefore a concentration technique should be performed when intestinal schistosomiasis is suspected and no eggs are found by direct examination.

- Concentration techniques may also be required:
  - To detect Strongyloides larvae, the eggs of Taenia, cysts of G. lamblia, and to make it easier to detect small parasites, e.g. small fluke eggs, or the oocysts of intestinal coccidia prior to staining.
  - To check whether treatment has been successful.
  - To quantify intestinal parasites.

- The following techniques are commonly used to concentrate faecal parasites in district laboratories:
  - **Sedimentation techniques** in which parasites are sedimented by gravity or centrifugal force, e.g. formol ether concentration method

which is the most frequently used technique because it concentrates a wide range of parasites with minimum damage to their morphology.

- **Floatation techniques** in which parasites are concentrated by being floated in solutions of high specific gravity, i.e. solutions that are denser than the parasites being concentrated. Examples include the zinc sulphate method and saturated sodium chloride method. Unlike the formol ether sedimentation technique, a single floatation technique cannot be used to concentrate a wide range of parasites because of differences in the densities of parasites and the damage that can be caused by floatation fluids to some parasites

- **Choice of concentration technique depends on** :
  - Why the technique is being performed, the species of parasite requiring concentration, and how well its morphology is retained by a particular technique.
  - The number of specimens to be examined and time available. –
  - The location, e.g. field or laboratory situation and equipment available.
  - Experience of staff performing the technique.
  - Health and safety considerations.

A. **Formol ether concentration technique**: This is recommended for use in laboratories because it is rapid and can be used to concentrate a wide range of faecal parasites from fresh or preserved faeces. Risk of laboratory acquired infection from faecal pathogens is minimized because organisms are killed by the formalin solution. The technique, however, requires the use of highly flammable ether or less flammable ethyl acetate.

Principle: In the Ridley modified method, faeces are emulsified in formol water, the suspension is strained to remove large faecal particles, ether or ethyl acetate is added, and the mixed suspension is

centrifuged. Cysts, oocysts, eggs, and larvae are fixed and sedimented and the faecal debris is separated in a layer between the ether and the formol water. Faecal fat is dissolved in the ether. The require reagent is formol water 10%V/V.

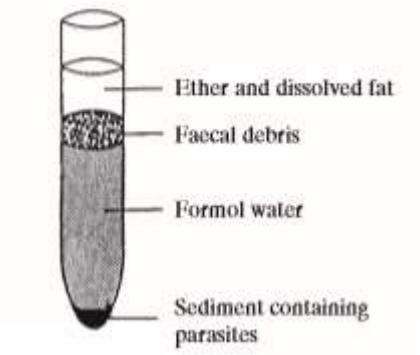

Fig. 1.2 Formal ether sedimentation concentration technique, after centrifugation

B. **Zinc sulphate floatation technique:** zinc sulphate technique is recommended for concentrating the cysts of G. lamblia and E. histolytica/E. dispar, and the eggs of T. trichiura species. Other nematode eggs are concentrated less well. The technique is not suitable for concentrating eggs or cysts in fatty faeces. Adequate safety precautions should be taken because faecal pathogens are not killed by zinc sulphate.

Principle: A zinc sulphate solution is used which has a specific gravity (relative density) of 1.180–1.200. Faeces are emulsified in the solution and the suspension is left undisturbed for the eggs and cysts to float to the surface. They are collected on a cover glass. The require reagent is Zinc sulphate solution, 33% w/v.

C. **Saturated sodium chloride floatation technique**:The saturated sodium chloride technique is a useful and inexpensive method of concentrating hookworm or Ascaris eggs, e.g. in field surveys. The technique is the same as that described for the zinc sulphate floatation technique.

♣ **Staining:** as many parasitic organisms cannot be cultured, microscopic examination is the mainstay of diagnostic parasitology. Examination after proficient staining of fresh and unconcentrated specimens, as well as preserved

and or concentrated specimens with permanent stained preparations, most often provides for rapid and accurate diagnosis. A variety of reagents and stains are available for these purpose, and each laboratory must decide which ones to use to best serve its patient population. In addition most specimens are transported in fixatives and preservatives.

There are three categories of chemicals used to preserve stool, prepare slides for staining and stain the preparation:

1. Fixatives
2. Preparatory reagents
3. Stains

- **Temporary stains**
    1. Eosin
    2. Saline
    3. Acridine Oragne
    4. Lugol's Iodine
    5. Thomson's stain
    6. Sargeant's stain
    7. Burrow's stain
- **Permanent stains**
    1. Auramine Phenol
    2. Field's stain (A/B)
    3. Giemsa stain
    4. Iron Hematoxylene (A/B)
    5. Trichrome
    6. Modified Trichrome
    7. Modified Ziehl – Neelsen

- **Sodium Acetate -** Acetic Acid - Formalin Fixative (SAF)

  SAF fixed material is suitable for:

  - ☐ Direct examination
  - ☐ Concentration and
  - ☐ Permanent staining

- **Field Stain A & Field Stain B**

- ☐ Enables rapid staining of fixed thin films.
- ☐ This particular method is very useful for staining films of unformed feces, fecal exudates, duodenal aspirates etc.

- **Lugol's Iodine (Aqueous)**
  - ☐ Temporary Stain for Protozoa.
- **Iron Haematoxylen Solution A/B**

Method: preparation of Working Iron Haematoxylin Solution

- ☐ Mix equal volumes of the two solutions and filter.
- ☐ Allow to stand at least two hours.
- ☐ Parasite stain blue if used immediately after preparation
- ☐ Mature stains stain light blue with grey back ground
- ☐ If a slide appears cloudy, then dehydration has been inadequate.
- ☐ Agitation in the final alcohols can improve the clarity of the smear.

- **Trichrome For Protozoa**
  - ☐ May be used to stain fresh faeces, prefixed faeces or cultured organisms.
  - ☐ The method varies slightly depending on the sample preparation used.

♣ **Immunological diagnosis (Antibody and antigen detection**

Pertaining to diagnosis by immune reactions. Is based on the detection of:

A. **Antibody (Ab) from person's serum**

Ab is produced in response to a particular parasitic infection

B. **Antigen (Ag) detection**

- ☐ Ag. Is excreted by parasites and can be found in the serum, urine, CSF, feces or other specimens.
- ☐ Antigen tests provide evidence of present infection

Why Immunodiagnostic techniques are required?

- Parasites live in the tissue of internal organ and cannot easily obtained for examination.
- Parasites can be found in specimens only in certain stages of infection,
  - ☐ E.g. in the acute stage not in the chronic stage.

- Parasites are present intermittently or in too few numbers to be easily detected in the specimens.
- The techniques used to detect parasites are complex or time consuming.

Immunodiagnosis is particularly used for the following types of parasites

- South American trypanosomiasis , Chronic stage
- African trypanosomiasis, when parasitaemia is low
- Leishmaniasis
- Filariasis
- Amoebic liver abscess
- Trichinosis
- Toxoplasmosis
- Toxocarisis
- Hydatid disease
- Schistosomiasis
- Malaria

Self-Check 3	**Written Test**

Directions: Answer all the questions listed below. Use the Answer sheet provided in the next page:

1. Macroscopic diagnosis technique involves detection_____ stage of parasite. (2 points)
2. List the chemicals used for temporary staining in parasitology lab. (5 points)
3. List the chemicals used for permanent staining in parasitology lab. (5 points)

4. Describe wet mount technique. (5 points)
5. Immunological diagnosis technique detects _____and _____? (2 points)
6. List at least five parasites for which we can use immunological diagnostic technique? (5 points)
7. Briefly explain why the immunological diagnosis method is required. (4 points)

Note: **Satisfactory rating - 16 points** **Unsatisfactory - below 16 points**
You can ask you teacher for the copy of the correct answers.

Answer Sheet

Score = _____
Rating: _____

Name: _____ Date: _____

Short Answer Questions

1. _____

2. _____

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7. _____
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Information sheet 4	Life cycle, morphological stage and classification of parasites

4. Life cycle, morphological stage and classification of parasites

4.1. Introduction:

Parasitism: A parasite is an organism that is entirely dependent on another organism, referred to as its host, for all or part of its life cycle and metabolic requirements. Parasitism is therefore a relationship in which a parasite benefits and the host provides the benefit. The degree of dependence of a parasite on its host varies. An obligatory parasite is one that must always live in contact with its host. The term free-living describes the non-parasitic stages of existence which are lived independently of a host, e.g. hookworms have active free-living stages in the soil.

4.2. Terms used to describe parasite hosts

- Definitive host: This is the host in which sexual reproduction takes place or in which the most highly developed form of a parasite occurs. When the most mature form is not obvious, the definitive host is the mammalian host.
- Intermediate host: This is the host which alternates with the definitive host and in which the larval or asexual stages of a parasite are found. Some parasites require two intermediate hosts in which to complete their life cycle.
- Reservoir host: This is an animal host serving as a source from which other animals can become infected. Epidemiologically, reservoir hosts are important in the control of parasitic diseases. They can maintain a nucleus of infection in an area.

4.3. Features of parasites

- Smaller than their host
- Outnumber of the host
- Have short life span than their host
- Have greater reproductive potential than their host

4.4. Classification of parasites

4.4.1. According to their habitat

- **Ecto-parasites: parasites** living on or affecting the skin surface of the host e.g. Lice, tick, flea, bed-bugs, etc---

- **Endo -parasites**: parasites living with in the body of the host. E.g. leishmonia species, Ascaris lumbricoides etc.

4.4.2. According to their dependent on the host

- **Permanent** (Obligate) parasites: the parasite depends completely up on its host for metabolites, shelter, and transportation e.g. malaria.
- **Temporarily** (facultative) parasite: the parasite is capable of independent existence in addition to parasitic life e.g. stronglyloids sterecolaris, Naegleria fowlery etc. …

4.4.3. According to their pathogencity

- **Pathogenic parasites**: It causes disease in the host e.g. E. histolytica
- **Non-pathogenic** (Commensally) **parasite**: the parasite derives food and protection from the host but not harms the host. E.g. Entamoeba coli.
- **Opportunistic parasites**: parasites, which cause mild disease in immunologically healthy individuals but they cause, sever disease in immuno – deficient hosts. e.g. pneumocystis carnii, Toxoplasma gondii, Isospora belli ,etc

4.5. Source of parasitic infections

- **Contaminated soil**: polluted soil with human excreta is commonly responsible for exposure to infection with Ascaris lumbricodes, Trichuris trichuria and hookworms.
- **Contaminated water**: water may contain
  a) Viable cysts of amoeba, flagellates and T.Solium eggs
  b) Cercarial stage of human blood flukes
  c) Cyclopes containing larvae of dracunculus medinesis,
  d) Fresh water fishes, which are sources of fish tapeworm and intestinal flukes infection.
  e) Crab or cray fishes that are sources for lung fluke and
  f) Water plants which are sources of Fasciolopsis buski
  g) Insufficiently cooked meat of pork and beef which contains infective stage of the parasite e.g. Trichenilla spirals. Taenia species

h) Blood sucking arthropods: - these are responsible for transmission of e.g.

- Malaria parasites
- Leishmania
- Trypanosoma
- Wuchreria (Filariasis)

2. Animals (domestic or wild animals harboring the parasites)
   - Dog – hydatid cyst, cutaneous larva migrans
   - Herbivores animal – Trychostrongylus species.
3. Human beings – Another person's his clothing, bedding or the immediate environment that he contaminated e.g. Amoeba, E.vermicularis, H.nana
4. Sexual intercourse e.g. Trichomonas vaginalis
5. Auto infection e.g. S.stercoralis, E.vermicularis, and taemia solium.

4.6. Mode of transmission

4.6.1. Direct- mode of transmission

The parasite does not require biological vectors and or intermediate hosts and requires only a single host to complete its life cycle. It may require mechanical vectors direct mode of transmission can be classified as.

4.6.2. **Horizontal direct mode of transmission**:

Mainly transmitted through

- Feco-oral route
- Sexual intercourse
- Blood transmission
- Direct skin penetration
- Air born

4.6.3. **Vertical direct mode of transmission occurs from mother to child through**

- Congenital /transplacental
- Transmammary (breast milk)

4.7. Indirect- mode of transmission

The parasite has complex life cycle and requires biological victor and /or one or more intermediate hosts for transmission.

4.8. Host parasite relation ship

4.8.1. Effects of parasites on their hosts

- Consumption of the nutritive e.g. hook worm-sucks blood, D.latum remove B_{12}
- Obstruction of passages – e.g. Ascaris lumbricoides
- Bleeding e.g. schitosome eggs.
- Destruction of tissues e.g. Trophooites of E. histolytica, causes necrosis of liver, leishmania donorani – bone marrow destruction
- Compression of organs e.g. Hydatid cysts in liver, brain cause presure
- Release of toxic substances e.g. rupture of E. granulosus result anaphylactic shock
- Opening pathway to secondary infections e.g. ulcer. Eg D. medenesis for bacteria
- Allergy development e.g. Bite of arthropod
- Transmission of pathogens to man e.g. lice transmitte rickettsia
- Predisposition to malignancy. Eg Bilharziasis
- Chronic immune stimulation leading to unresponsiveness to infections.

4.8.2. Host susceptibility factors

4.8.2.1. Host factors

- Genetic constitution
    - Age
    - Sex
    - Level of immunity: - natural and acquired immunity
    - Nutrition (malnutrition or under nutrition)
    - Intensity and frequency of infections

* Presence of co-existing disease or conditions which reduced immune response e.g. pregnancy, HIV,
* Life style and occupation

4.8.3. Parasite factors

- Strain of the parasite and adaptation to human host
- Parasite load (number of parasite)
- Site (occupied) in the body
- Metabolic process of the parasite, nature of the waste products or toxins

4.9. Escape mechanism of parasite from the immune system.

- Site e.g. intracellular parasites e.g. T. curzi, leishmania and plasmodia are to some extent protected from the action of antibodies and forming cysts as T.gondii and larva of T.solium, E.granulous and Trichinella spiralis,
- Avoidance of recognition e.g. Recognition of some parasites as self-cell. Masking their selsves, eg. Schistosomes. Variation of antigens e.g. African trypanosomes. This types of relationship is known as molecula mimicry
- Suppression of immune response e.g. plasmodium, Toxoplasm, Trypanosome and Trichinella are able to suppress the ability of the host to response immunologically and increasing other infections results
    * Combine with the antibody and preventing it from attaching to the parasite
    * Induce B or T-cell tolerance either by blocking antibody or by depleting specific antigens.
    * Activating specific suppressor cells

4.10. Nomenclature of Human parasites

All animals and plants must have names by which they can be distinguished. Although common names are frequently, used for this purpose. These are not universally understood, partly because of language barriers and partly because

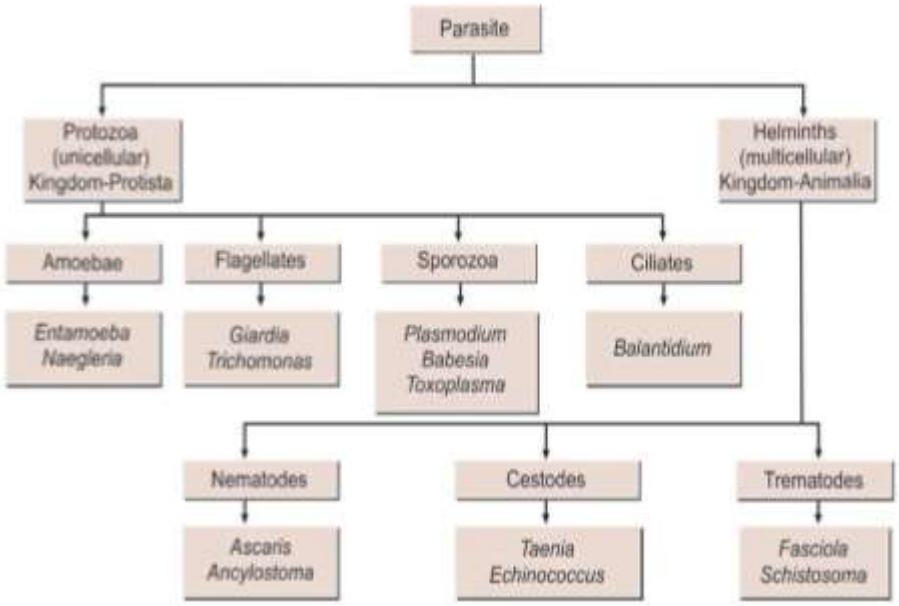
of a common name not necessarily applied to the same organism in different countries. To overcome this difficulty, a binomial scientific name is used. Based on the international code of zoological nomenclature.

The first name in the binomial is that the genus to which the organism belongs, and the second is that of the species this combination of in designating an animal or plant species is termed binomial nomenclature.

Taxonomic classification of medically important parasites of men belongs to the kingdom of Animalia and most of parasites are members of three phyla.

1. Phylum Nemathelminths
2. Phylum Platyhelminths
3. Phylum protozoa

The following flow chart shows the general classification medically important parasites.



Flow chart 1.1. Nomenclature of parasites

4.11. **The life cycles of parasites**:

The life cycles of parasites describes the cycle of development of the parasites that Involves passing through a number of developmental stages either in the host or the environment.

The life cycle a parasite has three phases (components) that are very important for the successful survival of a parasite:

- Growth and maturation

- Reproduction
- Transmission.

The life cycle can be **simple (direct)** or **complex (indirect**) depending on how many hosts it required to complete its cycle.

- **Simple life cycle or direct:** only one host is required to complete the life cycle. Transmission is through contaminated food (grass), water, air, contaminated material or directly from person to person through sexual intercourse.

Fig. 1.3. The direct life cycle of Trichstrogyles.

- **Complex (indirect**): two or more hosts are required to complete the life cycle.

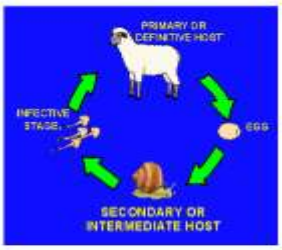


Fig. 1.4. Indirect life cycle involving more than one host.

4.12. Medically important helminthes

4.12.1. General features of helminthes:

- The word ‘helminths’ is a general term meaning ‘worm’. All helminths are multicellular eukaryotic invertebrates with tube-like or flattened bodies. Many helminths are free-living organisms in aquatic and terrestrial environments whereas others occur as parasites in most animals and some plants. Parasitic helminths are an almost universal feature of vertebrate animals; most species have worms in them somewhere. They are parasitic metazoans from the phyla Platyhelmintha (flatworms), Nematoda (roundworms). That means they are multicellular parasites belong to the kingdom Metazoa.The term

'helminths' (Greek helmins'worm') originally referred to intestinal worms, but now comprises many other worms, including tissue parasites as well as many free -living species. The size of the adult worms varies in size (6mm->30m). Their life cycles may be simple or complex. Pathology, clinical sign and symptoms depend on the location of the organisms. This may be caused by adults, larva, or egg (in case schistosomiasis).Laboratory diagnosis mainly depends on detection and identification of egg, larva or embryo and rarely adults in case of Ascariasis).

4.12.1. Classification of helminthes: Helminths, which occur as parasite in humans, belong to two phyla:

- Phylum Nemathelminths: It includes Nematoda
- Phylum Plathyhelmiths (flatworms). It includes two classes:
    - Class Cestoda (tapeworms)
    - Class Trematoda (flukes or digeneans)

4.12.2. Phylum Nemathelminths

Class: Nematoda

Introduction

Nematodes are the most common helminthes parasiting humans, and include intestinal nematodes as well as blood and tissue nematodes. The most common nematodes of medical importance are those inhabiting the intestinal tract. Most of these have a direct life cycle and their presence may be confirmed by detecting eggs in stool.

The filarial are among the most important of blood and tissue nematodes. They are long, slender, round worms that parasitize the blood, lymph, subcutaneous and connective tissue of humans. They are transmitted by insect vectors and most produce larvae called microfilariae.

4.12.3. General characteristics of nematodes

- Non segmented cylindrical or round worms
- Possess a shiny cuticle which may be smooth, spine or ridged.
- Mouth is surrounded by lips or papillae
- Sexes are separated with the male & female

- They live in the tissues or intestinal tract
- Intestinal nematodes are feco – oral route and soil.

4.12.4. Intestinal round worms (Nematodes)

General characteristics

- Adult worms live in the intestinal tract
- Female worms are oviparous (lay eggs)
- Humans are the only or the most significant hosts.
- Most species are soil transmitted
- Before becoming adults in their human host, the larvae of A.lumbricoides, S.stercoralis, and hookworms have heart lung migration.

4.13. *Ascaris Lumbricoides* (round worm)

A. lumbricoides has a worldwide distribution. It is particularly common in the tropics and subtropics in places where environmental sanitation is inadequate and untreated human faeces are used as fertilizer (night-soil). Habitat: Adult in the small intestine. Egg in the faeces.

- Morphology: Adult pinkish color
- Male about 15cm in size curved tail and two copulatory spi cules of equal size.
- Female – 2 – 25cm long with straight tail eggs.

Fig. 1.5. Male and female worms

There are five types of Ascaris eggs.

a. Fertilized egg with double shell

- Size – 70µm, shape oval or sometimes round.
- Shell – the two layers are distnict, rough, brown color, covered with little lumps external shell.

- Color brown external shell, and the contents are colorless or pale yellow.
- Content: a single rounded granular central mass.

b. Unfertilized egg with double shell.

- Size: 80 - 90µm
- Shape: more elongated (elliptical)
- Shell: brown, puffy external & thin internal shell.
- Content: full to large round very refractile granules.

c. Semi – decorticated fertilized egg

- Similar to **type (a) above** , but without the external shell
- Content: a single rounded colourless granular central mass
- Color: colorless or very pale yellow

d. Semi – Decorticated unfertilized egg

- Shell: a single smooth thin colorless (double line).
- Content: large rounded colorless refractile granules.

e. Embryonated egg.

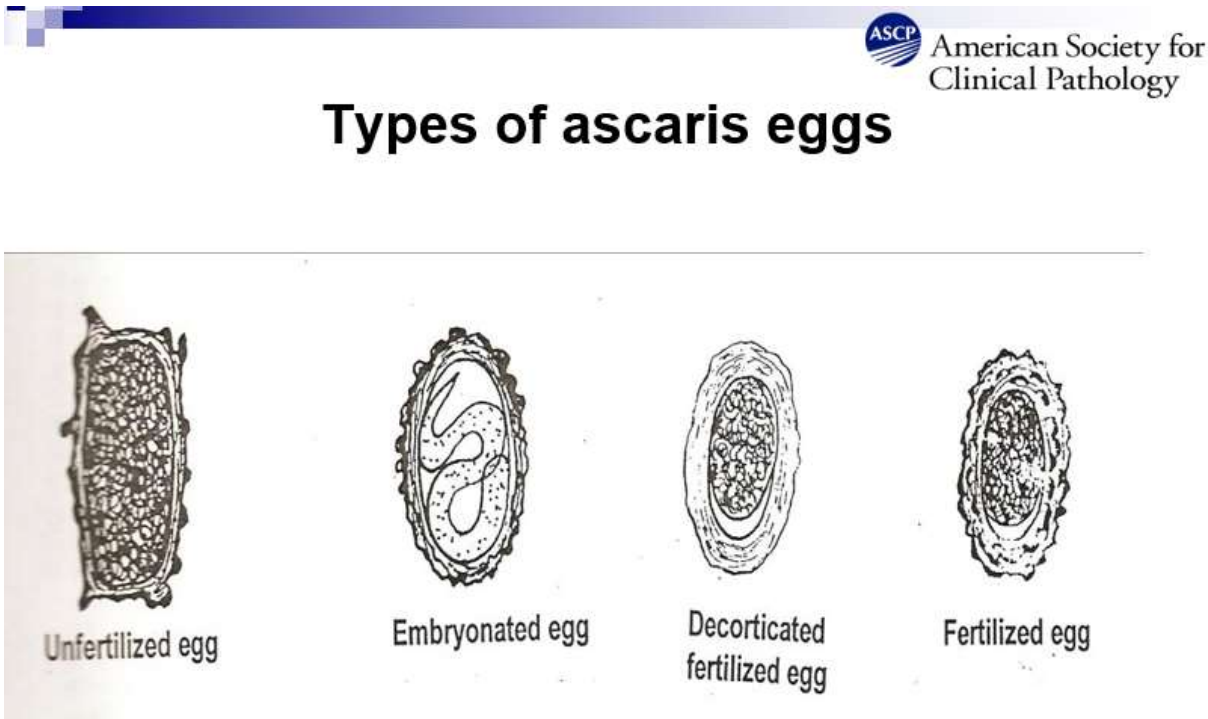


Fig. 1.6. types of eggs of A. lumbricoides

4.13.1. Mode of transmission

The infective stages is the egg containing second stage, rhabditiform larvae by ingestion in contaminated food or drink, from contaminated hand.

Life cycle: *Ascaris lumbricoides* is spread by faecal pollution of soil.

Embryonated egg: is an infective stage(egg containing 2nd stage larva) Infection may occur through: Ingestion of food or water contaminated with embryonated eggs, eating soil (geophage) frequently seen in children, putting contaminated finger or toys with infective egg in to mouth, rarely by inhalation of eggs carried in air.

Larva: After human ingests an embryonated egg, it would hatch larvae. The larvae invade the mucosa of the intestine and are then carried through lymphatic to the portal vein and finally to the lungs. The larvae mature in the lungs, penetrate the alveolar walls, and move up the bronchial tree to the throat, where they are swallowed.

Adult worms: the larva matured to Adult in the small intestine, where the female reportedly can lay up to 200,000 eggs per day after mating or without mating. The eggs are passed from the host with the feces. After eggs are passed with the feces, they require a period of 2 weeks' incubation in the soil before they are viable and are capable of causing an infection. After eating the eggs that have contaminated vegetables harvested from the soil, the eggs hatch in the intestine and then enter the circulatory system by means of the hepatic portal circulatory system. This circulatory route takes the blood containing the newly hatched larvae directly through the heart and into the lungs (Figure below).

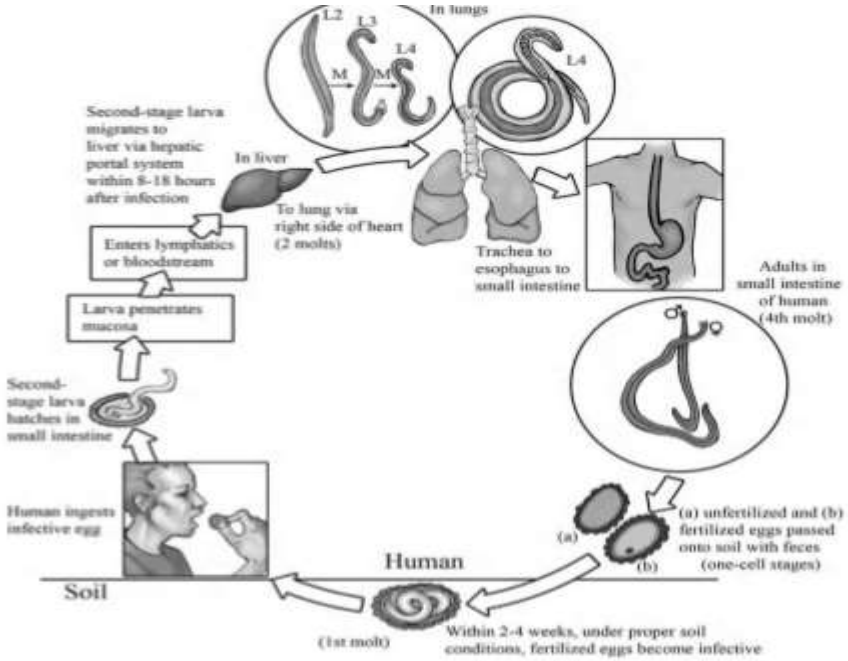


Fig.1.7. Life cycle of Ascaris lumbricodes

4.13.2. Pathogenesis:

1. by larval stage: offensive pneumonia results from lung tissue damage happens with migratory larvae during heart-lung migration
2. Pathogenesis adult worm stage: Bowel obstruction (intestinal obstruction) due to wandering many adult worms.
3. During feeding by adult worms: Parasite secretes trypsin inhibitor, prevents host from digesting proteins.
4. Migration of adult worms in the human body: Aberrant migration of “irritated” adult worms to; common duct, liver, Pharynx, peritoneum

4.13.3. Clinical Features: disease caused by A. lumbricoides is called as Ascariasis. Ascaris often causes mild or asymptomatic illness. Clinical manifestation is causes by both the migrating larvae and the adult worms.

The clinical manifestation is divided in to **three phases:**

The lung phase: occurs 5-6 days after infection

Pneumonitis (Loffler's pneumonia): due to penetration of lung capillaries by larval stage causing hemorrhage.

Intestinal phase: occurs 2-3 months after infection.

The most important clinical findings are:

- Abdominal pain due to migratory worms
- Diarrheal diseases
- Mal absorption of nutrients by adult worms
- stunted growth of children due to depletion of essential elements
- Cognitive impairment
- Intestinal obstruction by large number of adult worms. Wandering worms causes serious diseases such as, Intestinal perforation (see fig 7) by migratory worms and blacking bile ducts by adult worms.
- Liver abscesses due worms in the liver.

Fig. 1.8*. Intestinal obstruction and perforation by adult worms of A. lumbricoides*

4.13.4. Laboratory Diagnosis: Most often is made by the identification of either or both fertilized and unfertilized eggs of *A. lumbricoides*. Adult worms may also be identified, as they may pass from anybody opening (orifices) including the anus, nose, or mouth. Larvae may also be found in sputum or gastric washings from infected individuals.

Thus, laboratory finding is often finding and identification of eggs in the stool through:

- **Direct wet mount**: adequate for detecting moderate to heavy infections
- **Concentration technique:** may be used in light infection, Sodium chloride floatation technique & Formol-ether concentration techniques are used.
- Larvae can be identified in sputum or gastric aspirate during the pulmonary migration phase.
- Finding adult worms macroscopically passed in stool, or through mouth, nose or anus.

4.13.5. Treatment: Mebendazole, 200 mg, for adults and 100 mg for children, for 3 days is effective.

4.13.6. Prevention and control: prevention of infection by washing hands before eating, trimming finger nails and avoiding eating uncooked foods such as vegetables. In addition, preventing soil become faecally polluted and avoiding the use of night soil as a fertilizer. Treatment of infected individuals and health education and mass de-worming programmes for elementary school children, repeated at 3-6 month intervals, have been advocated in areas of high prevalence.

4.14. Trichuris trichuria

Trichuris trichiura is an intestinal nematode affecting an estimated 795 million persons worldwide. Also known as whipworm due to its characteristic shape, Trichuris can be classified as soil-transmitted helminths because its life cycle mandates embryonic development of its eggs or larvae in the soil. It is the second most common nematode found in humans, behind Ascaris. Morphology of *T. trichuria*: Adults: whip-like shape, anterior 3/5th of the worm resembles a whip & the posterior 2/5th is thick. Males are 30-45 mm in size and coiled tail. Females are 35 - 50mm with straight thick tail. Eggs 50-54 µm (micrometer) in Size. The shape resembles a "tea tray" or barrel- shaped with a colorless protruding mucoid plug at each end (look arrow) with eggs Shell

thick and smooth having two layers & bile stained. It is yellow brown and the content is a central granular mass which is unsegmented ovum inside

Fig. 1.9. Egg of *T. trichuria*

Life cycle:

Following ingestion of infective eggs the larvae in the small intestine and penetrates villi. After about a week, the larvae leave the small intestine and migrate to large intestine (caecum) where they develop in to mature worms.

- After mating, the female worms lay eggs which are passed in the faeces.
- In damp warm soil, the larvae develop and after 2-3 weeks each egg contains an infective larva.
- The eggs can remain infective for several months in moist warm soil but they are unable to withstand desiccation. (see fig. 1.10 below)

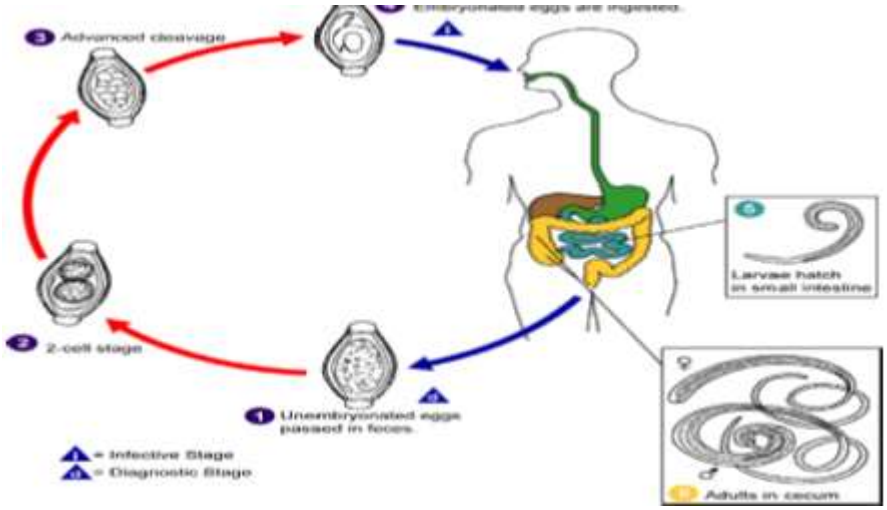


Fig. 1.10 lifecycle of T. trichuria

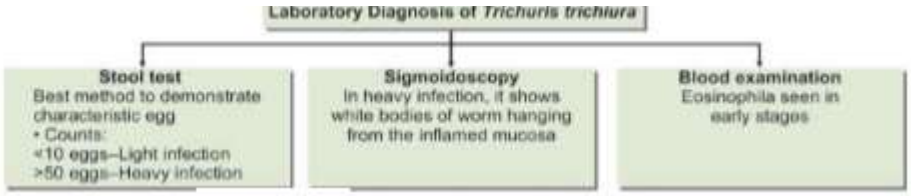
Clinical features: Clinical features are largely determined by dose of infection and worm burden. Often, less than 10 worms are asymptomatic (99% asymptomatic) while heavy worm burden results in mechanical damage to the intestinal mucosa. Infection with adult worms results in chronic profuse mucoid and bloody diarrhea with abdominal pain and edematous prolapsed rectum. Anemia from blood loss and iron

deficiency, malnutrition, weight loss, and sometimes death. Each adult worm sucks about 0.005 ml per day.

Fig. 1.11. Prolapsed rectum in trichuriasis.

Laboratory diagnosis

Both infective stages: embryonated eggs while the diagnostic stage is unembryonated egg. Stool Examination: the characteristic barrel-shaped eggs are found in stools. The degree of infection can be assessed by egg counts. Less than 10 eggs per smear in direct stool preparation is considered light infection and More than 50 per smear as heavy infection (Flowchart below).



Flow chart 1.2: Laboratory diagnosis for T. trichuria

Treatment: Mebendazole (100 mg 12 hourly for 3–5 days) Albendalzole (single dose of 400 mg) are effective with cure rates of 70–90%.

Prevention and control:

- Hand wash before and after toilet.
- Sanitary disposal of faeces in latrine.
- Treatment of infected individuals

4.15. *Entrobius vermicularis*

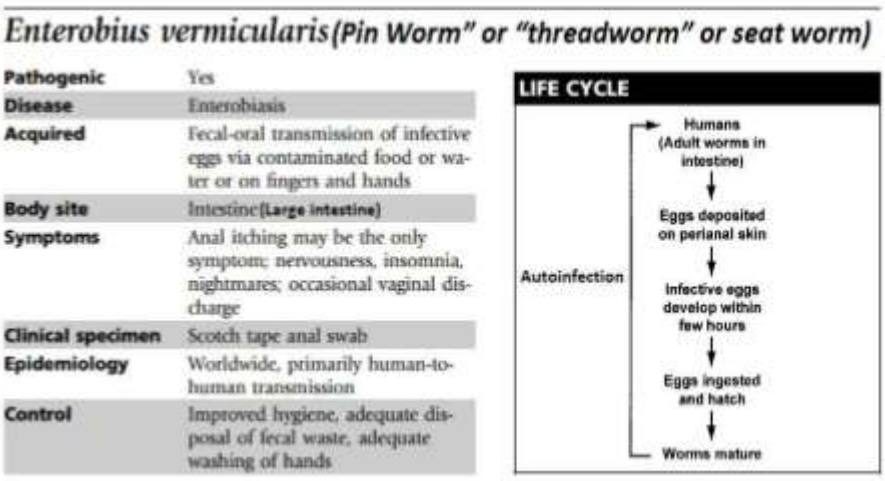
Entrobius vermicularis is a tiny worm living in the intestine. The common name is Pinworm, Seat worm, thread worm. E. vermicularis is considered to be world's most common parasite, which specially affects the children.

Habitat: Adult: in caecum, appendix and adjacent portions of the ascending colon while gravid female will be found around rectum (anus) to lay eggs.

Transmission: person to person through handling & sharing of contaminated clothes or bed linens and through surfaces in the environment that are contaminated with pinworm eggs.

- Self (autoinfection): Ingestion of eggs due to scratching of perianal area with fingers leading to deposition of eggs under the nails. This type of infection is mostly common in children. This mode of infection occurs from anus to mouth. Children who suck their fingers are more likely to be infected. Eggs are infectious upon leaving the host.

Life cycle: see below



Enterobius vermicularis (Pin Worm" or "threadworm" or seat worm)

Pathogenic	Yes
Disease	Enterobiasis
Acquired	Fecal-oral transmission of infective eggs via contaminated food or water or on fingers and hands
Body site	Intestine (Large intestine)
Symptoms	Anal itching may be the only symptom; nervousness, insomnia, nightmares; occasional vaginal discharge
Clinical specimen	Scotch tape anal swab
Epidemiology	Worldwide, primarily human-to-human transmission
Control	Improved hygiene, adequate disposal of fecal waste, adequate washing of hands

Fig 1.12. Life cycle of Entrobius vermicularis

Morphology:

Adults: are short, white, tapering at each end worms with pointed ends, looking like bits of white thread. Adults are yellow white in color. Males are 25 mm in length &Coiled tailed while Females are 8-13mm with thin pointed tails.

Fig. 1.13. Adult Entrobius vermicularis

Eggs: are deposited on perianal skin & occasionally in faeces. They are 50-60µm in size and oval with flattened in one side.

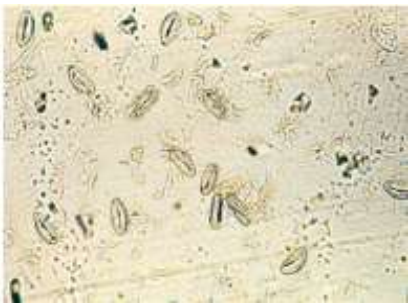
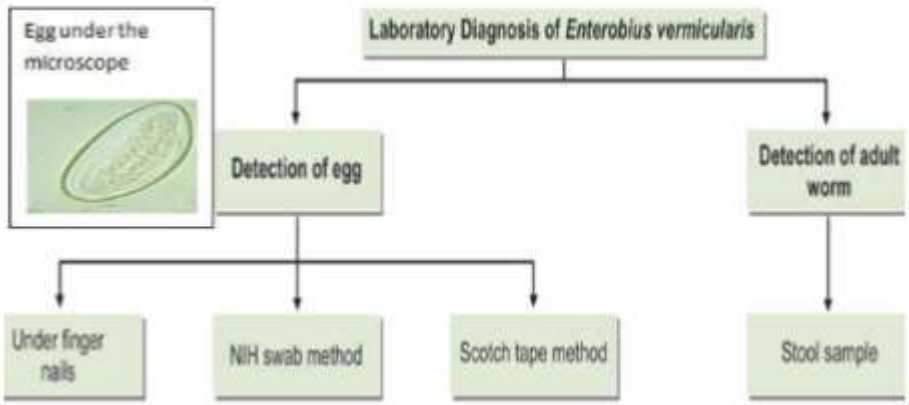


Fig. 1.14. Eggs of Entrobius vermicularis

Laboratory Diagnosis: Diagnosis depends on finding eggs or adult worms. This is normally done by sampling the perianal and perianal skin with cellulose tape (Scotch tape), which is applied sticky side down to the skin. The tape is transferred to a glass slide and examined under the microscope for eggs or adult worm.



Flow chart 1. 3. Laboratory diagnosis of E. vermucularis

Treatment: Pyrantel pamoate (11 mg/kg once, maximum 1 g), Albendalzole (400 mg once) or Mebendazole (100 mg once) can be used for single dose therapy, while piperazine has to be given daily for one week.

Prevention and control: Keeping personal and family hygiene. Frequent hand washing. Finger nail trimming and regular bathing. And frequent washing of night clothes and bed linen.

4.16. *Strongyloides stercoralis*:

Strongyloides stercoralis is the smallest nematode known to cause human infection and it is called dwarf thread worm.

Transmission:

- The main modes of transmission are through penetration of skin by filariform larva.
- Ingestion of food or water contaminated with filariform larva(oral route- causes Wakana syndrome) and autoinfection with rhabiditform larva.

Unique Characteristics of Strongyloides stercoralis:

- Parasitic males are absent
- Parasitic females are present in the sub-mucosa of small intestine which produces eggs parthenogenically (laying eggs without mating with male worm).
- Can develop in to free living generation in the soil outside the human host
- Has internal autoinfection (re-infection before larvae exit from the host)

Habitat:

The adult worm is found in the small intestine (duodenum and jejunum) of human.This parasite is a facultative parasite and has both free living and parasitic generations are present. Infective stage is Filariform larvae and diagnostic stage is Rhabditiform larvae. **Life cycle:** Adult: only females are parasite to human and they buried in the mucosal epithelium of the small intestine. Free living male and female worms mate on external environment.

- Eggs: are laid in the sub mucosa of small intestine. They are not found in feces/stool.

- Larvae: Rhabditiform larvae are passed in the faeces to the external environments and these motile larvae will be observed in stool. Filariform larvae are infective stages which are found in soil & water.

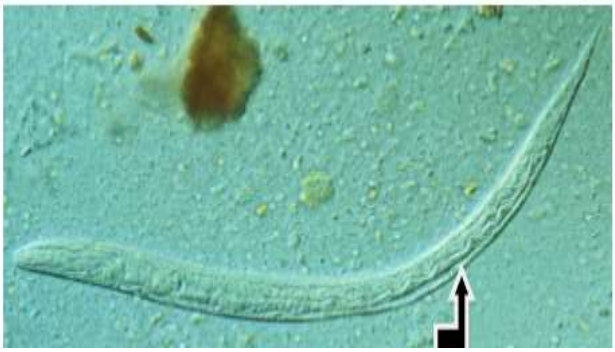


Fig.1.15. Rhabditiform larvae of Strongyloides stercoralis in stool

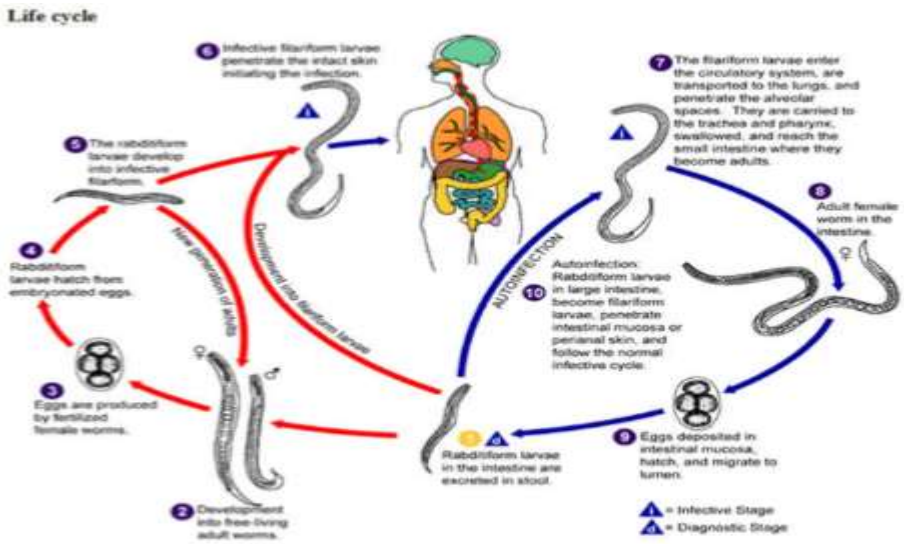


Fig. 1. 16. Life cycle of S. stercolaries

Clinical feature: It is usually asymptomatic. People with weaker immune systems such as elderly people & children are more susceptible.

There are three clinical phases:

- Cutaneous Manifestations: Large numbers of larva produce itching erythematic &allergic response at the site of infection within 24 hours of invasion by filariform larvae.

- Pulmonary Manifestations: when the larva escape from the pulmonary capillaries into the alveoli, hemorrhages in the alveoli and bronchioles may occur. Bronchopneumonia may be present, which may progress to chronic bronchitis and asthmatic symptoms in some patients. Larva of Strongyloides may be found in the sputum of these patients.
- Intestinal Manifestations: The symptoms may resemble those of peptic ulcer or mal-absorption syndrome. Mucus diarrhea is often present. In heavy infection, the mucosa may be honeycombed with the worm and there may be extensive sloughing and causing dysenteric stools.
- Other manifestations are protein losing enteropathy and paralytic illus.

Laboratory Diagnosis:

- Microscopy Direct wet mount of stool: Demonstration of the rhabditiform larvae in freshly passed stools is the most important method of specific diagnosis.
- Concentration methods of stool examination: Stool may be concentrated by Formol ether concentration examined for larvae more efficiently. Larvae may sometimes be present in sputum or duodenal aspirates and jejunal biopsies. Peripheral eosinophilia (>500/µmL of blood) is a constant finding. However, in severe hyper infection, eosinophilia may sometimes be absent.

Treatment: For all cases either symptomatic or not, should be treated to prevent severe invasive disease. Ivermectin (200 mg/kg/2 days) is more effective than albendazole (400 mg daily/3 days). For disseminated strongyloidosis, treatment with ivermectin should be extended for at least 57 days.

Prevention and control: Wearing protective foot wear and contact with infective soil and contaminated surface waters and treatment of infected individuals.

4.17. **Hook worms**:

Hook worms are hematophagous (blood feeders) nematodes. Common name: hookworms. Scientific name: Two species. Ancylomstoma duodelnale and Necator americanus.

Infective form: Filariform larva.

Habitat: - Adults are found in Jejunum and less often in the duodenum of man. - Eggs are found in the faeces; not infective to human (no infective stage).

- Rhabditiform & filariform larvae: free in soil and water.

- Morphology: Eggs: shape is oval. The egg shell is very thin & appears as black line. Eggs contains an ovum which appears segmented usually 4-8 blastomeres (undifferentiated immature cells witch finally develop to larvae stage in the eggs)

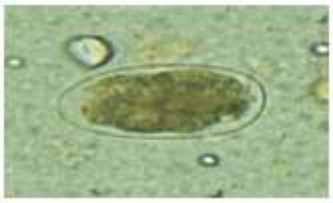


Fig. 1.17. Ova of Hook worm

Life cycle of hookworms:

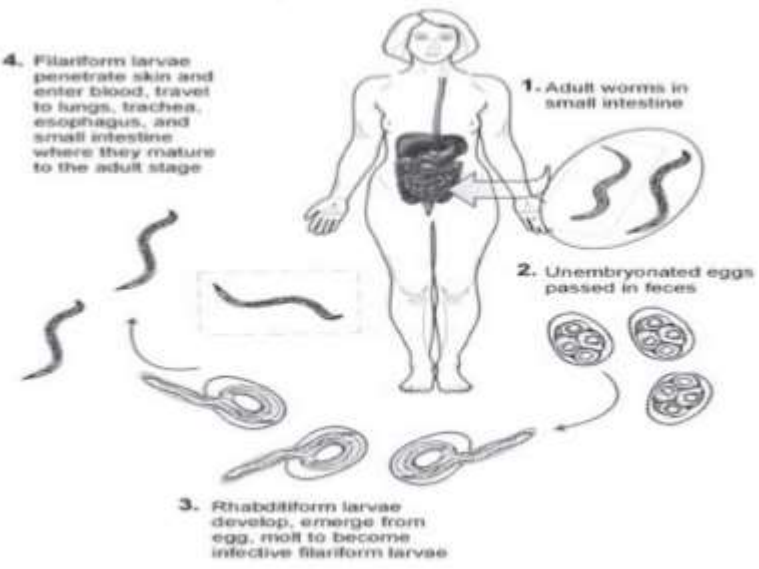


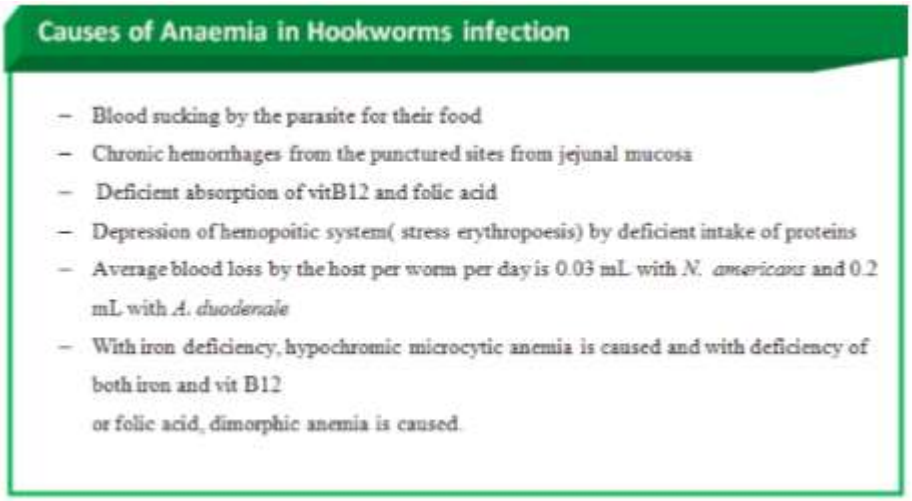
Fig. 1.18. Life cycle of Hook worm

An infective stage is filariform larva and diagnostic is egg. This is different from S.stercoralis. Transmission is same as that of S. stercoralis.

Clinical features: The name of disease is called hookworm infection. Intestinal inflammation & progressive iron/protein-deficiency anemia are the typical disease. About 90% of individuals with hookworm infection are asymptomatic. High loads of the parasite(20 – 100 worms) coupled with poor nutrition (inadequate intake of protein & iron) eventually lead to anemia which is a major clinical outcome of infection.

In general, hook worm causes:

- malnutrition, stunt growth & poor mental development in children
- Anemia leads to weakness & fatigue in adults.
- Other clinical features are same as S. stercoralis.



Laboratory diagnosis: Finding eggs in faeces by microscopic identification of eggs in the stool is the most common method. A.duodenale & N.americanus eggs morphologically indistinguishable by microscope.

- Freshly passed faeces should be examined.
- If the stool is more than 12 hours old, larva maybe seen inside the egg. –
- If the stool is more than 24 hours old, the larva will hatched and mislead with Strongyloides larva.
- The indirect method is blood examination reveals microcytic, hypochromic anemia and eosinophilia.

Treatment: A single 500 mg dose of Mebendazole often achieves a low cure rate, with a higher efficacy with a single, 400 mg dose of Albendalzole. Cutaneous larva migrants should be treated empirically with either albendazole 400 mg daily for three days, or ivermectin 200 mcg/kg/d for 1-2 days.

4.18. Blood and Tissue Nematodes (Filarial worms)

- General features: Filarial worms are threadlike (filum: thread) nematodes. The nematode genera of the super family filarioidea (order Spirurida) are included under the collective term filariae, and the diseases they cause are designated as flariasis. In the life cycle of filariae infecting humans, insects (mosquitoes, black flies, horse flies etc.) function as intermediate hosts and vectors, whereas human are definitive hosts. Hence, these parasites show complex life cycles involving more than one host. This indicates that it is difficult to control parasites involving more than one host. Generally, it is easy to control parasites which have direct life cycles than those having complex life cycles. The length of the adult stages of the species varies between 2–50 cm and the females are larger than the males. The females give birth to larvae stage called microfilariae. These are about 0.2 – 0.3 mm long and can be sheathed microfilariae (covered by external protective cover) or unsheathed microfilariae (uncovered). They can be detected mainly in the skin or in blood. Based on the periodic appearance of microfilariae in peripheral blood, they can be periodic filarial species or non-periodic ones showing continuous presence. The periodic species produce maximum microfilaria densities either at night (nocturnal periodic) or during the day (diurnal periodic). The insect species that actively bite during the day or night function as intermediate hosts accordingly to match these changing levels of microfilaremia.

Life Cycle of Filariae

Insect: → Ingestion of microfilaria with a blood meal → development in thoracic musculature with two moltings to become infective larva → migration to mouth parts and tranmission into skin of a new host through puncture wound during the next blood meal.

Human: → Migration to definitive localizations and further development with two more moltings to reach sexual maturity.

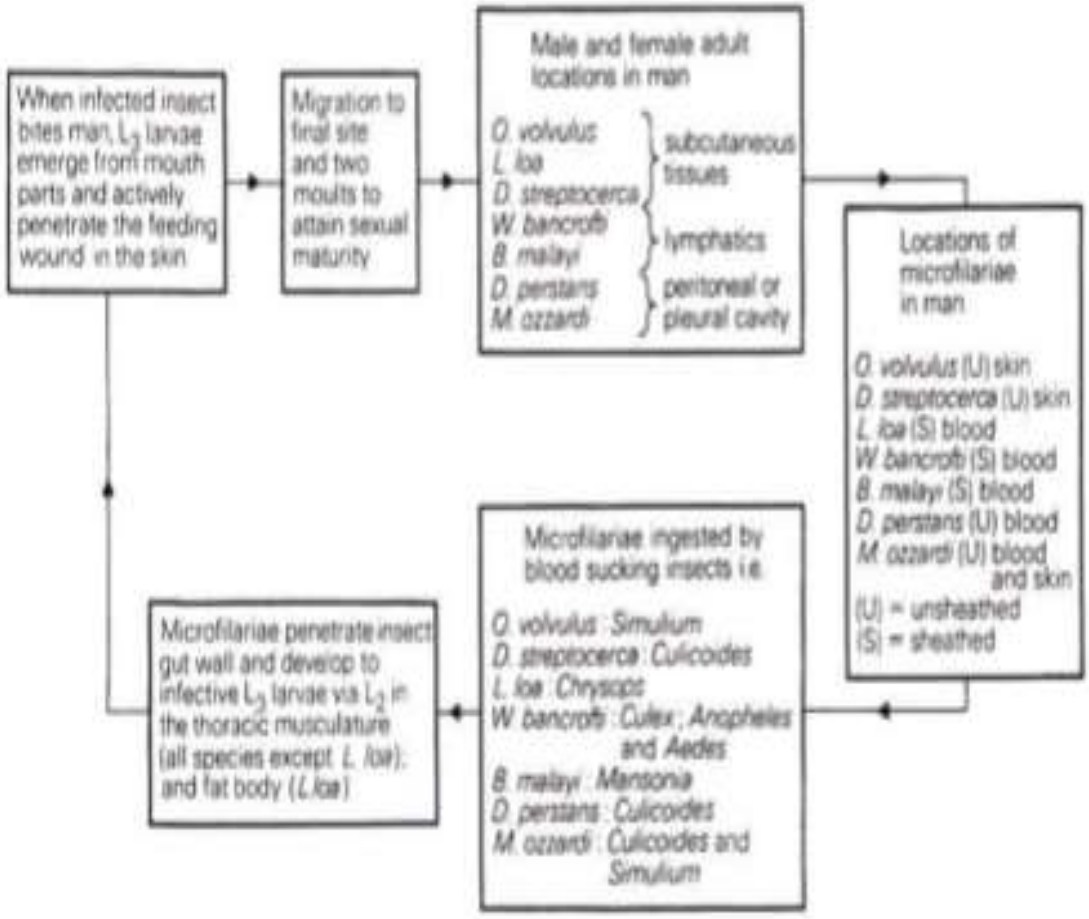


Fig. 1.19. Life cycles of all filarial worms

4.18.1. Wuchireria banchrofti and Brugia species: They are causative agents of lymphatic flariasis or elephantiasis. The intermediate hosts of W. banchrofti and B.malayi are various diurnal or nocturnal mosquito genera. The development of infective larvae in the insects is only possible at high environmental temperatures and humidity levels; in W. banchrofti the process takes about 12 days at 280C. After penetration to human from the mosquito, the filariae migrate into lymphatic vessels where they develop to sexually mature. Microfilariae (Mf) will appear in the blood after three months at the earliest (B. malayi, B. timori) or after seven to eight months (W. banchrofti) of infection. The adult parasites survive for several years in the infected individuals.

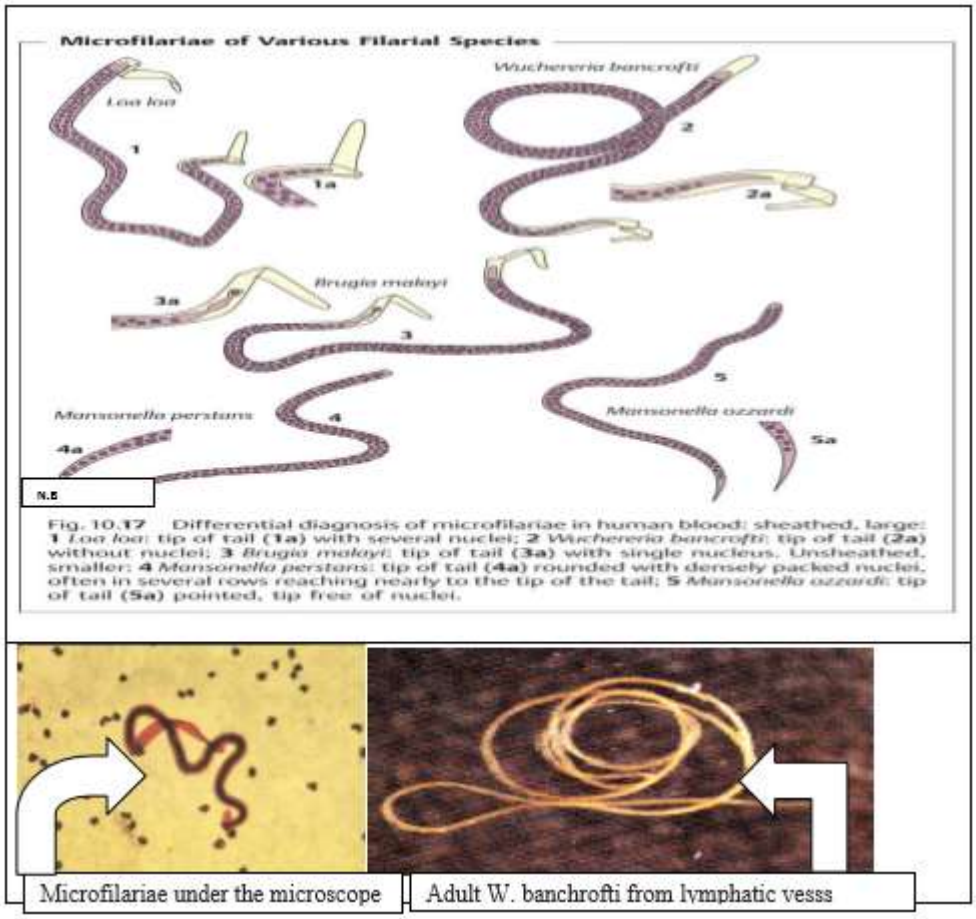


Fig 1.20. Morphology of filarials

Life cycle: Generally, the life cycle of W. banchrofti filarial worms are shown below. The infective stages are L1 larvae while the diagnostic stages are L3 larvae.

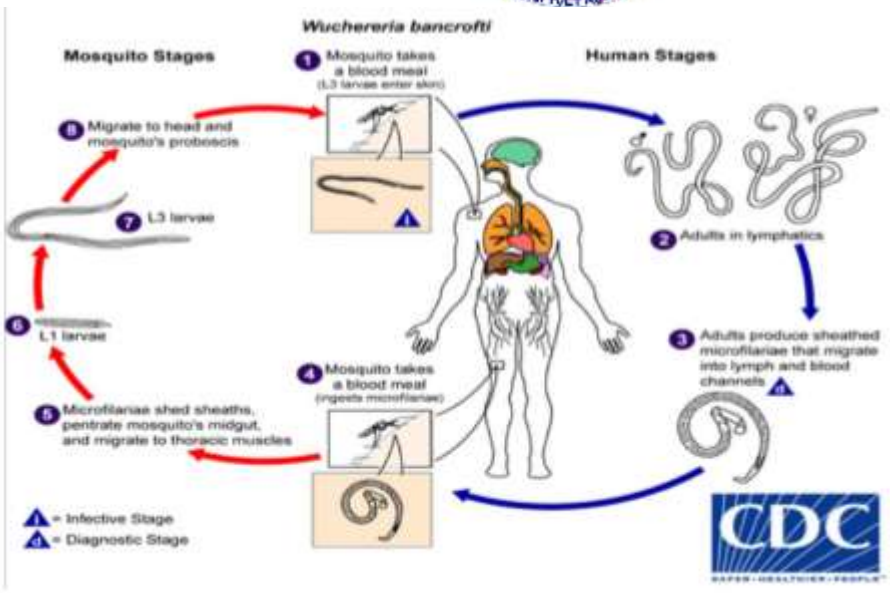


Fig 1.21. Life cycle of W. bancrofti

Pathogenesis and clinical manifestations: The pathologies caused by W. banchrofti and Brugia species are very similar. The initial symptoms can appear as early as incubation period although in most cases the incubation period is five to 12 months or much longer. The different courses taken by such infections can be showed as follows. Lymphatic flariasis most often consists of asymptomatic microfilaremia. Some patients develop lymphatic dysfunction causing lymph edema and elephantiasis (frequently in the lower extremities) and additionally with W. banchrofti, hydrocele and scrotal elephantiasis. Episodes of febrile lymphangitis and lymphadenitis may occur. Persons who have newly arrived in disease-endemic areas can develop afebrile (having no fever) episodes of lymphangitis and lymphadenitis. An additional manifestation of filarial infection is pulmonary tropical eosinophilia syndrome, with nocturnal cough and wheezing, fever, and eosinophilia. Laboratory Diagnosis: The standard method for diagnosing active infection is the identification of microfilariae in a blood smear by microscopic examination. The microfilariae that cause lymphatic filariasis circulate in the blood at night (called nocturnal periodicity). Blood collection should be done at night to coincide with the appearance of the microfilariae, and a thick smear should be made and stained with Giemsa or hematoxylin and eosin. Concentration techniques can be used to increase the sensitivity of the diagnosis.

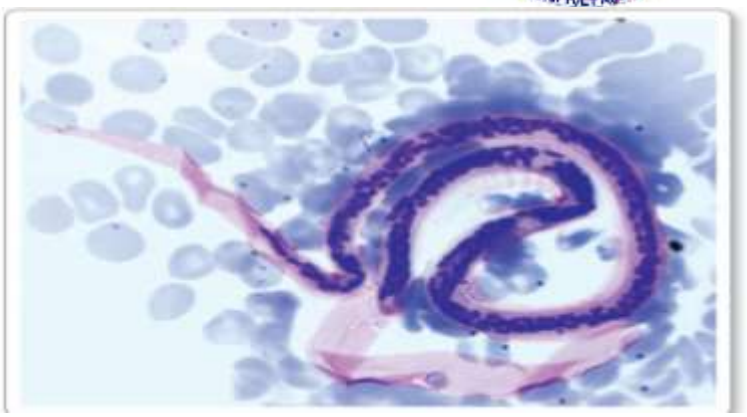


Fig. 1.22. Microfilaria in blood

Serologic diagnosis provide an alternative to microscopic detection of microfilarie for the diagnosis of lymphatic filarialsis. Patients with active filarial infection typically have elevated levels of anti filarial antibody in the blood and these can be detected by using serologic tests.

Treatment W. Banchrofti: The main aim of treatment is to kill the adult worms by diethyl carbamazine (DEC). General measures: include rest, antibiotics, antihistamines, bandaging and surgical measures for elephantiasis.

Prevention and control: Mosquitoes control, avoid mosquito bite, treating patients and health information for the community.

4.18.2. Onchoreca vovulus :

Onchoreca vovulus causes Onchocerciasis(common name: river blindness). The adult worms live (habitat) in nodules in subcutaneous connective tissue of infected persons. This filarial species causes onchocerciasis; a disease that manifests mainly in the form of skin alterations, lymphadenopathy, and eye damage, which latter is the reason for the special importance of the disease. Life cycle Humans are the only definitive host whereas day-biting female black flies of the genus Simulium(black flies) are intermediate hosts. The vector Simulium species breed in ‘fast flowing rivers; and therefore, the disease is most common along the course of rivers hence, the disease named as ‘river blindness. The female black flies are ‘pool feeders’ and suck in blood and tissue fluids after accumulated at cutting site. Microflariae from the skin and lymphatics are ingested and develop within the vector and become infective third-stage larvae, which migrate to its mouth parts. The normal

incubation period is about 6 days in vector. Infection is transmitted when an infected Simulium bites a person.

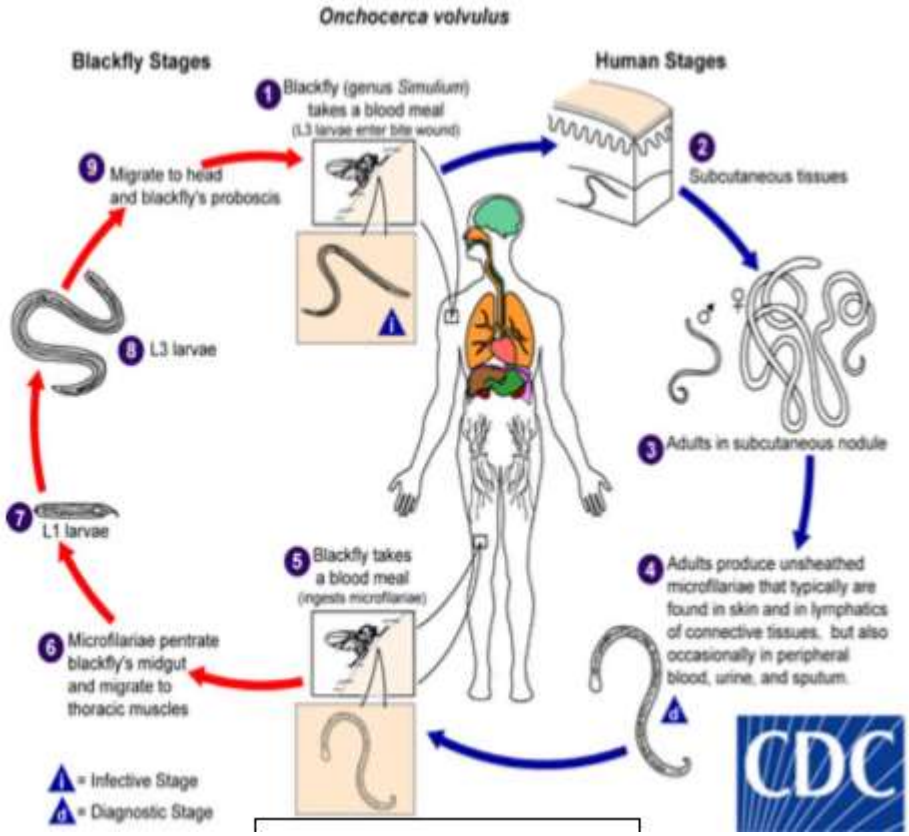


Fig. 1. 23. Life cycle of O. volvulus

Pathogenesis and clinical manifestations:

Pathogenesis depends on the host's allergic and inflammatory reactions to the adult worm and microflariae. Pathological reactions are produced by adult and microfilariae parasites. These reactions are influenced by the immune status of infected individuals. Reactions to adult parasites: enclosure of adult filariae in fibrous nodules (onchocercomas), usually 0.5–2 cm (sometimes up to 6 cm) in diameter in the subcutis along the iliac crest, ribs, scalp, etc., more rarely in deeper tissues. Nodulation occurs about one to two years after infection and is either asymptomatic or causes only mild symptoms. Reactions to microfilariae: microfilariae appear in the skin about 12–15 (seven to 24 months of incubation period). Initial symptoms occur after about 15–18 months: the clinical features are pruritus, loss of skin elasticity with drooping skin folds, papules, depigmentation, and swelling of lymph nodes; blood eosinophilia may also be present.

Eye changes: “snowflake” corneal opacities, in later stage sclerosing keratitis, the main cause of blindness, chorioretinitis (inflammation of the choroid layer behind the retina) and ocular nerve atrophy; tendency toward bilateral damage and finally results in eye blindness.

Laboratory Diagnosis: The microflariae may be demonstrated by examination of skin snip from the area of maximal microflarial density such as iliac crest or trapezius region, which is placed on a slide in water or saline. The specimen is best collected around midday. This method is specific and most accurate. Microflariae may also be shown in aspirated material from subcutaneous nodules. In patients with ocular manifestations, microflariae may be found in conjunctival biopsies. Adult worms can be detected in the biopsy material of the subcutaneous nodule.

Fig. 1.24. Skin sample

Treatment: Chemotherapy with Ivermectin is the main stay of treatment. Ivermectin is given orally in a single dose of 150µg/kg either yearly or semiannually. A 6 week course of doxycycline is microfilaria static, rendering the female worm sterile as it targets the wolbachia endosymbiont (which help the parasite for further existence) of filarial parasites. Surgical excision is recommended when nodules are located on the head due to the proximity of the worm to the eyes.

Prevention and control: Protective clothing and application of repellents tothe skin can provide some degree of protection from black fly bites. WHO involving repeated applications of insecticides to streams and rivers with the aim of selective eradication of the developmental stages of Simuliidae in western Africa have produced impressive regional results,

4.18.3. Dracunculus medinensis

Most common in areas of limited water supply where individuals acquire water by physically entering water sources. The disease is known as Dracunculus

medinensis and Common name: Medina worm or Guinea worm Medina worm is found Nile valley, India and areas where wells are used for water in supply Africa but currently it is in the verge of eradication Habitat: Adults in subcutaneous tissues of man/reservoir animals Adult: thread like, cylindrical esophagus.

- Male: About 3 cm in length posterior end coiled and 2 unequal spicules.
- Female: 30 to 100 cm in length, swollen anterior end, hooked posterior end and inconspicuous vulva near anterior

Fig.1.25. Adult stage of Dracunculus medinensis

Larva (or embryo): Rhabditiform esophagus tapering and long tail (1/3 bod

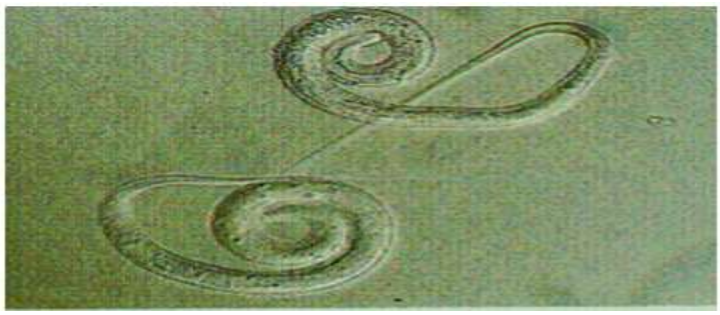


Fig. 1.26. Larvae of Dracunculus medinensis

Life cycle: A blister is formed from the female worm's production of embryos released beneath the skin, due to a burning pain that comes with this, the victims often immerse their legs in water for relief.

- With the sudden drop in temperature that follows, the blisters usually rupture, releasing the worms.
- These worms may release thousands of infective juveniles at this time, which enter the water

- Once within the copepod, the infective juvenile larvae moves into the hemocoel where they develop into 3rd stage juveniles.
- These get consumed when humans drink water with infected copepods.

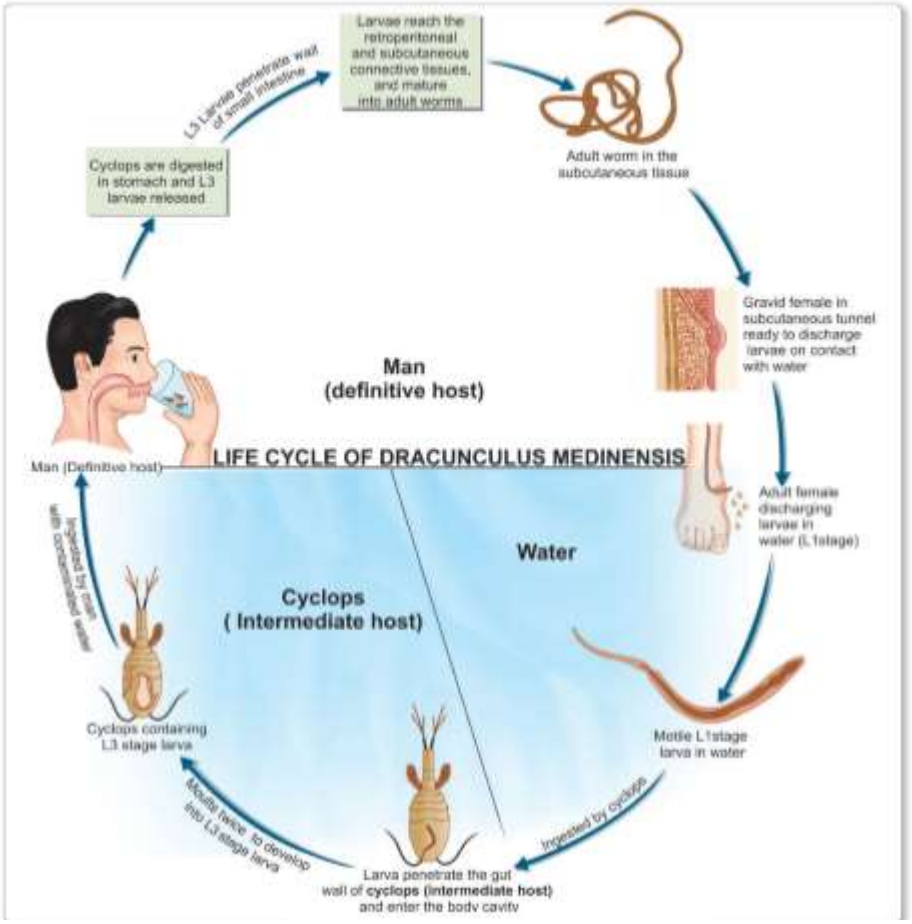
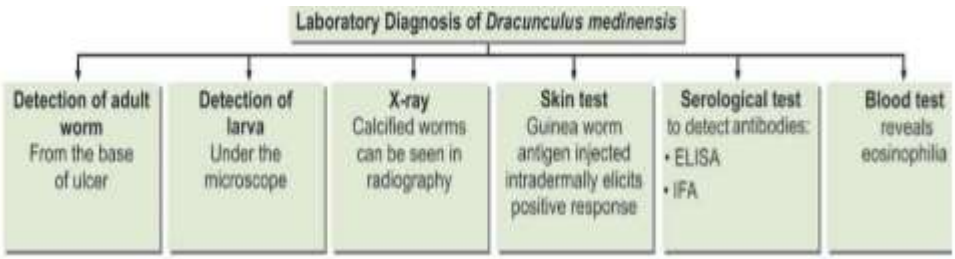


Fig. 1.27. Life cycle of Dracunculus medinensis



Flow chart: 1. 4. Laboratory diagnosis for Dracunculus medinensis

Prevention and control: preventing people with an open Guinea worm wound should not enter ponds or wells used for drinking water, boiling drinking water, filtered through tightly woven nylon cloth, or treated with a larvae-killing chemical

4.19. Phylum Plathyhelmiths (flat worms):

4.19.1. Class Cestoda (tapeworms)

General features: Various tapeworm species can parasitize in the small intestine of humans. Cestoda species are hermaphrodites and consist of the head (scolex or "holdfast"), followed by an unsegmented germinating section (neck) and a posterior chain of segments (proglottids). There are no digestive (alimentary) system, so nutrients are taken up through the absorptive integument. The life cycle of Cestoda require one or two intermediate hosts. Humans can also be infected by larval stages of various tapeworm species of cysticerci or metacestodes. These stages develop in body tissues and generally cause considerably greater pathological damage than the intestinal cestodes stages. Cestodes have tape - like, dorsoventrally flattened and segmented bodies. The scolex (head) carries suckers and some also have hooks. They are monoecious (no separate male and female organisms) and body cavity is absent. They are oviparous and reproduction via parthenogenesis (self-fertilization), thus the egg outputs rates are higher in this case than sexually reproducing parasites of separate male and female organisms.

4.19.2. Taenia species: The Taenia species cause a disease known as Taeniasis (intestinal manifestation) and cysticercosis (tissue manifestation). The two known species are *Taenia saginata* and *T. solium. T. solium* is 3–4 m long and is and smaller than *T. saginata*. The scolex of *T. solium* has a rostellum armed with two rows of hooks in addition to the four suckers which similar to T. saginata.

■ Taeniosis is a small intestine infection of humans caused by *Taenia* species. In the case of *T. saginata*, the intermediate hosts are cattle, in the musculature of which metacestodes (cysticerci) develop and can be ingested by humans who eat raw beef. The infection runs an inapparent course or is associated with mild intestinal symptoms. The metacestodes of *T. solium* develop in the musculature of pigs, or through accidental infection in humans as well (CNS, eyes, musculature, skin), causing cysticercosis. *T. saginata asiatica* is closely related to *T. saginata*, but its metacestodes parasitize mainly in the livers of pigs and ruminants. ■

Morphology: Egg: 33-40µm in size while the shape is round. It has dark yellowish-brown shell and light yellowish gray in content. The shell is thick, Smooth, radially

striated (embryophore).The content is round granular mass enclosed by a fine membrane with six hooklets (this is the diagnostic features of this parasite. Gravid proglotides: are those detached when fully develop & pass through the anus independently. It attains an opaque white color with 20mmX6m in size. Inside the gravid segments, the number of lateral uterus branches is usually 7–13 in T. solium which is less than that of T. saginata (usually >15). Pathogenesis and clinical manifestations of *T. saginata*. Taeniasis which is a morphological changes in intestine (villus deformation, enterocyte proliferation, cellular mucosal infiltration, etc.) and functional disturbances. Blood eosinophilia may occur sometimes. The infection takes an asymptomatic course in about 25% of cases. Symptoms of infection include nausea, vomiting, upper abdominal pains, diarrhea or constipation and increased or decreased appetite. Infection does not confer levels of immunity sufficient to prevent rein. Pathogenesis and clinical manifestations of *T. solium*: causetaeniasis in the intestine which has no or only mild symptoms, similar to infections with T. saginata. Cysticercosis is caused by T. solium cesticerci larvae encysted in brain or tissues of the central nervous system (neurocysticercosis) or of the eye (ocular cysticercosis) are among the more severe forms of the infection. In the CNS, the metacestodes are usually localized in the cerebrum (ventricle, sub-arachnoidal space), more rarely in the spinal cord; they can cause epilepti form convulsions, raised intracranial pressure, and other neurological symptoms. The cysticerci can also develop in subcutaneous tissues, in the heart, and in the skeletal muscle Causative agent of *T. solium* taeniasis and cysticercosis.

Life cycle: The life cycle is similar, except that T. saginata uses cattle as intermediate host and *T. solium* uses the pig as intermediate host, in which the metacestode (Cysticercus cellulosae) develops to infectivity within two to three months.

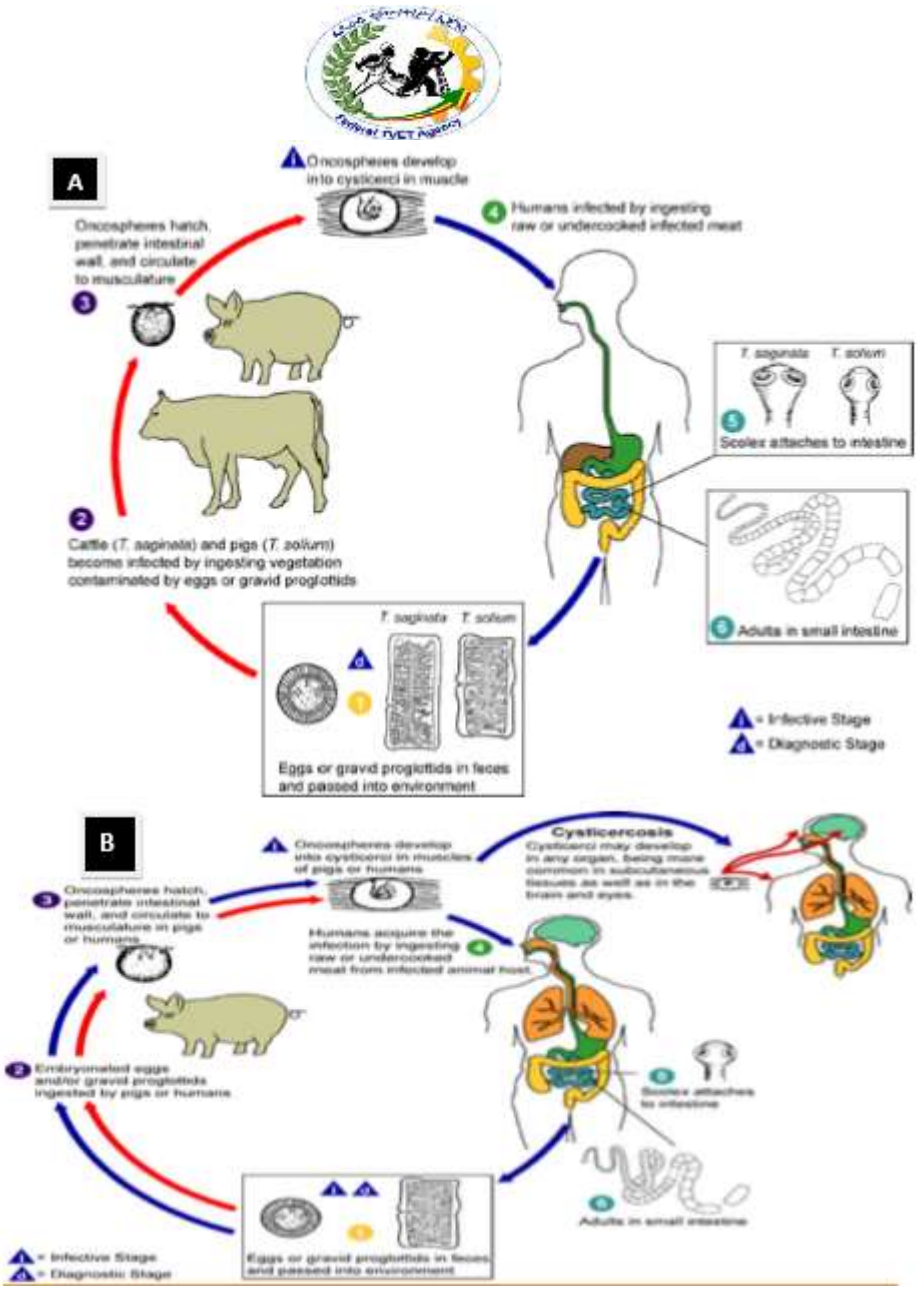


Fig. 1.27. Life cycle of intestinal Taenia species. A. intestinal case, B. cesticercosis to brain

Laboratory diagnosis: Detecting eggs in faeces is the first step. Macroscopic Identification of gravid segments/ proglotides in underclothing/ bedding/ in faeces. Scolex can be recovered from clothing or passed in faeces; T.saginata ova on perianal skin (cellotape slide is the best methode) T. solium (cysticercosis): Finding calcified larvae in histological or X-rays examination (by X-ray technicians).

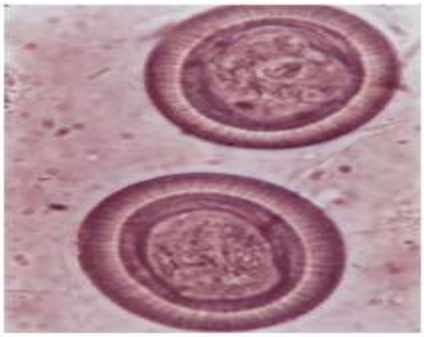


Fig. 1.28. Eggs of T.saginata and T.solium

Treatment: Praziquantel in adults 2.5-10mg/kg given in a single dose. Albendalzole for adults 6.6mg/kg or two doses each of 200mg/day on 3consecutive days (this drug should not be usedin children less than 2years old or during pregnancy.

4.19.3. Hymenolepis nana (Dwarf Tapeworm):

Hymenolepis nana (common name: dwarf tapeworm),1–4 cm long (rarely 9 cm) and 1mm wide, is a small intestinal parasite that occurs worldwide. The highest prevalence being found in warm countries and in children. The final hosts are rodents and humans. Infection results from ingestion of eggs, from which oncospheres hatch in the small intestine, penetrate into the villi, and develop into larvae (cysticercoids). The larvae then return to the intestinal lumen, where they develop into adult tapeworms within two to three weeks. Alternatively, H. nana develops in a cycle with an intermediate host (insects: fleas, grain beetles, etc.). The closely related species Hymenolepis diminuta (10–60 mm) is not as frequent in humans. The developmental cycle of this species always involves intermediate hosts (fleas, beetles, cockroaches).

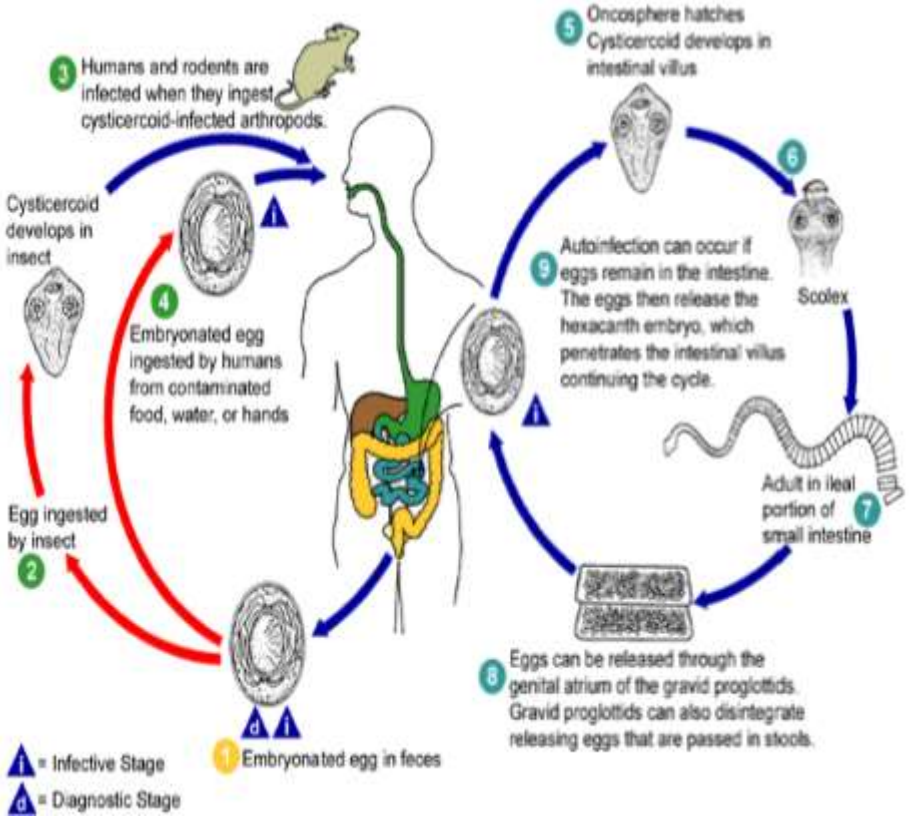


Fig. 1. 29. Life cycle of H.nana

Clinical manifestations: Infections gastrointestinal distress.

Laboratory diagnosis: Finding eggs in feces Size: 35-50µm.

- Shape: oval, almost round.
- Shell: double; thin external membrane and internal membrane often thicker at the poles.
- Thread like polar filaments coming from both poles.
- Color: color less or very pale gray
- Content: Rounded mass (embryo) with six retractile booklets arranged in fan shaped.

Fig. 1. 30. Egg of H.nana

Treatment: Praziquantel or albendazole are the drugs of choice.

Prevention and control: Preventive measures include general hygiene and treatment of infected persons void eating raw or insufficiently cooked meat. Inspecting meat for larvae and provide latrine for proper waste disposal. Protection of cattle from grazing on faeces or sewage polluted grass. Avoiding open field defecation and treating infected persons & providing health education may break the life cycle of these parasites.

4.19.4. Echinococcus

Echinococcus

Causative agent of echinococcosis

■ The most important species of the genus *Echinococcus* are *Echinococcus granulosus* (intestinal parasite of Canidae) and *E. multilocularis* (intestinal parasite of fox species, dogs, cats, and other carnivores). Both species occur in Europe. Their metacestodes can cause cystic echinococcosis (CE, hydatid disease) or alveolar echinococcosis (AE) in humans. Humans are infected by peroral ingestion of *Echinococcus* eggs, from which in CE, liquid-filled cystic metacestodes (the hydatids) develop, particularly in the liver and lungs. In AE the metacestodes primarily parasitize the liver, where the metacestodes proliferate like a tumor and form conglomerates of small cysts; secondary metastatic spread to other organs is possible. Clinical imaging and immunodiagnostic methods are used for diagnosis. Treatment involves surgery and/or chemotherapy. ■

Parasite species. *Echinococcus* species are small tapeworms that parasitize the small intestine of carnivores and produce eggs that are shed to the environment by the host. Pathogenic larval stages (metacestodes) develop following peroral ingestion of such eggs by the natural intermediate hosts (various mammalian species), as well as in humans and other accidental hosts (which do not play a role in the life cycle). Four *Echinococcus* species are currently known, all of them pathogenic for humans (*Echinococcus granulosus*, *E. multilocularis*, *E. vogeli*, and *E. oligarthrus*).

Life cycle : see below

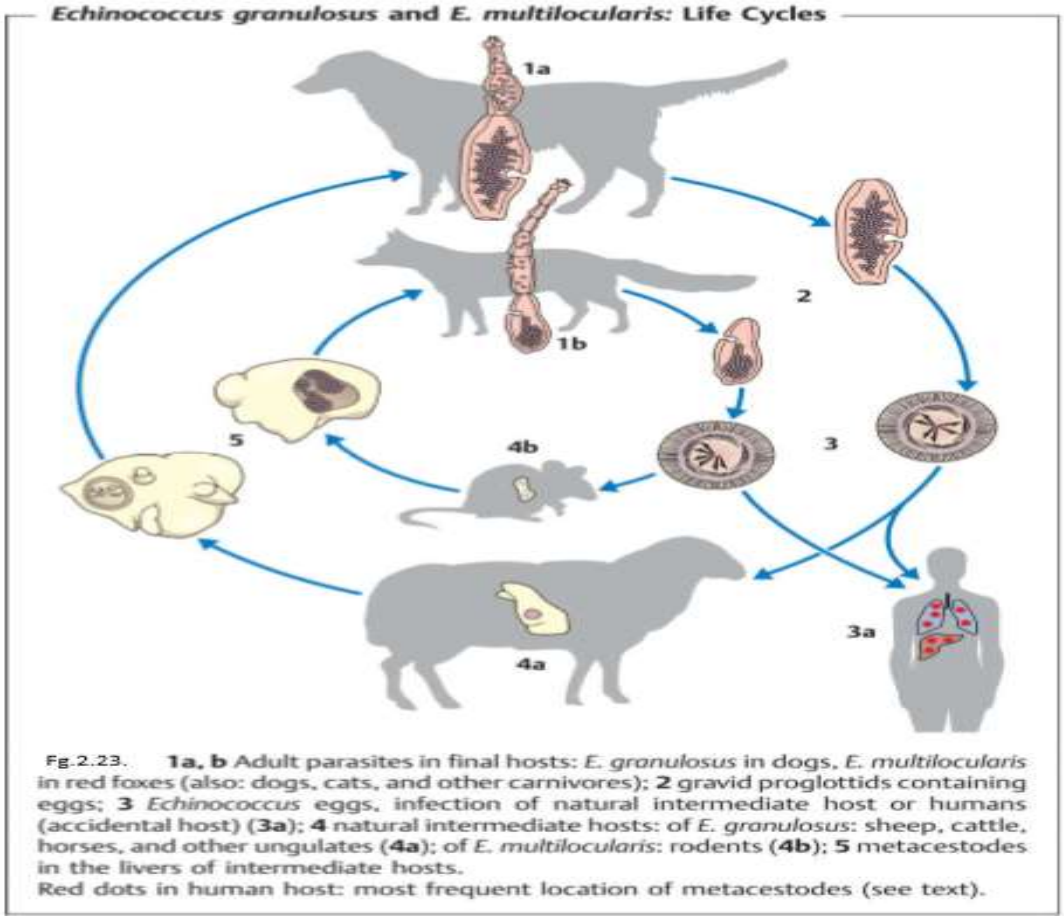


Fig 1. 31. Life cycle of E. granulosus and E. multiiocularis

Laboratory diagnosis: Microscopic diagnosis is impossible. But diagnosis is based on detection of cysts using imaging techniques (ultrasonography, computer tomography, thoracic radiography, etc.) in connection with serological antibody detection. Specific antibodies occur in about 90–100% of patients with cystic hepatic echinococcosis, but in only about 60–80% of cases with pulmonary echinococcosis. Diagnostic cyst puncture is generally not advisable due to the risks described above (secondary echinococcosis, anaphylactic reactions).

4.19.5. Trematodes (Flukes) or Platyhelminthes

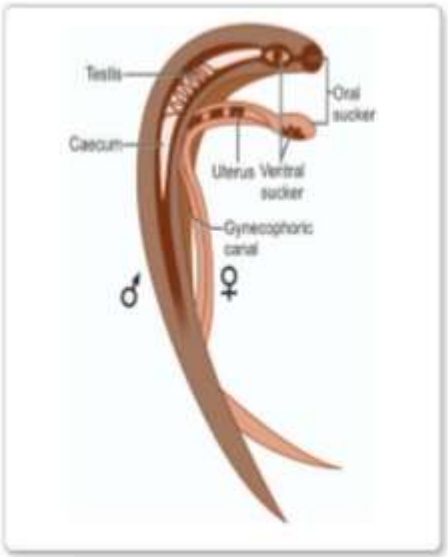
General Characteristics: They vary in size from the species just visible to the naked eye, like Heterophyes to the large fleshy flukes, like Fasciola and Fasciolopsis. Most of the trematode species that parasitize humans are dorsoventrally flattened with an oval to lancet shape, although others have different shapes such as the threadlike schisotosomes. Suckers (trema: hole,

opening) serve as attachment organs: an oral sucker around the mouth connected to the esophagus and the blind-ending intestine, and a ventral sucker. The body surface of adult trematodes is covered by a cellular tegument (composed of an outer annucleate, syncytial layer of cytoplasm connected by cytoplasmic strands to inner nucleated portions) through which substances can be absorbed from the environment. Most species are hermaphroditic, only the schisotosomes have separate sexes. Snails are the first intermediate hosts; some species require arthropods or fish as a second intermediate hosts.

Schistosoma species

Schistosomosis (bilharziosis) is one of the most frequent tropical diseases with about 200 million infected persons. The occurrence of schistosomosis depends on the presence of suitable intermediate hosts (freshwater snails). Human infections result from contact with standing or slow-moving bodies of water (freshwater) when *Schistosoma* cercariae penetrate the skin. *Schistosoma hematobium* causes urinary schistosomosis; *S. mansoni, S. japonicum, S. intercalatum,* and *S. mekongi* are the causative agents of intestinal schistosomosis and other forms of the disease. Diagnosis can be made by detection of either *Schistosoma* eggs in stool or urine or of specific antibodies in serum.

- They are unisexual (diecious)
- They lack a muscular pharynx
- Their intestinal caeca re-unite after bifurcation to form a single canal
- They produce non-operculated eggs
- They have no redia stage in larval development
- The cercariae have forked tails and infect by penetrating the unbroken skin of definitive host



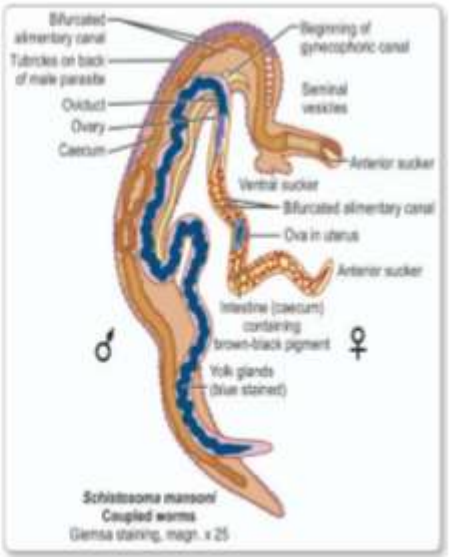


Fig. 1.32. Adult worms of schistosoma species.

History and Distribution: In 1902, Manson discovered eggs with lateral spines in the feces of a West Indian patient that led to the recognition of this second species of human schisotosomes. It was, therefore named S. mansoni. It is widely distributed in Africa including Ethiopia. **Habitat:** Adult worm lives in the inferior mesenteric vein.

Morphology: S. mansoni resembles S. haematobium in morphology and life cycle, except the adult worms are smaller and their integuments studded with prominent coarse tubercles. In the gravid female, the uterus contains very few eggs, usually 1–3 only. The prepatent period (the interval between cercarial penetration and beginning of egg laying) is 4–5 weeks. The egg has a characteristic lateral or apical spine and are non operculated with yellowish brown.

Life cycle: Definitive host**:** humans are the only natural definitive hosts.

Intermediate host: snails of the genus Biomphalaria.

Infective form: Fork-tailed Cercaria.

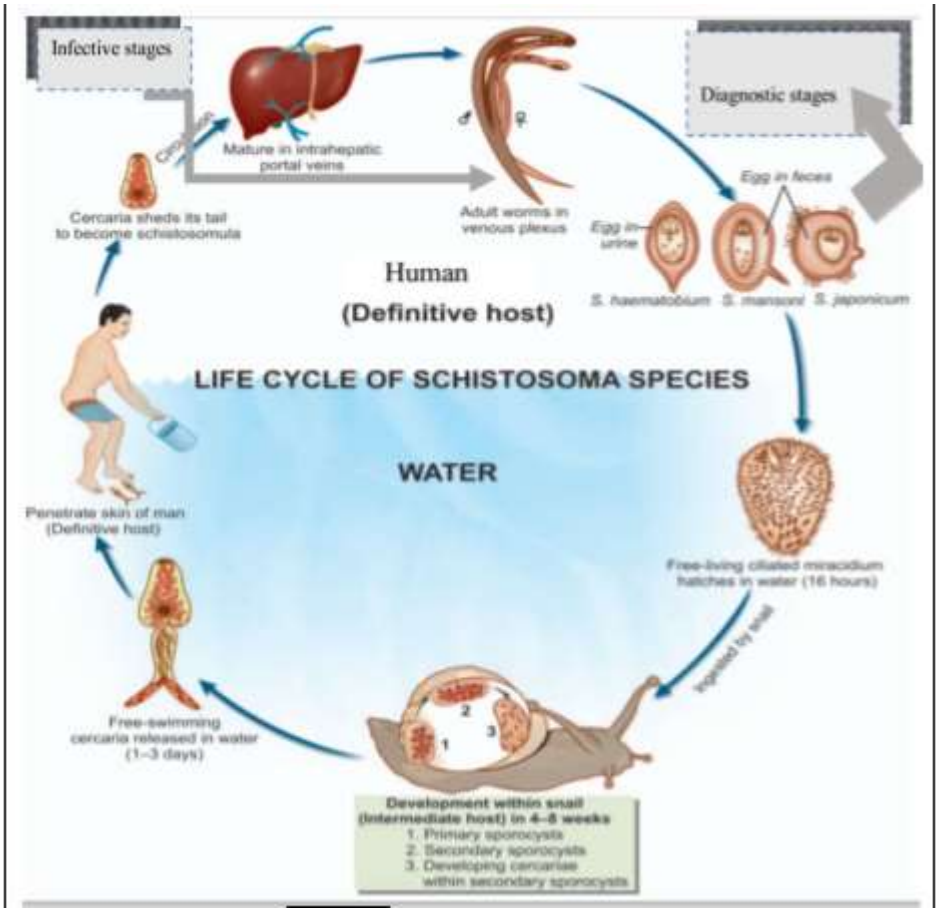


Fig 1.33. Life cycle of schistosoma spp.

Description of the life cycle in brief:

Migration of Schistosomes in the Human Body

Infection → schistosomula penetrate subcutaneous tissues → find venous capillaries or lymph vessels → migrate through the venous circulatory system into the right ventricle of the heart and the lungs → travel hematogenously into the intrahepatic portal vein branches where development into adult worms takes place as wells as male-female pairing just prior to sexual maturity → retrograde migration of pairs into mesenteric veins or to the vesical plexus.

Pathogenesis and clinical manifestations: The infection can be divided into the following phases: Cutaneous phase: penetration of cercariae into the skin, either without reaction or especially in cases of repeated exposure with itching and skin lesions (erythema, papules), which disappear within a few days. Acute phase: about two to 10 weeks after a severe initial infection, the symptoms may include fever, headache, limb pains, urticaria, bronchitis, upper abdominal pain, swelling of the liver, spleen and lymph nodes, intestinal disturbances, and eosinophilia (Katayama syndrome). Due to release of Schistosoma antigens, the serum antibody levels (IgM, IgG, IgA) rise rapidly and immune complexes are formed that can cause renal glomerulopathies. These symptoms persist for several days to several weeks. Normally, Schistosoma eggs are not yet excreted at the beginning of this phase (see prepatent periods). In low-level infections this phase is usually in apparent or subclinical. Chronic phase: the most significant phase in pathogenic terms begins after an incubation period of about two months with oviposition by the Schistosoma females. A large proportion (up to 50%) of the eggs laid remain in human body tissues, not only near the worms (urinary bladder, intestine), but also in more distant localizations due to hematogenous spreading (mainly to the liver and lungs, more rarely to the CNS, the skin, and other organs), where they lodge in small vessels.

- The main forms of schistosomiasis are differentiated according to the localization of the lesions:
  - Intestinal schistosomiasis (intestinal bilharziasis). The agents are mainly S. mansoni and S. japonicum, also S. mekongi (rare). Incubation period is from four to 13 weeks (acute phase) or months to years (chronic phase). The course of an initial infection is rarely symptomatic, in apparent and subclinical courses being the rule. Manifestations in the chronic phase are restricted almost entirely to large intestine with hyperemia, granulomatous

nodules, papillomas (bilharziomas), ulcerations, hemorrhages, and increasing fibrosis, abdominal pain and bloody diarrhea.

- Other forms: hepatosplenic form the causative agents are mainly S. japonicum, less frequently S. mansoni. The fibrotic form is caused by eggs deposited around the branches of the portal vein in the liver ("pipestem" fibrosis according to Swimmers) and results in circulatory. Anomalies, portal hypertension, splenomegaly, ascites, hemorrhages in the digestive tract, and other symptoms. Pulmonary schistosomiasis is observed mainly in severe S. mansoni infections, more rarely in infections with other species (including S. haematobium). Cerebral schistosomiasis is relatively frequent in S. japonicum infections (2–4%).

- Cercarial dermatitis. Cutaneous lesions (itching, erythema, urticaria, papules) in humans, caused by (repeated) skin penetration of cercariae parasitizing birds. The infection occurs worldwide in freshwater or brackish water and is known as "swimmer's itch." The symptoms generally abate after a few days. The cercariae of schisotosomes from humans can cause similar, although usually milder, symptoms.
- Urinary schistosomiasis (urinary bilharziosis). Causative agent: S. haematobium. Incubation 10–12 weeks or longer, morbidity rate as high as 50–70%. Hematuria (mainly in the final portion of urine), micturition (the discharge of urine) discomfort, hyperemia, increasing fibrosis, 1–2 mm nodules, necroses, ulcers and calcification of the bladder wall, pyelonephrosis and hydronephrosis, urethral strictures, lesions in the sexual organs. In some endemic areas, an increased incidence of urinary bladder cancer has been associated with the S. haematobium infection.
- Laboratory Diagnosis: Following the prepatent period, i.e., four to 10 weeks incubation period at the earliest, the eggs can be detected in stool specimens or in urine sediment. The eggs can also be found in intestinal or urinary bladder wall biopsies.
    - Immunodiagnostic methods are particularly useful for detecting infections before egg excretion begins (important for travelers returning from tropical

regions!). Detection of microhematuria with test strips is an important diagnostic tool in bladder schistosomiasis.

- Clinical examination with portable ultrasonic imaging equipment has proved to be a highly sensitive method of detecting lesions in the liver and urogenital tract in epidemiological studies.

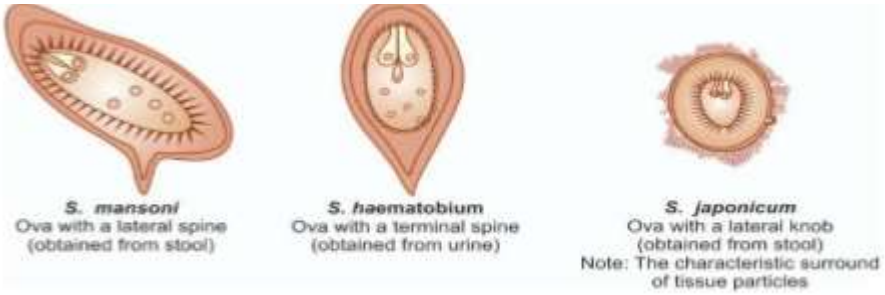


Fig. 1.34. Eggs of schistosoma spp.

- **Treatment:** The drug of choice for treatment of schistosomiasis is praziquantel, which is highly effective against all Schistosoma species and is well tolerated.
- **Prevention and Control:** Current schistosomiasis control strategies are based mainly on regular drug therapy of specific population groups. Morbidity, mortality, and egg excretion rates are clearly reduced by such programs. Hygienic and organizational measures (construction of latrines, improvement of water supply quality, etc.) aim to reduce Schistosoma egg dissemination and contact with contaminated bodies of water. Individual preventive measures in Schistosoma-contaminated areas include avoidance of skin contact with natural or artificial bodies of water (freshwater). Drinking water that could be contaminated with cercariae must be decontaminated before use by boiling, chlorination, or filtration.

4.20. Medical Protozoology

- **General characteristics:** Parasitic protozoa are eukaryotic, single-celled microorganisms belonging to kingdom protista. They are varied from 1–150 µm in size and enclosed by a trilaminated cell membrane. The single protozoal cell performs all functions. They possess one, rarely two nuclei (and multinuclear reproductive forms). Reproduction is asexual by binary or multiple fission of the cell, or sexual. The cellular construction of the protozoa is generally the same as in other eukaryotes but they also exhibit some special features. During the course of evolution some protozoa (Giardia, Entamoeba) have lost the mitochondria secondarily, except several genomic traits that were laterally transferred to the nuclei.

  The apicoplast, present in some species of Apicomplexa, is a residual of a former plastid typical for their ancestors. Some protozoa contain specialized organelles, such as glycosomes (exclusively in trypanosomatids), hydrogenosomes (in trichomonads).

  Most of the protozoa are completely nonpathogenic but few may cause major diseases such as malaria, leishmaniasis, and sleeping sickness. Protozoa like *Cryptosporidium parvum* and *Toxoplasma gondii* are being recognized as opportunistic pathogens in patients affected with human immunodeficiency virus (HIV) and in those undergoing immunosuppressive therapy.

  Protozoa exhibit wide range of size (1–150 µm), shape, and structure; yet all possess essential common features.The differences between protozoa and metazoa are given in table 1 below.

Table 1. Difference between protozoa and Helminths

Variable	Protozoa	Metazoa
Morphology	Unicellular;a single 'cell-like unit'	Multicellular;a number of cells, makingup a complex individual
Physiology	A single cell performsall the functions:reproduction,digestion, respiration,excretion	Each special cell performs aparticular function
Example	Amoeba	Tapeworm

- **The special and common terms in protozoology:**
  - Chromatic body: extra nuclear chromatin material (e.g., as found in Entamoeba histolytica cyst).
  - Karyosome: it is a DNA containing body, situated peripherally or centrally within the nucleus and found in intestinal amoeba, and E. coli.
  - Kinetoplast: non-nuclear DNApresent in addition to nucleus is called kinetoplast. It is seen in trypanosomes. Flagellum originates near the kinetoplast.point of origin of flagellum is called as basal body
  - Cilia: these are fine, needle-like filaments, covering the entire surface of the body and are found in ciliates, e.g. balantidium coli
  - Trophozoite: active feeding and growing stage of the protozoa is called the trophozoites. It derives nutrition from the environment by diffusion, pinocytosis and phagocytosis

The protozoan reproduction system

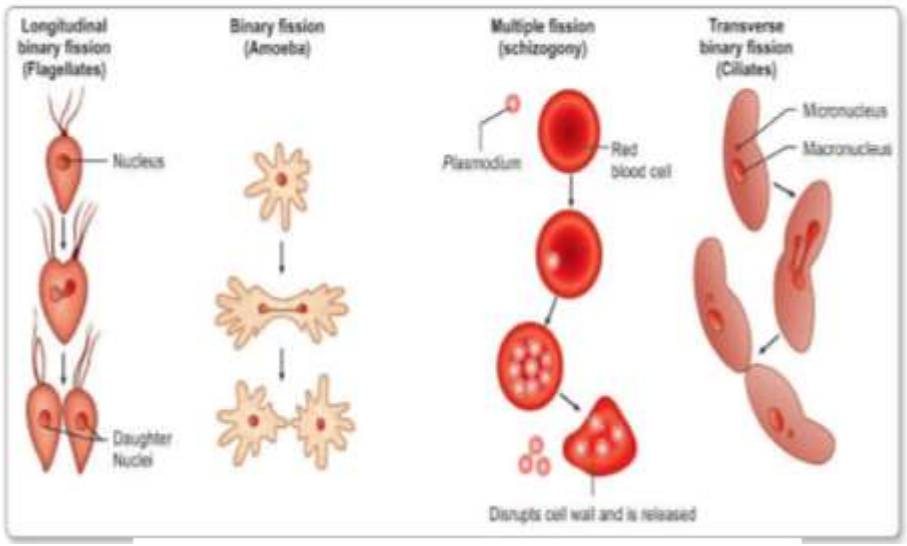


Fig. 1.34. Modes of reproduction of protozoa

Classification of protozoa

Phylum	Subphylum	Superclass	Class	Subclass	Order	Suborder	Genus
Sarcomasti-gophora	**Mastigophora (having one or more flagella)**		Zoomastigopho-rea		Kinetoplastida	Trypanosomatina	*Trypanosoma Leishmania*
					Retortamonadida		*Retortamonas Chilomastix*
					Diplomonadida	Enteromonadina	Enteromonas
						Diplomonadina	*Giardia*
					Trichomonadida		*Trichomonas Dientamoeba*
	Sarcodina (pseudopodia present)	Rhizopoda	Lobosea	Gymnamoe-bia	Amoebida	Tubulina	*Entamoeba Endolimax Iodamoeba*
						Acanthopodina	*Acanthamoeba*
					Schizopyrenida		*Naegleria*
Apicomplexa			Sporozoea	Coccidia	Eucoccidia	Eimerina	*Crypto-sporidium Isospora Sarcocystis Toxoplasma*
						Haemosporina	*Plasmodium*
				Piroplasmia	Piroplasmida		*Babesia*
Ciliophora			Kinetofragmino-phorea	Vestibuliferia	Trichostomastida	Trichostomatina	*Balantidium*
Microspora			Microsporea		Microsporida	Apansporoblas-tina	*Enterocytozoon Encephalito-zoon Microsporum*

4.20.1. Entameba histolytica

Of the various amebic species that parasitize the human intestinal tract, *Entamoeba histolytica* is significant as the causative agent of the worldwide occurring entamebosis, a disease particularly prevalent in warmer countries. The vegetative stages (trophozoites) of *E. histolytica* live in the large intestine and form encysted stages (cysts) that are excreted with feces. The infection is transmitted by cysts from one human to another. The trophozoites of *E. histolytica* can penetrate into the intestinal wall and invade the liver and other organs hematogenously to produce clinical forms of amebosis, most frequently intestinal ameboses (amebic dysentery) and hepatic amebosis ("amebic liver abscess"). Diagnosis of an intestinal infection is primarily confirmed by detection of the parasites in stool. If an invasive, intestinal or extraintestinal infection with *E. histolytica* is suspected, a serological antibody test can also provide valuable information. Morphologically, *E. histolytica* is indistinguishable from the apathogenic *Entamoeba dispar* (collective term for both species: *E. histolytica*/*E. dispar* complex).

The Parasites: the causative agent of amoebiasis is the pathogenic Entamoeba histolytica. This species is morphologically identical with a non-pathogenic Entamoeba dispar. They can be differentiated by means of zymodeme and DNA analysis and with monoclonal antibodies.

- **histolytica Occurs in 3 forms:**
    - Trophozoites

- Precyst
- Cyst

Trophozoites: is the vegetative or growing stage of the parasite (Fig. 33). It is the only form present in tissues. It is irregular in shape and varies in size from 12–60 µm (average 20 µm). It is large and actively motile in freshly-passed dysenteric stool. The parasite, as it occurs free in the lumen as a commensal is generally smaller in size, about 15–20 µm and has been called the minuta form. **Cytoplasm:** Outer ectoplasm is clear, transparent, and retractile. Inner endoplasm is finely granular, having a ground glass appearance. The endoplasm contains nucleus, food vacuoles, erythrocytes, occasionally leucocytes, and tissue debris.

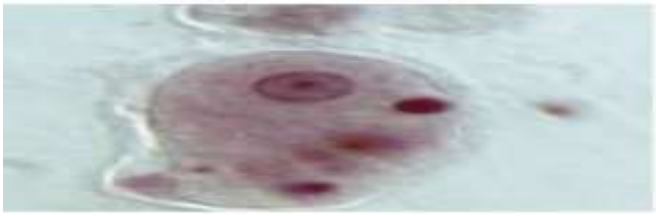


Fig 1.35 E. histolytica trophozoite

Pseudopodia are finger-like projections formed by sudden jerky movements of ectoplasm in one direction, followed by the streaming in of the whole endoplasm. Typical amoeboid motility is a crawling or gliding movement and not a free swimming one. The direction of movement may be changed suddenly, with another pseudopodium being formed at a different site, when the whole cytoplasm flows in the direction of the new pseudopodium. The cell has to be attached to some surface or particle for it to move. Pseudopodia formation and motility are inhibited at low temperatures.

Nucleusis spherical 4–6 µm in size and contains central karoyosome, surrounded by clear halo and anchored to the nuclear membrane by f ne radiating fibrils called the linin network, giving a cartwheel appearance. The nucleus is not clearly seen in the living trophozoites, but can be clearly demonstrated in preparations stained with ironhemotoxylin. The nuclear membrane is lined by a rim of chromatin distributed evenly as small granules.

The trophozoites from acute dysenteric stools often contain phagocytized erythrocytes. This feature is diagnostic as phagocytized red cells are not found in any

other commensal intestinal amoebae. The trophozoites divide by binary fission in every 8 hours. Trophozoites survive up to 5 hours at 37°C and are killed by drying, heat, and chemical sterilization. Therefore, the infection is not transmitted by trophozoites.

Even if live trophozoites from freshly-passed stools are ingested, they are rapidly destroyed in stomach and cannot initiate infection.

- Precystic Stage: Trophozoites undergo encystment (cyst formation) in the intestinal lumen. Encystment doesn't occur in the tissues nor in feces outside the body. Before encystment, the trophozoites extrudes its food vacuoles and becomes round or oval, about 10–20µm in size. This is the precystic stage of the parasite (Fig. 33 B).It contains a large glycogen vacuole and two chromatid bars. It then secretes a highly retractile cyst wall around it and becomes cyst.

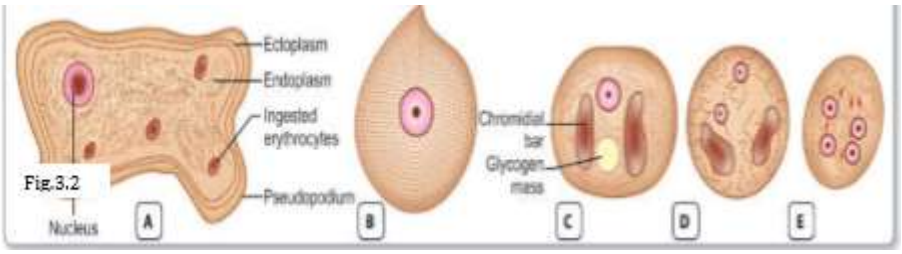


Fig 1. 36. **A *E.histolytica trophozoite B.* precystic stage C. uninuclear cyst D. binuclear cyst E. mature quadrinuclear cyst**

Cystic Stage: The cyst is spherical in shape about 10–20 µm in size. The early cyst contains a single nucleus and two other structures—a mass of glycogen and 1–4 chromatoid bodies or chromidial bars, which are cigar-shaped refractive rods with rounded ends (Fig. 33 B.). The chromatoid bodies are so called because they stain with hematoxylin, like chromatin. As the cyst matures, the glycogen mass and chromidial bars disappear and the nucleus undergoes 2 successive mitotic divisions to form 2 (Fig. 33 B above) and then 4 nuclei. The mature cyst is, thus quadri-nucleate(Fig. 33 B above). The cyst wall is a highly refractive membrane, which makes it highly resistant to gastric juice and unfavorable environmental conditions. The nuclei and chromidial bodies can be made out in unstained films, but they appear

more prominently in stained preparations. With iron hemotoxylin stain, nuclear chromatin and chromatoid bodies appear deep blue or black, while the glycogen mass appears unstained. When stained with iodine, the glycogen mass appears golden brown, the nuclear chromatin and karysome bright yellow, and the chromatoid bodies appear as clear space, being unstained.

Life cycle: E. histolytica passes its life cycle only in 1 host-human. - Infective form: Mature quadri-nucleate cyst passed in feces of convalescents (patients) and carriers. The cysts can remain viable under moist conditions for about 10 days.

Mode of transmission: Man acquires infection by swallowing food and water contaminated with cysts. As the cyst wall is resistant to action of gastric juice, the cysts pass through the stomach undamaged and enter the small intestine.

Excystation: When the cyst reaches caecum or lower part of the ileum, due to the alkaline medium, the cyst wall is damaged by trypsin, leading to excystation. The cytoplasm gets detached from the cyst wall and amoeboid movements appear causing a tear in the cyst wall, through which quadri-nucleate amoeba is liberated. This stage is called the metacys. Metacystic **trophozoites:** The nuclei in the metacyst immediately undergo division to form 8 nuclei, each of which gets surrounded by its own cytoplasm to become 8 small amoebulae or metacystic trophozoites. If exystation takes place in the small intestine, themetacystic trophozoites do not colonize there, but are carried to the caecum. In most of the cases, E. histolytica remains as a commensal in the large intestine without causing any ill effects. Such persons become carriers or asymptomatic cyst passers and are responsible for maintenance and spread of infection in the community. Sometimes, the infection may be activated and clinical disease ensues. Such latency and reactivation are the characteristics of amoebiasis.

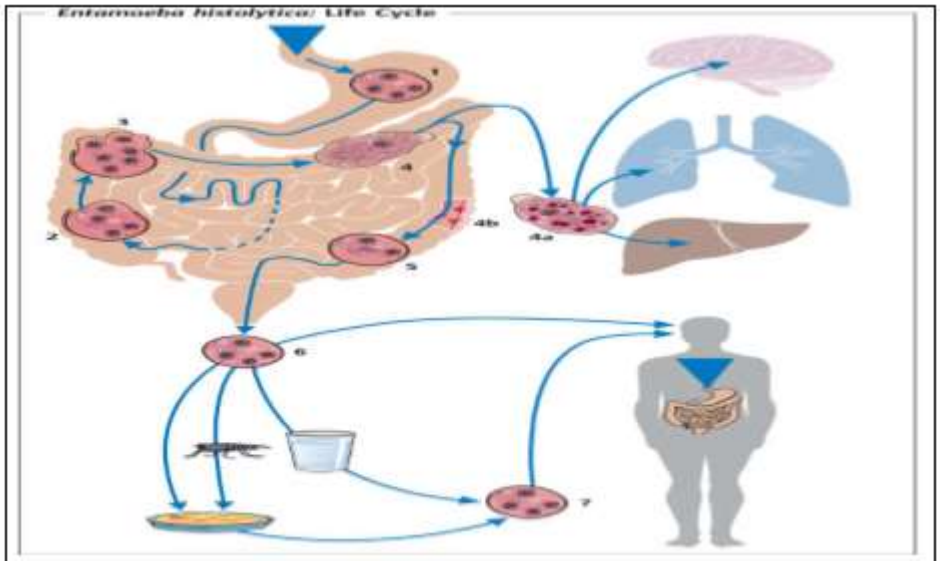
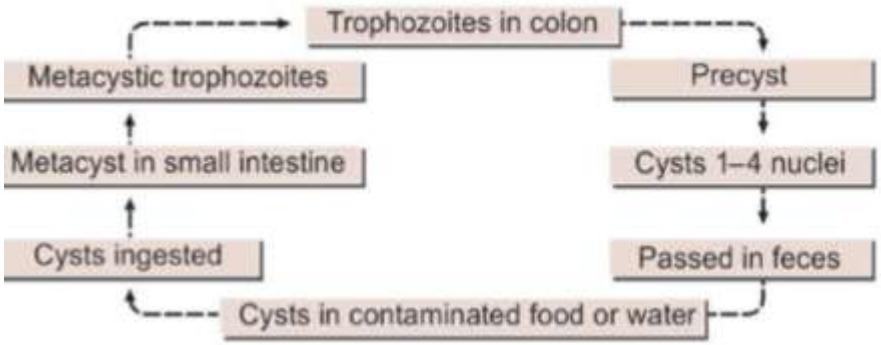


Fig 1. 37. C. Life cycle of E. histolytica.



Flow chart 1.5 Life cycle of E. histolytical

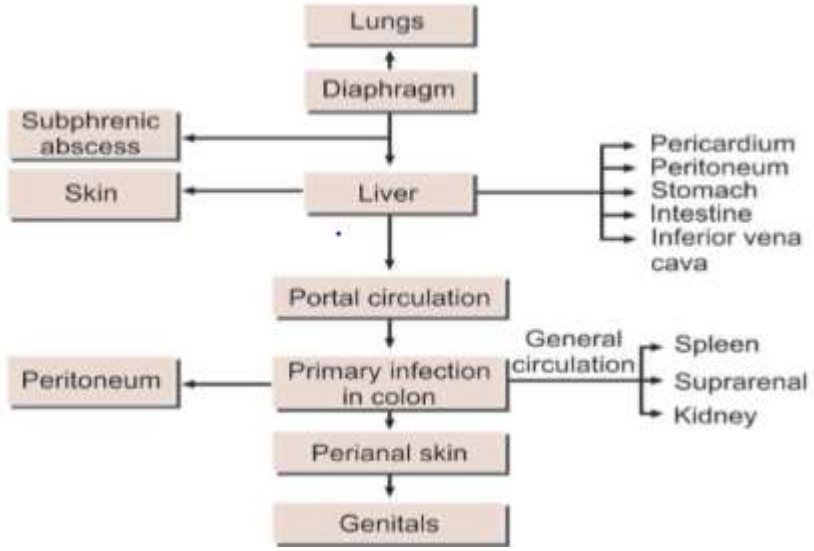
Important points on life cycle stages: Trophozoites are the multiplying, feeding and pathogenic stage of E histolytica. And cysts are transmission (infective stages), dormant, none dividing and none pathogenic stages E histolytica. Both trophozoites and cysts are diagnostic stages. Clinical manifestation:E. histolytica causes intestinal and extra-intestinal amoebiasis. Incubation period is highly variable. On an average, it ranges from 4 days to 4 months. Amoebiasis can present in deferent forms and degree of severity, depending on the organ affected and the extent of damage caused.

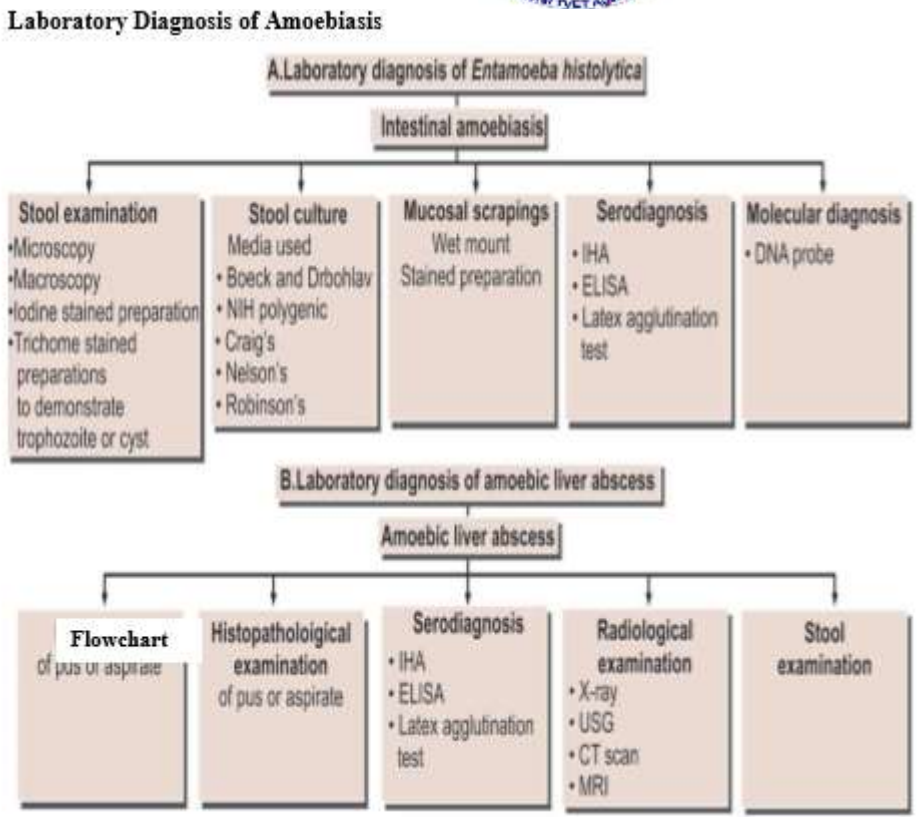
Intestinal Amoebiasis: The lumen-dwelling amoebae do not cause any illness. They cause disease only when they invade the intestinal tissues. This happens only in about 10% of cases of infection, the remaining 90% being asymptomatic. The following table shows summary of intestinal amoebiasis:

Lesions in chronic intestinal amoebiasis

- Small superficial ulcers involving only the mucosa.
- Round or oval-shaped with ragged and undermined margin and flask-shaped in cross-section.
- Marked scarring of intestinal wall with thining, dilatation, and sacculation.
- Extensive adhesions with the neighboring viscera.
- Formation of tumor-like masses of granulation tissue (amoeboma).

Extra-intestinal Amoebiasis: the following shows sites of extra-intestinal amoebiasis:





Flow chart 1.6 laboratory diagnosis of Amoebiasis

Table 2. Differential features of *E.histolytica and other non- pathogenic intestinal Entameoba*

	E. histolytica	*E. coli*	*E. hartmanni*
Trophozoite			
Size (μm)	12–60	20–50	4–12
Motility	Active	Sluggish	Active
Pseudopodia	Finger-shaped, rapidly extruded	Short, blunt slowly extruded	Finger-shaped, rapidly extruded
Cytoplasm	Clearly defined into ectoplasm and endoplasm	Differentiation not distinct	Clearly defined into ectoplasm and endoplasm
Inclusions	RBCs present, no bacteria	Bacteria and other particles, no RBCs	Bacteria and other particles, no RBCs
Nucleus	Not clearly visible in unstained films	Visible in unstained films	Not visible in unstained films
Karyosome	Small, central	Large, eccentric	Small, eccentric
Nuclear Membrance	Delicate, with fine chromatin dots	Thick, with coarse chromatin granules	Coarse chromatin granules
Cyst			
Size (μm)	10–15	10–30	5–10
Nuclei in mature cyst	4	8	4
Glycogen mass	Seen in uninucleate, but not in quadinucleate stage	Seen up to quadrinucleate stage	Seen in uninucleate, but not in quadinucleate stage
chromidial	1–4 with crounded ends	Splinter like with angular ends	Many with irregular shape

4.20.2. Luminal flagellates

Most luminal flagellates are nonpathogenic commensals. Two of them cause clinical diseases:

- *Giardia lamblia*, which can cause diarrheal diseases and
- *Trichomonas vaginalis*, which can produce vaginitis and arthritis. They are grouped under **phylum Sarcomastigophora**. The following **note** focuses on intestinal lumen dwelling flagellate and hemoflagellates.

Group	Parasites	Habitat
Lumen-dwelling flagellates	*Giardia lamblia*	Duodenum and jejunum
	Trichomonas vaginalis	Vagina and urethra
	Trichomonas tenax	Mouth
	Trichomonas hominis	Large intestine (caecum)
	Chilomastix mesnili	Large intestine (caecum)
	Enteromonas hominis	Large intestine (colon)
	Retortamonas intestinalis	Large intestine (colon)
	Dientamoeba fragilis	Large intestine (caecum and colon)
Hemoflagellates	*Leishmania* spp.	Reticuloendothelial cells
	Trypanosoma brucei	Connective tissue and blood
	Trypanosoma cruzi	Reticuloendothelial cells and blood

4.20.2.1. **Giardia lambilia:** A protozoan parasites first described by Dutch scientist Antonie von Leeuwenhoek in his own stools in 1681.It is named 'Giardia' after Professor Giard of Paris and 'lamblia' after Professor Lamble of Prague, who gave a detailed description of the parasite.

Habitat: *G. lamblia* lives in the duodenum and upper jejunum and is the only protozoan parasite found in the lumen of the human small intestine.

Morphology: It exists in 2 forms.

- trophozoites (or vegetative form): it is in the shape of a tennis racket (heart shaped or pyriform shaped) and is rounded interiorly and pointed posterior. It is bilaterally symmetrical and possesses 1 pair of nuclei, 4 pairs of flagella, 4 pairs of Blepharoplast from which the flagella arise, 1 pair of axostyles run along the mid line and two parabasal or median bodies, lying transversely posterior to the sucking disc. The trophozoites are motile, with a slow oscillation about its long axis, often resembling falling leaf.

- Cyst (or cystic form): it is the infective form of the parasite. The cyst is small and oval, measuring 12 µm x 8 µm and is surrounded by a hyaline cyst wall. Its internal structure includes 2 pairs of nuclei groupedat one end. A young cyst contains 1 pair of nuclei. The axostyle lies diagonally, forming a dividing line within cyst wall. Remnants of the flagella and the sucking disc may be seen in the young cyst.

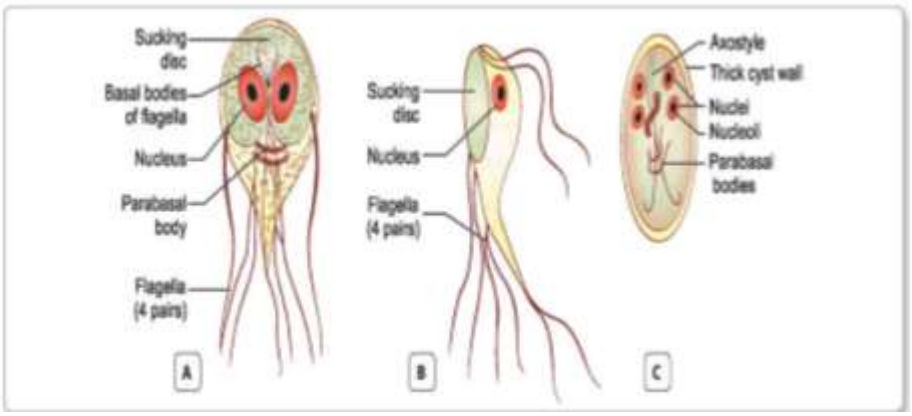


Fig. 1.37. A. trophozoite of G. lambalia B. lateral view C. cyst

Life cycle: The trophozoites live on the small intestine mucosa (less frequently on the gallbladder mucosa as well). They resemble a pear split lengthwise, are their dorsal side is convex; the anterior part of the ventral side forms a concave adhesive disk. Reproduction is by means of longitudinal binary fission of the trophozoites, which are able to produce variant specific surface proteins. G. lambilia produces oval cysts with four nuclei, flagella, and clawshaped median bodies. The cysts (and, less frequently, trophozoites) are excreted in stool.

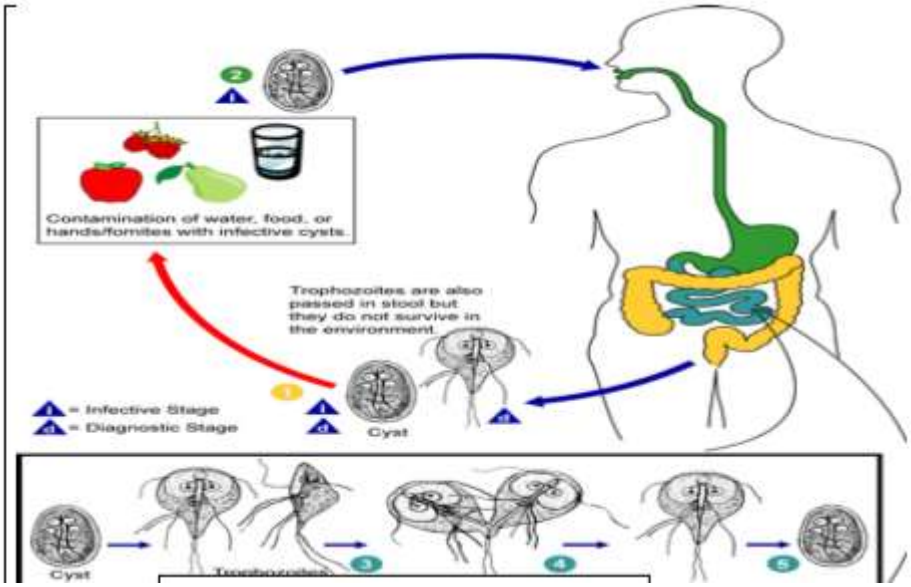
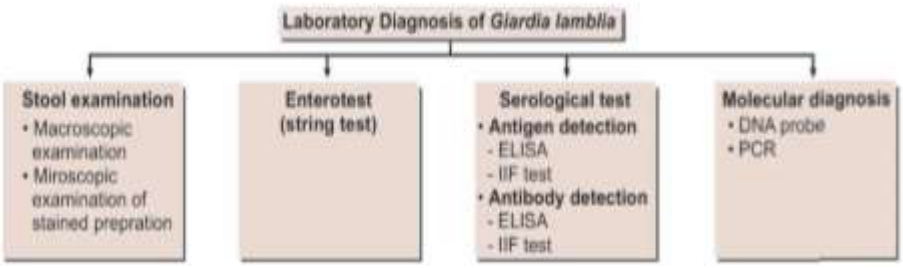


Fig. 1.38. Description of life cycle of G. lambalia

Clinical features: It does not invade the tissue, but remains tightly adhered to intestinal epithelium by means of the sucking disc. They may cause abnormalities of villous architecture by cell apoptosis and increased lymphatic infiltration of lamina propria. Diarrheal diseases (mucus diarrhea, fat malabsorption (steatorrheic), flatulence and adnominal discomfort.

Laboratory Diagnosis:



Flow chart 1.7. Laboratory diagnosis of G.lambalia

Treatment: Metronidazole (250 mg, thrice daily for 5–7 days) and tinidazole (2 g single dose) are the drugs of choice. Cure rates with metronidazole are more than 90%. Tinidazole is more effective than metronidazole. Furuzolidone and nitazoxamide are preferred for children, as they have fewer adverse effects.

4.20.3. Trichomonas vaginalis

Trichomonas vaginalis is a frequent flagellate species that occurs worldwide and is transmitted mainly by sexual intercourse. It causes vaginitis in women and urethritis in men.

Morphology: T. vaginalis exists only in trophozoites stage. Trichomonas vaginalis is a pear shaped protozoon about 10–20 lm long and 2–14 lm wide. Five flagella emerge from a basal body at the anterior pole, four freely extend forwards and one extends backwards, forming the outer edge of the undulating membrane, which reaches back only just beyond the middle of the cell. T. vaginalis colonizes the mucosa of the urogenital tract and reproduces by longitudinal binary fission. It is a motile with a rapid jerky or twitching type movement.

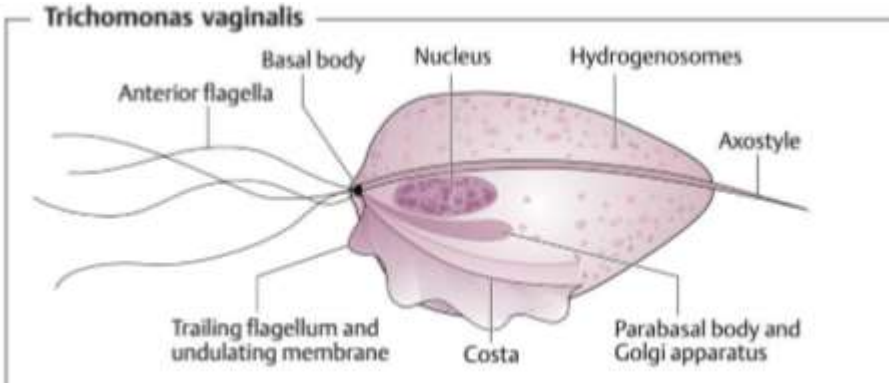


Fig. 1.39. Morphology of T.vaginalis

Habitat: In females, it lives in vagina and cervix and may also be found in urethra, and urinary bladder. In males, it occurs mainly in the anterior urethra, but may also be found in the prostate. Diagnosis: A fresh specimen of vaginal or urethral secretion is mixed with physiological saline solution and examined under a microscope for trichomonads. The trichomonads are readily recognized by their typical tumbling movements. The round trichomonad forms, by contrast, are hardly distinguishable from leukocytes. Can be easily identified as jerky like movement.

Treatment: It is always necessary for both sexual partners to receive treatment. Effective drugs include metronidazole preparations for oral application. In women vaginal application includes metronidazole and tinidazole. These substances are contraindicated in early pregnancy.

Preventive measures include loyalty and use of condoms during sexual intercourse.

4.20.4. Hemoflagellates

General Characteristics: The blood and tissue flagellates belong to the family Trypanosomatidae. The family consists of 6 genera, of which 2 genera Trypanosoma and Leishmania are pathogenic to humans.

Zoological Classification of Flagellates		
Phylum	:	Sarcomastigophora
Subphylum	:	Mastigophora
Class	:	Kinetoplastidea
Order	:	Trypanosomatida
Family	:	Trypanosomatidae

They live in the blood and tissues of man and other vertebrate hosts and in the gut of the insect vectors. Members of this family have a single nucleus, a kinetoplast, and a single flagellum. Nucleus is round or oval and is situated in the central part of the body. Kinetoplast consists of a deeply staining parabasal body and adjacent dot like blepharoplast. Flagellum is a thin, hair like structure, which originates from the blepharoplast. The portion of the flagellum, which is inside the body of the parasite and extends from the blepharoplast to surface of the body, is known as axoneme. A free flagellum at the anterior end traverses on the surface of the parasite as a narrow undulating membrane. Hemo-flagellates exist in two or more of four morphological stages. The names are **amastigote, promastigote, epimastigote and trypomastigote**. The names of the stages are formed by the suffix mastigote, combined with various prefixes, referring to the arrangement of the flagella in relation to the position of the nucleus and point of emergence from the cells.

Staining characteristics of trypanosomes: For smears of body fluids, Wrights stain, Giemsa stain, and Leishman's stain are suitable for identifying internal structures. The cytoplasm appears blue, the nucleus and flagellum appear pink, and the kinetoplast appears deep red. For tissue section, hematoxylin-eosin staining is done for demonstrating structures of the parasite. All

members of the family have similar life cycles. They all require an insect vector as an intermediate host. Multiplication in both the vertebrate and invertebrate host is by binary fission. No sexual cycle is known.

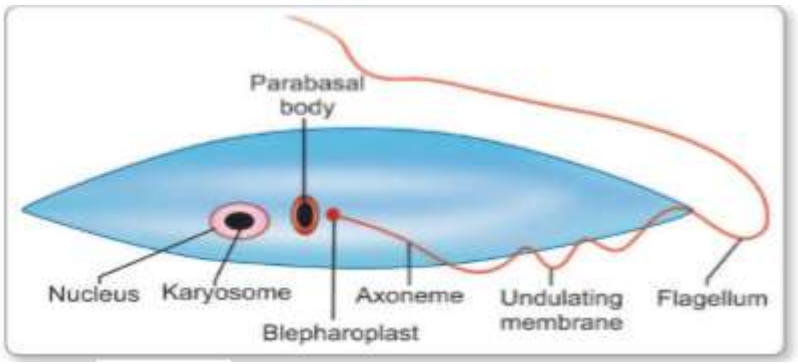
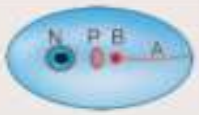
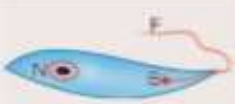
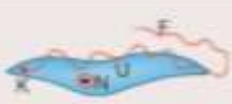


Fig. 1.40. Basic morphology of hemoflagellates.

The following table shows different between different morphological stages of Hemoflagellates.

Table: 3 differences between various stages of Hemoflagellates.

	Amastigote	Promastigote	Epimastigote	Trypomastigote
Morphological characteristics	Rounded or ovoid, without any external flagellum. The nucleus, kinetoplast, and axial filaments can be seen. The axoneme extends upto the anterior end of the cell	Lanceolate in shape. Kinetoplast is anterior to the nucleus (antenuclear kinetoplast) near the anterior end of the cell, from which flagellum emerges. There is no undulating membrane	Elongated, with the kinetoplast placed more posteriorly, though close to and in front of the nucleus (juxtanuclear kinetoplast). The flagellum runs alongside the body as a short undulating membrane, before emerging from the anterior end	This stage is elongated, spindle-shaped with a central nucleus. The kinetoplast is posterior to the nucleus (postnuclear kinetoplast) and situated at the posterior end of the body. The flagellum runs alongside the entire length of the cell to form a long undulating membrane before emerging as a free flagellum from the anterior end
Seen in	*Trypanosoma cruzi* and *Leishmania* as intracellular form in vertebrate host	It is the infective stage of *Leishmania*, found in the insect vector as well as in cultures *in-vitro*	It is the form in which *Trypanosoma brucei* occur in salivary gland of the vector tsetse fly and *Trypanosoma cruzi* in the midgut of the vector reduviid bug. **Note:** This stage is lacking in *Leishmania*	This is the infective stage of trypanosomes found in arthropod vector and in the blood of infected vertebrate. **Note:** This stage is lacking in *Leishmania*
Schematic illustration				

Three distinct Kinetoplastida cause human disease:

- Leishmania species (leishmaniasis)
- African trypanosomes (African sleeping sickness)
- Trypanosoma cruzi (Chagas' disease), and)

4.20.4.1. Leishmania species (leishmaniasis)

They are the causative agent of Leishmaniasis and are obligate intracellular protozoa of the genus Leishmania. They were named after Leishmania, who first described it in London in May 1903. They are reproduces by longitudinal binary fission. Biological insect vectors as intermediate hosts & human as definitive host. The species are morphologically indistinguishable, but they can be differentiated on the basis of on their clinical features, geographical distribution, and serologic tests. Generally, human infection is caused by about 21 of 30 species that infect mammals.

These includes:

L. donovani complex:

- *L. donovani,*
- *L. infantum,*
- *L. chagasi*

L. mexicana complex:

- *L. mexicana*
- *L. amazonensis*
- *L. venezuelensis*

L. braziliensis complex:

- *L. braziliensis*
- *L. Peruviana*

L. guyanensis complex:

- *L. Guyanensis*
- *L. panamensis*

L. tropica,

L. major

L. aethiopica

Leishmaniasis can easily classified clinically as **visceral leishmaniasis, cutaneous leishmaniasis, Mucocutaneous leishmaniasis and Diffused cutaneous leishmaniasis**.

In Ethiopia: Four species of Leishmania are found, namely:

- *L. aethiopica,*

- *L.major*
- *L. tropica*
- *L. donovani*

In Ethiopia: – L donovani is the causative agent of visceral leishmaniasis

– Cutaneous leishmaniasis is caused by three parasites:

- *L. aethiopica,*
- *L. major*
- *L. tropica*

Promastigote - Elongated, with flagella (10-20 µm long)
- Occur extracellularly in the insect midgut & in artificial culture
- Motile

Amastigote - Round (3-7 µm diameter)
- Occurs intracellularly, inmammalian
- Non-motile

Transmission and life cycle

- Common mode of transmission.
  - Bite of infected female sandfly
    - Genera *Phlebotomus* **in Old world**
    - *Lutzomyia in* **New world**
- Uncommon modes of transmission:
  - Congenital transmission,
  - Blood transfusion,
  - Rarely, inoculation of cultures.

- Infective stage: Promastigotes
- Diagnostic stage: amastigotes
- Definitive host: human
- Intermediate host: sand flies (Genus Phlebotomus)

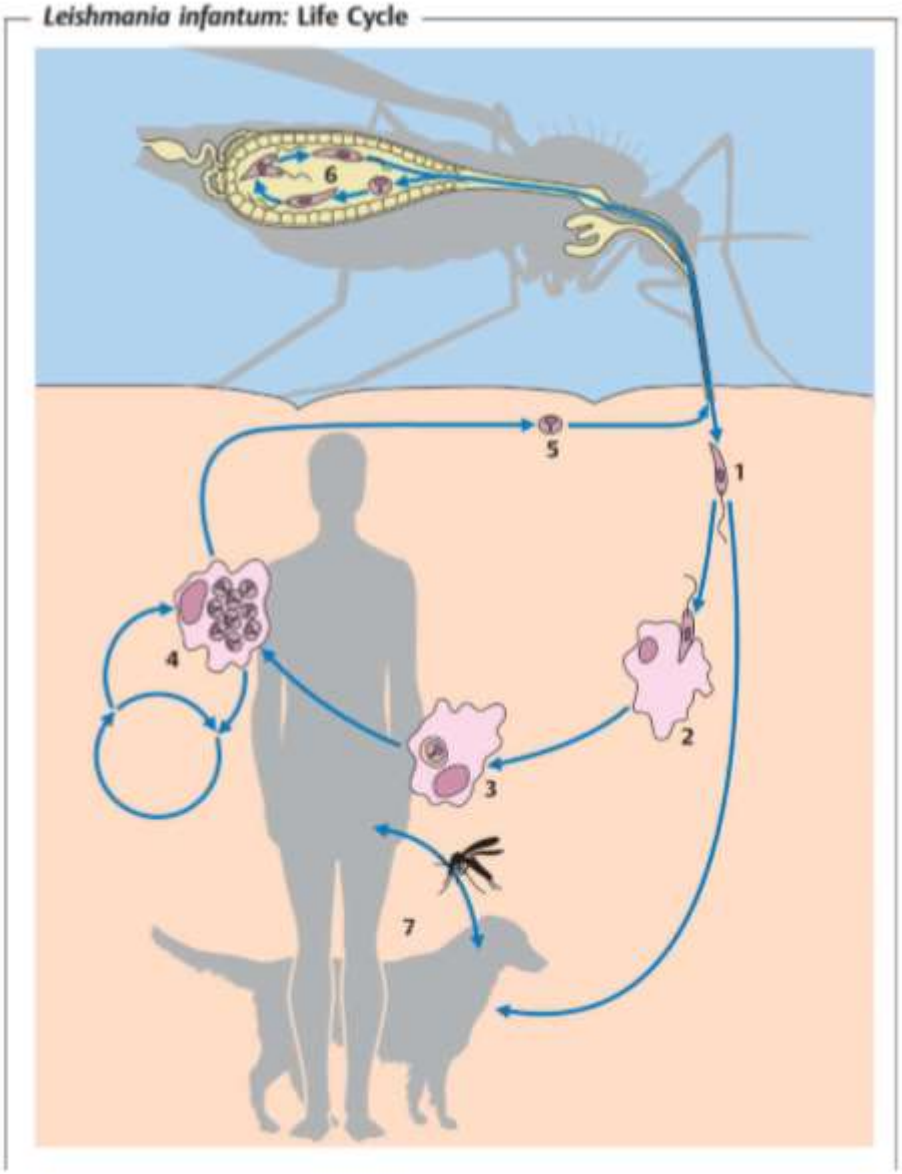
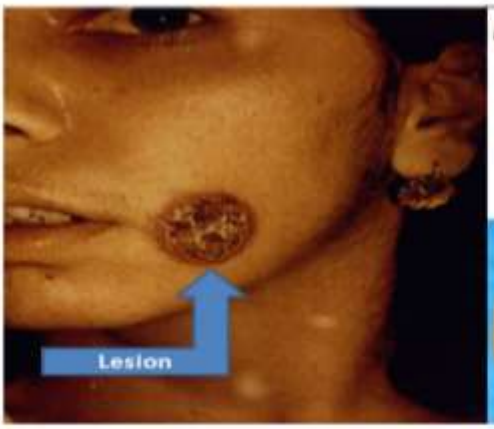


*Fig. 1.41. .**Inoculation of promastigotes stages (infective stage) by sand fly; 2 ingestion of parasites by phagocytes (Langerhans cells, dendritic cells, macrophages); 3 amastigote form in parasitophorous vacuole of a macrophage; 4 reproduction of amastigotes forms in a macrophage; 5 ingestion of amastigote forms by sand fly with blood meal (diagnostic stage); 6 transformation into promastigote form and multiplication in insect; 7 dog as reservoir host.***

Clinical manifestations:

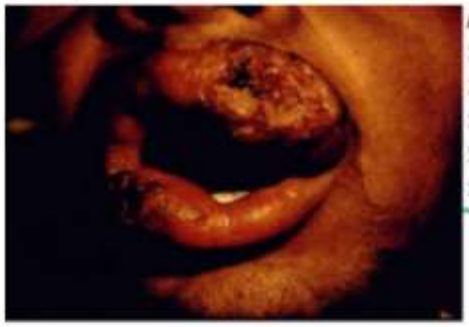
1. Old World cutaneous Leishmaniasis caused by



L. Tropica

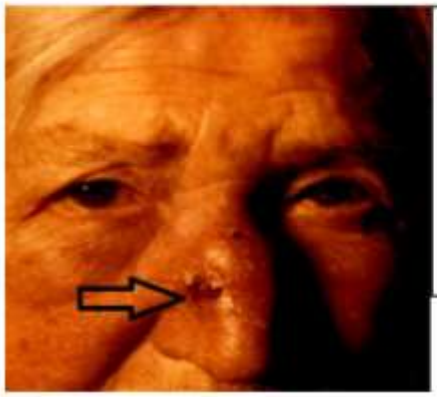
- Dog are reservoir hosts
- It is called dry ,urban ,chronic, old world oriental sore
- A 'dry painless lesion' 5-70mm diameter will be seen on checks

2. L. major



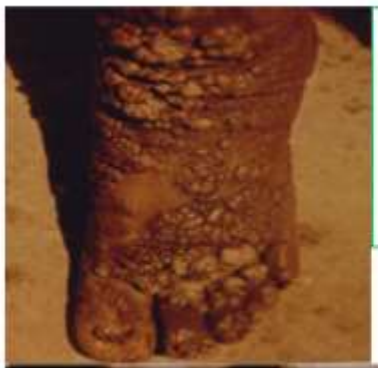
L. Major

- rural (rodent reservoir)
- wet oriental sore
- Early papules is inflamed (5-10mm)
- Develop to large uneven ulcer
- Self-healing (3-6mths)

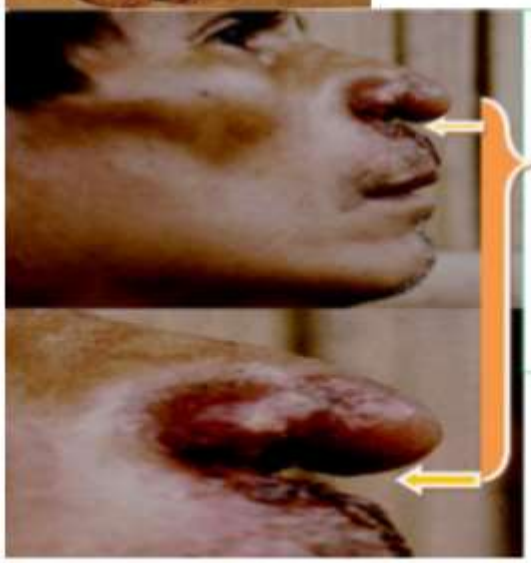


- ***L. aethiopica***
  - highlands of Kenya and Ethiopia
  - Similar to oreintal sore
  - Self-heal 1-3 yrs
  - Can cause DCL in patients
  - also cause MCL

- **Diffuse Cutaneous Leishmaniasis:**



- Caused by *L.aethiopica*and *L. amazonensis*
- Skin lesion develop over large areas of the body
- Scaly, not ulcerated, nodules
- Chronic and painless



- Disfiguration is often extreme with complete destruction of the
- nasal septum, perforation of the palate and damage of the tissues of the lips and larynx

- **Visceral Leishmaniasis (VL)**: Caused by *L. donovani*. Reticuloendothelial system affected:spleen, liver, bone marrow, lymph nodes.

Symptoms include:

- Fever, malaise (as mild sickness or depression), weakness
- wasting despite good appetite
- Spleen and hepatomegaly, enlarged lymph nodes
- Epistaxis (nose bleeding) and bleeding of gums
- Depressed hematopoiesis (blood cell production abnormalities) severe anemia
  - leucopenia
  - Thrombopenia

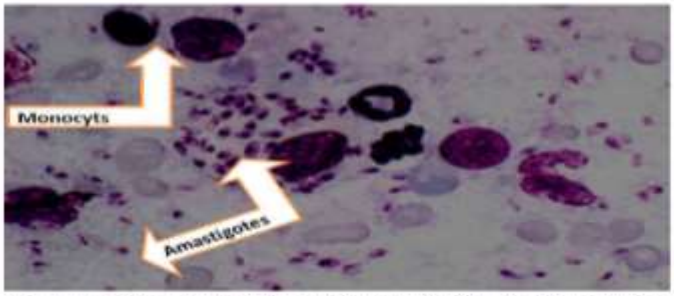
Laboratory Diagnosis:

Cutaneous, Mucocutaneous and diffused cutaneous Leishmaniasis

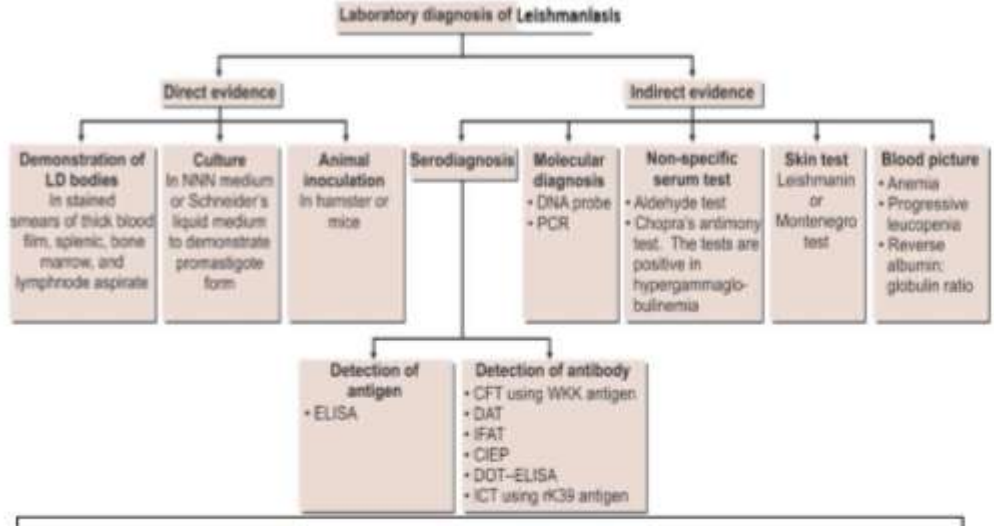
Four Laboratory investigation methodes:

1. Detecting amastigotes from scrapings, biopsy, aspirates
2. Culture from ulcer material
3. Leishmanial test
4. Serology

1. Detecting amastigotesfrom scrapings, biopsy, aspirates: Collection and examination of skin slit smears for amastigotes. It should be taken from the inflamed raised swollen edge of an ulcer or nodule, not from its base or center which usually contains only necrotic tissue.



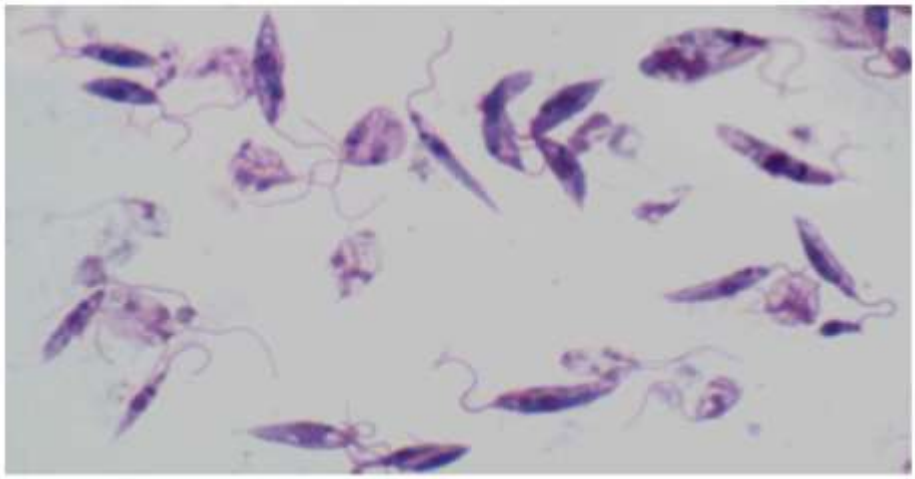
The above figure shows Microscopic examination of slides after Giemsa stain



Flow chart 1.8. Laboratory diagnosis of leishmaniasis

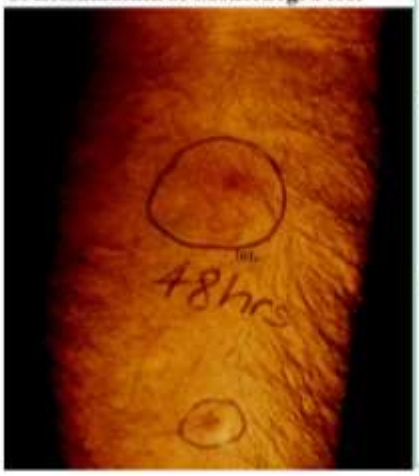
2.Culture **from ulcer material**

- Done when cutaneous leishmaniasis is suspected and parasites cannot be found in smears
- Material for culture is best obtained by injecting and then aspirating a small quantity **of sterile physiological saline in and out of** the hardened margin of the ulcer
- A few drops of the final aspirate is used to inoculate the culture medium
  - **Novy –Nicolle- MacNeal (NNN)**



Promastigote stages seen following *in vitro* culture.

3. Leishmainin or Montenegro test



- It is delayed type hypersensitivity skin test
- intradermal inoculation of leishmanin
  - suspension of whole or killed promastigotes
  - preferably from local area
  - include negative control
- *Positive reaction:* when the area of indurations + erythema of 5mm in diameter or more indurations in 48-72 hours

4. Serological Tests

Because of the poor antibody response in cutaneous leishmaniasis, serological tests have no advantages or little value in diagnosis. This is due to cellular response which is the basis of the leishmanin skin test. In muco-cutanous leishmaniasis, antibodies can be found in the serum.

Diagnosis **visceral leishmaniasis**

1. Demonstration of parasite in tissues by
   - light microscopic examination of the stained specimen,
   - culture
   - animal inoculation
2. Detection of parasite DNA in tissue samples
3. Immunodiagnosis by detection
   - Antigen detection
   - antibody detection

Demonstration of amastigotes from different specimens

light microscopic examination of the stained specimen to recover amasigote from aspirates

	Aspirate	%positive
1.	Spleen	95-98%
2.	Bone marrow	64-86%
3.	Enlarged lymph node	About 64%
4.	Buffy coat (India)	67-99%
5.	Buffy coat (Africa)	About 50%

So, it's important to note that the positivity rates of aspirates are more blood smears for leishmaniasis diagnosis.

Immunodiagnosis

1. Antigen detection	2. Antibody detection
➢ more specific than Antibody based tests ➢ Ag detected quite early ➢ the amount of detectable Ag tends to decline rapidly following chemotherapy ➢ useful in the diagnosis of disease in cases where there is deficient antibody production (as in AIDS patients)	➢ **Direct agglutination test (DAT)** ➢ is a rapid and reliable screening test for VL ➢ sensitivity of 91-100% and specificity of 72-100% ➢ some cross reaction with leprosy, Chagas disease, malaria and schistosomiasis may occur ➢ may also yield positive result for long time after complete cure

3. New K39 antigen strip test: to diagnose visceral leishmaniasis has high sensitivity and specificity for active kala-azar. The nitrocellulose strips used in the test are impregnated with recombinant K39 antigen can be used to test peripheral (finger prick) blood samples.

4. Rapid latex agglutination tests: are quicker and easier to perform and interpretable than the Direct agglutination Teats. Equal volumes of test serum and sensitized dyed latex particles are mixed on a cavity microscope slide and rotated for up to 2 minutes. A positive test is shown by agglutination. Its sensitivity is around 100% & specificity 98%.Thus, yields positive result for long time after complete cure and some cross reaction with malaria, Tuberculosis or typhoid fever may also occur.

5. Hematological investigations including:

- Measurement of the hemoglobin
- Total and differential white cell (leucocytes) count: leucopenia-low WBCs,
- Platelet (thrombocyte) count: thrombocytopenic- low platelet count
- a raised ESR(erythrocyte sedimentation rates)
- Plasma albumin levels are greatly reduced, total protein raised in patients with VL(visceral Leishmaniasis)

Treatment: Sodium stibogluconate (Pentostam), Pentamidine isethionate,amphotericin B **Prevention and control:** Early detection by serological diagnosis (VL) and treatment of infected persons:

- Health information dissemination
- Personal protection from sand fly bites by insect replants and avoiding endemic areas especially at times when sand flies are most active
- Use of pyrethroid impregnated bed nets and curtains
- Vector control etc.

4.20.5. Trypanosomiasis

Etiologic agents:

- Trypanosoma brucei complex
- African trypanosomiasis (sleeping sickness)
- Trypanosoma cruzi – American trypanosomiasis (Chagas' disease)

Important features: These species may have amastigote, promastigote, epimastigote, and trypomastigote stages in their life cycle. In human trypanosomes of the African form, however, the amastigote and promastigote stages of development are absent. Typical trypanosome structure is an elongated spindle-shaped body that more or less tapers at both ends, a centrally situated nucleus, a kinetoplast posterior to nucleus, an undulating membrane arising from the kinetoplast and proceeding forward along the margin of the cell membrane and a single free flagellum at the anterior end.

African trypanosomiasis Trypanosoma gambiense & Trypanosoma rhodesiene are causative agents of the African typanosomiasis, transmitted by insect bites. The vector for both is the tsetse fly.

- **Life cycle**: During a blood meal on the mammalian host, an infected tsetse fly (genus Glossina) injects metacyclic trypomastigotes into skin tissue
  - The parasites enter the lymphatic system and pass in to the blood stream transform into blood stream trypomastigotes , are carried to other sites throughout the body, reach other body fluids (e.g., lymph, spinal fluid), and continue the replication by binary fission.
  - The tsetse fly becomes infected with blood stream trypomastigotes when taking a blood meal on an infected mammalian host
  - In the fly's midgut, the parasites transform into procyclic trypomastigotes, multiply by binary fission , leave the mid gut, and transform into epimastigotes

- The epimastigotes reach the fly's salivary glands and continue multiplication by binary fission and transform into metacyclic trypomastigotes .

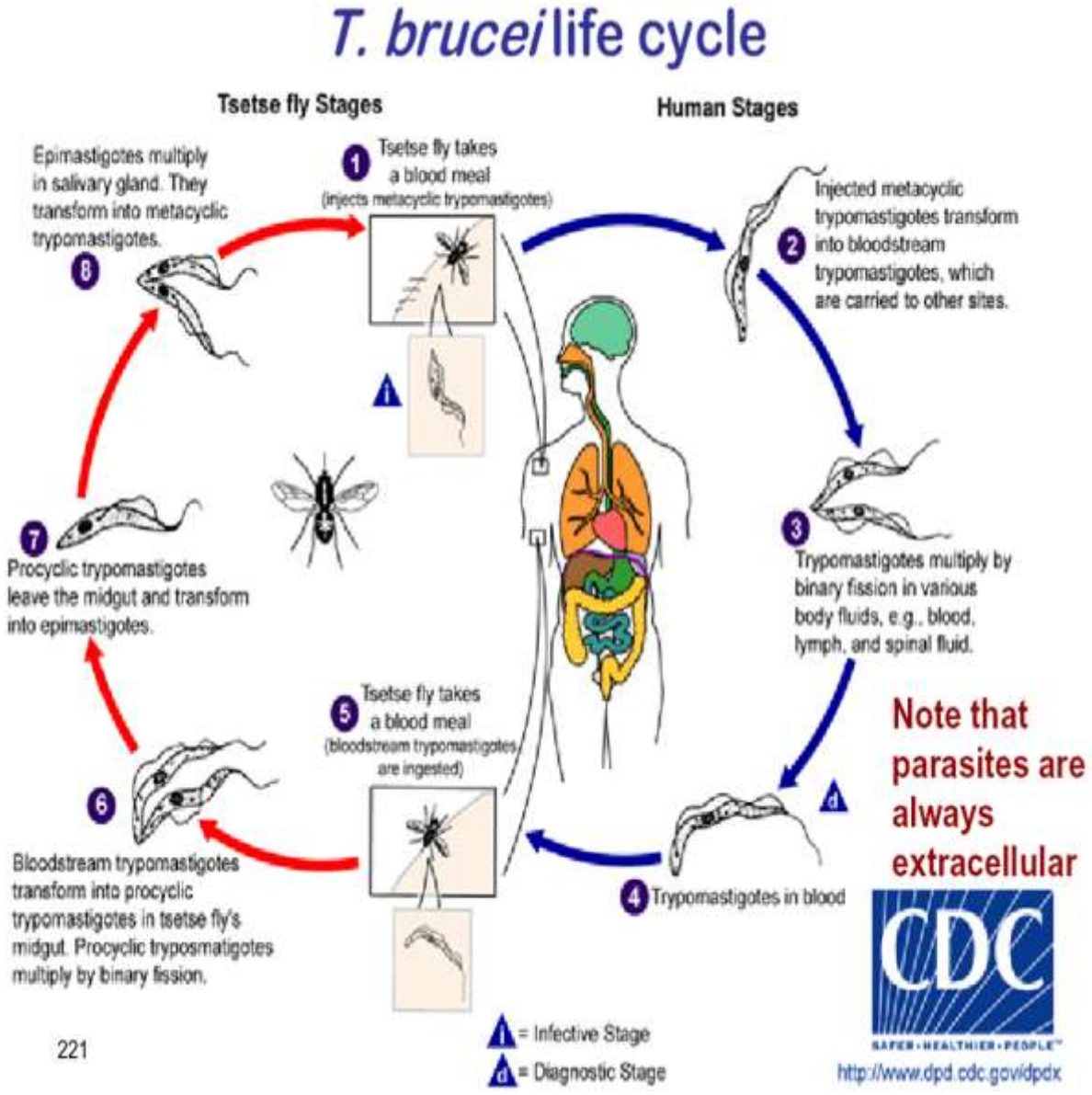
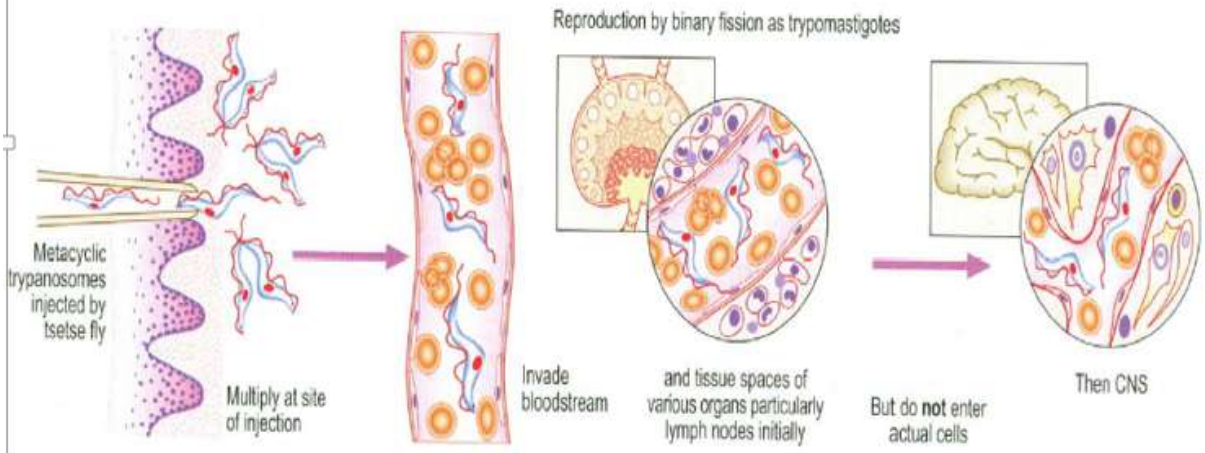


Fig 1. 42. Lifecycle African trypanosomiasis

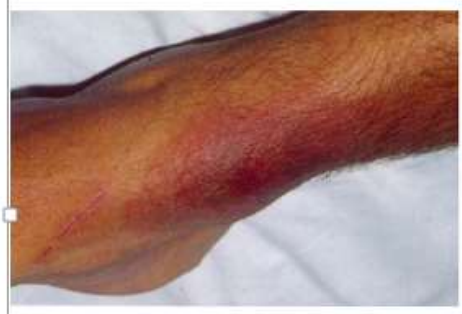
- **Pathogenesis:**
    - An exact pathogenesis of sleeping sickness is not known, although immune complexes and inflammation have been suspected to be the mechanism of damage to tissues
    - The immune response against the organism help to eliminate the parasite but it is not protective
- Antigenic variation
    - Trypanosomes are covered with a glycoprotein surface coat called variable surface coat glycoprotein (VSG)
    - VSG is major component 'surface coat " covering blood steam trypomastigotes
    - Immunoglobulin directed against these VSG recognize the trypanosomes and destroy it until the organism vary their VSG
    - Periodically (every 5-7 days) the parasite will express a different VSG gene which is antigenically distinct from the previously expressed VSG
    - It also causes severe depression of cell mediated and humoral immunity to other antigens
    - Results in death
- **Clinical feature:**

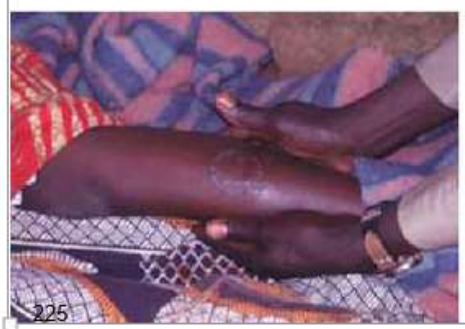
T. brucei in the human host



Sleeping sickness

Disease course and symptoms





First stage

- Trypanosomes multiply in the tissue around the initial bite site
- This often results in a characteristic local inflammation the trypanosomal **"chancre".** Usually not painful
- From there they enter the blood and lymphatic system

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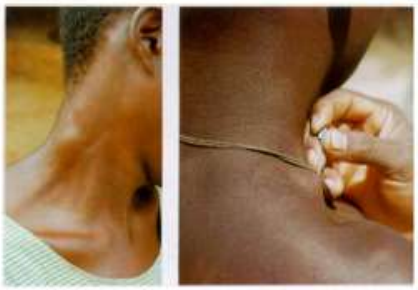
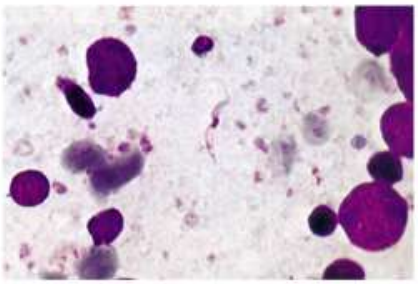
Sleeping sickness

Disease course and symptoms

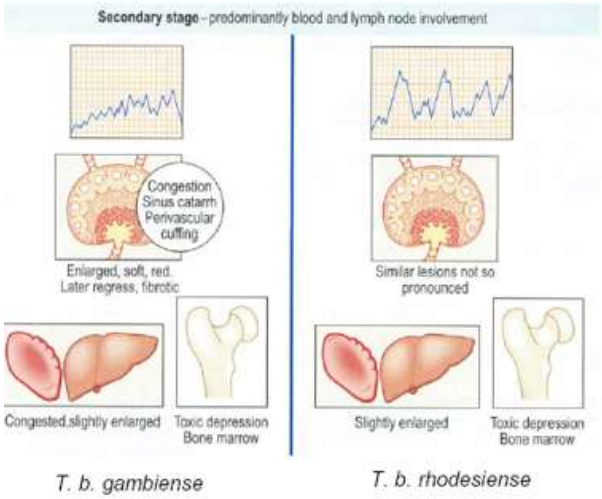




- Enlargement of the lymphatic glands (especially in the posterior triangle of the neck) can be an early sign of the disease (**Winterbottom sign**, not as common in rhodesiense infection).
- Aspiration of swollen gland often reveals parasites.

Sleeping sickness

Disease course and symptoms

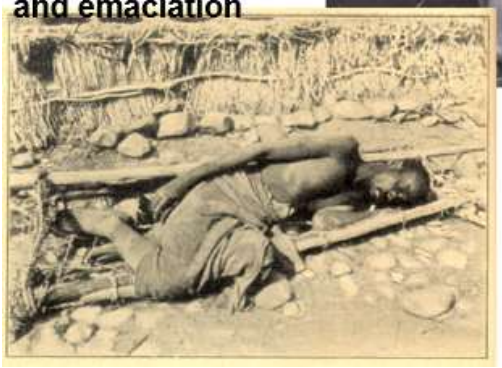


- After 1-2 week period of asymptomatic incubation, parasites invade blood leading to fever and headache
- Once parasites enter blood stream fever sets in (low and irregular in gambiense and high and periodic in rhodesiense)
- General toxic symptoms include headache, facial edema, nausea and vomiting, back and bone pain
- Symptoms at this stage are rather mild in gambiense but can be severe in rhodesiense with often fatal outcome

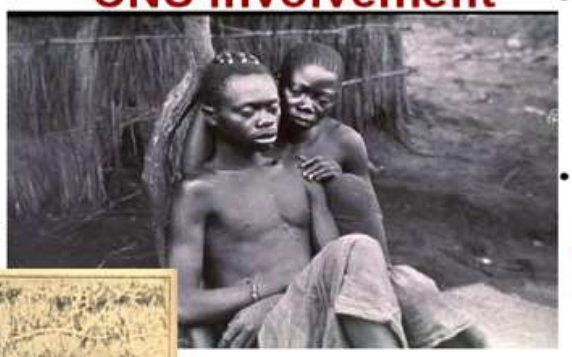
Sleeping sickness

Disease course and symptoms

Fatigability , confusion , drowsiness , somnolence (uncontrollable urge to sleep day time which may alternate with night time insomnia), wasting and emaciation



CNS involvement



- In later stages of infection parasites pass the blood brain barrier and infect the CNS
- Presence of parasites leads to **meningo-encephalitis** with progressive neurological involvement, which ultimately ends in coma (sleeping sickness)
- Untreated trypanosomiasis is always fatal

Sleeping sickness

Disease course and symptoms

Neurological complications can occur as a result of infection and, as seen here, patients may be immobilized for their own safety.

- The progressive encephalitis can cause severe dementia with sometimes aggressive behavior
- Disease progression especially CNS invasion is much faster in rhodesiense
- Gambiense can take a year or two while rhodesiense usually passes the blood brain barrier within a month

Gambiense and Rhodesiense Sleeping sickness

- **West African**
  - *T.b.gambiense*
  - West & Central Africa
  - Tsetse fly (*Glossina*, palpalis group)
  - Reservoir = human
  - Parasitemia = low/variable
  - Clinical features
    - Early: fever, myalgias, lymphadenopathy, edema,
    - Late: CNS; seizures, coma
    - Duration; months-years
  - Mortality; 100% if unRx
  - Asymptomatic carriers common

- **East African**
  - *T.b. rhodesiense*
  - Rift valley & eastern Africa
  - Tsetse fly (*Glossina*, morsitans group)
  - Reservoir = wild animals
  - Parasitemia = high
  - Clinical features
    - Early: severe fevers, edema, weakness, emaciation
    - Late: rapid demise
    - Duration; weeks - months
  - Mortality; 100% if unRx
  - Asymptomatic carriers rare

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Laboratory diagnosis

1. Microscopy

A. Examination of blood for trypanosomes

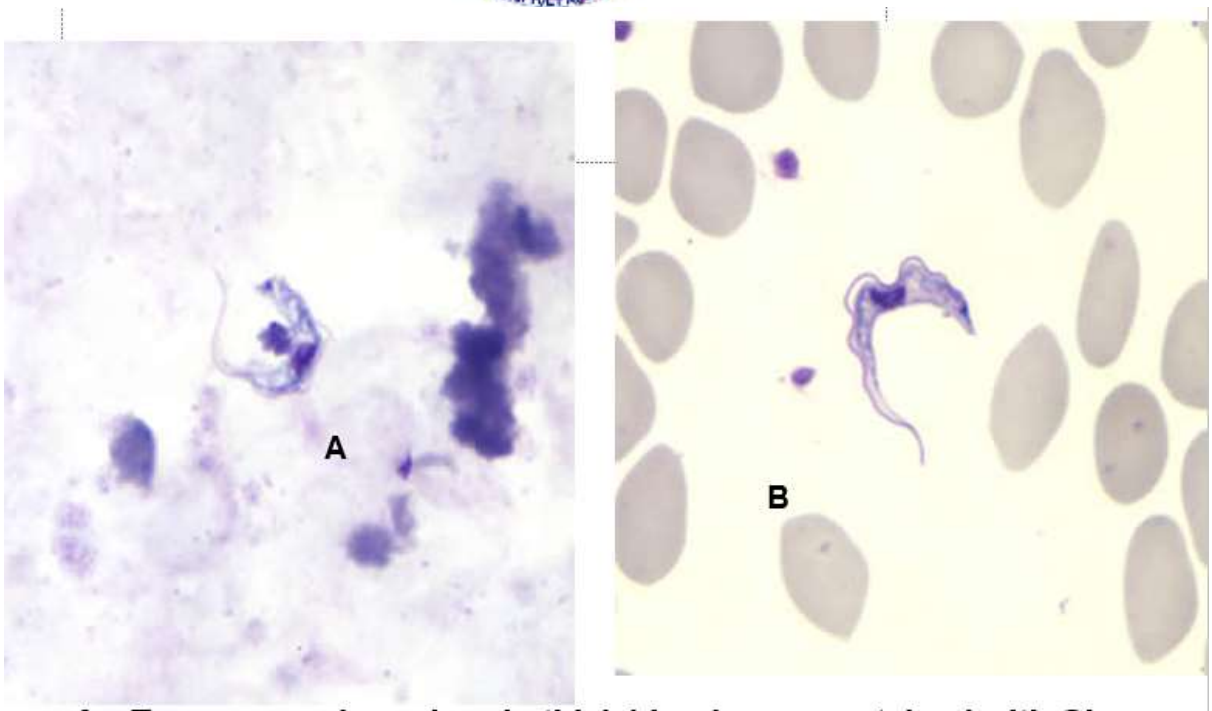
B. Examination of lymph gland aspirates

C. Examination of chancre fluid for trypanosomes

D. Examination of CSF for trypanosomes

2. Serology

3. Molecular techniques : polymerase chain reaction (PCR).



A: *Trypansoma brucei* sp. in thick blood smears stained with Giemsa.
B: *Trypanosoma brucei* sp. in thin blood smears stained with Giemsa
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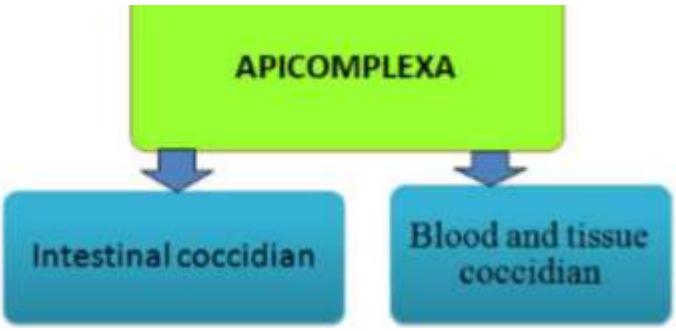
Treatment : Pentamidine isethionate or Suramin

Prevention and Control

1. Detecting and treating human infections at early stage
2. Identifying and killing/ restricting movement of animal reservoir hosts in endemic areas
3. Health information dissemination
4. Sterile breeding technique
5. Vector control:
    - ☐ By spraying vehicles with insecticide as they enter and leave the tsetse fly infested area,
    - ☐ By selectively clearing the bush and wood areas especially around water holes, bridges, and along river blanks
    - ☐ By using and maintaining insecticide impregnated tsetse fly traps

4.20.6. Blood and Tissue Coccidians

General features: they are **Coccidians** are **apicomplexa** parasites



- During part of their life cycles, most apicomplexans invade and replicate within the host cells
- Both asexual and sexual reproduction are involved
- The basic life cycle may be said to start when an infective stage (sporozoite) enters a host cell, and divides repeatedly to form numerous merozoites
- Micronemes and rhoptries are specialized membrane bound apical organelles that play a major role in interactions with and invasion of host cells
- Micronemes contain adhesive proteins that are important in attaching to host cells or the substratum
- The force needed for zoite motility and invasion is provided by a motor protein complex including a myosin unique to apicomplexans
- The apicomplexa have complex life cycles that are characterized by three distinct processes: **Sporogony, merogony and gametogony**
- Sporogony occurs immediately after a sexual phase and Consists of an asexual reproduction that culminates in the production of sporozoites
- Sporozoites are an invasive form that will invade host cells and develop into forms that undergo another asexual replication known as merogony.
- Therefore, blood and tissue sporozoa are found inside blood, blood forming organs or tissues.

Seven species infecting humans are but our main focus is Plasmodium species and we give short description on C. parvum and T. gondii.

1. Plasmodium species 2. Babesia 3. Cryptosporidium parvum 4. Isospora belli

5. Cyclospora cayetenesis 6. Sarcocystis 7. Toxoplasma gondii

4.20.6.1. Plasmodium species

They are causative agent of Malaria: an acute and/or chronic infection caused by protozoans of the genus Plasmodium.

Five plasmodium species causing human malaria

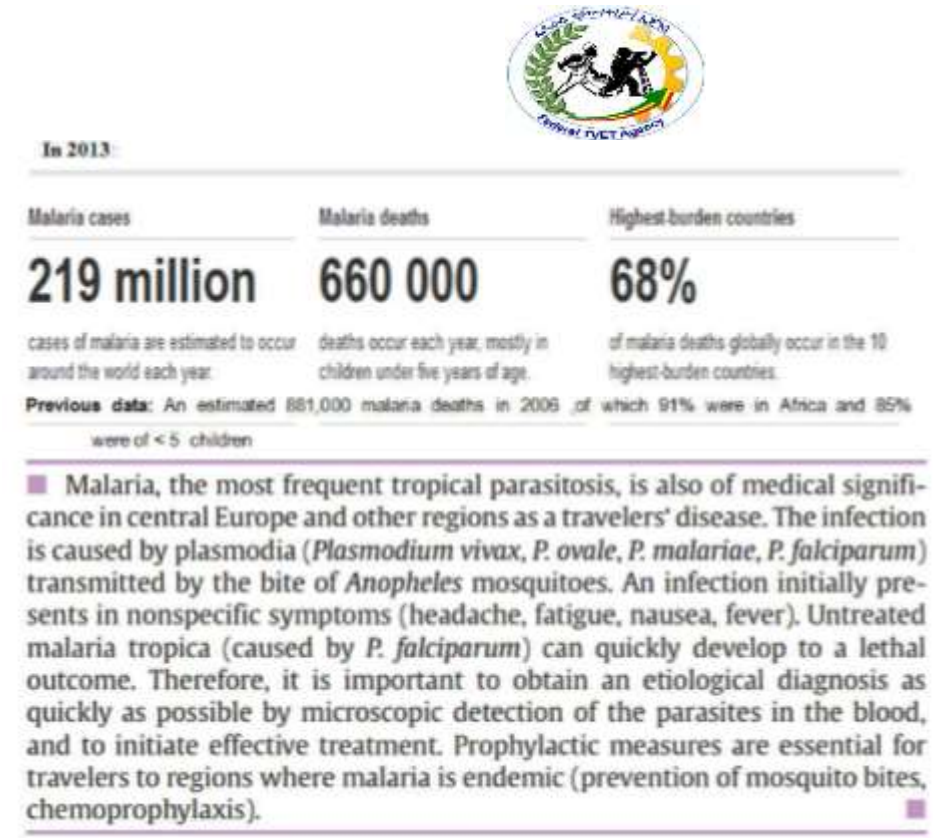
1. *Plasmodium falciparum*
2. *P. vivax*
3. *P. malariae*
4. *P. ovale*
5. *P. knowlesi*

General feature of Plasmodium species:

They are intracellular obligate parasites (liver cell & RBC). The life cycle is alternation of generation by alternation of hosts and requires two hosts:

- Sexual and asexual reproduction

Malaria is a highly fatal disease of all known to kill human beings.



In 2013

Malaria cases	Malaria deaths	Highest-burden countries
219 million	**660 000**	**68%**
cases of malaria are estimated to occur around the world each year.	deaths occur each year, mostly in children under five years of age.	of malaria deaths globally occur in the 10 highest-burden countries.

Previous data: An estimated 881,000 malaria deaths in 2006 ,of which 91% were in Africa and 85% were of < 5 children

Malaria, the most frequent tropical parasitosis, is also of medical significance in central Europe and other regions as a travelers' disease. The infection is caused by plasmodia (*Plasmodium vivax, P. ovale, P. malariae, P. falciparum*) transmitted by the bite of *Anopheles* mosquitoes. An infection initially presents in nonspecific symptoms (headache, fatigue, nausea, fever). Untreated malaria tropica (caused by *P. falciparum*) can quickly develop to a lethal outcome. Therefore, it is important to obtain an etiological diagnosis as quickly as possible by microscopic detection of the parasites in the blood, and to initiate effective treatment. Prophylactic measures are essential for travelers to regions where malaria is endemic (prevention of mosquito bites, chemoprophylaxis).

Morphological Stages

1. Sporozoite: develops in the mosquito salivary gland
2. Hepatic schizont: actively dividing, multinucleated, parasite form in hepatocytes
3. Trophozoite: metabolically active form living within the RBC. Sometimes called the ring form
4. Erythrocytic schizont: multinucleated stage in RBC resulting from asexual multiplication of trophozoites. Each schizont contains a species determined number of merozoites
5. Merozoite: infective of stage for RBC and its schizont components that break out of hepatocyte or RBC
6. Gametocyte: morphologically distinctive sexual (male or female) form which develops from some trophozoites in RBCs

The life cycle

The infective stage: sporozoites.

The diagnostic stages: Trophozoites, schizonts and gametocytes.

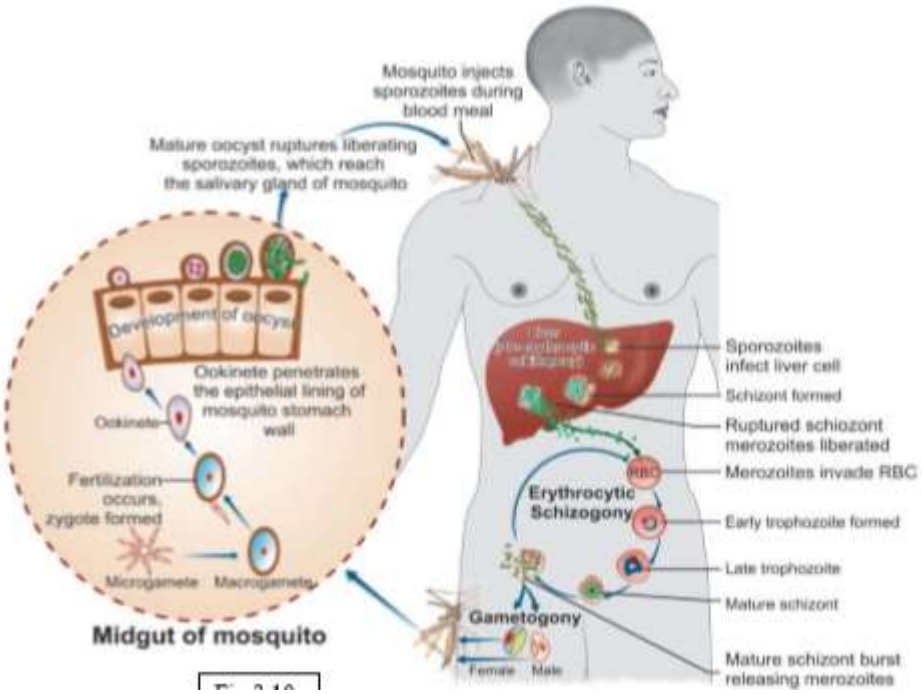
- Human as intermediate hosts-asexual reproduction takes place
- Female Anopheles mosquitoes are definitive hosts-sexual reproduction takes place.

- Male anopheles mosquitoes never take blood because

(1) They rely on flower nectars to feed on

(2) Their proboscis are blunt so that cannot pierce human skin.

Therefore, it is only the female one which is responsible for the transmission of malaria from infected person to non-infected person.

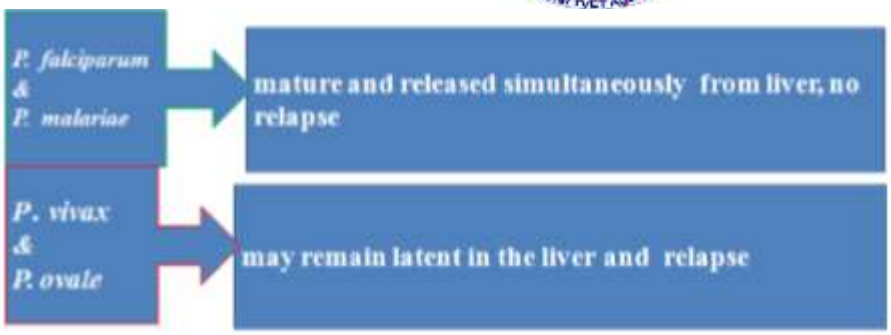


Fig, 1.43. ***Life cycle of plasmodium spp.***

Development in the Mosquito (Sexual Development and Sporogony)

This developmental stage is shown in detail in Fig. 9.**17b** and will only be described briefly here. In the mosquito midgut, each microgamont develops into (in most cases) eight uninucleate, flagellate microgametes and the macrogamont is transformed into a macrogamete → fusion of a microgamete and macrogamete to form a motile zygote (ookinete) → the ookinetes occupy the space between the epithelial layer and basal membrane of the midgut → morphological transformation into oocysts (40–60 µm) → in oocyst nuclear proliferation and production of thousands of sporozoites → sporozoites emerge into the hemolymph and migrate through the body cavity to the salivary glands, from where they can be transmitted to a new host. The duration of the cycle in the mosquito depends on the plasmodial species and the ambient temperature; at 20–28 °C, it takes eight to 14 days.

Note that:



The major clinical manifestations of malaria are as follows:

Clinical Complications of Malaria

P. falciparum
1. Cerebral coma
2. Anemia
3. Pulmonary edema
4. Renal Failure
5. Shock
6. Lactic acidosis
7. Hypoglycemia
8. Tropical splenomegaly
9. Pregnancy
   a. Maternal death
   b. Stillbirth
   c. Low birth weight
   d. Anemia

P. vivax (P. ovale)
1. Splenic rupture
2. Anemia (mild)
3. Debilitating fevers
4. Higher TNF-α per parasite

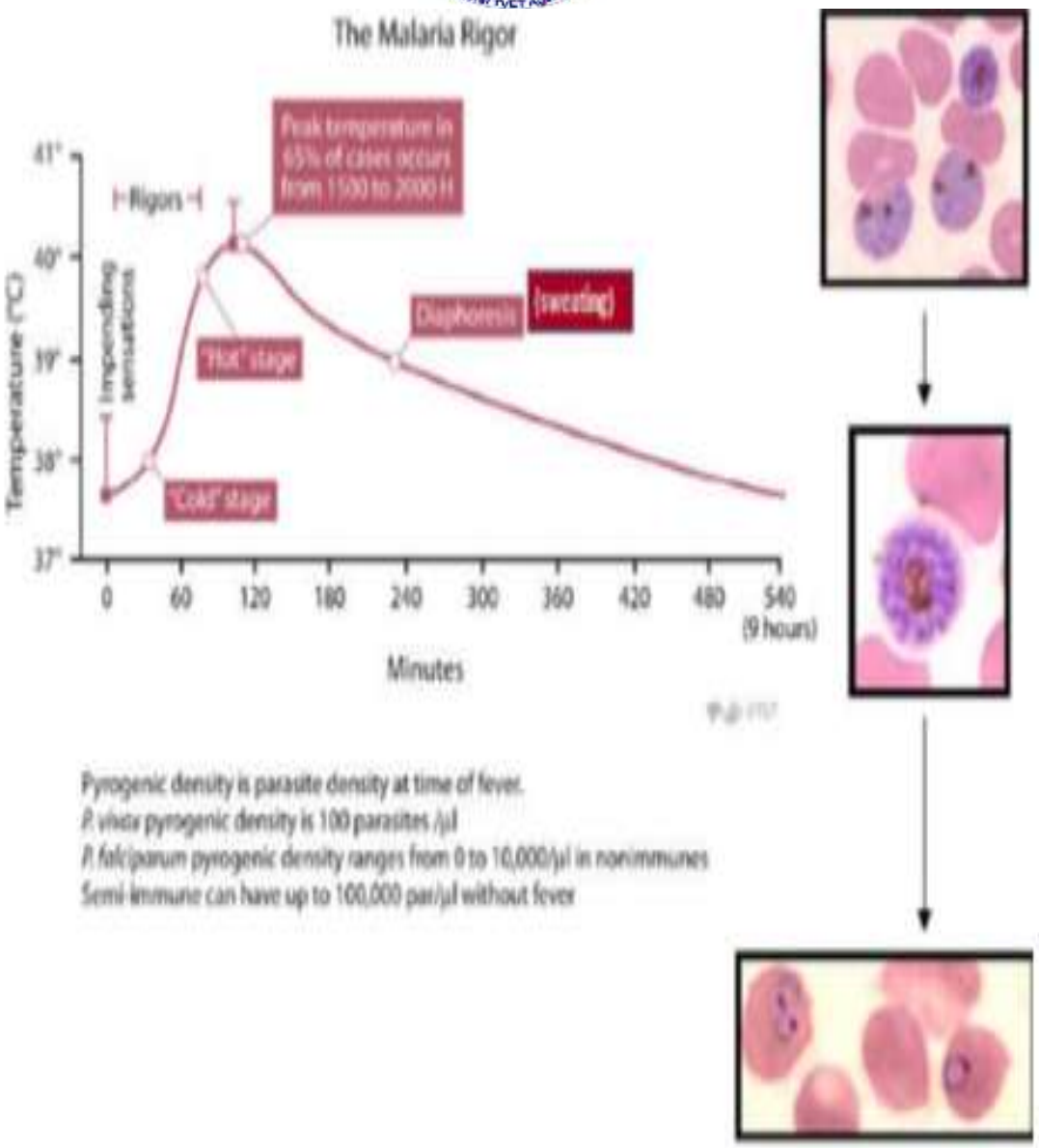
P. malariae
1. Immune complex
2. Glomerulonephritis, leading to nephrotic syndrome

Malaria paroxysm preceded by prodromal period from 2-3 days before 1stparoxysm. This includes: malaise, fatigue, headache, muscle pain, nausea, anorexia (i.e., flu-like symptoms) can range from none to mild to severe.

When generalized, the manifestations can be of three alternative episodes:

- Prodromal Symptoms (the cold stage, hot/heat stage and the sweating stage).

This cab ne presented by the following graph:



1. The cold stage: manifestations of cold and shivering for some minutes to an hour.
    - feeling of intense cold
    - vigorous shivering, rigor
    - lasts 15-60 min
2. Hot stage
    - feeling of intense heat
    - dry burning skin will be observed
    - severe headache
    - Lasts 2-6 hours

3. Sweating stage: this is the final stage when our body takes action to equilibrate the internal and external temperature by reducing excess heat via sweating. It is called homeostasis. **Symptoms include:**
    - profuse sweating, declining temperature, exhausted, weak
    - sleep which lasts for 2-4 hours Among malaria parasites known to infect human, P. falciparum is the dangerous one to kill human beings on the earth. The following shows its complications.

Severe Falciparum Malaria

Complications	Features Indicating Poor Prognosis
➢cerebral malaria	➢impaired consciousness
➢blackwater fever	➢repeated convulsions
➢anemia	➢respiratory distress
➢hypoglycemia	➢shock
➢GI and liver syndromes	➢acidosis/hyperlactemia
➢pulmonary edema	➢hypoglycemia
➢algid malaria (shock)	➢jaundice or other liver malfunctions
	➢renal impairment
	➢high parasitemia (>500,000/mm^3)

The rest species are not life threatening and some are self-limiting.

Table 4. Comparison of the characteristics of plasmodia causing human malaria

	P. vivax	*P. falciparum*	*P. malariae*	*P. ovale*
Hypnozoites	Yes	No	No	Yes
Erythrocyte preference	Reticulocytes	Young erythrocytes, but can infect all stages	Old erythrocytes	Reticulocytes
Stages found in peripheral blood	Rings, trophozoites, schizonts, gametocytes	Only rings and gametocytes	As in vivax	As in vivax
Ring stage	Large, 2.5 µm, usually single, prominent chromatin	Delicate, small, 1.5 µm, double chromatin, and multiple rings common, Accole forms found.	Similar to vivax, but thicker	Similar to vivax, more compact
Late trophozoite	Large irregular, actively amoeboid, prominent vacuole	Compact, seldom seen in blood smear	Band form characteristic	Compact, coarse pigment
Schizont	Large filling red cell	Small, compact, seldom seen in blood smear	Medium size	Medium size
Number of merozoites	12–24 in irregular grape-like cluster	8–24 grape-like cluster	6–12 in daisy-head or rosette pattern	6–12 irregularly arranged
Microgametocyte	Spherical, compact, pale blue cytoplasm, diffuse nucleus	Sausage or banana-shaped pale blue or pink cytoplasm, large diffuse nucleus	As in vivax	As in vivax
Macrogametocyte	Large, spherical, deep blue cytoplasm, compact nucleus	Crescentic, deep blue cytoplasm, compact nucleus	As in vivax	As in vivax
Infected erythrocyte	Enlarged, pale, with Schuffner's dots	Normal size, Maurer's clefts, sometimes basophilic stippling	Normal, occasionally Ziemann's stippling	Enlarged, oval fimbriated, prominent Schuffner's dots
Duration of schizogony (days)	2	2	3	2
Prepatent period (days)	8	5	13	9
Average incubation period (days)	14	12	30	14
Appearance of gametocyte after parasite patency (days)	4–5	10–12	11–14	5–6
Duration of sporogony in mosquito (25°C) (days)	9–10	10–12	25–28	14–16
Average duration of untreated infection (years)	4	2	40	4

Laboratory diagnosis Techniques: two methods are routinely performed for malaria diagnosis:

1. Microscopy examination: Collection of Blood Specimen: Collect sufficient quantity of blood (50 µl or one drop of finger prick blood). The collection sites include:
    - Capillary blood from finger prick , toes, or ear lobes are best ones
    - Venous blood.(EDTA anticoagulant)
    - In obstetric practice, cord blood and placental impression smears can be used

    Time of collection
    - Collect when the patients feels febrile.
    - Collect before anti-malaria drugs are given to the patients
    - Note: that a negative test DOES NOT rule out malaria and repeated tests are recommended in all doubtful cases
    - **Thin and thick blood film**s can be prepared.

Immuno-chromatographic tests for malaria antigens

- Are based on the capture of the parasite antigens from the peripheral blood
- Uses either monoclonal or polyclonal antibodies against the parasite anti targets.
- RDTs do not require a laboratory, electricity, or any special equipment.
- **Targets**
  1. Histidine-rich protein 2 of *P. Falciparum,*
  2. Pan-malarial *plasmodium* aldolase, and
  3. Parasite specific lactate dehydrogenase (pldh)

1. Histidine-rich protein of P. falciparum (PfHRP2): – It is a protein produced by the asexual stages and gametocytes of P. falciparum which remain in the blood for at least 28 days after the initiation of antimalarial therapy. Thus, detection of this protein from patient's blood may not indicate current infection. Because this proteins are released from dead RBCs and parasites.
2. Parasite lactate dehydrogenase (pLDH)
   - Is a soluble glycolytic enzyme produced by the asexual and sexual stages of the live parasites
   - Present in and released from the parasite infected erythrocytes
   - Found in all 4 human malaria species, and different isomers of pLDH for each of the 4 species exist. With pLDH as the target, a quantitative immuno-capture assay
3. Plasmodium aldolase
   - Is an enzyme of the parasite glycolytic pathway
   - Expressed by the blood stages of P. Falciparum as well as the non-falciparum malaria parasites
   - Monoclonal antibodies against plasmodium aldolase are pan-specific in their reaction
   - Have been used in a combined 'P.F / P.V' ICT test that targets the pan malarial antigen (PMA) along with pfhrp2

2. RDTS general Procedures 50 µl of blood from finger prick(one drop) is required.

   A blood specimen is mixed in a buffer. The labeled Ag – Ab complex migrates up the test strip.

- Labeled antibodies pre deposited –
- Finger prick blood is mixed with buffer solution with haemolysing compound & specific antibody
- If the target Antigen is present, antigen /antibody is formed
- Antigen –antibody complex migrates up the test strip by capillary action towards test specific reagents, which have been pre-deposited during manufacture –
- Buffer added to wash hemoglobin and permit visualization of any colored line on the strip
- Performance of the test is assessed in divers clinical situation:
- some RTD detect two parasites
- Advantages over microscopy:
    - Simpler to perform & interpret
    - Do not require electricity, special equipment & training in microscopy
    - Community volunteers can be taught the procedure in hrs.
    - RDTs detect circulating antigen may detect Pf antigens even when the parasites are sequestered
- Disadvantages over microscopy:
    - Highly sensitive, false positive result
    - Cannot detect density of the parasite
    - Expensive
    - Positive for circulating antigens
    - Not quantitative method, doesn't tell us the number of parasites
    - Do not differentiate between species: P. vivax , P. ovale P. malariae
    - Specific to malaria parasite only, cannot detect other haemoparasites

Prevention and Control

- Treatment of infected individuals
- Use of bed nets

- Use of Personal repellents and use of insecticides and Health education

Key points of *Plasmodium* and *Babesia*

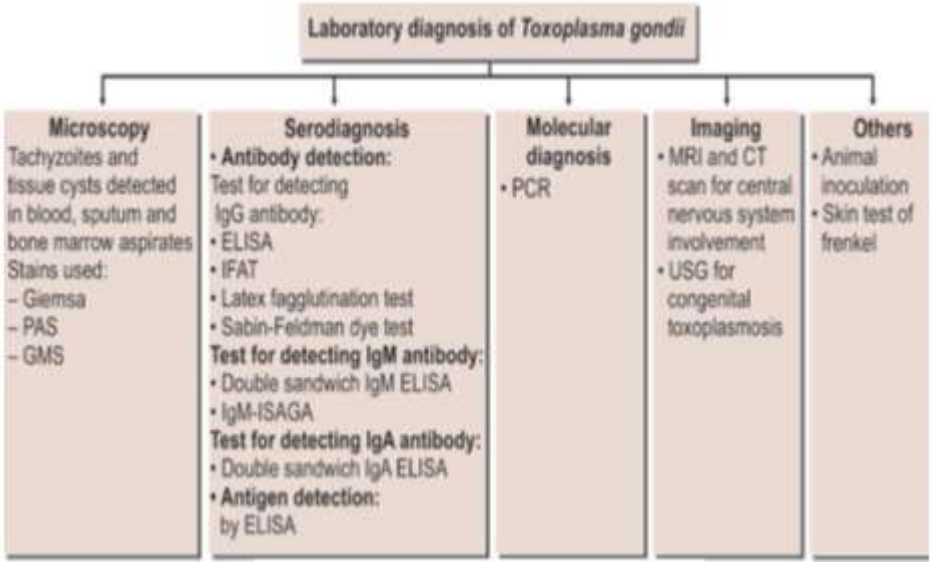
- **Malaria parasite** belongs to the genus *Plasmodium*.
- Four species of *Plasmodium* cause malaria in man—*P. vivax*, *P. falciparum*, *P. malariae*, and *P. ovale*.
- **Definitive host:** Anopheles mosquito (sexual phase of life cycle)
- **Intermediate host:** Man (asexual phase of life cycle)
- **Infective form**: Sporozoites present in salivary gland of mosquito.
- *P. vivax* and *P. ovale* cause benign tertian malaria, *P. falciparum* causes malignant tertian malaria and *P. malariae* causes benign quartan malaria.
- Acute falciparum malaria is the most dangerous and fatal form and is due to heavy parazitization of RBCs which cause blockage of capillary and venules by cytoadherence.
- **Clinical features:** Typical picture of malaria consist of periodic bouts of fever with rigor followed by anemia and splenomegaly. Febrile pasoxysms comprise of cold stage, hot stage, and the sweating stage.
- TSS is a chronic benign condition resulting from abnormal immunological response to malaria.
- Relapse of malaria occurs in *P. vivax* and *P. ovale* infection due to persistence of dormant stage hypnozoites in liver. Recrudescence occurs commonly in *P. falciparum* and *P. malariae* due to persistance of parasite in circulation at a subclinical level.
- **Diagnosis**: By demonstration of parasite in thick and thin smear of peripheral blood and also by detection of malaria antigen by rapid ICT.
- **Treatment**: Chrooquine sulfadoxine, and pyrimethamine along with primaquine. In chlroquine resistance, quinine or artemesinin are used.
- *Babesia species* comprising *B. microti*, *B. divergens*, and *B. bovis*, are intraerythrocytic sporozoan parasite resembling plasmodia. They cause opportunistic infections in humans.
- **Mode of transmission:** Through bite of Ixodid ticks.
- **Reservoirs:** Rodents and cattle.
- **Clinical features**: Mild and self-limiting. In immunocompromised patients, it causes anemia, jaundice, hemoglobinuria, respiratory failure, etc.
- **Diagnosis**: By examination of stained blood films for intraerythrocytic parasites, reticulocytosis, increased SGPT, alkaline phosphatase, hemoglobiniuria.
- **Treatment**: Atovaquine + azithromycin. Alternatively, clindamycin and quinine may be given.

4.20.7. Toxoplasma gondii

Toxoplasma gondii is the causative agent of a zoonosis that occurs worldwide with high prevalences (up to 80% depending on region and age). Humans are infected by ingesting oocysts excreted by the definitive hosts (cats) or by eating unprocessed meat containing *Toxoplasma* cysts. If a women contracts toxoplasmosis for the first time during pregnancy, diaplacental transmission of the pathogen to the fetus is possible with potential severe consequences (for example malformations, eye damage, clinical symptoms during childhood). There is, however, no risk to the fetus from mothers who had been infected before their first pregnancy and have produced serum antibodies (about 35–45%). Latent infections can be activated by immunodeficiencies (e.g., in AIDS patients) and may result in cerebral or generalized symptomatic toxoplasmosis. Serological surveillance in pregnant women is important to prevent prenatal infections.

Key points of *Toxoplasma gondii*

- Obligate intracellular parasite.
- Exists in 3 forms: trophozoite, tissue cyst, and oocyst.
- **Definitive host:** Cat family (enteric cycle).
- **Intermediate host:** Human (exoenteric cycle).
- Human infection occurs by ingestion of food containing oocyst and tissue cyst.
- Congenital infection can also occur.
- **Clinical feautes:** Acute encephalopathy, fever, chorioretinitis, lymphadenopathy, myocarditis, hepatosplenomegaly.
- Disseminated infection in AIDS.
- **Diagnosis:** By demonstration of parasite in tissue specimen, ELISA, IFAT, Sabin-Feldman dye test IgM-ISAGA.
- **Treatment:** Congenital infection is treated with pyrimethamine and sulfadiazine. For primary prophylaxis Trimethoprim-sulfamethoxazole is the drug of choice



Flow chart 1.8. Laboratory diagnosis of T.gondii

4.20.8. Isospora

The causative agent of human isosporiosis is *Isospora belli*. After peroral ingestion of sporulated oocysts and release of sporozoites, further development (schizogony, gamogony) takes place in the epithelium of the upper small intestine, leading finally to oocyst formation. In AIDS patients, encysted sporozoites have been found in various extraintestinal organs (lymph nodes, liver, gallbladder, spleen).

I. belli can cause severe clinical symptoms, especially in AIDS patients, for example persistent diarrhea, steatorrhea, cholecystitis, weight loss, and fever. Diagnosis is made by detection of unsporulated oocysts (20–30 µm long) in stool ( ) or of developmental stages in intestinal biopsies. High-dosed cotrimoxazole is the recommended therapy.

Key points of *Cryptosporidium parvum*

- Sexual and asexual cycle in single host.
- **Infective form:** Sporulated oocyst in food and water.
- **Clinical features:** Self limited diarrhea with abdominal pain in healthy persons. Chronic persistent watery diarrhea in immunocompromised hosts.
- **Diagnosis:** Demonstration of round oocyst in stool by direct microscopy, fluroscent microscopy, and modified acid-fast stain.
- **Treatment:** Supportive therapy with electrolytes and fluids and early antiretroviral therapy in AIDS patients.

4.20.9. Microspora

Genus	Species	Habitat and infection caused
Enterocytozoon	*E. bieneusi*	Small intestine epithelium (leading to diarrhea, and wasting). Also found in biliary tract of patients with cholecystitis. Rarely spreads to respiratory epithelium
Encephalitozoon	*E. intestinalis*	Small intestine epithelium (causing diarrhea and wasting). Also causes sinusitis, cholangitis, and bronchiolitis
	E. hellem	Conjuctival and corneal epithelium (causing keratoconjuctivitis). Also causes sinusitis, respiratory tract disease, and disseminated infection
	E. cuniculi	Small intestine epithelium (causing diarrhea). Corneal and conjuctival epithelium (causing keratoconjuctivitis). Rarely, may cause hepatitis and renal infection
Pleistophora	*P. ronneafier*	Skeletal muscle (causing myositis)
Brachiola	*B. vesicularum* *B. connori*	Skeletal muscle (causing myositis) Muscles (smooth and cardiac)
Trachipleistophora	*T. hominis* *T. anthropophtheria*	Cornea and conjuctival epithelium (leading to keratoconjuctivitis). Also causes myositis Brain
Vittaforma	*V. corneae*	Corneal stroma (causing stromal keratitis)
Nosema	*N. ocularum*	Corneal stroma (causing stromal keratitis)
Microsporidium	*M. ceylonensis* *M. africanum*	Corneal stroma (causing stromal keratitis)

Self-check 4	Multiple choice

Directions: choose the best answer for all the questions listed below. Use the Answer sheet provided in the next page:

1. One is **not true** characteristic of helminthes:
   A. Majority occur in parasitic forms larva, eggs or adults
   B. They are metazoans
   C. diagnosis is by detecting
   D. life cycle simple or complex
2. Which of the following is/are not class of helminthes?
   A. Cystodes
   B. Nematodes
   C. Trematodes
   D. None
3. The large intestine can be infected by adult stage of _____and _____.
   A. Trichuri trichuria
   B. Hook worm
   C. Ascaris lumbricoids
   D. Loa loa
4. For which of the following parasites the larval stage undergo heart lung migration.
   A. Trichuri trichuria
   B. Entribium vermicularis
   C. whip worm
   D. none
5. One of the following **doesn't** belong to geo helminthes:
   A. Ascaries lumbricoids
   B. Strongliodes stercoralis
   C. Hook worm
   D. Entribium vermicularis
6. Egg stage are extremely resistant to adverse environments and chemicals, and remain viable in soil and dust for up to 10 years for which nematode?
   A. Ascaries lumbricoids
   B. Strongliodes stercoralis
   C. Hook worm
   D. Entribium vermicularis
7. An infective stage for hook worm is;
   A. Rhabidity form larva
   B. Filariform larva
   C. embriyonated eggs
   D. adult
8. One of following is hematophagous:
   A. S.stercolaries
   B. A. lumbricoides
   C. T. trichuria
   D. pinworm
9. Egg stage **is not** a diagnostic stage for one of the following:
   A. *S.stercolaries*
   B. A. lumbricoides
   C. T. trichuria
   D. pinworm
10. Diagnostic stage for *S.stercolaries* is:

A. Rhabidity form larva C. embriyonated eggs
B. Filariform larva D. adult

11. Close relationship between two different organisms from which one benefits and the other neither benefits nor suffers.

A. Commensalism B. Mutualism C. Parasitism D. Relationship

12. Any animal that carries a parasite that can cause infections in humans

A. Intermediate host B. Definitive host C. Reservoir host D. None

13. Sources of Exposure to most intestinal parasitic Infections is though

A. Direct skin penetration B. Sexual intercourse C. Feco-oral route D. Blood transfusion

14. Vertical Direct Mode of Transmission of the parasite is from the mother to child through:

A. Direct skin penetration C. Congenital
B. Sexual intercourse D. Blood transfusion

15. Which of The following is **not** the reason to study Life cycles of Parasites?

A. Control D. Fundamental research
B. Treatment E. None
C. Epidemiology

16. Which of The following is not Parasite factors to cause the disease

A. Strain of the parasite to human host C. Metabolic processes of the parasite
B. Parasite load Site D. None

17. How do Parasites Cause Inquiry to their Host?

A. Competition for the host's nutrients C. Toxins and secretions
B. Destruction of host tissues andTissue changes D. ALL

18. Which of the following is the infective stage of plasmodium to human?

A. Gametocyte C. Merozoite
B. Sporozoite D. trophozoite

19. Which of the following an infective stage of plasmodium to female anopheles mosquito?

A. Gametocyte C. Merozoite
B. Sporozoite D. trophozoite

20. Which of the following are not the basics properties of protozoan?

A. Movement B. Feeding C. Reproduction D. None

21. Trophozoites stage of the protozoa is?

A. Changed to vegetative form C. Resístant form

B. Commonly the pathogenic form D. None

22. Cysts ingested by the definitive host has to be changed to vegetative form is ?

A. Excystation B. life cycle C. Encystation D. Resístant form

23. Which of the following are not properties of Amoebic Trophozoites State?

A. Non-Motile C. Multiplying form

B. Actively feeding D. susceptible to destruction

24. Is the trophozoite which emerges from the cyst?

A. Encystation B. Excystation C. Metacyst D. Pre-Cyst

25. Finger like structure used for amoeboid motility in fresh warm stool specimen is ?

A. Cilia B. Flagella C. Pseudopodia D. Gilding

26. Transmission of Entamoeba histolytica is by Ingestion of -

A. Cyst from B. immature cyst from C. mature cyst from D. None

27. The Most common form of extra intestinal amebiasis Primarily infects….

A. Kidney B. Liver C. Lung D. Spleen

28. The infective stage of schistosomia is:

A. Cercaria C. rabidity form larva

B. Filariform larva D. eggs

29. A definitive host for plasmodium is

A. *Human* C. tsetse fly

B. *Female anopheles mosquito* D. sand fly

30. Which of the following exists only in trophozoite form?

A. *G. lambalia* C. *T. vaginalis*

B. *Leshmania* D. plasmodium

31. Which of the following is a diagnostic stage for the leshmania?

A. Promostigote C. epimastigote

B. Amastigote D. metamastigote

32. Which of the following stage doesn't exist for trypanosomes?

A. Promostigote C. epimastigote

B. Amastigote D. metamastigote

33. The most prevalent form of malaria in Ethiopia is caused by

A. P. vivax

B. P. malariae

C. P. ovale

D. P. falciparum

34. The most common prevention and control method of vector borne disease is/are:

A. Treating infected individuals C. vector control

B. Health education D. all

35. "Falling leaf" motility in fresh warm stool specimen is Trophozoite stage of?

A. *Giardia lamblia* *C.Trichomonas hominis*

B. *Enteromonas hominis* D. *Trichomonas tenax*

36. Which of the following are TRUE about *Giardia* transmition is?

A. ingestion of fecally contaminated food or water with cyst
B. Person to person
C. sexually
D. All
E. None

37. Which of the following is TRUE about susceptiblelity of *Giardia* Cysts are susceptible to…

A. 1% sodium hypochlorite C. ammonium disinfectants

B. 2% glutaraldehyde or quaternary D. boiling water

38. Which of the following is associated with the more chronic stage of *Giardia?*

A. Vitamin B12 malabsorption

B. Disaccharidase deficiency

C. Lactose intolerance

D. All

39. Which of the following is drug used for the Treatment *Giardia* is?

A. Mebindalzole B. Metronidazole C. Metazole D. ALL

40. *Trichomonas vaginalis* trophozoite stage transmitted by Which of the following ways:

A. sexual intercourse
B. communal bathing, sharing of wash clothes
C. toilet equipment seats
D. mother to child during birth
E. ALL

41. Common mode of transmission of *Leishmaniasis* is by Bite of infected female:

A. Mosquito B. Tsetse fly C. Sand fly D. Bean E. None

42. Which of the following is the diagnostic stage of trypanosomes is?

A. Promastigotes
B. Amastigotes
C. Epimastigote
D. Trypomastigote
E. Metacyclic Trypomastigote

43. Which of the following drugs are used for the Treatment of *Leishmaniasis?*

A. Sodium stibogluconate (Pentostam)
B. Pentamidine isethionate
C. amphotericin B
D. cryotherapy and thermotherapy
E. ALL

44. Common mode of transmission of African Trypanosomiasis is by Bite of infected …

A. Mosquito B. Tsetse fly C. Bug D. Sand fly E. None

45. Common mode of transmission of American Trypanosomiasis is by Bite of infected…

A. Mosquito B. Tsetse fly C. Bug D. Sand fly E. None

46. The developemental form of trypanosomiasis which is found in blood vessels & intercellular spaces of Lymph nodes, spleen, liver, Brain, CSF of vertebrate host is?

A. Promastigotes
B. Amastigotes
C. Epimastigote
D. Trypomastigote
E. Metacyclic Trypomastigote

47. The developemental form of trypanosomiasis which is found in the mid and fore gut of the tsetse flies is?

A. Promastigotes
B. Procyclic trypomastigotes
C. Amastigotes
D. Epimastigote
E. Metacyclic Trypomastigote

48. The developemental form of trypanosomiasis which is seen in the salivary gland of tsetse flies is?
    A. Procyclic trypomastigotes
    B. Promastigotes
    C. Epimastigote & Metacyclic trypomastigotes
    D. Amastigotes

49. During a blood meal on the mammalian host, an infected tsetse fly (genus Glossina) injects which stage of trypanosomia into skin tissue is?
    A. metacyclic trypomastigotes
    B. amastigotes
    C. Epimastigote
    D. Promastigotes

50. The tsetse fly becomes infected with blood stream, when taking a blood meal on an infected mammalian host the stage of trypanosomia is?
    A. Trypomastigotes B. Amastigotes C. Epimastigote D. Promastigotes

Note: **Satisfactory rating - 25 points** **Unsatisfactory - below 25 points**
You can ask you teacher for the copy of the correct answers

Score = _____
Rating: _____

Answer sheet

No.	Answers	No.	
1		26	
2		27	
3		28	
4		29	
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6		31	
7		32	
8		33	
9		34	
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24		49	
25		50	

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Prepared By

No	Name	Educational Back grund	LEVEL	Region	College	Email	Phaone Number
1	Kalicha Boru	Laboratory	B	oromia	Nagelle HSC	boru9683@gmail.com	0912493885
2	Furo Beshir	Laboratory	A	Harari	Harar HSC	nebi.furo@gmail.com	0911739970
3	Motuma Chali	Laboratory	B	oromia	Nekemte HSC	lammiifcaalii@gmail.com	0938456753
4	Abdirahman Mahad	Laboratory	A	Somali	Jigjiga HSC	abdirahman7584@gmail.com	0911044715
5	Adisu Tesfaye	Laboratory	B	Somali	Jigjiga HSC	adistesfaye21@gmail.om	0931747320
6	Kebebe Tadesse	Laboratory	B	BGRS	Pawi HSC	no	0926841290
7	Tagel Getachew	Laboratory	A	Harari	Harar HSC	tagegetachew@gmail.com	0915746748