LECTURE NOTES

For Medical Laboratory Technology Students

Parasitology

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Preface

The problem faced today in the learning and teaching of Parasitology for laboratory technicians in universities, colleges, health institutions, training health centers and hospitals emanates primarily from the unavailability of textbooks that focus on the needs of Ethiopian students. This lecture note has been prepared with the primary aim of alleviating the problems encountered in the teaching of Medical Parasitology course and in minimizing discrepancies prevailing among the different teaching and training health institutions. It can also be used in teaching any introductory course on medical parasitology and as a reference material.

This lecture note is devoted to providing general aspects of parasitology in addition to covering human parasites in two major groups -the protozoa and helminths- including their distribution, habitat, morphology, life cycle, pathogenicity, prevention and control, laboratory diagnosis and their relevance to Ethiopia. It has also appendices, which discuss the collection of laboratory specimens, preservatives of stool sample, frequently used parasitological diagnostic methods and reagent preparation. Finally, it contains a glossary, which summarizes important terminologies used in the text. Each chapter begins by specific learning objectives and after each objective and after each class of parasites review questions are also included.

No systemic study has been conducted on the prevalence of human parasites in different ecological zones of Ethiopia but past surveys indicate the presence of all parasites except some that are found in the Far East, South East Asian and Latin American countries and which require specific intermediate hosts. This lecture note tries as far as possible to summarize local literatures that deal with parasite prevalence

in Ethiopia so that it may address itself particularly to the needs of Ethiopian students.

We welcoming the reviewers and users input regarding this edition so that future editions will be better.

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Contents

Overview

The earliest book devoted to Parasitology was published in 1684 by Redi, who provided descriptions of reproductive organs and eggs of *Ascaris*. In 1817 Lancisi recorded studies of mosquitoes and Vague Surmises about their role in the cause of intermittent fevers. Goldfuss, in 1817, first used the word ''protozoa,'' which was given modern meaning in 1845 by Siebold, Leeuwenhoek (1632-1723) had devised and used simple microscopes, but achromatic objectives in a compound microscope were not used in England until 1824. Gross, in 1849, was the first to describe an amebic parasite in man *Entamoeba gingival* and Losch identified *E. histolytica* in 1875. Then came the discovery of mosquito hosts for filariae by Manson (1877 -1878) and *Plasmodia* by Laveran in 1880, transmission of babesiosis by ticks by Smith and Kilburne in 1894, trypanosomes and their transmission by tsetse flies by Bruce (1895 - 1896), and mosquito transmission of plasmodia by Ross (1897- 1898).

After these discoveries, the science of parasitology expanded rapidly. With the aid of microscopes, morphological characters of various parasites were first studied and species and group characteristics were determined. Then several stages of the organisms were related to one another in life-cycle sequence. This provided important information on extrinsic as well as intrinsic development and paved the way for epidemiologic studies. Moreover, the relationship between parasite to host provided a background for studying the pathogenesis of infection in man and reservoir hosts and, indirectly, for understanding clinical aspects.

Recent investigations have been largely concerned with the ecology of parasitic infections, anatomy and physiology as revealed by electron microscopy, metabolism of the parasite in its host, immunologic phenomena, and the rationale of chemotherapy. Meanwhile, practical methods are being developed to control these infections and to reduce human exposure to them.

A landmark of great significance was the publication in 1978 of a two-volume work by B.H. Kean, K.E. Mott and A.J. Russell, which brought together in English translation as the articles on the major discoveries and earliest descriptions by workers in tropical medicine and parasitology. Having set the historical background; now we will see the importance and the scope of Parasitology.

Parasitology is a science that deals with an organism that lives in or on another organism in order to have shelter and /or nutrition. Medical parasitology study parasites that is capable of causing disease in humans. In the context of this ecture note, the term parasite refers to organisms, which belong to protozoa (i.e., Rhizopoda, flagellets ciliates, sporozoans, coccidians and Microsporidian) Helminths (Nematodes, Cestodes and Trematodes).

Human welfare has suffered greatly because of parasites. A lot of economic loss occurs as a result of infection of domestic animals by parasite, which causes diseases such as fascioliasis and trypanosomiasis.

The study of parasitology has more importance to developing countries where the social and economic conditions require great deal of improvement in terms of better clothing, shelter, food, provisions of wells

and latrines and sewages and other waste disposal facilities together with the means of controlling vectors.

Most of the developing countries lie within the tropics. The tropical or the semi-tropical nature of places where most of the people of the developing nations live not only provides better environmental conditions for larval development of parasites than that of temprate regions where most of the developed countries are found, but also provides better conditions for the multiplication of vectors.

This lecture note will discuss specific parasite of medical importance, including the information necessary to assist in the diagnosis.

General Objectives

- Understand the concepts of parasitism, the relationships between parasites and hosts,

  between parasites and environment and the cultural and socioeconomic factors affecting the transmission of parasites
- Know the general epidemiological aspects of parasites that affect human
- Apply simple preventive measures for specific parasites
- Know the life cycle of specific parasites and identify the important parasitic agents affecting human health
- Be able to prepare reagents necessary for parasitology lab.
- Use effectively the basic laboratory equipment
- Apply the necessary procedures for the diagnosis of parasites in the medical laboratory and reporting of results properly
- Apply the basic methods of specimens collection, preservation and processing

  Keep up the basic laboratory safety regulation

CHAPTER ONE

Introduction

Specific Learning Objectives

At the end successful student will be able to:

1. Identify organisms which parasitizes man
2. Define common terms used in Medical Parasitology
3. List the various environmental, cultural and socioeconomic factors that affect the distribution of parasites
4. Explain effect caused by parasites
5. Describe the classification and characteristics of parasite groups
6. Explain mode of transmission, source of infection, and portal of entry of parasites

1.1 Definition of Terms Used in Parasitology

Parasitology:- is a science that deals with parasites.

Medical Parasitology:- Is the study of parasites that causes disease in man.

Parasite:- is an organism living temporarily or permanently in or on another organism (host) from which is physically or physiologically dependant upon other.

Nature of Parasites- A parasite could be unicellular, worm or an arthropode.

Features of Parasites

1. Smaller than their host,
2. Outnumber the host,

3. Short life span than their host, and
4. Have greater reproductive potential than their host.

Association of Organisms

When there is an association between two organisms their relation will be one of the following type:

1. ***Mutualism*;-** Mutual benefit is derived from the association.
2. ***Symbiosis:-*** Permanent association between two different organisms, so dependant on each other, that their life part is impossible.
3. ***Commensalism:-*** When the parasite benefited from the host while the host neither benefited nor harmed.
4. ***Parasitism:-***One organism live at the expense of the other, The later usually suffers from the association.

Parasites can be Classified:-

I. According to their habitat:

1. *Ectoparasites:* parasites living on or affecting the skin surface of the host. E.g. lice, tick, etc.
2. *Endoparasites*: Parasites living within the body of the host. E.g. *Leishmania* species, *Ascaris lumbricoides, etc.*

II. According to their dependence on the host:

1. *Permanent (obligate) parasites:* The parasite depends completely upon its host for metabolites, shelter, and transportation. This parasite can not live outside its host. E.g. *Plasmodium* species, *Trichmonas vaginalis, etc.*

2. *Temporary (facultative) parasite***:** The parasite is capable of independent existence in addition to parasitic life. E.g. *Strongyloids stercolaris, Naegleria fowleri, etc.*

III. According to their Pathogenicity:

1. *Pathogenic parasites:-* It causes disease in the host.
   E.g., *E. histolytica*
2. *Non-Pathogenic (commensal) parasite**:-***The parasite derives food and protection from the host without causing harm to the host. E.g. *Entamoeba coli*
3. *Opportunistic parasites**:-*** Parasites which cause mild disease in immunologically healthy individuals, but they cause severe disease in immuno-deficient hosts.
   E.g. *Pneumocystis carnii, Toxcoplasma gondii, Isospora belli*

Host :- Hosts are organism which harbors the parasite.

Types of Hosts:-

1. ***Definitive host:-*** Depending on the parasitic species, *it is either* a host which harbors the adult stage of a parasite or most highly developed form of the parasite occurs; or sexually mature stages of a parasite and fertilization takes place in it, e.g., man is the definitive host of *Taenia saginata*. When the mature or most highly developed form is not obvious the definitive host is the mammalian host, e.g., human is the definitive host for trypanosomes that cause African trypanosomiasis.
2. ***Intermediate host****:-* Is a host harboring sexually immature or larval stage of a parasite and in which no fertilization takes place in it.
   E.g. Cow is the intermediate host for *Taenia saginata*

Amplifier host- Intermediate hosts in which parasites under go multiplication.

3. ***Reservoir host***:- A wild or domestic animal which harbors a parasite and acts as sources of infection to humans.
4. ***Carrier host:-*** A host harboring and disseminating a parasite but exhibiting no clinical sign.
5. ***Accidental (Incidental) host:***- Infection of a host other than the normal host species. A parasite may or may not continue full development in this host.

Vector:- Any arthropod or other living carrier which transports a pathogenicmicroorganisms from an infected to non-infected host.

A. ***Biological vectors***:-Those vectors that complete the life cycle a parasite
E.g. *Anopheles* (Vector of *Plasmodium*), *Phlebotomus* (Vector of *Leishmania*), *Glossina* (vector of *Trypanosoma*), *Simulium* (Vector of *Onchocerca*), etc.

B. ***Mechanical (Parathenic or transport) Vectors***: They are passive carriers of parasites, not essential in the life cycle. E.g. House fly and Chocroach as a mechanical vector for Amoebae, *Giardia*, etc.

Diagnostic Stage:- A developmental stage of a pathogenic organism that can be detected in stool, blood, urine, sputum, CSF or other human body secretions.

Infective Stage:- The stage of parasite at which it is capable of entering the host and continue development within the host.

Infection:- Invasion of the body by any pathogenic organism (except)arthropods and the reaction of the hosts tissue to the presence of the parasite or related toxins.

Infestation:- The establishment of arthropods upon or within a host.

Zoonosis:- Diseases of animals. Today this term is applied for those diseases that are transmittable to man.

Biological Incubation (Prepatent) Period:- It is time elapsing between initial infection with the parasite and demonstration of the parasites or their stages in excreta, blood, aspirate and other diagnostic material.

Clinical Incubation Period:- It is the interval between exposure and the earliest manifestation or infestation.

Autoinfection:- An infected individual acts as a source for hyperinfection to himself.

Superinfection (Hyperinfection):- When an individual harboring the parasite is reinfected by the same parasite.

Retroinfection:- A retrograde infection caused by the newly hatched larva of *E. vermicularis* from the perianal region to reach the colon, where the adolescent form of the parasite develop.

1.2 Sources of Exposure to Parasitic Infections

A. ***Contaminated soil***:- Soils polluted with human excreta is commonly responsible for exposure to infection with *Ascaris lumbricoides, S.stercolaris, Trichuris trichuria* and hook worms.

B. Contaminated water:- Water may contain

(a) Viable cysts of Amoeba, flagellates and *T. solium* eggs,

(b) Cercarial stages of human blood fluke,

(c) Cyclops containing larva of *Dracunculus medinesis*,

(d) Fresh water fishes which are sources for fish tape worm, and intestinal flukes infection

(e) Crab or cray fishes that are sources for lung fluke and

(f) Water plants which are sources for *Fasciolopsis buski*.

C*. *Insufficiently cooked meat of pork and beef which contains infective stage of the parasite.

E.g., Trichenilla spiralis, *Taenia* species.

D. Blood sucking arthropods:-These are responsible for transmission of: e.g.,

1. Malaria parasites by female anopheles mosquito
2. *Leishmania* by phlebotomus
3. *Trypanosoma* by tsetse fly
4. *Wuchreria* by *Culicine* mosquito

E. Animals (a domestic or wild animals harboring the parasite), e.g.

1. Dogs are direct sources for human infection with the hydatid cyst caused by *E. granulosus* and cutaneous larva migrans caused by *Toxocara canis,*
2. Herbivores animals commonly constitute the source for human infection with *Trychostrongylus* species.

F*. *Human beings:-Another person his clothing**,** bedding or the immediate environment that he contaminated are directly responsible for all or a considerable amount of infection with a pathogenic amoeba *E. histolytica, E. vermicularis, H. nana* **.**

G. S**exual intercourse** :- e.g., Trichomonas vaginalis

H. Autoinfection **:-** e.g., *S. stercoralis, E. vermicularis,* and *T. solium*

1.3 Mode of Transmission

1.3.1 Direct mode of Transmission:-

The parasite dose not require biological vectors and/or intermediate hosts and require only a single host to complete its life cycle. It may use mechanical vectors for transmission.

Direct Mode of Transmission can be classified as:

I. **Horizontal Direct Mode of Transmission:** Transmission is mainly effected through:- Feco-oral route:

Most intestinal parasites transmitted in this way.

- Sexual intercourse
- Blood transfusion
- Direct skin penetration (soil transmitted helminthes)

II. ***Vertical Direct Mode of Transmission:***

Transmission of the parasite is from the mother to child through:

- Congenital / transplacental
- Transmammary (breast milk)

1.3.2 Indirect Mode of Transmission

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

1.4 Route of Transmission

The infective stage of the parasite may be transmitted in the following ways:

I. By ingesting infective stage of parasites:

1. In food, water or from hands that have been contaminated with faeces,
   E.g. *E. histolytica, E. vermicularis*
2. In raw or undercooked meat, e.g. *T. saginata, T. solium, T. spiralis*
3. In raw or undercooked fish, crab, or water vegetation e.g. intestinal flukes
4. Water containing Cyclopes e.g., *D. medinensis*

II. Penetration of Skin When in Contact with

1. Faecally polluted soil, e.g., *S.stercoralis*, Hook worms
2. Water containing infective stages of the parasite
   E.g., Cercaria of Schistosome species.

III. Through Insect Bite

e.g, filarial worms, *Trypanosoma* species, *Plasmodium* species , *Leishmania* species

IV. Sexual Contact, e.g., *Trichomonas vaginalis*

V. Transmammary, e.g., *S. stercoralis*

VI. Inhalation of contaminated air, e.g., E. vermicularis, *P. carnii*

VII. Transplacental, e.g., *T. gondii*

VIII. Kissing, e.g., *Trichomonas gingivalis, Trichomonas tenax*

1.5 Host Parasite Relationship

1.5.1 Effects of Parasites on their Hosts

A Parasite can affect the host in a number of ways such as:-

1. Consumption of the nutritive elements of the host
   E.g. Hookworm –sucks blood, *D. latum* selectively remove V B_{12}.
2. Obstruction of passages
   E.g., heavy infection with adult *Ascaris* may cause intestinal obstruction
3. Bleeding e.g. Schistosomes eggs
4. Destruction of tissues: e.g. Trophozoites of *E. histolytica* causes necrosis of liver, *Leishmania donovani* results marked destruction of marrow elements.
5. Compression of organs, e.g. Hydatid cysts in liver, brain cause pressure

6. Release of toxic substances, e.g., Rupture of *E. granulosus* cyst result anaphylactic shock
7. Opening path way to secondary infections e.g. Ulcer formed as a result of *D. medinensis* infection exposes to Bacterial, Viral infection
8. Allergy development, e.g., Bite of arthropode
9. Transmission of pathogens to man, e.g., lice transmitting *Rickettsia*
10. Predisposition to malignancy-e.g., Infection with bilharziasis predisposes to maliganacy
11. Chronic immune stimulation leading to unresponsiveness to infections.

1.5.2. Host Susceptibility Factors

Not all parasitic infection causes disease of clinical significance. Both host and parasitic factors are involved.

1.5.2.1 Host Factors

1. Genetic constitution
2. Age
3. Sex
4. Level of immunity: natural and acquired immunity.
5. Nutrition (malnutrition or under nutrition)
6. Intensity and frequency of infections
7. Presence of co-existing disease or conditions which reduces immune response. e.g. Pregnancy, HIV
8. Life style and occupation

1.5.2.2 Parasite factors

1. Strain of the parasite and adaptation to human host
2. Parasite load (number of parasite)
3. Site (s) occupied in the body

4. Metabolic process of the parasite, particularly the nature of any waste products or toxins produced by the parasite during its growth and reproduction.

1.5.3. Escape mechanism of parasite from the immune system

That parasitism is wide spread in almost all species of animals would imply that parasites have developed the capacity to escape or render ineffective the host internal defense mechanisms. Parasites can evade the host immune responses by variety mechanisms:

1. *Site*

Intracellular parasites as *T. cruzi*, *Leishmania* and the intracellular stage of *Plasmodia* are to some extent protected from the action of antibodies as are those forming cysts as *T. gondii* and larva of *T. solium*, *Echinococcus* and *Trichinella spiralis*.

Parasite living in macrophages as *Toxoplasma, T. cruzi* and *Leishmania* are able to avoid or inactivate the lysosomal enzymes, which are the cells weapons of offences against microbial organisms.

2. Avoidance of recognition :

This can be accomplished by:

2.1. Production of successive waves of progeny with different surface antigens
(i.e., variation of antigens) as in African trypanosomes.

2.2. Molecular mimicry: Certain parasites are recognized as self and consequently do not stimulate immunologic reactions in their host. Thus *Schistosome* worms are capable of masking their foreigners by acquiring a surface layer of host antigens which possibly protect them from antibody damage. These are called “eclipsed” antigens, since these antigens by resembling those of the host are not recognized as foreign and therefore are hidden

from the immune recognition. This phenomenon of antigen sharing between a parasite and a host is called Molecular mimicry.

3. *Suppression of immune response*:

Several parasitic species e.g, *Plasmodium*, *Toxoplasm*, *Trypanosoma* and *Trichinella* are able to suppress the ability of the host to respond immunologically. This sometimes, results in an increase in the severity of any viral or bacterial infection also present. Immuno-supression is due to production by the parasite of large quantities of soluble antigens which:

3.1. Combine with the antibody and preventing it from attaching to the parasite

3.2. Induce B or T-cell tolerance either by blocking antibody forming cells or by depleting the stock of mature antigen-specific lymphocites (clonal exhaustion).

3.3. Activating specific suppressor cells (T-cells or macrophages).

1.6 General Life Cycles of Parasites

1.6.1. Direct Life Cycle

A parasite that can complete its life cycle in a single host.

E.g., *S. stercoralis, Hook worms, G. lamblia, E,. histolytica*, etc.

1.6.2. Indirect Life Cycle:

When a parasite requires an intermediate host or vector to complete its development.

E.g., *Plasmodium* species*, Leishmania* species *, Taenia* species. etc.

1.7 Types of Specimen Used For Parasitological Examination

Stool *:-*e. g., intestinal nematodes, cestodes, trematodes and protozoa.

Blood :- e.g., Haemoparasites

Urine :- e.g., *S. hematobium, T. vaginalis,*

Sputum :- e.g., *P. westermani.*

Skin :- *e.g., L. aethopica, O. volvulus, D. medinensis* and *E. vermiculari*

Cerebro-Spinal fluid*:-* e.g., *Trypanosoma rhodisense* and *Naegleria fowleri.*

Bone marrow:- e.g., *L. donovani* and *T.gondii*

Lymphgland aspirates:- e.g *Trypanosoma rhodisense, L..donovani and T. gondii*

Liver aspirate *:*e.g.,*E.histolytica*, *L..donovani and T.gondii*

Spleen aspirate*:-* e.g *L..donovani and T.gondii*

Muscle biopsy*:-* e.g., *T. spiralis*

Rectal scraping*:-* e.g., Schistosoma species

Duodenal aspirate*:-* e.g., *G. lamblia, F. hepatica* and *S. stercoralis*

Bronchial biopsy *:-* e.g., *P.carnii*

Perianal swab:- e.g**.,***E.vermicularis*

1.8 Classification of Parasites

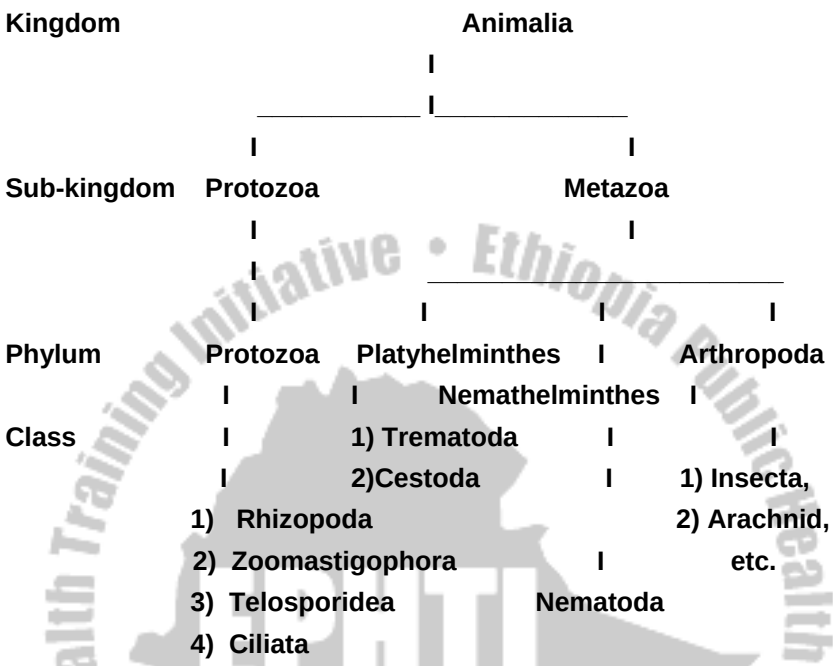
Nomenclature

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, consisting of a generic and a specific designation based on the International Code of Zoological Nomenclature. The first name in the binomial is that of 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 man belong to the kingdom of Animalia and most parasites are members of three phyla:

- Phylum Protozoa
- Phylum Platyhelminths and
- Phylum Nemathelminths.

Basic Classification of Parasites of Medical Importance



1.9 Major Differences between Parasitic Protozoa and Metazoa

Differences	Protozoa	Metazoa
1. Number of cells	Unicellular	Multicellular
2. Mode of multiplication	Asexual(withexception)	Sexual(with exception)
3. Infection caused by	Multiplication	Accumulation
4. Rate of Multiplication	Fast	Slow
5. Longivity	Short	Long

Review Questions

1. What is the difference between?
    a. Commenslalism and parasitism
    b. Obligate and temporary parasite
    c. Definitive and intermediate host
    d. Biological vector and mechanical vector
2. List the sources of exposure to parasite.
3. Mention possible parasites that are found in the following specimens
    a. Stool
    b. Blood
    c. Urine
    d. Sputum
    e. CSF
4. Identify the possible sources of specimen for the following parasites
    a. Schistosoma mansoni
    b. E. vermicularis
    c. Trypanosome species
    d. Filaria worms
    e. Giardia lamblia
5. Parasites that have indirect life cycle are more difficult to control. Why?

CHAPTER TWO

Medical Protozoology

Specific Learning Objectives

At the end, successful students will be able to

1. To recognize the general epidemiological aspects of protozoa.
2. Identify pathogenic and non-pathogenic protozoa.
3. Discuss the characteristics of each class of protozoan parasite in general and each parasite in particular.
4. Illustrate the life cycle of each parasite.
5. Apply the necessary procedures for the diagnosis of protozoan parasites and be able to identify them in the procedures used.

Introduction

Protozoa consists of a vast assemblage of single cell micro-organisms that are placed in the subkingdom, or phylum protozoa. They are made of a mass of protoplasm differentiated in to cytoplasm and nucleoplasm .The cytoplasm consists of ectoplasm and endoplasm. The ectoplasm function in protection, locomotion, ingestion of food, excretion, respiration. The endoplasm is concerned with metabolism. It contains the nucleus and many organelles. Reproduction and maintenance of life is performed by the nucleus. The protozoa of medical importance to humans include Amoebas, Flagellates, Ciliates, Coccidia, sporozoa and *Microsporidia*.

Many protozoan species are not pathogenic. However, they may be difficult to differentiate from pathogenic species. For this reason the laboratory technician must be familiar with characteristic of pathogenic as

well as non-pathogenic species. Protozoa may colonize or infect intestinal tract, pharynx, and the uro-genital tract of humans. The majority of this parasite belongs to the Amoeba or Flagellate; however infection with Ciliate, Coccidian or Microsporidian parasite may also be encountered. These organisms are generally of world wide distribution and almost are acquired by fecal- oral contamination. In review of stool specimens examined for intestinal parasite in United State, non pathogenic protozoa were detected in 10.8% of specimens. There is no such similar review in Ethiopia.

The protozoa of blood and tissues include the sporozoan parasites *Plasmodium, Babesia and Toxoplasma gondii*; the hemoflagellates *Leishmania* and *Trypanosoma*; and the free living amoeba *Naegleria* and *Acathamoeba*. *Pnumocystis carnii* is also included as a sporozoan tissue parasite, although it will likely be classified as fungus based on recent studies of ribosomal RNA sequence.

The major clinical manifestations of the protozoa causing blood stream infection (malaria and babesia) are secondary to the destruction of RBCs or slugging of infected RBCs in the microvasculature of the brain and other organs.

The protozoa causing tissue infections cause significant damage to specific organs such as the eyes (toxoplasmosis, acanthamoeba, keratitis), the brain (toxoplasmosis, amoebic meningoencephalitis, African sleeping sickness), the heart (toxoplasmosis, chagas disease),or gastrointestinal tract (chagas disease). *Pneumocystis carnii* primarily causes pneumonia; however invasion of other sites such as the eye have been reported.

As a parasite protozoa play a double role; they can attack man and cause disease or they can affect him economically by attacking domestic

animals. Malaria is still the world's most important disease. Trypanosomes have made many a grazing land in Africa inaccessible to livestock.

The general procedures utilized for diagnosis of the protozoa vary according to where the parasite is found in the body. The malarial parasites and blood or tissue flagellates (*Trypanosome* or *Leishmania)* are usually detected in stained smears of blood or tissue. In the case of the blood or tissue flagellates cultivation procedures and animal inoculations are often important tools. The intestinal and atrial parasites, with few exceptions, may be found in stool as a motile trophozoite stage or a non-motile, resistant cyst stages.

An increasing number of parasites are being associated with human immonodeficiency virus (HIV) / AIDS; where most of them belong to protozoa. They include:

- *Cryptosporidium parvum*, *I.bellie* and *Cyclospora catayenesis*, causing enterities with secretory diarrhea
- *Microsporidia* species causing a diarrhea with wasting, eye disease, and disseminated disease
- *Pneumocytis carnii*, causing life threatening pneumonia
- *Blastocystis hominis* which can cause severe enteritis

Other parasites causing opportunistic infections in those infected with HIV, include: *Leishmania* species – emerging as a major pathogens in HIV persons

- *T.gondii*, causing cerebral, toxoplasmosis
- *Acanthamoeba* species, causing ulceration of skin and infections in other tissues
- *T. cruzi* causing meningoencephalitis

2.1 Class Rhizopoda (Amoebae) (Rhiza = root, pod= foot) Amoebae

Protozoan parasite belongs to the class Rizopodea characteristically move by pseudopodia which present the organ of locomotion. Seven ameba, are belonging to the order amoebida, are found in man. One of them is found in the oral cavity and the remaining six species are found in the large intestine, these include: *Entamoeba histolytica, E.dispar, E. Coli, Endolimax nana, Iodamoeba butschlii and Entamoeba polecki;* of these only one, i.e. *E.histolytica* is pathogenic to man, *E. nana* and other amoebae may coexist in the large gut as commensals. *E. gingivalis* is commonly found carious teeth disease gum and tonsils.

All human intestinal amoebae have: 1) a trophozoite from which is motile organism, feed, and reproduce, and, 2) a cystic form which is the non-feeding, non motile, dormant stage of protozoa. Among amoeba, *E. gingivalis* has only a trophozoite from. The trophozoite stage consists of a shapeless mass of moving cytoplasm which is divided into granular endoplasm and clear ectoplasm. Digested food substances are stored as glycogen and chromatoid bodies. Amoeba reproduce asexually by simply dividing into two (binnary fission).

Before going into structural details here for each of them, their nuclear character for identification is considered:

E. histolytica, E. coli and the mouth ameba *E gingivalis*., have conspicuous peripheral chromatin, arranged on the inner surface of nuclear membrane; where as such chromatin material is lacking in the remaining other three amoebae. The nuclear membrane in *E. histolytica* is delicate and the chromatin substance on the inner side of the nuclear membrane appears as fine beads, which are uniform in size and evenly

arranged. The central chromatin substance is called the Karyosome. It is small and has a halo around it.

In *E. coli*, the nuclear membrane is thicker and the chromatin on the inner surface of the membrane is distributed irregularly in the form of coarse plaques. The karyosome in this case is coarser and eccentrically placed. This is called *''Coli type''* of nucleus.

The special feature of E. nana is the large karyosome located in the center or slightly eccentric. In *I. butschlii*, the karyosome is large but it is surrounded by a ring of achromatic granules giving a halo effect around

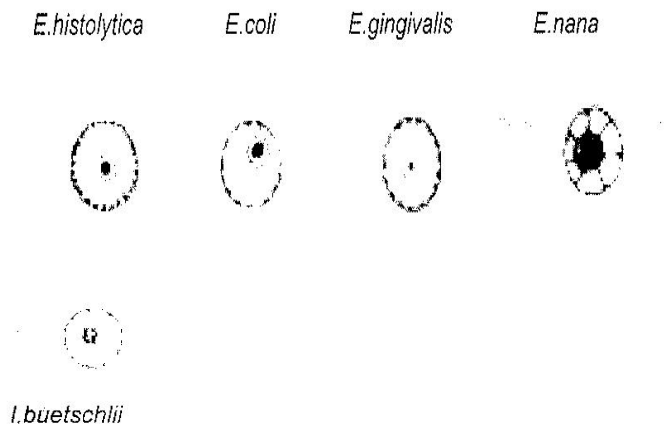


Figure 2.1.

Nuclei of the different species of amoebae (From Dey TK and DeyNC. Medical Parasitology, 9th ed. India, 1984.)

Entamoeba histolytica

Geographical Distribution:- Cosmopolitan distribution, mainly in the tropics and subtropics, and is mainly related to inadequate personal hygiene environmental sanitation, lack of safe water supply and poor socioeconomic situation.

Habitat:-***Trophozoite***:- Large intestine, liver abscesses and other extra-intestinal organs

Cyst:- found in the stools of chronic dysenteric patients and carriers.

Morphology

Trophozoite:

Size:- 12 to 35μm, Usually as long as 3 or 4 red blood cells .

Shape:- elongated form when actively motile and rounded form when at rest.

Motility:- Active, Progressive , directional amoeboid motility in fresh warm stool specimen.

Pseudopodia:- Finger like, broadly rounded end.

Cytoplasm:- Well differentiated into ectoplasm and endoplasm.

- May contain ingested host's red blood cells in dysenteric specimens

Nucleus:- Single nucleus, not visible in the motile form but in iodine stained smear clearly seen.

Cyst:- Size: 12-15μm (1½-2 red blood cells)

Shape: spherical

Nuclei: 1-4 nuclei

Nuclear membrane: thin, regular and circular lined with fine chromatin granules internally , and small, compact central karyosome.

Cytoplasm: Yellowish-gray and granular in iodine stained smear.

Stored food: Sausage shaped chromatoidal bars with blunt ends and glycogen mass in immature cysts with one or two nuclei.

Life cycle:

Entamoeba histolytica requires a single host to complete its life cycle. When mature tetra-nucleated cyst from contaminated food or drink or form hands contaminated with feces is ingested it excysts in the small intestine to produce metacystic trophozoite by a process of binary fission. The immature trophozoites migrate to the colon and grow to become mature trophozoite stage, multiply by binnary fission to invade the mucus membrane of the large intestine. Some times it can perforate the intestinal wall causing extra-intestinal amoebiasis. The trophozoite stage may pass with diarrhea or dysentery.

After a period of growth and multiplication, encystment occurs in the large intestine. In the process of cyst formation, the trophozoite discharge undigested food appears spherical in shape and condense to become pre-cyst. The pre-cyst secrets cyst wall to form a mono-nucleated cyst which is followed by a nuclear division to produce a bi-nucleated and then a tetra-nucleated mature cyst. Cyst and precyst will also pass in semi- formed or formed stool, where cyst is infective if it is ingested by any means of transmission.

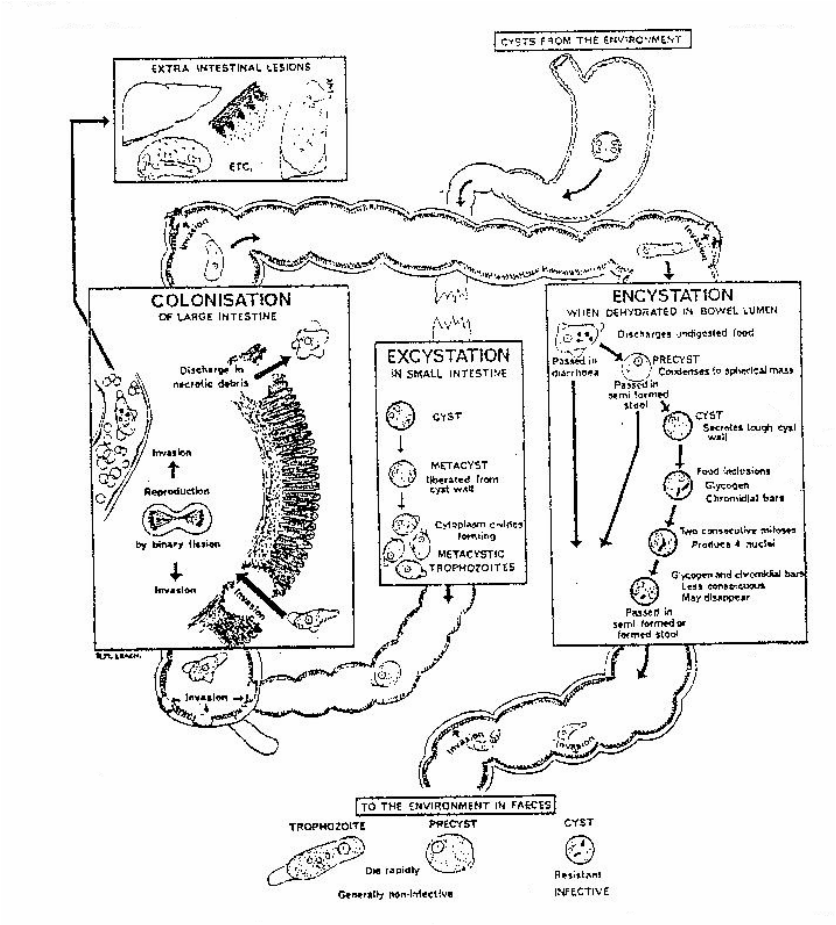


Figure 2.2.

Life cycle of *E.histolytica.* (From Jeffrey HC and Leach RM. Atlas of Medical Helminthology and Protozoology, 1975.)

Mode of Transmission

Infective stage: Tetra-nucleated mature cyst.

Man acquires infection of *E.histolytica* from:

- Ingestion of food or drink contaminated by infective cyst.

Clinical Features and Pathology

May be asymptomatic or exhibit amoebic dysentery or extra-intestinal amoebiasis in the liver, brain, spleen, lung, etc. Amoebic dysentery occurs when *E histolytica* trophozoites invade the wall of the large intestine and multiply in the submucosa, forming large flask shaped ulcers. The amoeaba ingest red cells from, damaged capillaries.

Amoebic Liver Abscess:

Occasionally amoeba is carried to the liver in the portal circulation and form abscesses, usually in the right lobe. There is pain & tenderness over the liver, wasting and fever with chills & night sweats. Patients with large or multiple abscess may become jaundiced and anemic.

Formerly a pathogenic invasive strain & a non pathogenic strain, of *E. histolytica* were thought to exist. The two 'strains' have now been recognized as separate species. *E. histolyrtica* is the invasive pathogenic and *E. dispar* has been designated the non- invasive non-pathogenic species. The two species are morphologically identical.

Prevention and Control

1. Cooking of food and vegetables
2. Hand washing after defecation and before eating
3. Safe water supply (treatment, boiling, filtration, etc.)
4. Control of mechanical vectors

5. Avoid use of night soil as a fertilizer proper sanitary disposal of faeces.
6. Treatment of infected individuals and health education.

Laboratory Diagnosis

Laboratory diagnosis of intestinal amoebiasis is based on:

1) Examination of a fresh diarrheic or dysenteric faecal specimen or rectal scraping for motile amoebae using saline, or
2) Examination of formed or semi-formed faeces for cyst stages. Such stool can be examined by direct saline and/or iodine smear, and Zinc sulphate floatation or centrifugal floatation method.
   - Charcot-Leyden crystals, representing the crystallized contents of granules from eosinophil leukocytes may also be found in a fecal smear.
   - Specimens must be examined without delay otherwiseidentification of the trophozoites becomes impossible because the amoeba lose their motility, extrude vacoules containing red cells, round up
   - With the recognition that *E.histolytica* is morphologically identical but genetically distinct form *E.dispar*, cysts, formerly reported as *E.histolytica* should be now reported as *E.historlytica* / E.dispar.

Differential Diagnosis of Amoebic Dysentery and Bacillary Dysentery

	Amoebic dysentery	Bacillary dysentery
Odor	Offensive	Odorless
Color of blood	Dark red	Bright red
Exudate	Few pus cells	Many pus cells
E.*histolytica*-	Present	Absent
Consistency	Mucus adherent to the	Not adherent

	container	
	Has charcoat -Leyden crystals	No crystal
Appearance -	Fecal matter with stratum of blood and mucus seen over the surface	Hardly any fecal matter consists of blood and mucus
Bacteria	Numerous, motile	Scanty, non motile

Relevance to Ethiopia

E. histolytica has a worldwide distribution but it is more common in developing countries. The parasite is very common amongst children. General parasitological survey indicates that the infection rate is variable. Of the seven species of amoeba which inhabit the human intestinal tract, Only *E.histolyica* is pathogenic to man, as the agent of amoebiasis. Slightly over 80, 000 new cases of amoebiasis were reported by the Ethiopian MOH in 1988-89, based on outpatient reports. The infection was common in all age groups, and even 3,716 children under 1 year were treated for this disease (MOH, Addis Abeba, 1991).

A wider range in prevalence has been reported from community surveys in other parts of the country. Extremely high *E. histolytica* infection rates (55%) were found among the isolated Saysay shifting cultivators in the Blue NileGorge in Welega. In a survey of 1,850 school children in 50 farming communities on the central and northern plateaus covering five administrative regions, the parasite was found in 94% of the communities, with prevalence rates ranging from 3% to 50 %; on the average, 19% of the school population were infected (Lo CT, et al. 1980). Twelve percent of 698 school-aged children in 17 rural communities in the highlands of Showa were infected (Kloos H et al. 1980).

Low to intermediate levels of amoebiasis prevalence have been reported from towns, apparently due to the effect of urbanization on transmission; 0.5% of 468 school children in Addis Ababa and 2.2% of 90 school

children in Debre Zeit were found to be infected. Rates were highest among poor children (2.8%). [Taticheff et al, 1981].

The highest prevalence of amoebiasis in Ethiopia was found the potential for nosocomial infections in Ethiopian health institutions (Editorial, Ethiop Med J, 1972).

In a relatively recent country wide survey of amoebiasis, a totalof 12,457 persons in 97 communities was stool examined by formol ether concentration technique. The cyst passsers, inschoolchildren and non-schoolchildren coimmunities were 15.0% and 3.5%, respectively. Slightly more females (18.4%) than males (14.2%) were infected among school children but the the difference was not significant non-schoolcommunities.There was a tendency but not a statistically significant decline of cyst –excretion with increasing age. So far as our survey goes, the influence of altitude on the prevalence of amoebiasis appeared not to be significant.Health education,improvement of sanitationand personal hygiene are suggested as realistic measures toreducethe transmissionof the parasite (Erko B, et al. Trop Geog Med, 1995).

The above stuidies did not differentiate between *E.histolytica* and *E. dispar*. A single study done in Wongi areas of central Ethiopia, where an increased incidence of amoebic infection has been reported, of the 29 amoebic isolates successfully stabilized, cloned and characterized bybSargeaunt's electrophoretic technique, 27 (93.1%)were of *E.dispar* zymodemes and two were (6.9%)wereof *E.histolytica* (Gatti S et al, Ann Trop Med Parasitol, 1998)

Entamoeba Hertmanni

Geographical Distribution: Cosmopolitan

Habitat: both trophozoite and cyst live in the small intestine

Morphology

Known as “small race” of *E.histolytica* because of similarity in their morphology. It feeds on bacteria not red blood cells.

Trophozoite

4-12μm, smaller than *E.histolytica*

Active but less vigorous directional motility by finger-like pseudopodia

Single nucleus with fine bead-like coarse chromatine granules on the thick nuclear membrane and has large centeral karyosome.

Cyst:- 5-10μm in size and spherical in shape.

1-4 nuclei; Minute rice grain shaped chromatoidal bars and glycogen mass in the immature cyst stage.

Life cycle

Similar to the life cycle of *E.histolytica.* It requires a single host and has five main developmental stages.

Tetranucleated cyst→Metacyst→Metacystic trophozoite→Trophozoite→Precyst→Uninucleated cyst

Mode of Transmission

Through contaminated food or drink, or from hands contaminated with faeces.

Pathology:- Harmless commensal

Laboratory Diagnosis:

- Finding the characteristic trophozoite and cyst stages in stool specimen. Differential *characters*:
- Cyst of *E.hertmanni* is similar to that of *E.histolytica* / *E*. *dispar* but the former is smaller in size.
- Cyst of *E.hertmanni* is also similar to that of *E.nana* but the later has 4-hole like nucleus and don't have chromatoid body

Entamoeba coli

***Geographical Distribution*:** Cosmopolitan.

Habitat

Both trophozoite and cysts in the large intestine of man

Morphology

Trophozoite:-

Size: 15-50μm(average 25μm), usually bigger than *E.histolytica*

Shape : Oval or elongated

Motility:- Sluggish, non -progressive and non-directional
- Short blunt pseudopodia

Cytoplasm: Ectoplasm and endoplasm not well differentiated.

Nucleus: Single nucleus, visible in the fresh state without staining.
Thick nuclear membrane lined with coarse chromatin granules and eccentric karyosome .

Inclusions: Bacteria, Yeasts, but never red blood cells.

Cyst:-

Size: 12-20μm a little larger than the cyst of *E.histolytica.*

Shape: rounded or slightly oval.

Nucleus: 1-8 nuclei; thick irregular nuclear membrane large, diffuse,often eccentric karyosome

Cytoplasm: bright pale yellow in iodine stained smear.

Life cycle

Similar to the life cycle of *E.histolytica.*

Octanucleated cyst Metacyst Metacystic Trophozoite Trophozoite precyst unincleated cyst binucleated cyst Tetranucleated cyst

Mode of transmission

Ingestion of contaminated food or drink by infective cyst.

Pathology:- Harmless commensal, may cause diarrhea in children.

Laboratory diagnosis:-

Finding the characteristic trophozoite and cyst stages in stool specimen. Can be differentiated from *E.histolytica by its larger size. The cyst of E.coli shows a greater variation in shape and size than those of E.histolytica.*

Entamoeba gingivalis

Geographical distribution: world wide distribution

Habitat: Oral cavity

Morphology:-Has trophozoite stage only, no cyst stage

Trophozoite:-

Size:-10-20 nm

Motility: sluggish

Cytoplasm: well differentiate into ectoplasm and endoplasm

Pseudopodia:- multiple

Nucleus:- single, delicate nuclear membrane lined with fine chromatin granules and Small central karyosome.

Life cycle:-It is reproduced by binary fission and transmitted from one person to another through kissing , droplets spray from the mouth,contaminated spoons or cups.

Pathology: non pathogenic commensal amoebae

***Laboratory Diagnosis*:-**Finding the characteristic trophozoite stage from swab of the oral cavity. It is the only species to ingest host's leukocytes and has numerous food vacuoles.It should be differentiated from *Trichomonas tenax which belong to flagellates and found in oral cavity.*

Endolimax nana

Geographical distribution: cosmopolitan.

Habitat: Trophozoite and cyst in the large intestine.

Morphology:-

Trophozoite

Size: 6-15μm (average 10μm)

Motility: multiple small rounded blunt pseudopodia moving slowly in all direction.

Cytoplasm: very granular with small vacuoles

Inclusion: Bacteria.

Nucleus: single large irregularly shaped eccentric karyosome and thick nuclear membrane with out lining of chromatin granules.

Cyst:-

Size: 8-10μm

Shape: oval

Nucleus: 1-4 nuclei large, irregular karyosome

Cytoplasm*:* Deep yellowish color in iodine smear.

***Life Cycle*:-**Trphozoite stage reproduces by binary fission and man acquires infection from contaminated food or drink with

mature cyst stage.

Laboratory Diagnosis:-Finding of the cyst and trophozoite stages in fecal smear.

Iodamoeba butschili

Geographical distribution: Cosmopolitan.

Habitat: both trophozoite and cyst in the large intestine.

Morphology:-

***Trophozoite*:-**

Size: 10-25μm

Shape: compact, leaf -like

Motility: sluggish by clear, rounded, finger like pseudopodia.

Nucleus: single, no chromatin granules on the nuclear membrane and has large karyosome surrounded by achromatic granules.

Inclusion: bacteria; large food vacuole packed with glycogen mass that stains deep brown with iodine solution.

***Cyst*:-**

Size: 8-10 μm

Shape: rounded, oval or irregular

Nucleus: single, very large oval karyosome surrounded with cluster of granules.

Vacuole: very large glycogen vacuole often taking up half part of the cyst and stains brownish red by iodine solution hence the name was given as Iodamoeba.

Life Cycle:-The trophozoite reproduces in the large intestine by binary fission. The infective stage is the mature cyst stage and man acquires infection from contaminated food or drink.

Pathology: It is non-pathogenic.

Laboratory diagnosis:-Finding the characteristic trophozoite and cyst stages in the direct fecal smear examination or using concentration technique.

Entamoeba polecki

Most commonly infects pigs and monkeys; but may cause mild diarrhea in human hhumaman

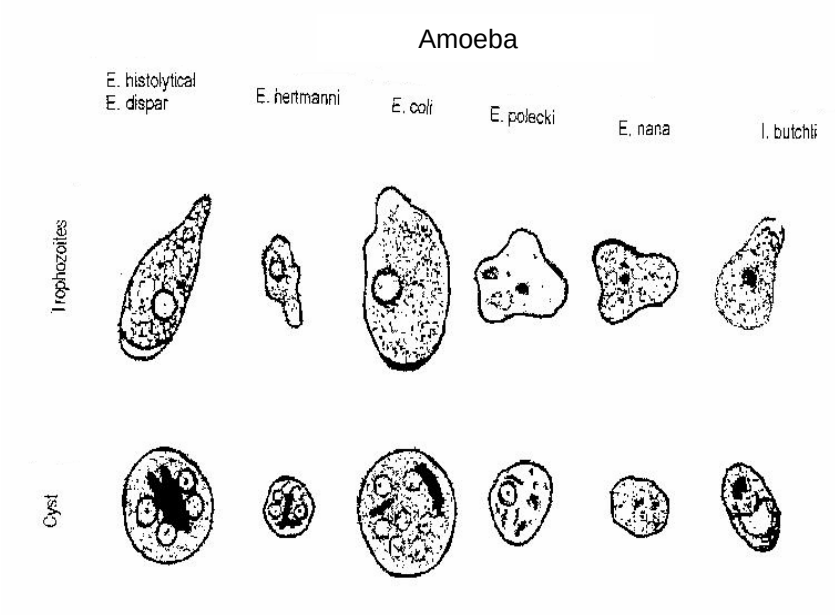


Figure 2.3.

Ameba found in stool specimens of humans. (From SmithJW,et al. Diagnostic Medical Parasitology : intestinal protozoa. American Society of Clinical Pathologist, 1976.)

2.1.2 Free living Pathogenic Amoebae

The free living amoeba constitute a large group, inhabiting fresh, brackish and salt water, moist soil and decaying vegetation. Some are coprozoic. For convenience this large and diverse group is separated into two groups on the bases of their ability to undergo transformation from an amoeba to a flagellate stage. *Naegleria* belongs to the family Vahkampfiidea, members of which are characterized as amoebaflagellates able to assume a temporary flagellate form while completely devoid of flagella in their amoeboid stage. *Acanthamoeba*, belonging to the family Acanthamoebidea, never produce flagella. Most cases of primary amebic meningoencephalitis can be ascribed to infection with *Naegleria*; less commonly Acanthamoeba is involved.

Naegleria fowleri

Geographical Distribution:-It is free living pathogenic amoebae with worldwide distribution.

This genus of amoeboflagellates has an amoeboid phase, which alternates with one possessing two flagella. The forms found in the tissues are ameboid, and in the tissue they are distinguished with difficulty from acanthamoeba. Most case have occurred during the hot summer months in young persons who within the preceding week swam or dived in fresh or brackhish water, lakes , hot springs and swimming pools have been apparent sources of the infection.

That the source of infection is not always aquatic is illustrated by a case report from Nigeria where the organisms were apparently inhaled during a dust storm by an 8-month old infant, from whose nasal mucosa and spinal fluid the organism were recovered prior to death. Another interesting report from Nigeria concerns a Muslim farmer thought to have

become infected during the ritual washing before prayer, which involved sniffing water up his nose.

Habitat: Nasal cavity, Central nervous system,

Morphology

Trophozoites

Size: 10-15μm

Cytoplasm - Well differentiated into ectoplasm and endoplasm

Motility - actively motile with broad pseudopodia elongated

Nucleus - Single with central karyosome

Inclusion -does not ingest host RBCs

Cyst - One nucleus and thin cystcyst wall which has no pore.

Flagellate

Shape - Oval

Nuclues - similar as above

Flagella - two flagella equal in length and longer than broad

Pathology

Causes primary or acute meningoencephalitis. Major symptoms include fever, stiff neck (meningitis), frontal headache, altered test (food or drink), vomiting, white blood cell count about 24,000 with many neutrophils.

Prevention and Control*:-*Infection can be prevented by avoiding contact, swimming and snuffing in waters of ponds, lakes, treatment of water and proper sewage disposal, treatment of infected individuals and health education.

Laboratory Diagnosis*:-*Only amoeboid trophozoite stage is found in man. Finding the amoeboid trophozoite stage in cerebrospinal fluid (CSF). It can be found in unstained or Giemsa stained CSF smear. The

CSF appears to be claudy, purulent and may contain eosinophils and red blood cells.

Acanthamoeba Species

Similar with that of N.fowleri except the following differences:

1. *Trophozoiutes are larger 15-25*μm
2. *Pseudopodea arefilamentous (spiky pseudopodia or acathopodia)*
3. *It does notforma flagellate stage in water*
4. *Cysts are thickwalled with many pores*
5. *It may encysting the tissue*
6. *Pathology:-* Causes secondary or chronic meningoencephalitis.. Symptoms include fever, headache, rhinitis, meningoencephalitis, conjunctivitis, corneal ulceration, keratitis and loss of vision
7. Notkilledbydessication

Review Questions

1. What are the differences among cysts and trophozote of *E.hisolytica, E.hertmani* and E.coli?
2 Which amoeba do not have cyst stage in its life cycle?
3 Explain the general prevention and control methods of amoeba.
4 List pathogenic amoeba.
5. Discuss the general mode of transmission and laboratory diagnosis of amoeba.
6. How do you differentiate amoebic dysentery from bacilliary?
7. What is the clinical significance of studying *Naegleria* and *Acanthamoeba* species.

2.2 Class: Zoomastigophorea

Mastigophora: Flagellates

Flagellates infecting man are divided into two groups.

1. The oro-intestinal and urogenital flagellates and
2. The Hemo-somatic flagellates.

The oro-intestinal and urogenital flagellates are found in the intestine, oral cavity and genital tract. Many of them are not pathogenic. They are classified in to 2 orders, namely; Protomonadida and Diplonadida, The former is characterized by one nucleus and flagella at the anterior end; where as the latter has a pair of nuclei and flagella, which are symmetrically distributed at the interior end.

The hemoflagellates are present in the blood and invade various tissues of the body; remain either in the intercellular fluid, bathing the cells; as in trypanosoma, or are engulfed by the Red cells and leucocytes as in *Leshmania*. Of the six genera the parasite athogenic to man belong to two genera under the family Trypansomatidae, these are *Trypanosoma* and *Leishmania*.

2.2.1 The Oro-intestinal and Urogenital Flagellates

General Characteristics

1. Uses flagellum as locomotory organell
2. Reproduce by simple binary fission
3. Complete their life cycles in a single host and a second host whom they infect is necessary for the continuation of the species.
4. Most are commensal forms except *G.lamblia, T.vaginalis* and *D.fragilis*
5. The infective stage may be either the trophozoite or the cyst stage

6. Except the species of *Trichomonas and Dienatamoeba fragilis*, all have both cyst and trophozoite stages.

Dientamoeba fragilis

Dientamoeba fragilis originally classified as an amoeba, is now considered an amoeba like flagellate more closely related to the genus *Trichomonas*. Electron microscopic studies have revealed that the internal structures are typical of flagellate.

Geographical Distribution: World wide

Habitat: In the large intestine.

Morphology: Has trophozoite stage only, No cyst stage.

Trophozoite*:-*

Size: 6-15μm

Motility: Either non-motile (most often), or very actively motile in very fresh fluid stools with fan-like multiple pseudopodia. It becomes non-motile under the cover slip or disintegrates immediately.

Cytoplasm: clear ectoplasm.

Nucleus: Usually one or two nuclei but 3or 4 nuclei may be found rarely. Karyosomes split into 4-6 granules.

Inclusion bodies: Bacteria

Life cycle:- The mode of transmission is uncertain but most likely is feco-oral nature. It is postulated that the delicate trophozoite is transported from person to person inside the protective shell of helminth ova such as *Entrobius vermicularis*. It reproduces asexually by binary fission. It is considered to be harmless commensal

Laboratory Diagnosis:-The trophozoite stage is highly fragile and disintegrates explosively in water immediately. Hence it needs

immediate examination of fresh stool specimen to find the trophozoite stage.

Chilomastix mesnili

Geographical Distribution: cosmopolitan but mostly prevalent in warm climates.

Habitat*:* Trophozoite and cyst live in the colon and caecum of the large intestine.

Morphology

Trophozoite:- Size: 6-20 by 3-10μm

Shape: Triangular and tapered at one end

Motility: spiral in one definite direction.

Cytoplasm:

- Spiral groove that makes asymmetrical flagellate
- cytostome (mouth-like cleft) at the rounded end.

Nucleus: one nucleus, easily visible in unstained preparation

Flagella: Six flagella. Three anterior free flagella, one delicate flagellum lying in the cytostome and two flagella on the lateral margin of the cytostome

Cyst*:-* Size: 6-8 by 4-6μm

Shape: pear or lemon shaped

Cystostome and remains of locomotory organelles can be seen.

Nucleus: single; Thick nuclear membrane with small central karyosome.

Life Cycle

Cyst→Excystation→Trophozoite→Binary fission→encystation→Cyst in the faeces

Trophozoite stage reproduces by binary fission. The infective stage is the cyst from contaminated food or drink. Excystation occurs in the large intestine and trophozoite multiplies by binary fission.

Pathology*:* It is commensal

Laboratory Diagnosis:-Finding the trophozoite and cyst stages in stool specimen. The trophozoite stage is very similar to *Giardia lamblia and Trichomonas hominis*; and needs careful identification.

Giardia lamblia

Also called *Giardia intestinalis* and *G.duodenale*

Geographical Distribution:- Cosmopolitan distribution in warm climate and is more prevalent in children than in adults. It is the most commonly diagnosed flagellate of the human intestinal tract. High prevalence occurs in young, malnourished children in large families, orphan asylums, and elementary schools.

Habitat: Upper parts of the small intestine mainly in the duodenum and jejunum.

Morphology:

Trophozoite*:*-Size: 10-21 by 5-15μm

Shape: pyriform (pear-shaped), i.e. rounded anteriorly and pointed posteriorly.

Motility: Progressive, rapid, tumbling and spinning often linked to a "falling leaf" type of motility in fresh liquid stools.

Bilaterally symmetrical

Covex dorsal surface and a flattened ventral side

Contents:

- Anteriorly there are two sucking discs each contains a nucleus, 4 pairs (8) flagella, Parabasal body and axonemes

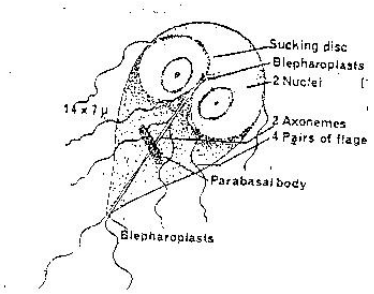


Figure 2.5. Trophozoite of G.lamblia. (From Jeffrey HC and Leach RM. Atlas of Medical Helminthology and Protozoology, 1975.)

Cyst :- Size: 8-12μm, oval shape with thick cyst wall.

Finely granular cytoplasm clearly separated from cyst wall.

2-4 oval nuclei at one pole, each with small, central karyosome.

Cytoplasm: clear when unstained; yellowish green or bluish in iodine solution.

Fibril: thread-like remains of flagella; axonemes and parabasal bodies folded as S-shaped placed length wise in the center of the cyst.

Life Cycle

Requires a single host to complete its cycle and reproduces by a simple longitudinal binary fission

Cyst ingested→excystation→Trophozoite→binary fission→Encystation→cyst in faeces

Infection occurs by ingestion of mature tetranucleated cyst with contaminated food, drink, finger, etc. Following ingestion, the cyst excyst in the upper part of the small intestin to form flagellates. They become attached to the intestinal wall by a sucking disc and absorb nourishment through their body surface. They multiply by longitudinal binar fission and some of them are carried down the intestinal tract to undergo encystation. The trophozoites and infective cysts are excreted in the faeces.

Clinical Feature and Pathology:-Major symptoms includes duodenitis, excess secretion of mucus or malabsorption of fat (steatorrhoea), sugar and vitamins, dehydration, diarrhoea, weight loss, poor appetite, vomiting, lethargy bile passage obstruction

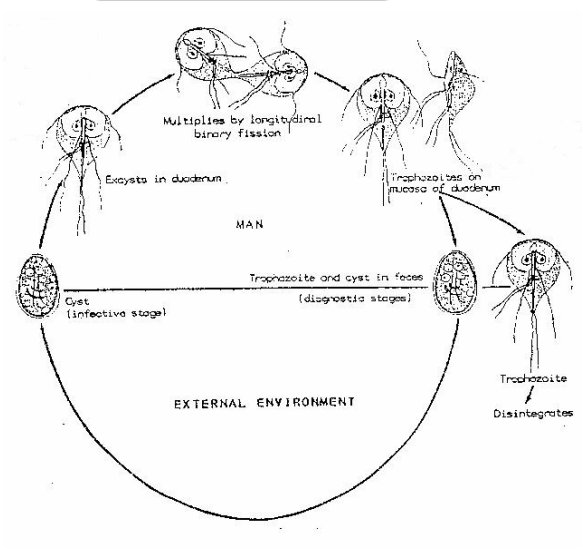


Figure 2.6. Life cycle of Giardia lamblia. **(**From Nasir NT. Review of Human Parasitology,2nd ed. The Scientific Book Center, Cairo)

Prevention and Control:

1. Improving personal, family and group sanitation and hygiene.
2. Avoid contamination of food, drink and hands with the faeces.
3. Safe water supply and latrine construction.
5. Treatment of infected individuals and health education.

***Laboratory Diagnosis*:-**Finding the trophozoite and cyst stages in stool specimen. The stool is usually offensive, bulky, pale, mucoid (fatty), diarrheic (watery) but there is no blood in the stool. Several specimens collected at different time need to be examined because trophozoites and cysts are excreted irregularly.

Intestinal and non-pathogenic flagellate that require differentiation from *G.lamblia* include: *C.mesnili* and *Pentatrichomonas hominis (*formerly *T.hominis).*

Trophozoites of the above mentioned flagellates can be easily differentiated from *G.lamblia* by their shape and movement (in fresh sample) and bcause they have only one nucleus (and fewer flagella). The only other trophozote that has two nuclei is *D.fragilis* but this organism has no flagella or median bodies and look likes a small amoeba.

Cyst of intestinal flagellates can be easily differentiated from those of G.lamblia because they are smaller and do not have the same characterstic appearance of G. lamblia (do not contain remains of flagella). *C.mensili* cysts are lemon shape and *D. fagilis* does not has cyst stage.

Relevance to Ethiopia

Infection by *Giardia lamblia* has a cosmopolitan distribution both in developed and developing nations. Infection rates ranging from 1% to 50% or so have been reported from various parts of the world. In African,

Asian and Latin American countries, about 200 million cases of *Giardia lamblia* infections have been estimated to occur annually. The infection may be endemic as in the tropics where it is a familial infection passed around by faecal-oral route, sporadic as in travelers, or epidemic as waterborne or institutional outbreaks (Helmut K and Zein AZ, 1993)

Giardiasis is wide spread in Ethiopia. A countrywide survey of giardiasis, using formal-ether concentration method, among school children and residents showed overall prevalence rates of 8.9% and 3.1% respectively. The corresponding rate for non-school children (5-19 years of age), however, was 4.4% showing that the School children are more significantly infected than their school children counter parts. *Giardia lamblia* infection was generally found to be more prevalent in children than in adult. Among children of school population those in their first decade of life were more affected (Hailu and Berihanu, 1995).

Although these and other prevalence data are not strictly comparable due to differences in sample selection and diagnostic methods used in different institutions, they indicate that while urbanization resulted in the reduction of the prevalence of giardiasis, it remains common infections in urban population. Infection rates reported here can not represent the actual prevalence rate giardiasis in Ethiopia as stool examination alone is not reliable to rule out infection of *Giardia* the cysts of which are excreted episodically.

Trichomonas hominis

Geographical Distribution*:* Next to *Giardia lamblia*, it is probably the most common and most cosmopolitan of the intestinal flagellates of man.

Habitat: Large intestine.

Morphology: has trophozoite stage only.

Trophozoite

Size: 10-15μm, pyriform (oval with two pointed poles) in shape

Motility: whirls and turns (jerky) in all directions, seeming to vibrate. Most resistant flagellate that remains motile even in old stool specimens.

Undulating membrane and costa reach 2/3 or full length of the body Nucleus: Single nucleus with central karyosome.

Flagellum: 3-5, usually 4 anterior free flagella and another flagellum on the margin of undulating membrane with a free trailing posterior end. Conspicuous cytostome opposite to the undulating membrane, has semi-rigid axostyle and parabasal body

Life Cycle:-The trophozoite stage reproduces by binary fission and requires direct host to host transmission through contaminated food and/or drink. It has high prevalence in children and more common in warm climates.

Pathology: It is non-pathogenic but may cause diarrhoea and infection can be prevented by personal hygiene and sanitation.

Laboratory Diagnosis: Finding the trophozoite stage in fresh stool specimen.

Trichomonas vaginalis

Geographical Distribution:-World wide distribution and mainly common in the temperate region.

Habitat:-In the genital tract of male and commonly in female, especially the vagina, cervix, urinary bladder, prostate and seminal vesicles.

***Morphology*:** Has trophozoite stage only.

Trophozoite

Size 15-25 by 5-12μm, is the largest *Trichomonas*.

Shape: pyriform

Moility: Jerky (on-spot), non-directional motility in fresh specimen.

Short undulating membrane: extending along one third of the body.

Nucleus: Single with uniformly distributed chromatin granules

Flagella: 4 anterior free flagella and one on the margin of the undulating membrane Axostyle may be split into several fibrils anteriorly.

Less conspicuous cytostome and has marked parabasal body.

Life Cycle:-The trophozoite stage reproduces by longitudinal binary fission and mode of transmission is usually via sexual intercourse but also by communal bathing, sharing of washclothes, toilet equipment seats and mother to daughter during birth.

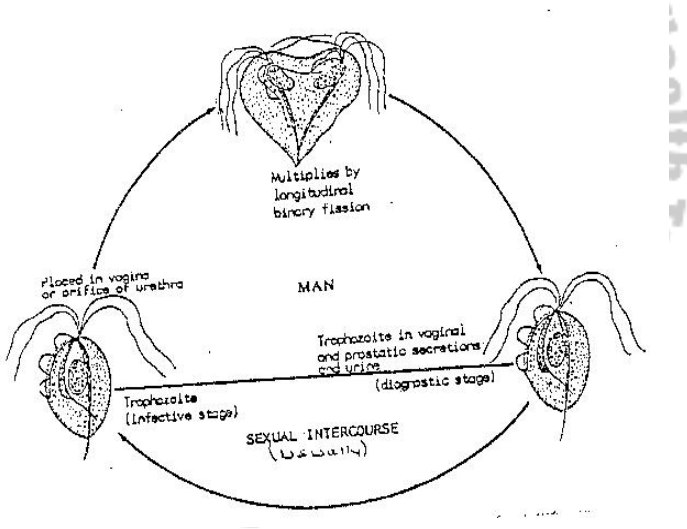


Figure 2.7. Life cycle of T.vaginalis **(**From Nasir NT. Review of Human Parasitology,2nd ed. Cairo: The Scientific Book Center.)

Pathology: Causes trichomoniasis. Major symptoms are Vaginitis, urethritis, prostatitis, chaffing of vulva, cervical erosion, burning sensation, yellowish prulent discharge, reversiable sterility in male.

Prevention and Control

1. Personal hygiene and sanitation
2. Simultaneous treatment of both partners.

Laboratory Diagnosis:-Finding the trophozoites in unstained or stained preparation of vaginal or urethral discharges, urine sediment, vaginal swab, prostate secretions.

Relevance to Ethiopia

Trichomonas vaginalis is fairly common in Ethiopia as the level of hygiene is very low. Forty of 216(15%) prostitute in A.A tested were positive in 1995. (Kloos &Zein Ahmed, 1995). Another study done in A.A revealed 20% of women attending antenatal clinic harbour this parasite (Duncan ME, et al. Ethiop J Health Dev, 1995). Recent Unpublished study conducted inJimmatown detect a prevalence of 12.3% inwoman attending gynecology OPD (Tariku L, 2002).

Trichomonas tenax

Geographical Distribution

World wide.distribution with high incidence in warm climates.

Habitat: oral cavity.

Morphology: Has trophozoite stage only.

Trophozoite:-Size: 5-12μm

***Shape*:** pyriform

Motility: active jerky motility

Undulating membrane: reaches two third of the body length.

Nucleus: Single

***Flagella*:** four anterior free flagella and one flagellum on the undulating membrane.

Thick axostyle extending a considerable distance behind the body. Has parabasal body

Life Cycle:-The trophozoite stage reproduces by binary fission and transmission is direct from mouth to mouth through kissing or communal use of contaminated food and drinking utensils.

Pathology*:*-It is non-pathogenic

Laboratory Diagnosis:-Finding the trophozoites in unstained or stained smear in swab taken from the oral cavity.

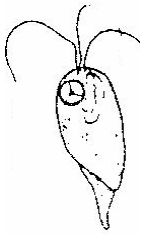
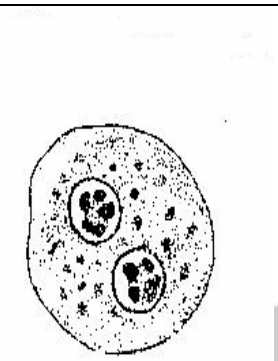
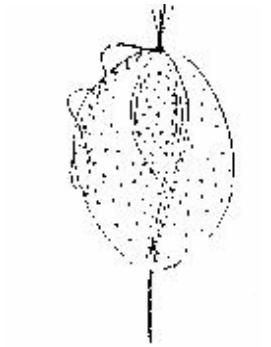
	T. hominis	*C.mesnili*	*G. lamblia*
t r o p h o z o l t e s			
Cyst	***No cyst***		

Figure 2.8. Flagellates.(From Smith JW, et al.Diagnostic Medical Parasitology:Intestinal Protozoa, Chicago.American society of Clinical Pathologist, 1976.

	D.fragilis	*T.vaginalis*
t r o p h o z o l t e s		
c y s t	***No cyst***	***No cyst***

Continuation of

Figure 2.8. Flagellates.(From Smith JW, et al.Diagnostic Medical Parasitology:Intestinal Protozoa, Chicago.American society of Clinical Pathologist, 1976.

Review Questions

1. Among the oro-intestinal flagellates which of them are more clinically important than others.
2. What is the typical diagnostic movement of *G.lamblia* trophozoite?
3. Write the sources of specimen for the following flagellates:
   a. *Giardia lamblia*
   b. *Trichomonas vaginalis*
   c. *Trichomonas tenax*
4. Which species have only trophozoite stage in their life cycle?
5. How do you differentiate among trophozoites of oro-intestinal flagellates?

2.3 Blood and Tissue Flagellates

The blood and tissue of humans may be infected by one of the several species of flagellate protozoa belonging to the family Trypomastidea. There are six genera but only two of them are responsible to cause disease to man. These are Genus *Leishmania* and genus *Trypanosoma*.

All of these organisms have developmental stages in blood sucking arthropodes (intermediate host) and in humans (definitive host), and may have a non human mammalian reservoir host.

Leishmania species are transmitted by Sandflies (*Phlebotomus*, *Lutzomia*) and the trypanosomes are transmitted by either the tsetse fly *Glossina* (for Afrcian trypanosomes) or *Triatomid* bugs (for American Trypanosomes). The hemoflagellates may occur in a variety of stages in the human host and the insect vectors.

General Characteristics

1. Reproduces by simple longitudinal binary fission
2. The infective stage is always the vegetative form.
3. Transmission occurs through biological insect vectors as intermediate hosts and man as definitive host
4. The species are morphologically indistinguishable, but they can be differentiated on the basis of on their clinical features primarily and also on their geographical distribution, serologic tests, cultural characteristics, vectors, reservoir hosts, biochemical tests, immunological tests, etc.
5. The different developmental forms are differentiated on the basis of
   a) Presence or absence of free flagellum
   b) Presence or absence of undulating membrane
   c) Position of the kinetoplast relative to the nucleus.

6. They have the following main body parts; Flagellum, Kinetoplast divided into blelopharoplast and parabasal body, axoneme, nucleus, undulating membrane,
7. Based on their development in the insect vector and their mode of transmission, Trypanosomes are grouped into two,

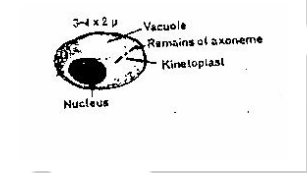
These are:-

I. Saliverian Group.The parasites develop in the mid and fore gut of their vectors and transmitted to man by inoculation of the parasites.

II. Stercorarian Group. The parasites develop in the hind gut of their vectors and transmitted to man by the contamination bited area with the faces of their vectors.

The following are the the main developmental forms

I. Amastigote /leishmania/ form



Spherical, no free flagellum, No undulating membrane, The only intracellular forms of all leishmania species and *Trypanosome cruzi*.

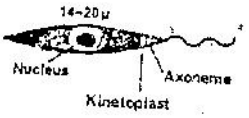
Figure. 2.9. Amastigote stage

II. Promastigote /Leptomonad/ forms

Fig. 2.10 Promastigote stage

Elongated, Single free flagellum, single nucleus, no undulating membrane, nucleus is.near the middle. The kinetoplast is just in the anterior portion. This form is found in the invertebrate host, and in culture media (of all *Leishmania* species) and in man as a transitional form for *Tryponosoma cruzi*.

III. Epimastigote /crithidial/ forms



Elongated body, single free flagellum, single nucleus, undulating membrane, the kinetoplast is just anterior to the nucleus. It is found in the invertebrate host and in culture media (of Trypanosome species).

Figure 2.11 Epimastigote stage

IV. Trypomastigote /Trypanosomal/ forms

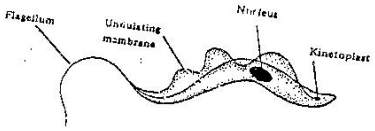


Figure 2.12. Trypomastigote stage

Pleomorphic, it can be as “U” or “C” shaped, single free flagellum, single nucleus, undulating membrane. The kinetoplast and axonemes are found at the posterior end relative to the nucleus.This form is found in the peripheral blood of vertebrates and is the diagnostic stage of *Trypanosome* species.

V. Metacyclic Trypomastigote /Trypanosomal/ Forms

Morphologically similar to trypomastigote stage but it is short and stumpy. It is the final developmental stage in the gut of the insect vectors and is the infective stage from the insect vector to man.

General life cycle of *Leishmania* species

Promastigotes inoculated into the skin when sandfly take a blood meal. The promastigotes are taken by macrophages and become amastigotes. Amastigotes multiply by binary fission.

Amastigotes are ingested by the insect vector when it takes a blood meal and becomes promastigote in the gut of the insect vector. The promastigotes multiply by binary fission and migrate to the head and mouth parts.

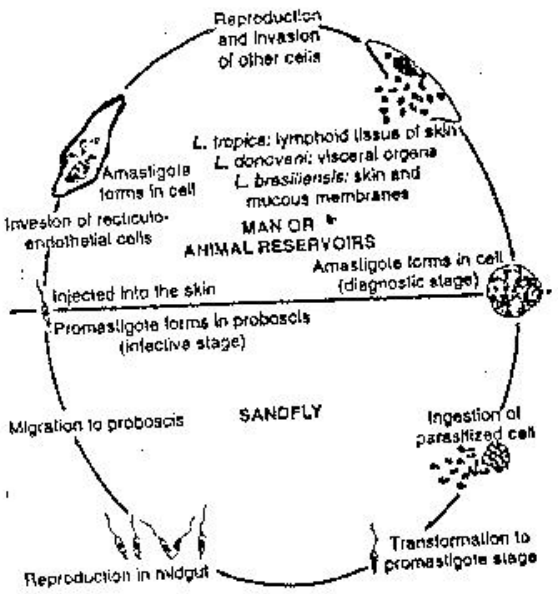


Figure 2.13. The life cycle of *Leishmania* species. (From StricklandGT.Hunter's tropical medicine 7th ed. Philadelphia:WB Saunders,1991.)

Leishmania tropica minor

Geographical Distribution*:*-Widely distributed in the urban area of Middle East, Europe, India, Eastern Mediterranean countries, Morocco, Algeria, Tunisia.

Habitat: -Amastigotes: In the endothelial cells of cutaneous tissues, lymph nodes, ulcers.
Promastigotes: In the gut of sandfly

***Morphology*:-**It has Amastigote and promastigote Stages only.

Life Cycle:- Definitive host: Man
Intermediate host: Female sandfly
Reservoir host: Dog, cats, mice, etc.

It requires a female *Phlebotomous* sandfly as a biological vector and man as its main definitive hosts to complete its life cycle. The vector inoculates the promastigotes in to the cutaneous tissue while taking a blood meal. The promastigotes are taken up by reticuloendothelial cells and develop into amastigotes within the cell. The amastigotes multiply and are ingested by a female sandfly vector when it sucks a blood meal. The amastigotes become (flagellated) promastigote in the midgut of the sandfly. The promastigotes multiply and fill the gut of the insect vector. Some of the vectors that are involved in this life cycle are *P.papatasi, P.sergenti and P.pernicious.*

Mode of Transmission

1. Inoculation by infected sandfly
2. Direct contact with the ulcer
3. Autoinfection

Pathology-

Causes cuaneous leishmaniasis (dry, urban, chronic, old world oriental sore). At the site of bite there is dry painless ulcer, 25-70mm in diameter, usually self-healing after 1-2 years often leaving disfiguring scar. Multiple unhealing lesion known as leishmaniasis recidivans may develop sometimes. The infection is usually heals and confers long lasting immunity to reinfection.

Leishmania tropicam major

Similar with *Leishmaniatropica minor* except with the following differences:

Geographical Distribution:-Wider distribution than *Leishmania tropica minor* and found in rural areas of sub-Saharan Africa from Senegal to central Sudan, Middle East, India, Pakistan, central Asia, North Africa. Infections are zoonotic.

Life cycle

Same as *Leishmania tropica minor* but man acquires infection upon invading enzootic areas because sand rat and the gerbils are also the main reservoir hosts. Important vectors are *P.papatasi, P.alexandri, P.caucasicus,* and *P.grove*

Clinical Feature and Pathology*:-*Causes moist (wet), rural, acute, Old world cutaneous Leishmaniasis or oriental sore. Forms a papule that develops to a large uneven ulcer or multiple lesions and is self-healing within 3-6 months. This infection protect against reinfection and against infection with *L.t.minor*. There is sporadic human infection.

Leishmania aethiopica

Similar with the above leishmania except with the following differences.

Geographical distribution

Southern Yemen, and the highlands of Ethiopia and Kenya

Life Cycle:-Man acquires infection by the bite of the infected female phlebotomus sandflies. The main vectors are *P.longipes* and *P.pedifer.* Infections are zoonotics with rocky hyraxes (*Procavia habessinica*) and tree hyraxes (*Heterohyrax brucei*) serving as reservoir hosts.

Pathology*:* Causes old world coetaneous and diffuse cutaneous leishmaniasis.

⇒ *Leishmania tropica minor, L. t. major,* and *L. aethopica* have similar prevention and control, and laboratory diagnosis, i.e.,

Prevention and Control *Leishmania* Species:

1. Avoid insect bites,
2. Control of insect vectors,
3. Protection of lesion from insect bites,
4. Avoid auto-infection /self-infection,
5. Treatment and health education.

Laboratory Diagnosis of Leishmania species:-

1. Amastigotes in stained smears taken from ulcers, lesions, nodules
2. Promastigotes in culture media.
3. Montenegroimmunologic/ leishmanin test.

Leishmania donovani

Geographical Distribution: India, Central Asia, China, Kenya, Sudan, Ethiopia, Somalia, Central and South America.

Habitat

Amastigotes: In the reticulo-endothelial cells of the visceral organs such as spleen, bone marrow, Lymph node, liver, kidney, lung, brain,
CSF, whit blood cells, intestine, etc.

Promastigotes: In the gut of phlebotomus in the old world and Lytzomyia in the new world

Morphology: Has both amastigote and promastigotes stages.

Life Cycle:

Reservoir hosts are rodents, dogs, fox, jackals.

Promastigotes are inoculated in to the subcutaneous tissues and taken up by macrophages. They become amastigotes and multiply. Large mononuclear cell invaded and the parasites are carried through the blood circulation to the visceral organs. When the sandfly takes a blood meal, these amastigotes are ingested into the gut of the insect vector and becomes promastigotes then they multiply. The parasites can be also transmitted through blood transfusion, sexual contact or congenitally.

Pathology: Visceral leishmaniasis or kala -azar. Major symptoms are fever, chills, sweating, cough, diarrhoea, vomiting, bleeding gums, weight loss,splenomegally, hepatomegally, lemphadenopathy, hypopigmentation of skin.

Prevention and Control

1. Personal protection from sandfly bites by using repellants, avoiding endemic areas especially when sandflies are active,
2. Insecticide spraying of houses and farm buildings
3. Destruction of stray dogs in area where dogs are reservoir hosts.
4. Treating infected person and health education

Laboratory Diagnosis

1. Amastigotes in aspirates of spleen bone marrow, enlarged lymph nodes, and in peripheral blood monocytes.
2. Promastigotes in culture media
3. Testing serum for leishmanial antibodies
4. Formal get test; is a non-specific screening test for marked increases in IgG

Relevance to Ethiopia

In Ethiopia, leishmaniasis is caused by four species of *Leishmania*, namely, *L. donovani,L. aethioplca.L.Major and L. tropica* . The former, *L. donovoni*, causes visceral leishmaniasis (VL) and is widely distributed throughout the lowlands of north-western Ethiopia (Asrat H and Frommel D, 1993). The latter three are agents of Cutanceous leishmaniasis (CL) with L. aethiopica prevailing in the highlands of Ethiopia. *P. martini* and *P. celiae* known vectors of Visceral leishmaniasis in south western Ethiopia (Mengesha et al, 1999).

Visceral leishmaniasis (VL) occurs in Ethiopia mainly in arid and semiarid lowlands below 1,300 m altitude. Many lowlands surrounding the central Ethiopian Highlands are known to be endemic areas. The disease affects the age group between 14 and 40 years. Mostly males are affected because of occupational activity (Helmut K and Ahmed Z, 1993).

Cutaneous leishmaniasis caused by *L. aethiopica* is endemic and widespread at altitudes between 1400 and 2700 m in most administrative regions. Prevalence rates of active infection between 5.5 and 40 per 1000 population were reported from villages in Shewa , Wello and Gamo Gofa administrative regions, with the highest rate in Ocholo village in Gamo Gofa. The ecology of Ethiopian Highlands cutaneous leishmaniasis is unique. *Phlebotomus longipes* and *P.pedifer*, the main

vectors, do not serve as vectors of leishmaniasis anywhere else. *Procavia habessinica* and *Heterohyrax brucei* are the main animal reservoir hosts for *L*. *aethiopica* (Helmut K and Ahmed Z, 1993).

Leishmaniasis and HIV Infection

Viceral leishmanisisis usually accompanied byimmunosuppression.The specific and the nono-specific immunosuppresion in VL is reported as a predisposing factor to in ntercurrent viral, bacterial, fungal and parasitic infection.

In ares where both leishmaniasis and HIV infection occur, Viceral leishmanisis (VL) is being increasingly reported in those with immunosupression caused by HIV. Frequently parasites infect not just the reticuloendothelial system but also the lungs, central nervous system, normal skin and blood. Parasites have been found in phagocytic cells in peripheral blood in upto 75% of patients (98%) in bone marrow aspirate.

There is a clear association between VL and immunosupression. In 1996 WHO epidemiological analysis, 90.4% of patients with *Leishmania*/HIV co-infection. Patients with *Leishmania* / HIV coinfection do not respond well to teatment. There are also reports of severe VL being caused by *Leishmania* strains normally of low or no virulence.

The risk of *Leishmania* / HIV co-infection is increasing as VL is becoming more urbanized, e.g. in the suburbs of cities such as Rio de Janeiro and Natal, and HIV infection is becoming progressively more common in rural areas, Kenya and Ethiopia.

In the study of VL/HIV co-infections in Ethiopia it was recommended that tests for *Leishmania* should be carried out when patients with HIV have fever of unknown origin, anemia, and enlarged spleen, liver or lymph nodes. Patients with VL who respond poorly to treatment or relapse

frequently should be investigated for HIV infection. In the Ethiopian study (endemic VL area), the clinical and laboratory findings were those associated with classical VL.

Severe diffuse cutaneous leishmaniasis and recurring cutaneous and mucocutaneous leishmaniasis are. laso being increasingly reported in persons with *Leishmania* /HIV coinfection (Monica C, 2000).

Leishmania Mexican complex

Geographical Distribution:-Central and southern America mainly in the rain forest of Mexico, Brazil, Guatemala, Venezuela.

Habitat:

Amastigote: Reticulo endothelial cells of the skin promastigote: In the gut of Lutzomyia sandfeies

Morphology: Has amastigote and promastigote

Life Cycle:-Same as the life cycle of Leishmania tropica except that the vectors are new world sand flies

Pathology:-Causes new world coetaneous leishmaniasis.

Relevance to Ethiopia*:-*This parasite is not recorded in Ethioipia.

Leishmania braziliensis complex

Geographical Distribution:-Tropical forests of South America and Central America. Reservoir hosts are rodents and some domestic animals

Habitat:

Amastigote:- In the reticulo-endothelial cells of muco-cutaneous tissues of nose, mouth, lips, larynx.

Promastigote:- In the gut of *Lutzomyia* sandfeies

Morphology: Has amastigote and promastigote stages.

Life cycle:-*Lutzomyia* sandfeies are the main vectors and man acquires infection from enzootic area.

Pathology:-Mucocutaneous leishmaniasis (espundia). Chronic ulceration of mucus membrane of the mouth nose, throat, etc. with destruction of bone and cartilage.

Leishmania Mexican complex and *Leishmania braziliensis complex* have similar prevention and control methods and laboratory diagnosis as presented below:

Prevention and Control

1. Avoid endemic areas
2. Avoid insect bites
3. Treatment of infected individuals
4. Health education

Laboratory Diagnosis

1. Amastigotes in stained smears taken from infected ulcers, lesions, sores and nodules
2. Promastigotes from culture media.
3. Immunologic tests

Relevance to Ethiopia:- The parasite is not recorded in Ethiopia.

Trypanosoma gambiense

Geographical Distribution: West and western central Africa, extending from Senegal across to Sudan and down to Angola.

Trypanosoma brucei complex (group)

Trypanosoma brucei brucei (infective to animal but not humans) and the human pathogens *Trypanosoma brucei rhodesiense* (causing acute trypanosomiasis) and *T.b. gambiense* (causing chronic trypanosomiasis) are morphologically indistinguishable.

Habitat:

Trypomastigotes: In blood vessels and intercellular spaces of Lymph nodes, spleen, liver, Brain, CSF etc.

Metacyclic trypomastigotes: In the mid and fore gut of the Glossina (tsetse flies)

Morphology: has trypomastigote and metacyclic trypomastigote stages

Life cycle*:* It requires two hosts to complete its life cycle, species of Glossina as an intermediate host and man and other animals as a reservoir host. It reproduces asexually by binary fission.

Trypanosoma gambiense is a saliverian trypanosome in which the trypomastigotes develop in the mid and fore gut of the insect vector so that infection is acquired through inoculation of the metacyclic trypomastigotes into the subcutaneous tissues with the saliva. In the blood vessels the metacyclic trypomastigots transforms into trypomastigots stage. There is multiplication of the parasites in the mammalian host and the insect vectors. The parasites can also be

transmitted through blood transfusion, and congenitally. Important vectors are *G.palpalis, G.fuscipes, and G.tachinoides.*

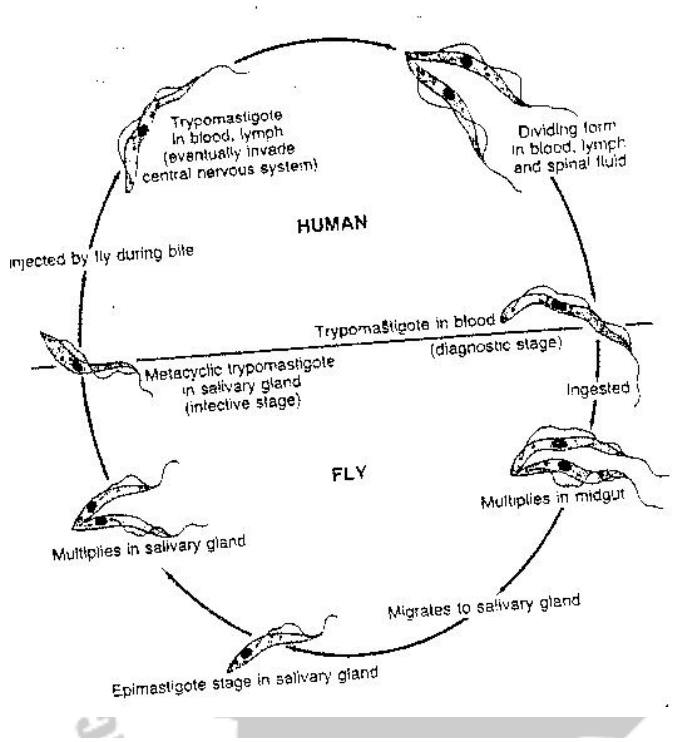


Figure 2.14. The life cycle of *T.b.gambiense and T.b.rhodesiense*

(From Strickland GT. Hunter's tropical medicine 7th ed. Philadelphia:WB Saunders,1991.)

Clinical Features and Pathology: Gambian trypanosomiasis or chronic African sleeping sickness. Major symptoms are chancer, fever, haedache, sweating, post cervical enlargement of the lymph node(winter bottom's sign), splenomegally, hepatomegally, meningoencephalilis,

inability to speak, progressive mental dullness, excessive sleeping, weight loss, coma and death if untreated.

Trypanosoma rhodesiense

Geographical Distribution: East Africa, Central Africa, and Southern Africa, extending from Ethiopia down to Botswana.

Habitat:

Trypomastigotes: Mainly in the blood and CSF, also in lymphnodes, spleen, Brain etc.
Metacyclic Trypomastigote: In the mid and fore gut of the insect vector.

Morphology: has trypomastigote and metacyclic trypomastigote stages that are morphological similar to trypomastigote and metacyclic trypomastigote stages of *T.gambiense* but posterior nucleated trypomastigote stages are common.

Life Cycle*:*-Similar to *T.gambiense* but the main reservoir hosts are game animals such as Antelops, Giraffe. The disease is transmitted naturally by wood land and savannah tsetse flies and also by blood transfusion. Important vectors are *G.morsitans, G,pallidipes, G.austeni, G.tachinoides, G.fuscipes.*

Pathology: Rhodesiense trypanosomiasis or acute African sleeping sickness. The major symptoms are similar to *T.gambiense* but has short incubation period and rapid loss of weight.It is a zoonosis and has low prevalence, sporadic form of infection, and more prevalent in male than in females.

Both *Trypanosoma gambiense and T.rhodesiense* have similar methods of prevention and laborarory diagnosis.

Prevention and Control

1. Vector control:
   -By spraying vehicles with insecticide as they enter and leave the tse-tse fly infested area,
   -By using and maintaining insecticide impregnated tse-tse fly traps,
   -By selectively clearing the bush and wood areas especially around game reserves, water holes, bridges, and along river blanks.
2. Detecting and treating human infections at early stage.
3. Restricting the movement of game animals to within fenced game reserves.
4. Health education.

Laboratory Diagnosis

1. Trypomastigotes in wet blood film -to observe motilit,
2. Thick or thin stained blood films or buffy coat from Micro-haematocrit or capillary tube centrifugal concentration technique.
3. Examination of aspirate from enlarged lymph gland, chancher fluid, CSF
4. Testing serum for anti-trypanosomal antibodies.

Relevance to Ethiopia

Animal trypanosomiasis (Nagna or gendi) always has been a problem in many parts of Ethiopia (Tedla S, A.A Introduction to Parasitology, 1986)

T.b. rhodesiense is singled out as the species occuring in Ethiopia (Baker JR McConnel, Ethiop Med J, 1974).

The species of vector of trypanosomiasis are to be found in Ethiopia are:

1) From the palpalis group- Glossina fuscipes fuscipes and G.tachniodes

2) From the moristans group- G.pallidipes and G.moristans sub moristans also called Ugandanesis.
3) From fusca group- G. longipennis

These *Glossina* species are confined to the southern and Western regions. The seven adminstrative regions infested are Shewa, Gojam, Welega, Illubabor, Kefa, Gamo Gofa and Sidamo. Although the belt is quite extensive in Ethiopia the Sleeping sickness foci are limited to Gambela (the areas along Baro, Gilo and Akobo rivers), Gamo Gofa (from Mursi-Bodi district), Kefa (from maji), and Welega (from the settlement area in the Anger-Didesa valley).The following domestic animals are commonly found in the tsetse belt of Ethiopia: Cattle, sheep, goats, donkeys, mules, dogs and chickens (Kloos H and Zein AZ, 1993).

Trypanosoma cruzi

Geographical distribution: Central and South America

Habitat:

Amastigotes: Intracellular forms in the reticuloendothelial cells and tissues of brain, muscles, Lymph nodes, liver, Spleen, bone marrow, etc.
Promastigotes: Transitional stage
Epimastigotes: In the mid-gut of the insect vector (bug)
Trypomastige: In the mid-gut of the vector and; in the blood circulation and intercellular spaces of man.
Metacyclic Trypomastigote: In the mid gut and in the faeces of the insect vector

Morphology:

Has all the developmental stages of haemoflagellates

Amastigote stages are similar to amastigote stages of *Leishmania* species. The Trypomastigote are monomorphic forms about 2μm in size with “C” or “U” shape.

Mode of Transmission of T. cruzi

1. Contact with the faeces of an infected blood sucking triatoma bug.
2. Blood transfusion.
3. Less commonly trans-placental transmission occurs with a fetus being infected from an asymptomatic mothers.
5. It can occur also if viable parasites (even very few) penetrate the skin, conjuctiva, or mucous membrane.

Life Cycle

In most infections, metacyclic (infective) trypomastigote contained in the faeces of an infected bug (Triatoma) penetrate the skin through the bite wound or enter through the conjunctiva of the eye or the membrane of the mouth or nose. The feace are deposited as the bug feeds or soon after. The trypomastgotes invade the reticuloendothelial cells near the point of entry and multiply intracellularly as amastigotes. The amastigotes develop into Trypomastigotes which are released into the blood when the cell ruptures. The trypomastigote become amastigotes and multiply, forming masses known as Pseudocysts.

Within the pseudocyst, a proportion of amastigote become elongateed and develop first into epimastigotes and then into trypomastigotes which ar released into the blood when the host cell ruptures. Some of these trypomastigites continue to circulate while the majority infect further tissue cells.

By way of the blood and lymphatic system, the parasites reach tissue cells of the heart, nerve, skeletal muscle, smooth muscles of the gastrointestinal tract and else where. The life cycle is continued when a triatomine bug vector ingests circulating Trypomastigotes in a blood meal. In the vector, the Trypomastigotes transform in to epimastigotes which multiply by binary fission in the gut of the vector .Finally metacyclic trypomastigotes are formed in the hind gut of the bug.

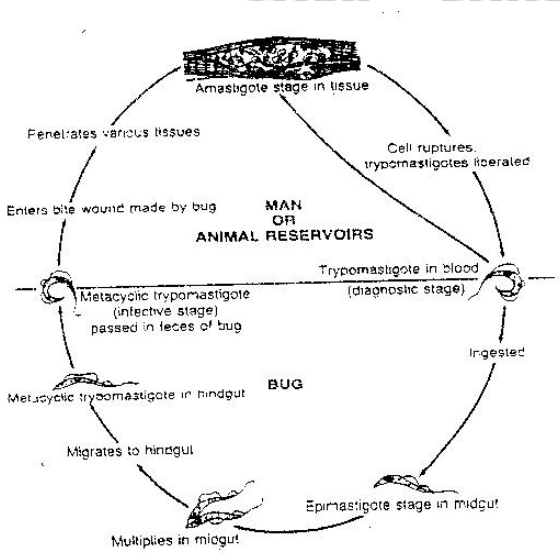


Figure 2.15. The life cycle of *Trypanosoma cruzi*. (From Strickland GT. Hunter's tropical medicine 7th ed. Philadelphia:WB Saunders,1991.)

Pathology*:* Causes chaga's disease.

Prevention and Control*:* Vector control, improvement of housing, treatment and health education.

Laboratory Diagnosis:-

1. Blood film -wet film for motility
   -thin and / or thick stained blood films
2. Xenodiagnosis (in chronic and subacute infections where their number in the blood is usually very few).
   Xenodiagnosis in this method uninfected, susceptible, laboratory reared triatomine bugs are starved for 2 weeks and then fed on the patients' blood. If trypanosomes are ingested they will multiply and develop into epimastigotes which can be found 25-30 days later in the faeces or rectum of the bug
3. Culture of blood on blood agar slopes in the later stages of infection when facilities for xenodiagnosis is not available.
4. Serological diagnosis to detect anti- *T.cruzi* antibodies

Review Questions

1. List hemo-somatic flagellates that are relevant to Ethiopia.
2. Discuss the general laboratory diagnosis of *Leismania* and *Trypanosoma* species.
3. Enumerate the vectors involved for the transmission of
   a. *Leishmania donovani*
   b. *Leishmania tropica minor and major*
   c. *Trypanosima gambiense*
4. Identify hemoflagellates for the presence or absence of the following developmental stages in their life cycle: Amastigote, promastigote, epimastrogte and trypomastiogte
5. Outline the general prevention and control of vector-transmitted diseases.
6. How can you differentiate between species of African sleeping sickness since both species have similar diagnostic morphology?

2.4 Class Telosporidea

Telosporidea

The members of the subphylum sporozoa do not possess any organells of the locomotion like flagella or cilia, but they show change of from by sluggish amoeboid movement. They reproduce asexually by a process of sporulation called schizogony, alternating with sexual reproduction by union or syngamy called sporogony.

The organisms of the subphylum sporozoa are divided into two classes; namely Telosporidea and Sarcosporidea. Telosporidea contains the order coccidiida, having the sub -orders coccidiidea and haemosporidiidea.

Coccidiidea:-These are intestinal sprozoa. In these case, the maturation oocyst occurs outside the body in the passed faeces or in the soil and infection takes place in the susceptible host by contamination through the oral route. The coccidiidea has the family Eimeriidae which has two genera: *Eimearia* and *Isospora*.

Haemosporiidea are parasite of blood and blood forming organs. They have sexual and asexual union in this case takes place in an insect, the definitive host, and the infection takes place in man or other vertebrates by the bite of an insect vector, usually a mosquito. The suborder Haemosporiidea contains three families: Babasiidae, Haemoproteidae and Plasmociidae.

General Life Cycle

Sporozoites→Trophozoites→Schizontes→Merozoites→ Gametocytes→Gametes→Zygote→Ookinet→Oocyst (Sporoblasts→Sporocysts).

2.4.1 Intestinal and Tissue Coccidian Parasites

Isospora belli:

Geographical Distribution*:* Widely distributed in the tropical and subtropical countries

Habitat*:* The epithelial cells/villi of the small intestine.

Morphology:

***Oocyst*:**

Size: 20-30μm

Shape: oval /flask shaped/

Colour: transparent or occasionally pale yellow.

Content: immature when released in the faeces and contain granular mass and two sporocysts each with four sporozoites when the oocyst gets mature, smooth thin and colourless with two layers of cyst wall.

Life Cycle

I.belli complete its life cycle in a single host. The infective stage is the mature oocyst containing sporozoites and following ingestion, with contaminated food or drink the parasites excyst and the sporozoites enter epithelial cells of the small intestine where they develop and multiply by schizogony (merogony). Merozoite infect new cells . Some merozoite form male and female gametes. Fertilization→ Zygotes. Zygotes→Oocyst. Oocysts are excreted in the faeces. Feces

containing oocyst contaminate water supply, food, etc. The oocysts are immature when passed with the faeces and maturation(sporogonic reproduction) is completed in the external environment. Sporozoites are produced in oocyst by sporogony.

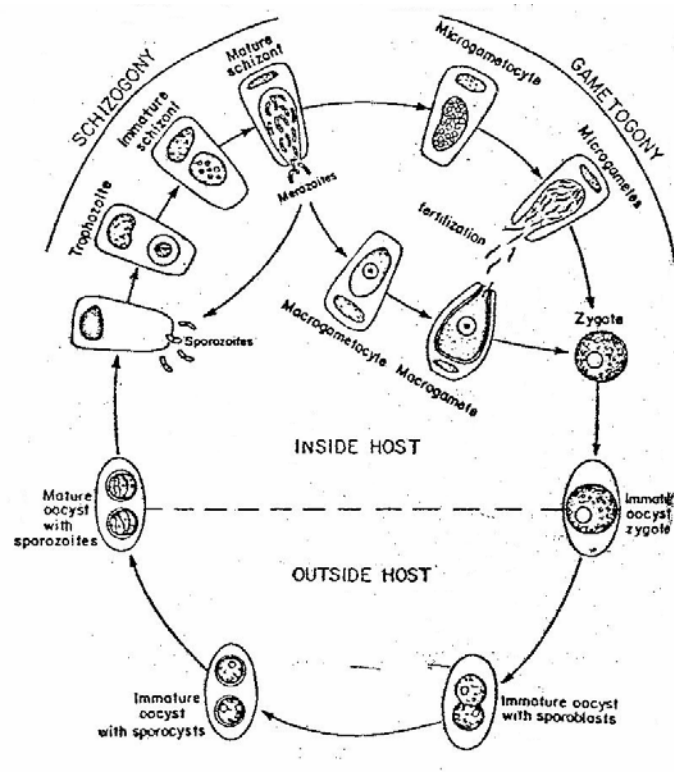


Figure 2.16. Life cycle of *I.belli*. (From Hegazi M. Applied Human Parasitology. 1st ed. 1994; Cairo, the Scientific Book Centers)

Pathology*:* Infection *I. belli* in the immunocompetent is generally associated with no disease or self limiting gastroenteritis, but in some cases the symptoms may be protracted with diarrhea, loss of weight or low grade fever. But in immunocompromized such as AIDS patient it is responsible for significant chronic diarrheal disease.

Prevention and Control:

Infection can be prevented by sanitation of food and drink, and Personal hygiene.

Laboratory Diagnosis:

Finding *I.belli* oocyst in the faeces

Usually only immature oocysts are found in the faeces but occasionally mature oocysts can be seen. In about 50% of infected patients Charcot Leyden crystalare are found in the faeces.

Cryptosporidium Parvum

Geographical Distribution: world wide

Habitat:- Just under the surface membrane/with in the brush-border/of the epithelial cells of the villi of the small intestine

Morphology:

Oocyst: size: 4-5μm

Shape: spherical Contents four elongated naked sporozoites, no sporocyst stage. It is infective when passed in feces.

Life Cycle:

Has similar life cycle to *I. belli* and man acquires infection from contaminated hand, food or drink. Its life cycle is similar with the life cycle of *I.belli*. It will also infects a wide range of domestic animals and wild life.

Pathology*:* It is an important cause of diarrheal disease in young children and toddlers in developing countries. In persons with abnormal immune response, particularly those with AIDS, infection with *C.parvum* can cause acute often fatal diarrheal disease and also respiratory disease (in disseminated) cryptosporidiosis. Autoinfection can occur with infective oocyst sporulating in the intestine.

Prevention and Control:

1. Sanitation of food and drink
2. Personal hygiene
3. Latrine construction.
4. Treatment of infected individuals and reservoir hosts

Laboratory Diagnosis: Finding the oocysts in watery and non-offensive stool sample prepared:

1) With modified Ziehl-Neelsen or safranin-methylene blue staining technique following Sheather's flotation or formol-ether concentration.
2) With acridine orange staining if fluorescent microscopic is available
3) Serological technique using ELISA

Cyclospora cayetanesis

Geographical Distribution:-It is thought to has a world wide distribution, It has only recently been recognized as a human pathogen.

It is transmitted by fecal-oral route with infective oocyst being ingested. Its oocyst is not infective when passed in stool. They require 3-5 days in the environment in which to mature. Water born outbreaks are of *C.parvum* and *C.cayetanesis* have been reported from tropical countries and elsewhere.

Life cycle:-Its life cycle is similar to *I.belli.*

Clinical Feature

It has been described as a cause of prolonged diarrhea in tropical countries and elsewhere. Increasingly, It is being reported as causing severe prolonged diarrhea in those infected with HIV. The specimens are usually watery and often have an offensive smell.

Laboratory Diagnosis

Finding oocyst in wet fecal specimen preparation. It is more easily identified in smears stained by the modified Ziehl-Neelsen Method following concentration by formol-ether oocyst concentration technique. The detail procedure is found in the appendix.

Relevance to Ethiopia

Among opportunistic protozoa parasites *C.parvum, I.belli,* and *C. catyenesis* oocyst were isolated from 6(11%), 4 (7.4%) and 2 (3.7%) of HIV infected patients with chronic diarrhea of JImma HIV/ AIDS patients respectively Mohammed A et al, Ethiop J Health Dev, 2002). Two studies done in Addis Abeba detected a higher prevalence of *C.parvum* (25-32%) than this done in Jimma town. *I belli* were detected in a higher rate (7.4%) than reported in Addis Abeba (1.4%) Of the protozoan infections, *Cryptosporidium parvum, Isospora belli and Cyclospora cayetenesis* are opportunistic infections that are consistently found in adult HIV/AIDS patients with chronic diarrhea. (Mengesha B,E Afr Med J, 1994 ; Fisseha B et al, E Afr Med J,1998),

Toxoplasma gondii

Geographical Distribution: world wide

Habitat:-In the reticulo-endothelial cells of heart, lymph nodes, lung, spleen, bone marrow, mononuclear leukocytes, brain, CSF, spleen, etc of man, domestic and wild animals.

Morphology: -There are five main developmental forms in the life cycle but only trophozoite (toxoplasm) and cyst stages are found in man but all stages occur in the felines (cats).

***Toxoplasm (trophozoite)*:-**Two forms

I. **Tachyzoite/endozoite:-**occurs in the early acute stage of infection. Size: 3μm by 7 μm Shape: crescent or oval in shaped, one end is rounded and the other end is pointed content: In Giemsa stain, paranuclear body- stains red, nucleus stains dark red and cytoplasm stains blue.
   Quickly multiplying forms that form pseudocyst (aggregation of a parasites inside a macrophage)

II. **Bradyzoites/cryptozoites:-**Occurs in the chronic stage of infection, develops slowly and multiplies in the tissues to form a true cyst.

***Cyst*:-**10-100μm and may contain about 3,000 trophozoites

Life Cycle:

Definitive host: Cat and Lynx

Intermidiate host: Man and other animals

The life cycle can be either heteroxenous (requiring two hosts) or monoxenous (one host). Both sexual and asexual reproduction occurs in cat but only sexual reproduction occurs in man. Mode of multiplication can be endodyogony (repeated division in to two by internal budding), ectomerogony (division in to several organisms simultaneously by

external budding), and endopolygony (division into several organisms simultaneously by internal budding.

Mode of Transmission

1. Ingestion of oocysts in food, drink, or hand contaminated with faeces of an infected cat.
2. Blood transfusion,
3. Transplacental/congenital
4. Ingestion of cysts in raw or under cooked meat
5. Organ transplantation

Pathology*:* Causes toxoplasmosis

Major symptoms: fever, headache, splenomegally, lymphadenopathy, hydrocephales abortion, still birth. CNS toxoplasmosis is quite common in HIV/AIDS patient with clinical presentation of hemiparesis and/ or loss of consciousness.

Prevention and Control:

1. Avoid contamination of hand, food and water with the faeces of cat
2. Not eating raw or under cooked meat such as pork, mutton, beef
3. Screening of blood and organ of individuals for the parasites
4. Treatment and health education

Laboratory Diagnosis

1. Identifying toxplasms in Giemsa stained histological sections, aspirates of lymphnode, bone-marrow, CSF, pleural fluid, peritoneal fluids and sputum.
2. Serologic tests such as Sabin-feldman dye test, ELISA, IFAT, CFT.

There has to be differenital diagnosis of toxoplasms from amastigote stages of *Leishmania species* and *T.cruzi.*

Pneumocystis carnii

Geographical Distribution: world wide

Habitat:-In the interstitial plasma cells of the lung (alveoli, epithelial cells) of man and other animals

Morphology*:-Trophozoite*

Foam-like mass in the lung

Amoeboid form

Has single nucleus

Cyst*:-* size: 4-5μm in diameter

Shape: spherical

Contents eight comma shaped sporozoites

Resembles a fungus

Life Cycle*:-*The complete life cycle of this parasite is unknown. The trophozoite stage reproduces by binary fission. Man acquires infection from droplets sprayed from the mouth.

b] Indirect fluorescent antibody test.
c] Detection of blood-born antigens by counter-immuno-electrophoresis.

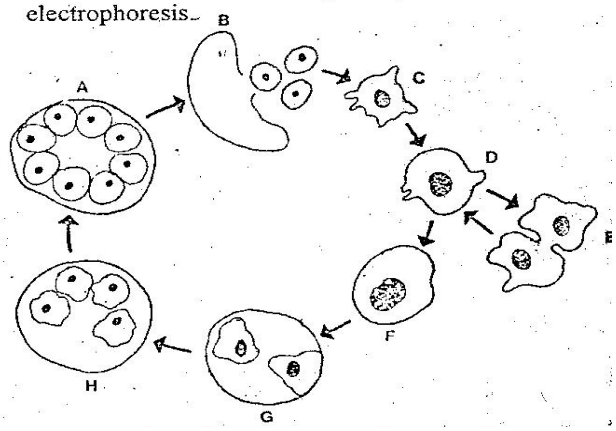


Figure 2.17. Life cycle of *P.carnii.* From Hegazi M. Applied Human Parasitology. 1st ed. 1994; Cairo, the Scientific book centers

Pathology: Causes a severe a typical interstitial plasma cell pneumonia, impaired ventilation, and death due to respiratory failure. Common in immunodeficient patients. *P.carnii* is a leading cause of death in patient with AIDS.

Prevention and Control:

1. Sanitation of food and drink
2. Treatment of infected individuals

Laboratory Diagnosis

1) Finding the trophozoites and cysts in Geimsa stained
   -sputum or bronchial biopsy
   -Bronchial alveolar lavage

2) Serological detection using PCR, IFT

Blastocystis Hominis

Blastocystis is a common inhabitat of the human intestine. Previously it was regarded as a non-pathogenic yeast; however, more recent studies confirm its identity as a sporozoan and place it in the family sporozoa.

B.hominis is more than a harmless commensal or it is a potential pathogen. It should be borne in mind when causes of intestinal disorder are sought, esoecially in the absence of other identified causes of diarrhea or gastrointestinal symptoms should be assessed for the presence of *B.hominis.*

It is found in stool specimens obtained from asymptomatic individuals as well as those with persistent diarrhea. Although it has been suggested that the finding of large numbers of B.homins (5 or more organisms /oil immersion field) in the stool of symptomatic patients consistent with its role in disease. It may also be detected in wet mounts or trichrome-stained smears of faecal specimen.

It has a round shape an about the size of an *E.coli* cyst but shows great variation in size. It has a peripheral cytoplasm, a central vacuole but no nucleus

Relevance to Ethiopia

There is only a few recent studies which determined the prevalence of B.hominis infection in Ethiopia. In one study done in AIDS patient in A.A, two cases among the 190 patients were detected (Fisseha B et al, Ethiop J Health Dev. 1999).

Review Questions

1. What is the importance of studying intestinal and tissue sporozoa?
2. Explain the laboratory diagnosis of *Isospora belli* and *C.parvum*?
3. Discuss the sexual and asexual reproduction of *T.gondii?*
4. List the diagnostic and infective stages of the following parasites.
   a. I. belli _____ _____
   b. C.parvum _____ _____
   c. P.carnii _____ _____
   d. T.gondii _____ _____
5. Illustrate the developmental stages of parasites listed above.

2.4.2 Haemosporidia (The Malaria Parasites)

Malaria is the most important of all protozoan disease; it annually infects over 250 million individuals and is a leading cause of illness and death in the developing world. In many endemic areas it is becoming increasingly difficult to control because of *Anopheline* mosquito vector and the parasite to develope resistance to various eradication and treatment options.

General Characteristics

1. Intracellular obligate parasites.
2. Man is intermediate host.
3. Female Anopheles mosquitoes are the definitive hosts.
4. Those species which infect human being are *P.vivax, P.falciparum, P.malariae* and *P.ovale*
5. Has no animal reservoir host except *P.malariae* in which monkeys are the reservoir hosts
6. Infective stage to man from the insect vector is sporozoites and to the insect vector from man is gametocytes.

Geographical Distribution

Malaria is endemic in 91 countries with about 40% of the world population is at risk. *Plasmodium falciparum* is the most prevalent species in the hotter and more humid regions of the world*. P.vivax* is the most widely distributed in the temperate, subtropics and some parts of the tropics. Unlike the other species, it is more common and well adapted to the temperate region than in the tropics.

P.malariae has much lower prevalence than *P.vivax, P.falciparum* and *P.ovale*. It is confined mainly to tropical Africa. Also it is found in South America and South west Asia. Infection rates in Ethiopia are about 60%,

40%, 1% and less than 1% for *P.falciparum, P.vivax, P.malariae* and *P.ovale,* respectively.

Habitat:

The parasite enters the blood and carried to the parenchyma cells of liver, where they multiply enormously. This is called the pre-erythrocytic or tissue phase. By rupture of the infected cells they enter the RBCs, the erythrocytic phase (Schizogony) and reach all the organs of the blood via the circulating blood, producing parasitaemia.

Morphology

There are sequential developmental stages with distinct morphological features that helps for species identification in Romanowsky stains of Peripheral blood films. This presented for each species below:

P.falciparum

Young Trophozoite (Ring forms)

Stage frequently found in blood film

Size: - Small rings, 0.15-0.5 diameters of RBCs which is unaltered in size.

Shape: small fine pale blue ring

Chromatin: 1 or 2 small red dots.

Often with double chromatin dot

May lie on red cell membrane (accole forms)

Pigment: absent:

Mature Trophozoite

Stage rarely seen in peripheral blood

RBC unaltered in size, sometimes stippled, pale

Shape: compact thin blue ring , comma or exclamation mark shaped

Chromatin: 1 or 2 red dots

Pigment: black or dense brown mass

Schizont

RBC unaltered in size , sometimes stippled, pale.

Size: parasite about 0.6RBC

Merozoites: 8-32; average 24

Pigment: clumped black

Not usually seen in peripheral blood

Gametocytes

RBC is distorted.

Fairly frequently found

Size: larger than red cell

Shape: crescent or banana or kidney or sickle shaped

Rounded forms may be seen if film dries slowly

Stippling

Maurer's cleft

Infected red blood cells

All age group of red blood cells are infected.

Often contain several parasites

Density*:* often high density of parasites

Plasmodium Vivax

Rarely more that 2% of cells infected

Young Trophozoites

Size : 0.3-0.5 diameter of RBCwhich is unaltered in size.

Cytoplasm-circle, thin

Chromatin : fine dot some times two

Pigment: absent.

Mature Trophozoites

Size : large (RBC enlarged,stippled)

Parasite- irregular amoeboid

Chromatin: Dots or threads of red colour

Pigment: Golden brown and scattered

Schizonts

RBC much enlarged

Size: Almost fills red blood cells

Shape: amoeboid or segmented, parasite large, filling enlaged ***RBC***

Cytoplasm: pale blue

Merozoites: 14-24; average 16

Pigment: Golden brown central loose mass

Gametocytes

RBC enlarged, stippled.

Parasite large, rounded filling enlarged RBC

Stippling

Schuffner's dot seen indistinctly around parasites.

Infected Red Blood Cells

Youngest erythrocytes are infected

Parasite Density: medium

Plasmodium Malariae

Young Trophozoite

Size: Up to 1/3 Red blood cell.

Cytoplasm -circle,thicker

Chromatin: one large red dot

Pigment: absent

Mature Trophozoite

RBC unaltered

Parasite- compact often band or rounded shape.

Chromatin: round do or red band

Pigment: dark brown or black pigment, often concentrated along one edge of the band

Band forms can be seen in thin films

Occasionally "birds-eye" ring form may be seen

Schizont

RBC unaltered

Size: Nearly fills red cells

Merozoites 6-12; average 8, sometimes forming rosette

Pigment: Brown aggregated in centre.

Gametocytes

RBC unaltered, parasite small round filling RBC

Infected Red Blood Cells

Oldest erythrocytes are infected

Stippling- None (Ziemanns dots often after prolonged leishmania staining

***Parasite Density*:** low density, rarely more than 1% of cells infected (easily missed in Laboratory diagnosis)

Plasmodium Ovale

Young Trophozoite

Size: 1/3 Red blood cell

Shape: Regular compact dense blue ring (can resemble P.malariae in thick films)

Chromatin : One medium sized red dot

Pigment: absent

Mature trophozoite

Size : Small

Shape: Round compact, very blue cytoplasm

Chromatin: One large red dot

Pigment: -small amount of brown pigment

Schizont

Size: small

Shape: segmented

Merozoites: 6-12; average 8 in a rosette

Pigment: Brown

Gametocytes

Shape: large, oval or round, dense blue

Pigment: Brown particles

Difficult to differentiate from late trophozoites

Infected Red Blood Cells

Slightly enlarged

Pale in colour

Oval in shape, fimbriated at one side

Stippling

Has schuffner's (or James) dots. Youngest erythrocytes are infected

Density*:* Medium Density.

Rarely more than 2% of cells infected

- About 20-30% of cells may become oval with fimbriated (ragged) ends

General Life Cycle of Malaria parasites:

-Malaria parasites require two hosts to complete their life cycle. Female Anopheles mosquitoes as the definitive host, where sexual reproduction (sporogony) takes place and human being as the intermediate host, where the asexual reproduction (schizogony) takes place.

- Sporozoites from infected female Anopheles mosquito are injected with the saliva into the blood circulation of man when the vector takes a blood meal. After circulating in the blood stream for not more than one hour, the sporozoites enter into the liver cells.
- Two cycles occur in man, in the liver as exo-erythrocytic schizogonic reproduction and in the red blood cells as erythrocytic schizogolnic reproduction. In the liver the parasites multiply and develop into schizonts. When mature the schizont, the liver cell rupture releasing large number of merozoites. The merozoites enter the red blood cells and develop to trophozoite stage. The trophozoite feeds on haemoglobin and forms malaria pigment (haemozoin). The trophozoite stage develops into schizontes.
- In the schizont nuclear division takes place to produce large number of merozoites that are released from the schizont to invades new red blood cells. After several erythrocytic schizogonic reproductions, the merozoites develop into gametocytes.

- To continue the life cycle, the gametocytes are ingested by a female Anopheles mosquito while taking a blood meal. In the stomach of the mosquito, the male and female gametocytes under go fertilization and produces a zygote. the zygote develops into a motile ookinete which penetrates the stomach wall of the mosquito to form an oocyst. Inside the oocyst large numbers of sporozoites are formed. The oocyst ruptures releasing the sporozoites that also enter into the salivary gland to be transmitted to another individual when the insect takes a blood meal.
- Recrudescence (due to small number of erythrocytic parasites remaining in the blood after a previous attack) occurs in *Plasmodium falciparum* and P*lasmodium malariae* over a long period of malariae infection.
- Relapse due to hypnozoite (dormant forms which precede schizont development in the liver occur in vivax malaria and less commonly in Ovale malaria.

Modes of Transmissions

1) Bite of infected female Anopheles mosquitoes. The main vectors in Ethiopia are *A.gambiae, A.funestus, A.nili, A. arebiansis* and *A.pharonensis*
2) Blood transfusion causes only erytrocytic infection.
3) Contaminated syringes and needles
4) Congenital / transplacental

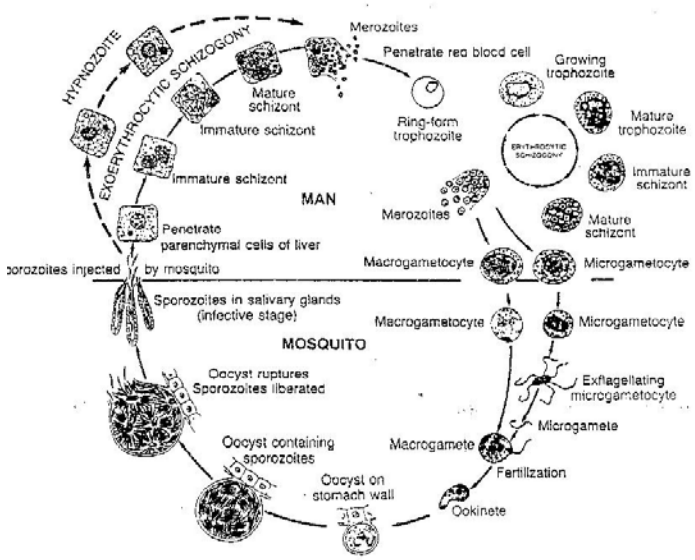


Figure 2.18. The life cycle of Plasmodium species. (From Strickland GT. Hunter's tropical medicine 7th ed. Philadelphia:WB Saunders,1991.)

Clinical Feature

Malaria pathogenicity is mainly releated to P. vivax causes Benign Tertian malaria and now called vivax malaria.

P.malariae causes Quartan malaria and nopw called malariae malaria.

P.falciparum causes malignant Tertian or subtertian malaria.

P. ovale causes Benign Tertian malaria.

Major symptom is malaria fever usually that occurs in three stages:

1. Cold stage: Rigor, headache, coldness and shivering
2. Fever stage: rise in temperature, up to 40.6^0c, severe headache, back and joint pain, vomiting, diarrhea
3. Sweating stage: perspiration, temperature falls, headache and pain relived until the next rigor.

The malaria fever is due to the rupture of the infected red blood cells containing mature schizonte stage releasing malaria pigment, toxins, metabolic by products, debris of red blood cells and merozoites that can infect other red blood cells

Level of Parasitaemia in the Red Blood Cells

P.falciparum. Up to 30-40% of the red blood cells can be infected, and it is considered as sever if more than 5% of RBC are infected.

P.vivax. 2% of the RBC can be infected

P.ovale. more than 2% of the RBC can be infected

P.malariae. up to 2% of the RBC can be infected

Number of Merozoites Released From a Single Pre-erythrocytic Schizonts

P.falciparum: 30,000 merozoites

P.vivax: 10,000merozoites

P.ovale : 15,000 merozoites

P.malariae : 15,000 merozoites

Factors for Malignance of P.falciparum

1. Rapid multiplication
2. Infected red blood cells become "stick"
3. Infects all age group of red blood cells
4. A single red blood cell can be infected by more than one parasites
5. Erythrocytic schizogonic reproduction takes place in the deep capillaries of organs such as brain, lung, heart, spleen, bone-marrow, placenta, intestine, etc.

Factors That Provide Protection against Malaria Infections are:

1) Glucose-6-phosphate dehydrogenase deficiency , Scickle cell anemia Ovalocytosis and Adenosine tri-phosphate deficiency in non-immune black males provides protection against *P.falciparum* infection
2) Thalassemia, Duffy blood group antigens (i.e., Fy^a and Fy^b) negative RBCs and Hemoglobin E provides protection against *P.vivax* infection.
3) Human fetal hemoglobin gives protection against all forms of malaria infection

Dominance of Malaria Parasites

P.falciparum repress the parasitaemia of *P.vivax* and *P.malariae*
P.vivax dominates over *P.malariae*

Prevention and Control

Prevention and control of malaria become difficult as a result of resistance of the parasites to the drugs and failure of control measures. Besides this population movement, climatic changes and economic problems are also considered currently as factors that related with malaria spread. However, the following are some of the measures to be taken as prevention and control measures;

1) Avoid mosquito bites by
   - Selecting healthy sites for houses and screening windows and doors with mosquito net.
   - Using mosquito bed nets
   - Wearing protective clothes such as long trouthers
   - Using mosquito repellents

2) Destroy adult mosquitoes by
   - Indoor residual regular effective spraying

3) Preventing breeding of mosquitoes by
   - Altering the habitat to discourage breeding
   - Flooding or flushing of breeding places
   - Drainage to remove surface water, filling in ponds, pot holes, etc.
   - Spraying breeding places with effective chemicals particularly with larvicides

4) Using drugs to
   - Prompt diagnosis and treatment of malaria cases
   - Prevent infections using chemoprophilaxis, especially in non-immune persons visiting or going to malarious areas or in persons with reduced immunity such as pregnant women.

5) Health education.

6) Blood screening for malaria before providing for those who need blood.

Laboratory Diagnosis

1) Malaria parasites are detected in thin or thick blood films stained by wright's stain, Giemsa stain, leishman stain or Field stain. Take blood films when the patient feels febrile because the parasites are usually most numerous in the blood towards the end of an attack of fever. Always collect the blood before anti-malarial drugs are taken.
   Field stain is recommended for smears stained straight away and Giemsa stain for smears to be stained after a few days.

2) Using a rapid immunodiagnostic tests such as ParaSight F, ICT malaria Pf / Pv and, OptiMALr. These are recently discovered groupof techniques, which proved tobe valuable and highly sensitive

and specific tests. All of themdepend on the “dipstick” format and they are termed collectively as”rapid test for malaria.

- The principleof these test kits depends on detection of P.falciparum Histidine Richprotien-2 antigen (Pf HRP-2)which is only produced by P.falciparum or by detection of Plasmodial Lactate Dehydrogenase enzyme(LDH) isoenzymes produced by both *P.falciparum* and *P.vivax*.
- Detection of PfHRP-2 can not differentiate between dead and live parasites, this antigen canpersist in the serum for months after parasitological clearance of the disease. On the other hand, identification of specific LDH verifies live parasite only and inturn it appears more specific fordiagnosisof active infection.

3) ELISA

4) PCR

Babesia

Apicomlexan parasites belonging to the genus Babesia have long been known as parasites of domestic and wild animals, causing at times inapparent infections but also causing such economically important disease as Texas cattle fever and malignant jaundice in dogs. Humans are accidental hosts. It is transmitted in nature by ixodid, or hard -bodied, ticks.It can be differentiated from malaria by the absence of pigment within infected erythrocytes. The organisms infect the red cells, in which they appear some what poleomorphic ring like structures. Most resembles ring stage of Plasmodia. The small parasites appearing much like *P.falciparium* can be differentiated from malaria parasite by the absence of pigment in the infected erythrocytes.

Human infection is diagnosed by identifying the intra-erythrocytic parasite in Giemsa -stained blood films. It can also be diagnosed by serologic test with the indirect immunoflorescent antibody test; which is the most useful in diagnosis.

Relevance to Ethiopia:-

Tropical Africa is generally endemic to falciparum malaria and due to the absence of Fya/Fyb duffy antigens. It is however reported to be allopatric to *P. ovale,* which is endemic in the western part of the continet. Based upon this geographical distribution. Western and south western Ethiopian lowlands lye at the eastern tip of the *P. ovale* belt. thus Setit Humera along with Gambela and Arbaminch, was identified as one of the endemic sites of ovale malaria.

The rise of the sibling species, *P. vivax.* in once *P. ovale* endemic area is considered to be related to the migration of settlers and agricultural laborers form the *P. falciparum: P. vivax* codominant north central Ethiopian highlands . On the other had the prevalence of *P. malariae* in Humera, Like the rest of Ethiopia, Is descrete and focal. The overall prevalence of *P. malaria* was shown to 1% and below.

Malaria in the lowlands has contributed to overpopulation, overcultivation, deforestation, soil erosion, and drought and famine in the *weyna dega* and dega zones of the Ethiopian Highlands. According to the 1984 census, about 37% of the Ethiopian population lived above 2,200 m, 45% between 1,500 and 2,200m, and the remaining 18% below 1,500 m. Encouraged by the initial success of the malaria Eradication Service in the 1960s and increasing economic opportunities, many highland people settled in, or seasonally worked in, the lowlands, a trend which accelerated during the government’s resettlement program in the mid-1980s and subsequent famines and military activities.

With the exception of perennial transmission which prevails around lakes and swamps and irrigation systems and riverine areas of the large rivers, particularly the Awash, Baro, Blue Nile, Didesa, Beles, Takeze, Wabe Shebele, and Omo, malaria is highly seasonal in the *kolla* and weyna dega *A. gambiae* s.l. is found in all administrative regions of Ethiopia.

This is the second most frequent and widely distributed vector of malaria in Ethiopia comprising 18.9% of the 19,352 specimens collected during the period 1984 – 1988.

An. funestus is the third most common vector of malaria in Ethiopia, comprising 3.2% of all the specimens collected during the period 1984-1988.

An. Nili is the least common species, and it is more localized, being confined to the southwestern, western, and northwestern parts of the country.

Anopheles culicifacies adenensis is the only species found in the Aseb port area along the Red Sea coast, where there is a known indigenous transmission of malaria.

Of the four *Plasmodium* species known to cause human malaria in Ethiopia, the two epidemilogically important species are *Plasmodium falciparum*, comprising 60% of all the cases of malaria, and P*lasmodium vivax*, constituting nearly 40% of all malaria cases. *Plasmodium malariae* comprises less than 1% of all cases and is most frequently reported from the Arba Minch area. *Plasmodium ovale* is rarely reported. The geographic distribution of all four parasites at the regional level was mapped by Gebremariam.

Plasmodium falciparum

P. falciparum causes the most frequent and fatal episodes of malaria in Ethiopia. It is the cause of severe and complicated malaria in which the case fatality rate is about 10% in hospitalized adults and up to 33% in children less than 12 years old. A study conducted on 1,261 subjects from eight different Ethiopian ethnic groups revealed that glucose-6-phosphate dehydrogenase deficiency was present only in the Anuak and Nuer of the southwestern lowlands, as well as in the Afar in the Danakil Depression, with a prevalence of 1.4%, 6.7%, and 6.3%, respectively. Only one case of sickle cell trait (Hb As) was found in the same study.

Plasmodium vivax

P. vivax is also widely distributed in Ethiopia and often precedes the transmission of *Plasmodium falciparum. P. vivax* is more common during the dry season, and whether this is due to active transmission or relapses has not been clearly determined. This species is not a major cause of mortality but is an important cause of morbidity due to the phenomenon of relapse. A study conducted in the Gambela area demonstrated that residents speaking Nilotic languages are more resistant to *P. vivax* infections than are the more recent arrivals to these lowlands, who speak semitic and Cushitic languages. Later studies established the absence of Duffy antigen in Nilotic speakers.

2.5 Class Ciliatae (Ciliates)

Balantidium coil

Geographical Distribution: World wide being more commonly found amongst those who keep pigs, and uses pig faeces as fertilizer vespecially in warmer climates. In Ethiopia it was reported from Debre Brahne.

Habitat: Trophozoite and cyst in the large intestine of pig and rarely man.

Morphology:

Trophozoite

Size: 50-200 μm by 40-70μm

Shape: Oval, with one pole more rounded than the other.

Motility: Rapid motility, crossing the field in a definite direction and sometimes turning in circle.

Cilia: Cover the whole body and many around the cytostome

Nucleus: a large kidney shaped macronucleus and a small micronucleus .

Cytostome: a sort of "mouth" that contracts and expands to draw in debris also has cytopyge

Cyst

Size : 50-70μm, very large cyst /the size of a round worm egg.

Shape: round

Shell: thin double wall

Nuclei: one large kidney shape macronucleus and one small Micronucleus beside the large nucleus.

Cytoplasm: granular, greenish, filled with inclusion bodies.

Life Cycle

As soon as the thick wall cysts are excreted in the feces, they are infective. Man acquires infection from contaminated food or drink or from hands contaminated with faeces. Following ingestion, the cyst excyst in the intestine producing trophozoites; where each cyst producing a single trophozoite. The ciliate multiply in the colon by simple binnary division often following conjugation. Conjugation takes place by a process, when the two trophozoite are found to be in state of union and enclosed in a cyst, with the exchange of nuclear material. This is followed by separation of the two rejuvenated individuals. Pig is the main reservoir host.

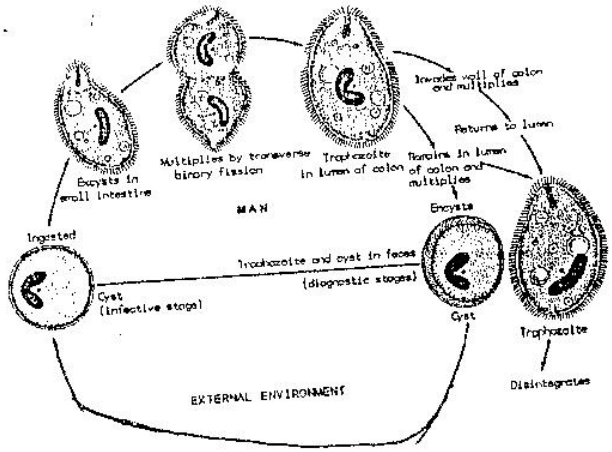


Figure 2.18. Life cycle of *B.coli*.(From Nasr NT.Review of Human Parasitology,2nd ed. Cairo: The Scientific Book Center)

Pathogenicity*:-* It is the only ciliate that parasitize humans. Causes balantidial dysentery. Infection with B.coli can be without symptoms unless the ciliates invade the intestinal wall. Invasion can cause

infammation and ulceration, leading to dysentrywith blood and mucus being passed with faeces.

Prevention and Control:-

1. Avoid contamination of food or drink.
2. Improving personal hygiene especially those who keep pigs.
3. Treatment and health education.

Laboratory Diagnosis:-Finding the tropozoites in dysenteric faecal specimens and the cysts in formed or semiformed faeces.

- In dysenteric specimens the ciliates usually contain ingested red cell.

Relevance to Ethiopia

The parasite is not common in Ethiopia A few cases have been reported in Debre Berhan.

Review Questions

1. What characters help to differentiate one plasmodium parasite from other species?
2. What is the significance of thin and thick blood in the laboratory diagnosis of malaria parasites?
3. What factors give natural protection against plasmodium infection?
4. Which plasmodium species is more malignant that others? Why?
5. Explain methods used to estimate parasitemia load of malaria parasites.
6. Identify a ciliate which infect human.
7. What is the diagnostic and infective stage of the above parasite?

CHAPTER THREE

Medical Helminthology

Helminths (Worms)

The word, helminths from Greek means "Worm" and originally referred to intestinal worms but it is more usually interpreted to include both parasitic & free-living species of round worms (phylum Nemathoda)"hair snakes" or gordiid worms (Phylum – Nematomorpha), tubellarians, flukes & tape worms (phylum-plathyhelminthes) & thorny - headed worms (phylum-Acanthecephala). The helminths are generally macroscopic, and the adult worms vary tremendously in size from barely visible to 10 meters in length.

The life cycles of helminths may be quite complex and include both direct and indirect cycles. The clinical sign and symptoms of helminthic infections depend on the location of the organisms and may be caused by adults, larva, or eggs. The host response to the presence of parasite may be prominent and often includes eosinophilia, especially in the early stages of infections when the parasites are in tissue. The final diagnosis is usually dependant on detection and identification of a mature or developmental (larva, embryo, egg) stage of the parasite. Occasionally the diagnosis may be made clinically or serologically.

The majority of helminths produce characteristics eggs that are passed in feces and serve as the chief means of diagnosing infections. The identification of eggs should be approached in a systematic manner taking into account the size, and shape of the egg, the thickness of the shell, the presence or absence of specialized structures such as spines,

knobs or opercula. The presence and characteristics of larva present within the eggs may be useful.

3.1 Platyhelminths

3.1.1 Class Cestoda (Tape worms)

Tape worm infection in man is less limited in their distribution than are human fluke infections. They are frequently not restricted to any specialized group of intermediate hosts, as species- of Mollusks that may be local in their distribution or confined by certain meteorological conditions. Some of them are dependent primarily on exclusively on man as a definitive host for the continuation of their life cycles (*T.segina, T.solium, H. nana D.latum*); on the other cases man is incidental to continued propagation of the parasite (*E.granulosus, H. duminuta and Diplidium caninum).* In all known tapeworm infection, except in certain varieties of sparganosis, in which exposure in topical, the portal of entry is mouth hence, strict care not to swallow raw or inadequately, cooked beef, pork or fish or food or water contaminated with faeces or vermin, will ensure protection of the individuals.

General characteristics:

1. Dorso-venterally flattened (leaf or tape-like)
2. Bilaterially symmetrical.
3. They are provided with a nervous system and an elaborate excretory apparatus.
4. Digestive system may be absent, or when present it is rudimentary and without anus. It obtains its nutrient by absorption through cuticle.
5. Respiratory, circulatory system and body cavity are absent.
6. Tape worms are hermaphrodites and have well developed reproductive system.

7. Each unit of chain (segements) is known proglottides. The entire chain of proglottids is called strobila
8. The body is divided into three main body regions; this are

Head (scolex): attachment organ and may have grooves, suckers, and rostellum armed with hooklets; this varies with species.

Neck: growth region, proglottids proliferate from this region.

Strobila: varies in number, shape, size, and maturity. It is divided into three regions:

a/ immature : sex organs are immature.

b/ mature: sex organs are fully mature.

c/ gravid : reduced or atrophied primary genital organs, uterus is filled with eggs

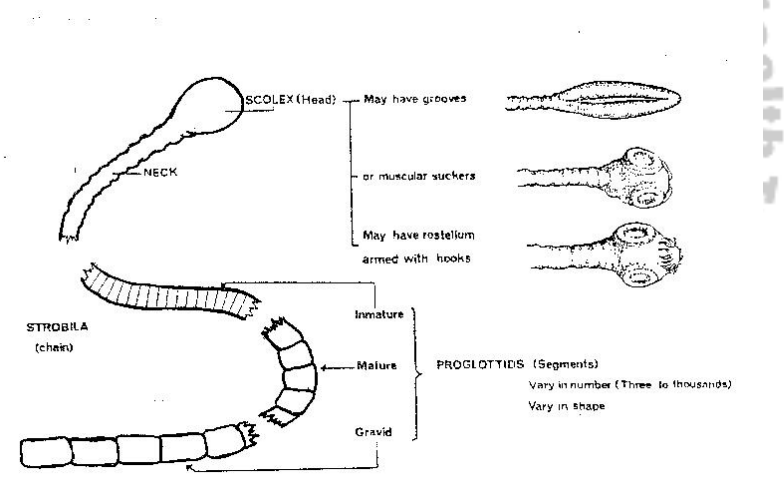


Figure 3.1. Body parts of tape worm. (From Jeffrey HC and Leach RM. Atlas of Medical Helminthology and Protozoology, 1975.)

N.B: Infection persists as long as the scolex and the neck region remain attached to the intestinal wall.

9. The entire body is covered with active homogenous, elastic, resistant and continuous cuticle/integument from one proglottid to the next through out the entire body.
10. Elaborate and well developed reproductive system with complete set of male and female genital organs which are found in a single worm.
11. Man is;
    a. The only or main definitive host for *T.saginata, T.solium, H. nana and D.latum*
    b. Intermediate host for *E.granulosus* and *E.multilcularis*
    c. Both as definitive and intermediate host for *H.nana* and *T.solium*

Based on the following differences criteria, cestodes are classified into two orders:

	Order- Pseudophillidea	**Order-Cyclophillidea**
Differences		
1. Scolex	- Spoonshaped, grooves	- globular with 4 suckers
2. Genital pore	- venteral	- marginal
3. Utrine pore	- Present(ventral)	- absent
4. Uterus	- coiled	- sacular tubular or branched
5. Ova	- operculated	- non-operculated
6. Onchosphere	- ciliated	- non-ciliated
7. Rostellum	- absent	- present
8. Progilottids	- broader than long	- longer than broader
9. Larval forms	- solid	- cystic

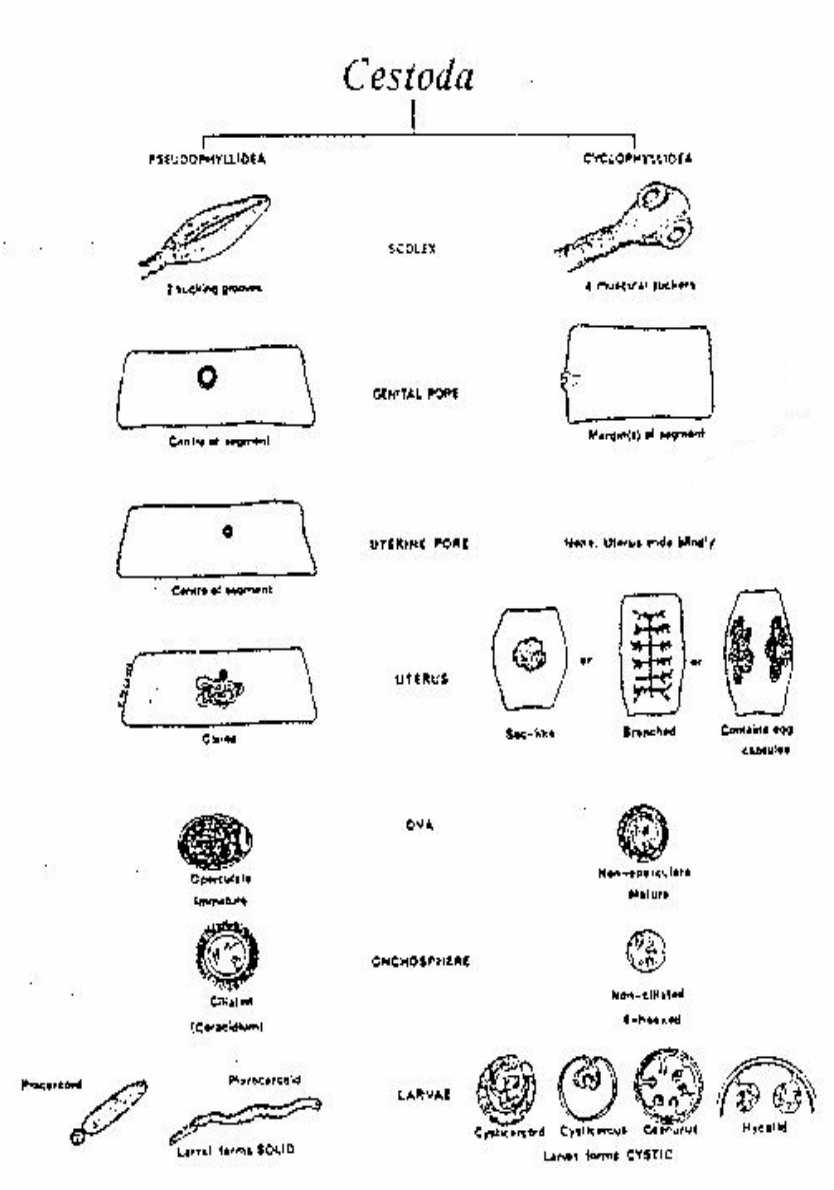


Figure 3.2. Criteria used for the differentiation of Pseudophyllidea from Cyclophilidea. (From Jeffrey HC and Leach RM. Atlas of Medical Helminthology and Protozoology, 1975.)

Egg: - Two type

1. Operculated, immature when voided to the external environment.
2. Non-operculated ,fully embryonated when voided to the external environment.

Larvae: -Generally two type

I. Solid : eg. Procercoid, Plerocercoid, cysticercoid
II. Cystic(true bladder): can be with:- Single scolex eg. Cysticercus; Many scolexes and/or with daughter cyst eg. hydatid cyst, coenurus cyst, etc.

Taenia saginata

(Beef tape worm)

Geographical Distribution

World wide distribution where cattle are raised and beef is eaten raw or under cooked. More common parasite of man unlike *Taenia solium.* It is very common in Ethiopia.

Habitat

Adult: In small intestine of man

Larvae: In muscular tissues of cattle

Eggs: In faeces of man or in gravid segments.

Morphology:

Adults: *Size*: Total worm: 3-10 m long

Mature segment: 1-2cm long.

Colour: ivory white

Scolex (head): quadrate, with four suckers, no looks, no rostellum

Strobila : 1000-2000 proglottides

Mature Proglottides:

- Broader than long
- Genital pores are arranged irregularly alternate on the lateral margin of each segment

Gravid proglottide

- Detach when fully develop and pass through the anus independently.
- 15-30; average 10 compound lateral uterine branches.

Larvae: Known as Cysticercus bovis
Found in skeletal and muscular tissues of cattle
Has four suckers and no rostellum and hooklets

Egg (Embryophore): -
identical with the egg of *T.solium.*
Size: - 33-40 μm
Shape: -Round
Colour: - Shell-dark yellowish-brown, content light yellowish gray.
Shell:-Thick, Smooth, brown, radially straited (embryophore)
Content: - A round granular mass enclosed by a fine membrane with six hooklets
Stains red (acid fast) in Ziehl-Neelsen staining technique, this character helps to differentiate from *T.solium* which do not have red color in such staining.

Life cycle

Requires two hosts to complete its life cycle. Man as a definitive host and cattle as intermediate host.

Egg(hexacanth embryo→larva(Cysticercus bovis) →Adult

Man acquires infection from raw or under cooked infected meat. Following ingestion, the larvae become attached to the wall of the small intestine with its suckers. Proglottides are formed from the neck region and the larvae grow into a long adult tapeworm. When fully developed the gravid segments become detached and the eggs are discharged only after the gravid segments have been separated from the worm. Gravid segments containing eggs and eggs from ruptured segments are passed in the faeces. Cattle ingest the eggs with contaminated grass while grazing. The embryos escape from the eggs and pass through the intestinal wall into a blood vessel. Through the blood circulation they are carried to muscles and develop into infective cystic larvae called cysticercus bovis.

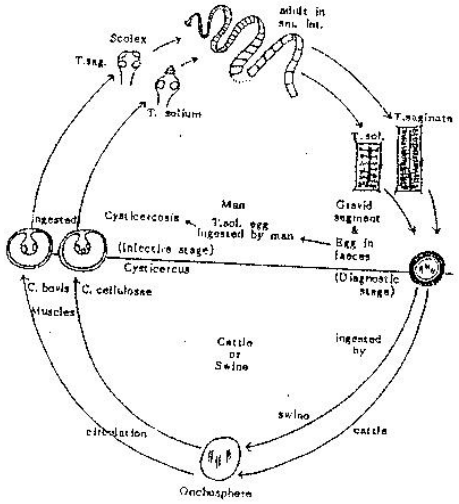


Figure 3.3. Life cycle of *Taenia* species.

(From Nasir NT.Review of Human Parasitology,2nd ed. TheScientific Book Center, Cairo)

Pathogenicity*:* - Causes taeniasis

Major symptoms are loss of appetite, weight loss, hunger, acute intestinal obstruction, eosinophilia, and discomfort by the crawling of segments through the anus. Proglottides of *T.saginata* have a strong tendency to crawl from the anus during the day when its host is active unlike *Entrobius vermicularis.*

Prevention and Control

1. Avoid eating raw or insufficiently cooked meat which may contain infective larvae.
2. Inspecting meat for larvae
3. Provide latrine for the proper waste disposal
4. Not using untreated human faeces as fertilize for pasture land
5. Protection of cattle from grazing on faeces or sewage polluted grass.
6. Treating infected person and providing health education

Laboratory Diagnosis

1. Detecting eggs in faeces. Morphologically eggs of *T.saginata* and *T. solium* are indistinguishable unless stained by AFB.
2. Identifying gravid segments and scolex recovered from clothing or passed in faeces. Single rectangular segments found in underclothes and bed clothes.

Relevance to Ethiopia

Taeniasis. High *T.saginata* infection rates in Ethiopia are due to the widespread custom in the agricultural highlands of eating raw beef, and the habit of defecating in open fields coupled with the tradition of allowing cattle to graze in such fields. Infections are under-reported. Owing to the inherent problem of missing many infections during routine stool examination. Estimates made by different investigators of the prevalence

of taeniasis in Ethiopia vary widely, from 2% -16% to over 70% (Kloos H et al, 1993, Yared M et al 2001).

Taeniasis is so common in the country and the tradition of self-treatment is so well developed that most people do not use the health services for diagnosis and treatment. Instead, Ethiopians use about dozens of traditional plant medicines, including Kosso (*Hagenia abyssinica*). Enkoko (*Embelia schimperi*), and Metere (*Glinus lotoides*) upon noticing proglottids in the feces or when experimenting abdominal discomfort, usually every 2 months (Shibru T, 1986).

Taenia solium

(Pork tape worm)

Geographical Distribution:-Widely distributed where human faeces reach pigs and pork is eaten raw or insufficiently cooked.

Habitat:

Adult: In the small intestine of man

Larva: In muscular tissues of pig

Egg: In the faeces of man and in gravid segment.

Morphology:

Adult

Size: Total worm 2-7m

Mature segment :0.5-1.5 cm

Colour: pale blue

Scolex (head): Quardate with four large deep suckers, rostellum with two rows of hooklets

Strobila:

800-1000 Proglottides

Immature proglottide are broader than long

Mature proglottide are nearly square and genital pores are arranged irregularly alternate on the lateral margin.

Gravid Proglottides:- 7 to 12, on average 10 lateral compound uterine branches. Small chains of 3-4 rectangular segments found in the faeces.

Larvae:-

Known as Cysticercus cellulosae

Found in skeletal and muscular tissues of pig

Has four sucers, rostellum and two raws of hooklets

Egg (embryophore): -morphologically identical with the egg of *T.saginata*.

Size: - 31-43 (m

Shape: -Round

Colour:- Shell-dark yellowish-brown, content light yellowish gray.

Shell: -Thick, Smooth, brown, radially straighten (embryophore)

Content: - A round granular mass enclosed by a fine membrane with 6 hooklets.

Does not stains red (acid fast) in Ziehl-Neelsen staining technique

Life Cycle:

The life cycle of *T.solium* is similar to that of *T. saginata* except pig serving as an intermediate host for the development of larvae known as *Cysticercus cellulosae.*

Egg(hexacanth embryo) →larva(Cysticercus cellulosae) →Adult

Man acquires infection from eating raw or under cooked pork that develops into adult in the intestine or from contaminated food or drink with faeces containing the eggs and develops into larval stage in visceral organs.

Mode of Transmission can be

- Eating raw or under cooked pork meat
- Eggs in food or drink
- Internal autoinfections

Pathology: Taeniasis and cysticercosis

Major symptoms are as a result of the adult worm. These include abdominal pain, loss of appetite, and infection with larvae cause cystic nodules in subcutaneous and muscles. If it is in the brain it causes epilepsy and other CNS disorders.

Prevention and Control:

1. Avoid eating raw or insufficiently cooked pork meat
2. Ensuring pigs do not have access to human faeces.
3. Inspecting meat for larvae
4. Treating infected person, providing health education and adequate sanitary facilities

Laboratory Diagnosis

1. Detecting eggs in the faeces which is morphologically indistinguishable from the egg of *Taenia saginata*.
2. Identifying gravid segments and scolex in the faeces after treatment.
3. Finding calcified larvae in histological or X-rays examination .

Relevance to Ethiopia

The parasite has not been reported from Ethiopia. The presence of *T.solium* in Ethiopia is doubtful at though some people keep pig the main reason for its possible absence is the fact that both Moslems & orthodox follow the religious prohibition against eating pork (Kloos H et al,1993).

Hymenolepis nana

(Dwarf Tape Worm)

Geographical Distribution:-

H.nana is widely distributed in countries with warm climates than in cold climates and fairly common in Ethiopia. Children are more commonly infected than adults.

Habitat:

Adult: small intestine of man, rat and mice

Cysticercoid larvae: in the intestinal villi of man, rat and mice.

Eggs: In the faeces of man, rat and mice

Morphology:

Adult

Size: 10-44 mm

Scolex with 4 suckers, short retractile rostellum with single crown of hooklets.

Stroblia: 100-200 proglottides, the size is inversely proportional to the number of worms present in the intestine of their host.

Mature Segment: Unilateral common genital pore

80-180 eggs in gravid segment

Egg:

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.

Colour: colour less or very pale gray

Content: Rounded mass (embryo) with six refractile hooklets arranged in fan shaped.

Life Cycle: *H. nana* has a direct life cycle with a human host serving as both definitive and intermediate host.

Egg(hexacanth embryo) →Cysticercoide larvae→ Adult

Eggs are the infective stages which are ingested contaminated hands, food and drink. Following the ingestion of eggs, the embryos are freed in the small intestine. They penetrate villi and develop into infective cysticercoid larvae. When fully mature, the larvae rupture out of the villi into the lumen of the intestine. They attach to the intestinal wall by their scolex and grow rapidly into mature tapeworms. Gravid segments detached and eggs are released in the intestine. Some of the eggs are passed in the faeces while others remain in the intestine to cause internal autoinfection. The eggs are infective when passed in the faeces.

Mode of Transmisssion: -

1. Ingestion of egg with contaminated food, drink of finger.
2. Autoinfection.

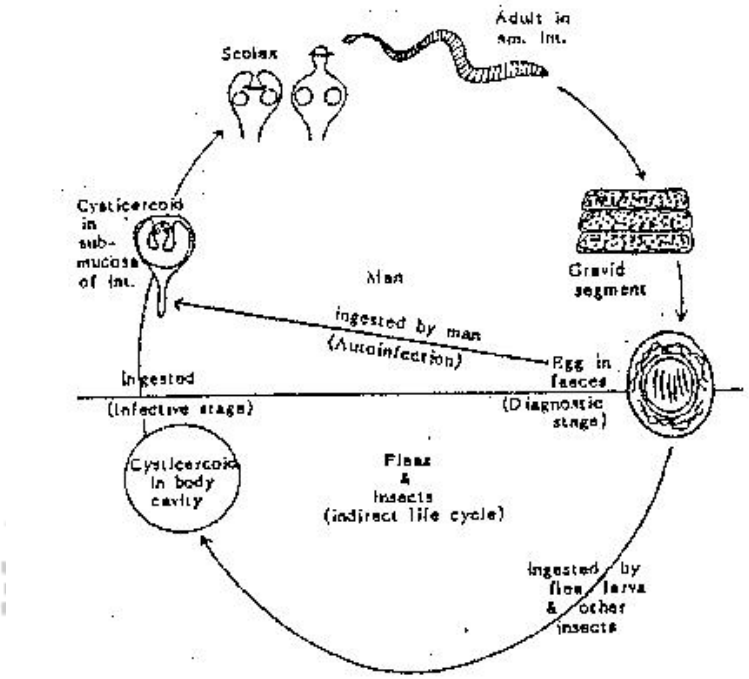


Figure 3.4. Life cycle of *H.nana* **(**From Nasir NT. Review of Human Parasitology,2nd ed. Cairo:The Scientific Book Center.)

Pathology: Although many *H.nana* tapeworms can be found in the same host due to internal autoinfection, the life span of the adult worm is only a few month. Symptoms of infection are rarely detected except in children when many tapeworms may cause abdominal pain, diarrhoea, anorexia and lassitude. Toxins released from the worms can cause allergic reactions.

Prevention and Control:

1. Personal hygiene, washing of hands before eating and after defecation
2. Sanitary disposal of faeces into latrines
3. Avoiding eating uncooked food
4. Protection of food, and drink from contamination with faeces
5. Treatment and health educations.

Laboratory Diagnosis

1. Usually eggs of the parasite in the faeces.
2. Some times adult worms in the faeces

Hymenolepis diminuta

(Rat tape worm)

Geographical Distribution: Cosmopolitan with sporadic human infection in the world. It is fairly common in Ethiopia.

Habitat:

Adult: Ileum of rat, mice and rarely man

Larva: body cavity of insects (fleas and cockroaches)

Egg: In the faeces of rat, mice and man

Morphology:

Adult -

20-60 cm

Scolex with 4 suckers and retractile rostellum without hook-lets.

Colour: Transparent or pale yellow

Stroblia: 800-1000 segments

Egg

Yellow-brown or bile pigmented.

70 by 60µm

Shell with double shell and with out thread like polar filaments.

Content: A rounded embryo containing six hooklets arranged in fan shape.

Life Cycle:

Requires two hosts to complete its life cycle. Intermediate hosts are fleas, beetles, cockroaches. Definitive hosts are rat, mice and man.

Egg(hexacanth embryo) →Cysticercoide larvae→Adult

Eggs in the faeces of the definitive hosts are ingested by the insect vectors and hatches releasing the oncosphere. The oncosphere migrate into the body cavity and develop into cysticercoid larva. Man acquires infection by accidentally ingesting the infected insect vector.

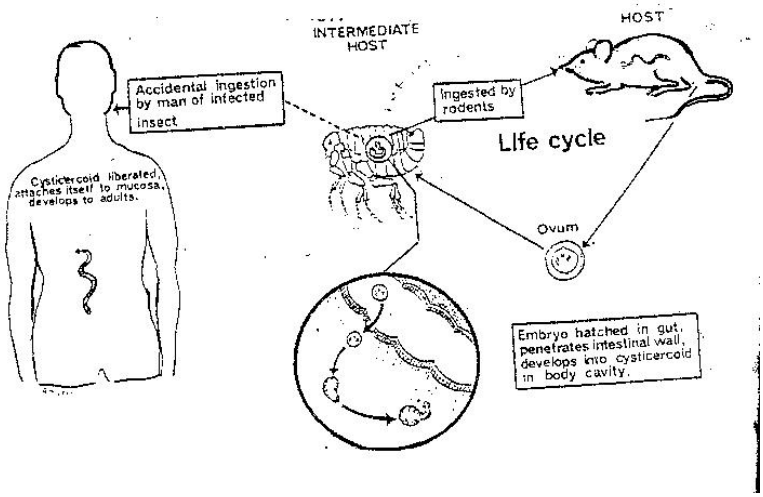


Figure 3.5. Life cycle of *H.dimunita*(From Jeffrey HC and Leach RM. Atlas of Medical Helminthology and Protozoology, 1975.)

Pathology: Causes hymenolopiasis and major symptoms are abdominal pain, diarrhoea, restlessness.

Prevention and Control

1. Avoid insect vectors
2. Avoid their reservoir hosts
3. Protection of food and drink from insect vectors
4. Health education

Laboratory Diagnosis

1. Eggs in the faeces

Relevance to Ethiopia

Hymenolepiasis. Both *Hymenolepis nana* and *H. diminuta* have a cosmopolitan distribution, but *H.diminuta* is relatively rare and its existence in Ethiopia has been revealed only recently. *H. nana* is fairly common in Ethiopia and most common in children and is easily transmitted from person to person through the feco-oral route. In a survey of schoolchildren in 26 towns and villages in Harerge, only 1 case of *H.diminuta* but 65 cases of *H. nana* (in 20 communities) were found. The highest recorded prevalence of H. nana in Ethiopia was 61% among school children in Kemise town in southern Wello. Of 50 communities in the central and northern highlands, 78% had positive cases, with a mean prevalence of 12%. Among the general population of the Lake Zeway islands and the outpatients of Zeway Health Centre, *H.nana* was found in 6.9% and 2.0%, respectively (Kloos M et al. 1993).

Echinococcus granulosus

(Hydatid worm)

Geographical Distribution:-Common in sheep and cattle raising countries mainly in Kenya, Middle East, North and South Africa, India, Australia. Also it is found in South and South East Ethiopia.

Habitat

Adult: mucus membrane of small intestine of carnivores such as dog, fox, Hydatid cyst/larvae: in the different body parts (liver, lung, brain, etc) of man and herbivorous animals.

Egg: in the faece s of dog, fox, and jackals

Morphology

Adult

Size: 3-6mm

Scolex with 4 suckers, rostellum with two rows of hooklets.

Stroblia: three proglottides. One immature, one mature and one gravid segment.

Egg:

morphologically indistinguishable from the ova of *Taenia* species

Size: 30-37µm

Larvae (Hydatid Cyst)

Contains brood capsules with many scolice, free brood capsules and scolice, hydatid sand, cystic fluid containing salt, enzymes and toxins.

Life Cycle:-

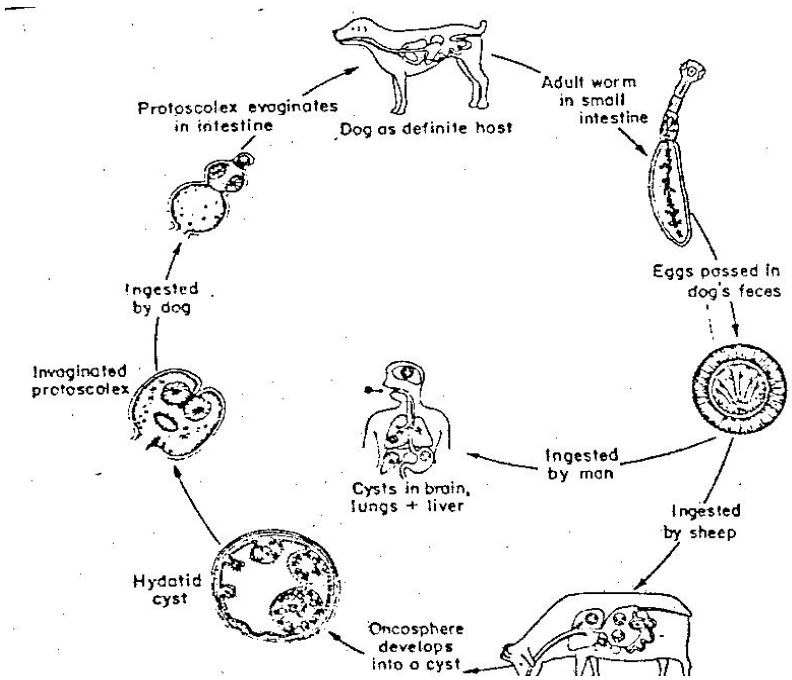


Figure 3.6. Life cycle of *E.granulosus*. (From Hegazi M. Applied Human Parasitology. 1st ed. 1994; Cairo, the Scientific book centers)

Requires two hosts to complete its life cycle. Carnivores such as dog, fox, jackles are the definitive hosts, man and herbivorous animals.

Egg (hexacanth embryo) →Hydatid cyst(larvae) → Adult

Man acquires infection from ingesting eggs in contaminated food, drink and fingers. The eggs hatche in the intestine and penetrate the intestinal wall and disseminated through out the body through the blood stream and become hydatid cyst. There is no development of the parasite as adult forms in man.

Mode of Transmission:

- Contaminated food, drink or finger with infected faeces of dog, fox, Jackals.
- Handling infected dogs.

Pathology: Causes hydatid disease.

Major symptoms are obstruction and pressure on vital organs, anaphylactic shock due to rupture of the cyst, Jacksonian epilepsy, jaundice, erosion and fracture of bones.

Prevention and Control:

1. Personal hygiene, washing of hands before eating
2. Avoid handling dogs
3. Avoiding eating uncooked food
4. Protection of food, and drink from contamination with faeces
5. Treatment and health education.

Laboratory Diagnosis

1. Histological examination to find larvae
2. X-ray examination to find larvae
3. Examination of cystic fluid for brood capsules and protoscoleces
4. Casoni's skin test

Relevance to Ethiopia

This parasite is common among the pastoral people in the South and South-East of Ethiopia. These people have close contact with their dogs. Although rarely indicate that settled agriculturalists as well as urban dwellers do have the parasite (Shibru T, 1986).

Diphyllobothrium latum

(Fish tapeworm)

Geographical Distribution:-Widely distributed in the lake areas of Europe, Asia, Far East, North America, South America and Central Africa.

Habitat

Adult: small intestine of man, cat, dogs, pig

Eggs: passed in the faeces of man

Larval forms:

Coracidium: free in water

Procercoid: body cavity of copepod / Cyclopes

Plerocercoid (sparganum) larvae: in the flesh of fresh water fish such as pike, perch, salmon, eel, barbel, ruff, trout

Morphology

***Adult*:** the largest tapeworm

Size: 10m or more

Grayish-yellow in colour

Scolex is elongated, spoon shaped, longitudinal suctorial groove/bothria/slits with no rostellum and hooklets.

Long and slender neck

Strobila*:* 3000-4000 proglottids

- Broader than long
- Mature and immature segments can not be distinguished.
- Genital pore and uterine pore open on the ventral surface

- Has coiled uterus opened by uterine pore for the passage of individual egg
- Gravid segments are retained by the worm, and eggs are discharged periodically but as they cease to function gradually disintegrate.

Egg: 58-76μm by 40-51μm
Broadly ovoid
Light golden yellow, Operculated
Thick shell
Contains immature embryo

Life Cycle: -

D.latum requires three hosts to complete its life cycle.

Definitive hosts: man dog, pig

Intermediate hosts: - Primary Intermediate hosts: Crustacean
- Secondary Intermediate hosts: Fresh water fish

Man acquires infection by eating raw or inadequately cooked fresh water fish.

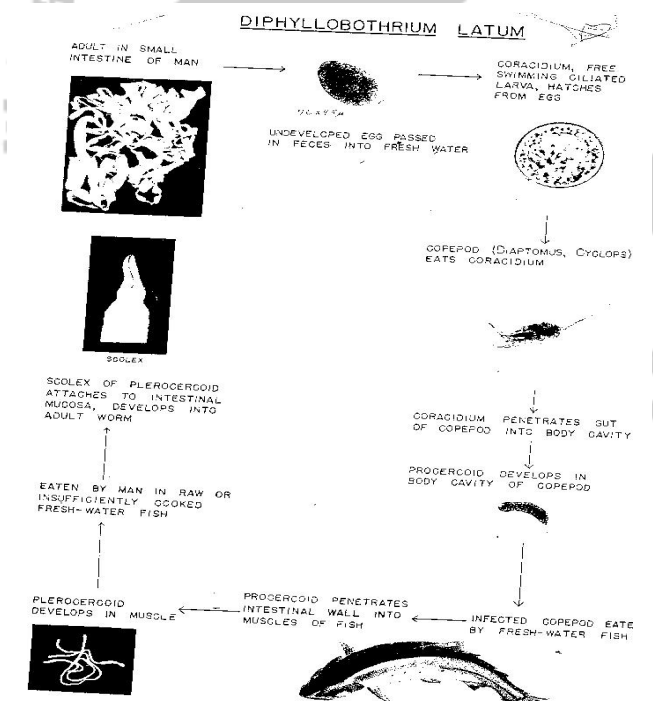


Figure 3.7. Life cycle of *D.latum* (From Brown HW and Neva FA, *Basic clinical parasitology*, 5th ed. USA: Applenton- century Crofts,1983).

Pathology: Competes for vitamin V 12 and and cause megaloblastic anemia.

Major symptoms are abdominal pain, diarrhea, constipation, loss of weight, intestinal obstruction, pernicious anemia and eosinophilia.

Prevention and Control:

1. Avoid eating raw or undercooked fish
2. Proper disposal of faeces
3. 0Fish inspection for larvae
4. Treatment of infected individuals and health education.

Laboratory Diagnosis

1. Eggs in the faeces
2. Scolex in the faeces
3. Adult worms in the faeces

Relevance to Ethiopia

A few people, particularly indigenous inhabitants of the lake Zway Islands, eat row fish as part of their diet, but stool exam. of 100 local fisherman didn't reveal any eggs of fish tapeworm, *D. latum (* Yared M, et al, 2001*).*

Dipylidium caninum

(Dog Tapeworm)

Geographical Distribution **:-**World wide distribution

Habitat:

Adult: mucus membrane of small intestine of carnivores such as dog, cat, Man Cysticercoid larvae: In the body cavity of insects

Egg: in the faeces of dog, cat, man

Morphology

Adult 20-60cm

Scolex with 4 suckers, retractile rostellum with several rows of hooklets.

Stroblia: many proglottides each with two sets of genital organs.

Egg: 5-15 eggs in capsule

40μm in size and yellowish brown in color

Larvae(Cysticercoid):-Evaginated scolex with several rows of hooklets

Life Cycle:-Requires two hosts to complete its life cycle. Carnivores such as dog, fox, and occasionally man are the definitive hosts, and fleas and other insects are intermediate host. Man acquires infection from accidentally ingesting infected insect vectors.

Pathology: Causes dipylidiasis

Prevention and Control: Similar to *E.granulosus*

Laboratory Diagnosis:-Finding gravid segments and eggs in the feces

Review Questions

1. eggs in contaminates food of drink?
2. List parasites that have auto infection in their life cycle.
   Enumerate the possible diagnostic stages of cestodes
3. Identify a cestode that does not require intermediate host in its life cycle.
4. What are the differences and similarities between *T.saginata* and *T.solium*?
5. Among the cestodes which of them are acquired by ingestion of
6. At makes *T.solium* more sever than *T.saginata*?
7. What are the basic differences between the two order of cestodes?

3.1.2 Class Trematoda (Flukes)

Trematodes or flukes are members of the platyhelminths and are generally flat, fleshy flat, leaf shaped worms. Trematods vary in size from species just visible to the naked eye, like *H.heterophyes* and *M.yokogawi* to large fleshy species, like *Fasciola* and *Fasciolopsis*. The most Characteristic external structures are the acceptable (i.e., suckers) which caused the early workers in this group to call them trematoda, i.e. "body with holes." They posses two muscular suckers: one oral, surrounding the opening to the primitive digestive tract, and one ventral sucker for attachment. The digestive system consists of a muscular pharynx and esophagus and bilateral ceca that end blindly near the posterior aspect of the worm.

The flukes may divide into two major categories based on their reproductive systems. The majority of flukes are hermaphrodites. The adult hermaphrodites contains both male and female sex gonads and produce operculated eggs. The S*chistosomes* constitute the second major category and include organism with separate sexes. The female *Schistosomes* deposits only non-operculated eggs.

Both S*chistosomes* and hermaphroditic flukes have similar lifecycles that include one or more intermediate hosts. Another means of classifying the trematodes that cause human infection is by the anatomic location of the parasite in the human host. Thus we have the intestinal trematodes, the liver and lung trematodes and the blood trematodes.

General Characteristics

1. Attached to their host by means of suckers. They have oral sucker which surrounds the mouth and ventral sucker on the ventral surface

2. The digestive system consists of a mouth and an esophagus which divides to form two intestinal caeca. In some species the caeca are branched. There is no anus.
3. For the development, eggs must reach water.
4. Adult flukes live in the bile duct (liver flukes) intestinal tract (intestinal flukes), portal veins (blood flukes) and lung (lung flukes) according to species.
5. Reproductive structure is similar to the tapeworm's
6. Require asexual and sexual generations to complete their life cycle in two or more hosts.

Developmental Forms of Tematodes

Egg:- Can be
-Embryonated or non-embryonated
-Operculated or spined

Miracidium: **-** is first larval stage, ciliated, swims freely in water
- is infective stage to the molluscan host

Sporocyst: **-** The second larval stage in the molluscan host.
- Produce either daughter sporocyst or redia from the germ cells

Redia: **-** The third larval stage in the molluscan host

This stage is absent in the life cycle of Schistosomes.

Cercariae: **-**The fourth larval stage sheded from the molluscan host.
Based on the morphology of the tail, it can be named as:-

1. Furcocercus (forked tail as in *Schistosomes species*),
2. Microcercus(short stumpy and rudimentary tail as in *Paragonimus westermani*),
3. Lophocercus(large fluted tail as in *Clonorichis, Heterophyes, Fasciola, Fasciolobsis, Metagonimus)* and

4. Pleurolophocercus(long tail with fin folds as in *Opisthorchis*)
   -Cercaria is the infective stage of *Schistosomes* to the definitive host

Metacercariae: -Is encysted cercaria with out a tail

This stage is the infective stage of flukes except *Schistosomes.*

3.1.2.1 Blood Flukes (Schistosomes)

General Characteristics:

1. Develop in the portal venous system and the adults flukes depending on species live in the veins that drains the intestine or urinary bladder
2. Sexes are separate(diecious)
3. They are cylindrical; other flukes have flat shape
4. No redia and metacercariae stages
5. Males are broader and females are filiform and larger than male
6. Male has gynaecophoric canal where the female resides after mating
7. Some fresh water snails serve as intermediate hosts, no requirement of secondary intermediate host .
8. Humans are the most significant definitive host. Cercariae is the infective stage from water bodies.
9. The immature stage that migrates in the body after infection is known as schistosomulum

Schistosoma mansoni

(Manson's blood fluke)

Geographical Distribution

S.mansoni is widespread in many African counties including Sudan, Kenya, Madagascar, South America, Middle East, Brazil, India. In Ethiopia, it is found at 2000m above sea level. It was reported from all administratoive regions. The major sites are small streams and lakes.

Habitat

Adult: In the mesenteric venous (haemorroidal) plexuses draining the large intestine (colon and rectum)

Larvae: In fresh water snails.

Egg: In the faeces, and rarely in the urine of man.

Cercariae:- In fresh water, Infective to man

Morphology:

Adults:

***Male*: -** Size: 10-14 mm

Marked tuberculated integument

6-9 testes

Intestinal caeca reunion in the anterior half of the body

Female:

Size: 7-17 mm; longer and thicker than male

Ovary: In the anterior half of the body

Uterus: short, contains only one or two eggs at a time.

100-300 eggs are laid per day per female worm

Egg: `Size : 114-17µm 45-68µm

Shape: Oval, with one well rounded pole and one more conical pole

Colour: pale yellow-brown

Spine: large, triangular lateral spine near the rounded end

Shell: smooth, very thin

Content: fully embryonated (developed miracidium) when discharged with the faeces

Life Cycle: Requires two hosts to complete if life cycle, man as a definitive host and species of fresh water snails known as Biomphilaria as an intermediate hosts.

Embryonated egg→Miracidium→Sporocyst→cercariae→ schistosomulum→ Adult

Man acquires infection when in contact with water containing infective cercariae. The cercariae penetrate unbroken skin, shed their tails and develop into schistosomulum which migrates through subcutaneous tissue into blood vessels. They are carried through the heart and lung. From the lung the schistosomul pass through the left side of the heart and enter the abdominal aorta o reach the portal circulation. The young flukes reach maturity and undergo mating.

Following mating, the paired flukes migrate to the inferior mesenteric vein which drains the large intestine. The female lays eggs in the venules and penetrate through to the lumen of the bowel to be excreted in the faeces. In the water, the miracidia hatch and swim in the water to infect Biomphilaria snails. In the snails the miracidia become sporcysts, reproduce and finally produce infective cercariae which are shed from the snails during day-light hours.

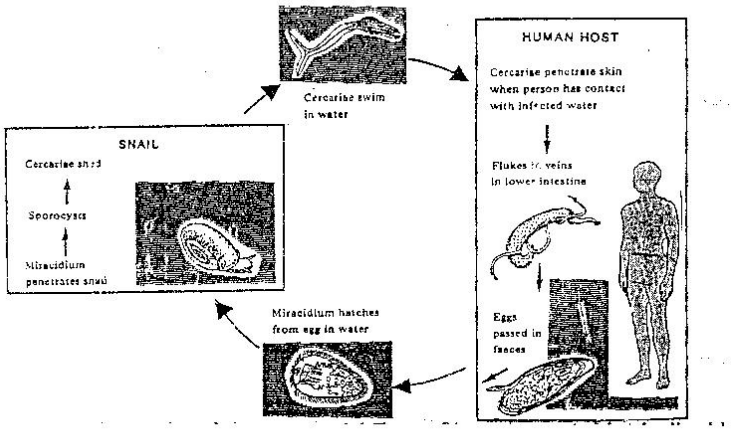


Figure 3.8. Life cycle of S.mansoni (From Cheesbrough M. Medical laboratory Manual for tropical countries, Vol. I, 2nd ed. Britain:The Bath Press.1987**)**

Pathology

Causes Intestinal schistosomiasis / bilharziasis/.

They may be irritation and a skin rash at the site of cercarial penetration (swimmer's itch). The majority of *S.mansoni* eggs penetrate the intestinal wall and are excreted in the faeces sometimes with blood and mucus. Host reaction to eggs lodged in the intestinal mucosa leads to the formation of granulomata, ulceration, thickening of the bowel wall.

A portion of the eggs reach the liver through the portal vein. In the liver , reaction to the eggs may eventually cause thickening of the portal vessels known as claypipe-stem fibrolysis. *Salmonella* infection in patient with *S.mansoni* tend to be chronic and prolonged.

Prevention and Control (General for all Schistosome species)

1. Avoid contact with water known to contain cercariae.
2. Safe water supply
3. Construction of bridges on streams and rivers
4. Providing safe recreational bathing and swimming sites
5. Avoid contamination of water with the faeces of man
6. Latrine construction and sanitary disposal of faeces and urine
7. Sitting settlements away from irrigation canals, drains, dams,
8. Destroying snail hosts and their breeding sites
9. Treatment of infected individuals and giving health education.

Laboratory Diagnosis

1. Finding the eggs in faeces by direct examination or more commonly by using concentration; occasionally eggs may also be found in urine often following faecal contamination. Mucus and blood are often present in the faecal specimen
2. Examining a rectal biopsy for eggs when they cannot be found in faeces.
3. Immunodiagnosis using ELISA, RIA, Latex `agglutination are helpful particularly in prepatent period, and in chronic and ectopic cases in which eggs are difficult to be demonstrated in the faeces. These assays detect circulating antibodies in the serum.

Relevance to Ethiopia

Schistosomiasis appears to have existed in Ethiopia since ancient times, as suggested by the evolution of the human Schistosomes in lakes region of East Africa and the discovery of high *Schistosoma mansoni* infection rates in isolated hunting and gathering populations in the south western and western parts of the country. Increasing population movements in recent decades and deteriorating living conditions have

facilitated the spread of schistosomiasis to areas where it was previously absent (Kloos H and Zein,1993).

The incidence of schistosomiasis in many developing countries is high. In Ethiopia, *S. mansoni* is more common than *S. haematobium*, being found at 2000 m above sea level. Although the right snail hosts have been collected at higher altitudes, no transmission takes place. It is believed that at this altitude the temperature is too low for larval development (Kloos H and Zein,1993).

In children the incidence of *S .mansoni* is low below the age of five. The infection is more common in rural than in urban communities. It is perhaps associated with means of water supply. Schistosomiasis is more important in developing countries, as are nearly all other parasitic diseases, not solely because of the low sanitary level, but also because of greater dependence on agricultural products produced mostly by irrigation and the fact that most people are engaged in agricultural practices (Kloos H and Zein, 1993).

The schistosomiasis surveys carried out by the Institute of pathobiology in 219 communities in all 14 administrative regions between 1978 and 1982 found 15% of 29,451 people infected with *Schistosoma mansoni*. The national schistosomiasis survey of 1988-89 among 27658 people in 291 communities reported an overall prevalence of 25%. Although the results of these two surveys are not strictly comparable due to the preference given by the latter to high-risk areas (agricultural development schemes, water resource development projects, and resettlement sites), comparison of rates in the same communities studied by both surveys shows and overall increase in prevalence during the 10 year interim period. Assuming a 20% prevalence nationwide, 10 million Ethiopians would be infected in 1992 (Kloos H and Zein, 1993).

Schistosomiasis is distributed in most administrative regions of the country. At present the disease is spreading and new transmission foci, including Addis Abeba are being reported (Melakebrhane, 1999).

The geographic distribution of *S.mansoni* infection and of its intermediate snail hosts in Ethiopia-*Biomphalaria pfeifferi* and *B.sudanica*--have been described by various investigators; *B. pfeifferi*, which prefers small streams and irrigation canals, is by far the most common snail host. The highly localized distribution of the infection is largely due to altitude, through its effect on temperature, rain fall topogaphy, population density, and the distribution and nature of surface waters (Kloos H and Zein, 1993).

About 90% of the known endemic communities are villages and small towns by small rivers and streams at intermediate altitudes between 1300 and 2,000m. The greatest concentrations of high-prevalence communities are in the lake Tana Basin and favorable areas in Welega, Arsi, and Harerge, around lakes Zeway, Abaya, and Chamo, and in the irrigation schemes in the upper part of the Awash Valley.At lower elevations high temperatures prevent the survival of B. pfeifferi, although high mineral content and instability of many water bodies during the dry season are contributing factors (Kloos H and Zein, 1993).

S. mansoni transmission is highly seasonal in Ethiopia, concentrated at the end of the dry season, before snails are flushed out of their habitats during the rains. It is during the hot, dry season that human contact with streams and lakes is most intense assuring intense transmission (Kloos H and Zein, 1993)

Infections are common in urban populations in towns with more than 10,000 residents' poor water supply and sanitation, and dependence on local streams put many urban dwellers at high risk of schistosomiasis infection (Kloos H and Zein, 1993).

Studies conducted among children in different parts of the country reported *S.mansoni* infection rates ranging from30 % t0 70%. Study done in three communities: Zarima, Gorgora and Dek in School attendingand non-attending youth revealed that the over all prevalence of the former is greater (66%-86%) than the latter (35-57%). A possibl;e explanation given for this occurrence is that, the school attenders by virtue of their knowledge of hygiene, wash themselves more frequently than the non-attenders and thus, are more risk of infection with *S.mansoni. (*Moges T et al. 2001)

Schistosoma haematobium

(Urinary schistosomiasis)

Common Name - Vesical blood fluke or Urinary schistosomiasis

Geographical Distribution

Widely spread in most parts of Africa, Madagascar, Middle East, Southern Europe, and Western Asia. In Ethiopia - Awash, Wabeshebelle, and Assosa.

Habitat:

Adult: In the vesical pelvic venous plexuses surrounding the urinary bladder, prostate, seminal vesicle and lower thirds of uterus.

Eggs: In the urine, rarely in faeces

Larval stages: Fresh water snails

Cercariae: Free swimming in fresh water, Infective stage.

Morphology

Adults

Male:

Size; 10-15 mm

Finely tuberculated integument

4-5 testes, the ventral sucker is larger

Intestinal caeca reunite in the middle body of the worm

Female:

Size: 20-25 mm, long and slender

Ovary: In the posterior half of the body

Uterus: 20-100 ova at a time.20-200 eggs per day per female worm

Appears darker than male due to the presence of blood pigment in the gut. The suckers are sub equal

***Egg*:**

Size : 120 - 170 µm by 40-70µm

Shape: oval, with one well rounded pole

Spine : Terminal spine at one pole

Shell: smooth, very thin except minute spines on the sucker

Colour: pale yellow-brown

Contain fully developed miracidium when laid

Shell is not acid fast in Ziehl-Neeelsen staining, but the egg shell of other terminally spined *Schistosoma* species is acid fast

Life cycle:

Embryonated egg→Miracidium→Sporocyst→cercariae→ schistosomulum→ Adult

The life cycle of *S. haematobium* is similar to the life cycle of *S.mansoni* with a few exceptions following mating; the paired flukes migrate via the inferior mesenteric veins into the vesical venous surrounding the bladder. The fresh water snail hosts are species of *Bulinus*. The main intermediate hosts are *B.truncatus, B.africanus and B.abyssinicus.* The latter is known to transmit in Ethiopia.

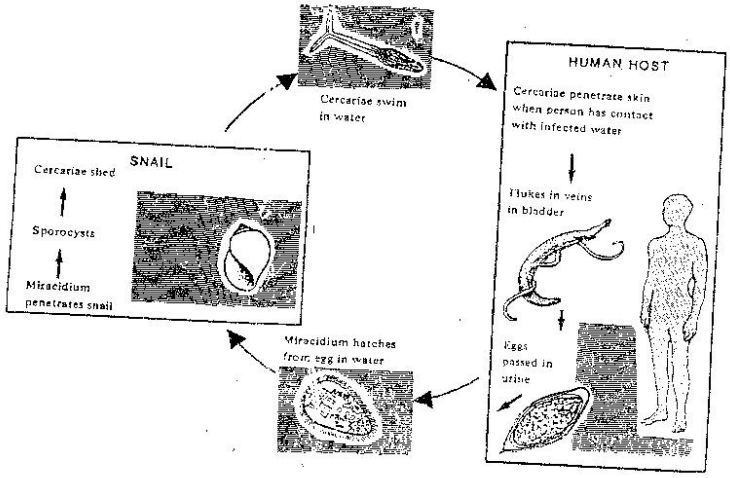


Figure 3.8. Life cycle of *S.haematobium*(From Cheesbrough M. Medical laboratory Manual for tropical countries, Vol. I, 2nd ed. Britain:The Bath Press.)

Clinical Features and Pathology

Causes Vesical or urinary schistosomiasis /bilharziasis.

1. Cercarial dermatitis (Swimmers itch) within 24hrs of infection an intense irritation & skin rash, may occur at the site of cercarial penetration.
2. Migration
   - Passage of cercaria in the lungs leads to minute hemorrhages & pneumonia
3. Ovipositor & tissue reaction
   - Some eggs trapped in the tissue bladder results hematuria

- About 20% of the egg remain inside the tissue of the bladder & this egg finally die & calcified giving rise to the so called sandy patch, appearance

4. In heavy infections eggs can be carried to other parts of the body
5. Following prolonged untreated infection
   - The ureter may become obstructed
   - The bladder wall thickened
   - Eventually, Obstructive renal disease kidney damage

Prevention and Control

Similar as above.

Laboratory Diagnosis:

1. Finding eggs or occasionally the hatched miracidia in urine.
   Urine contains blood and appears red or red-brown and cloudy. Eggs may not be present in the urine all the time; it is neccessary to examine urine collected over several days.
2. Less frequently detecting eggs in faeces, rectal biopsy or bladder mucosal biopsy when an infection is light.
3. Immunodiagnosis: a variety of serodiagnostic methods are currently available. These include: RIA, ELISA IHA. These methods are especially useful in diagnosis of ectopic schistosomiasis where no eggs can be detected in the urine or faeces, and cases with symptoms during the late prepatent period.

Differentiating non-viable from viable schistosome eggs

In assessing active infection or in judging whether treatment had been successful, it is helpful to know whether the schistosome eggs detected are viable or non-viable.

Although it is often possible to see flame cell movement in viable eggs.

Relevance to Ethiopia:-

In Ethiopia all the three known S.haematobium endemic areas--in the middle and lower parts of the Awash valley, in the lower Wabe Shebele Valley, and along the Sudanese border in Welega are below 800 m. Only in the Welega focus do the two schistosome species overlap. Community prevalence in the endemic areas ranged from 5% to 58% in the 1970s. Most infected communities and the highest prevalence rates were in the Awash valley . In the past, the only ethnic group infected with *S.haematobium* in the Awash Valley were indigenous Afar pastoralists living near swamps along the Awash River. Whereas in other endemic areas males are most highly infected, Afar females have rates almost twice as high as males, due to division of work involving the collection of food and fiber plants in and around the swamps, the only transmission sites (Shibru T, 1986).

Bulinus abyssinicus, an exclusive swamp breeder in Ethiopia in the past transmits *S.haematobium* in the Awash Valley and apparently in the Wabe Shebele Valley.

B africanus is the only transmitter in the Wellega focus. Although none of the other eight *Bulinus* species endemic in Ethiopia have been found naturally infected with *S. haematobium,* at least two of them*, B. truncatus and B. octaploidus*, are potential transmitters , based o laboratory finding and the fact that the former is the most important host snail in North Africa and South west Asia (Klooos et al.,1993).

Schistosoma japonicum

(Oriental blood fluke)

Geographical Distribution:

China, Philippines , Indonesia, Japan, Thailand, Philippine.

Habitat:

Adult: superior mesenteric portal veins of the small intestine of man

Eggs: In the faeces

Larvae: In amphibious snail hosts.

Cercariae: free swimming in fresh water; Infective stage

Morphology

Adult

Male -Size: 12-20 mm by 0.5-0.55mm

Non-tubercular body /smooth/

Intestinal caeca reunite at the posterior body part.

Female -26 mm by 0.3mm

Ovary lies at the middle part of the body

Uterus contain 50 or more eggs at a time

1500-3500 eggs per day per female worm

Egg: *Size:* 70-80μm

Shape: oval, almost round

Colour: transparent or pale-yellow

Spine: very small hook- like spine laterally

Contain a fully developed miracidium

Life Cycle

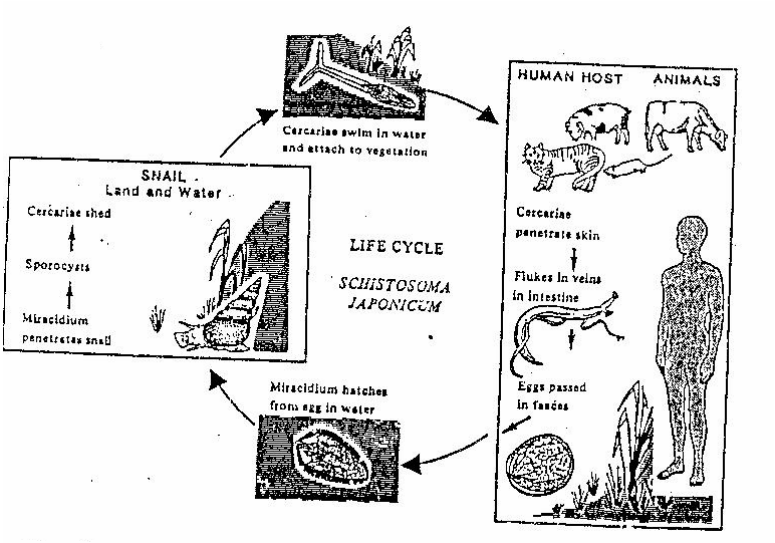


Figure 3.10. Life cycle of S.japonicum (From Cheesbrough M. Medical laboratory Manual for tropical countries, Vol. I, 2nd ed. Britain:The Bath Press.)

Embryonated egg→Miracidium→Sporocyst→cercariae→ schistosomulum→ Adult

It is similar to the life cycle of other schistosomes with the exception of that after mating they migrate to the superior mesenteric veins in the wall of the small intestine and the intermediate hosts are species of Oncomelania.

Pathology: About 2-60 days after infection a severe skin reaction to the products of young flukes and eggs may occur. It is known as Katayama reaction and takes the form of an acute illness with fever, muscular and abdominal pain, spleen enlargement, urticaria and eosinophilia. Although

this can also occur with other schistosomes infection, it is more common with *S.japonicum.*

The clinical feature and pathology of *S.japonicum* infection are similar to, but more often more severe, than those of *S.mansoni* infection. As the eggs are smaller than both *S.mansoni* and *S.haematobium*, they are readily conveyed in the portal system to the liver; in which in many cases becomes cirrhotic causing portal hypertension and enlargement of the spleen.

Prevention and control: Similar to the prevention and control methods of other Schistosomes

Laboratory Diagnosis

1. Eggs in the faeces, the feaces is usually mucoid and bloody
2. Eggs from rectal biopsy.

Schistosoma Intercalatum

Geographical Distribution

West and central Africa i.e. zaire, Chad, Congo, Cameroon, Nigeria, Tanzania

Habitat:

Adult: mesenteric portal veins

Eggs: In the faeces

Larvae: In snail hosts.

Cercariae: free swimming in fresh water; Infective stage

Morphology:

Adult

Male

Size: 11-14 mm by 0.3-0.4mm

Body covered with tubercules and fine spines

4-6 tests

Female

11-14mm by 0.15-0.18mm

Ovary lies at the middle part of the body

Uterus contain 5-50 eggs at a time

Egg

Resembles with the egg of S.haematobium but can be differentiated by acid fast stain.

Size: 175µm by 60µm

Shape: large, elongated

Colour: yellow-brown

Spine: long terminal spine

Contain a fully developed miracidium

Life Cycle

It is similar to the life cycle of other schistosomes but the intermediate hosts are species of *Bulinus*.

Pathology: Causes intestinal schistosomiasis.

Major symptoms are swimmer's itch at the site of insect bite, abdominal pain, fever, pain, splenomegally, urticaria, and eosinophilia, liver fibrosis, dysentery, and asites.

Prevention and Control: Similar to the prevention and control methods of other Schistosomes.

Laboratory Diagnosis

Finding

1. Eggs in the faeces, feaces is mucoid and bloody
2. Eggs from rectal biopsy.

3.1.2.2 Liver Flukes

General Features

1. Adults are large and live in the live or biliary duct
2. Testes are large and branched
3. Eggs are large and contain undeveloped ovum when passed in the faeces.
4. They are hermaphrodite

Fasciola hepatica

(sheep liver fluke)

Geographical Distribution

Cosmopolitan; Prevalent in most sheep and cattle raising countries, especially in the temperate countries, America, Europe, Asia, Africa, and in the highlands of Ethiopia.

Habitat:

Adult: In the bile duct of sheep, goat, cattle,

Egg: In faeces

All larva stages: Fresh water snail

Metacercaria: on water vegetations

Morphology:

Adult:

size: 30 mm by 12 mm

Shape: fleshy, flat, leaf-like

Colour: grey brown

Cone shaped prominant two “shoulders”

Branched intestinal caeca

Genital pore anterior to the venteral sucker

Tests in tendem and highly branched

Branched and fan-shaped ovary

Has no seminal receptacle

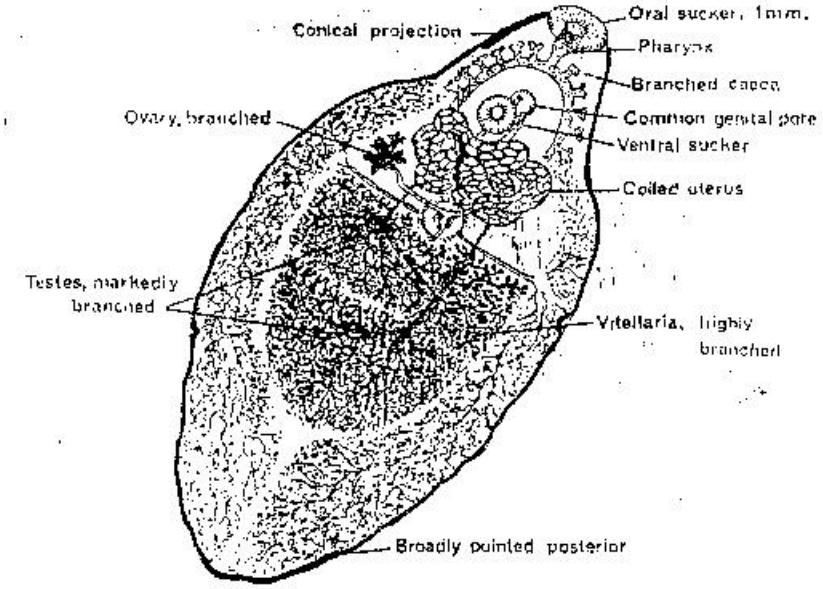


Figure 3.11. Adult stage of *F.hepatica*. (From Jeffrey HC and Leach RM. Atlas of Medical Helminthology and Protozoology, 1975.)

Egg:

Size: 130-150(m by 60-90

Shape: oval with rounded poles

Shell: smooth with a double line and marked operculum at one pole

Content: a mass of large indistinct cells with clear, granular nuclei

Colour: bile-stained or yellow to dark brown

Non-embryonated.

Life Cycle:

TRANSMISSION

1. Metacecareia ingested on aquatic plants ← 8. Cercaria leave snail

AQUATIC PLANTS

Cercaria encyst on aquatic plants

Become metacercaria

↓

HUMAN HOST SNAIL HOST

2. Metacercria excyst in duodenum
3. Immature flukes penetrate liver
4. Become immature fluke in biliary tract
5. Eggs produced and passed in faeces

7. Miracidium enter snail miracidia- sporocyst- → Multiply-rediae Redia-cercaria

FRESH WATER

6. Eggs hatch miracidium

Egg→miracidium→ sporocyst→Redia→ercariae→ metacercariae→Adult

Sheep, cattle, man and other herbivorous animals are definitive host and species of *Lymanae* snails are intermediate hosts. Following ingestion, the metacercariae excyst in the duodenum and the young flukes migrate in to the peritoneal cavity. They reach the bile ducts of the liver by penetrating through the liver capsule and become adult worms. Immature eggs are excreted in the faeces. The egg develops in fresh water and hatches miracidium which enter snails of the genus *Lymnaea*. In the snail, the miracidium develop in to sporocyst and produces generation of rediae and then cercariae. The cercaria is shaded from the snail host and undergoes encystation on water vegetation to become metacercariae. Man acquires infection by eating wild water cress or other water vegetation on which metacercariae have encysted.

Clinical Manifestation

- Light infections are usually asymptomatic
- In heavy infection
  - Local irritation during migration of the young warms to the liver
  - Fever, Sweating, abdominal pain,
  - Colic & Obstructive jaundice
  - Acute epigastria pain & abdominal tenderness
  - Persistent diarrhea
  - In the bile duct flukes cause inflammation

Prevention and Control

1. Avoid eating uncooked water plants
2. Treating infected animals
3. Destroying snail hosts
4. Sanitary disposal of faeces
5. Treating infected individuals and giving health education

Laboratory Diagnosis:

Finding 1. Eggs in the faces in chronic infection

2. Eggs in aspirates & in bile if eggs are absent in stool.

3. Serological diagnosis by testing serum for antibodies is particularly valuable in the early stages of infection when the immature flukes are migrating through the liver and causing serious symptoms but not yet producing eggs.

***Note**:* If eggs are found in human faces it must be confirmed that they are present due to a *Fasciola* infection & not from eating animal liver containing fascioliasis eggs (false fascioliasis)

False Fascioliasis - due to ingestion of animal liver containing Fasciola egg, with the passage of eggs in stool, is at time mistaken for actual infection

Rules out

keep the patient on liver free diet for three days. If egg is found in repeated exam the infection is true.

Relevance to Ethiopia

The parasite does not play an important role in human health in Ethiopia. There are only as few reported cases of the disease; even those reports may have been the result of finding eggs in the stools of people who had consumed infected liver of sheep or cattle. Sheep liver is considered to be a delicacy and is normally eaten raw. *F.hepatica* causes serious economic loss throughout the highlands in Ethiopia by infecting cattle and sheep. Human fascioliasis caused by *F.gigantica* is less common than *F.hepatica.*

Fasciola giagantica

(The giant liver fluke)

Geographical Distribution:-Widely distributed in tropical Africa including Ethiopia, and Far East, south and south East Asia. In some area, e.g., Egypt and Islamic republic of Iran *F.hepatica* and *F. gigantica* occur together.

Habitat:-Adult: In the bile duct of sheep, goat, cattle; it is a relatively commonparasite of hebivorous mammals

Egg: In faeces

All larva stages: Fresh water snail

Metacercaria: on water vegetations

Morpology: Similar to *F.hepatica* but the eggs are larder.

Adult:- Size 25-75 mm by 12 mm, larger and more lanceolate than *F.hepatica*

Branched intestinal caeca, short cephalic cone and large ventral sucker

***Egg*:** 160-190 μm by 70-90μm, operculated and non-embryonated

Life cycle

Egg→miracidium→ sporocyst→Redia→ercariae→ metacercariae→Adult

Sheep, cattle, man and other herbivorous animals are definitive host and species of *Lymanae* snails are intermediate hosts. The life cycle of *F.gigantica* is similar to the life cycle of *F.hepatica* except the species of snail hosts infected by *F.gigantica* are aquatic not amphibious.

Pathology: similar to *F.hepatica* but tess adapted to humans. However, its infection in cattle leads to considerable economic loss especially in some African countries.

Prevention and Control

1. Avoid eating uncooked water plants
2. Treating infected animals to reduce the egg out put.
3. Destroying snail habitats when feasible
4. Proper sanitary disposal of faeces
5. Treating infected individuals and giving health education
6. Cultivating watercress in water free from fecal pollution

Laboratory Diagnosis:

1. Eggs in the faeces
2. Eggs in aspirates of the duodenal fluid.

Clonorchis sinesis

(The Chinese Liver fluke)

Geographical Distribution:-Far East, Japan, Korea, Taiwan,
High infection rates are found especially in those parts of china where fish are cultured in pond that are fertilized with human or animal feaces.

Habitat:

Adult: bile duct of man and fish eating animals including cat, dog, pig, etc.

Eggs: In the faeces

Metacercariae: under the scale of fresh water fish

Morphology:

Adult- *Size:* 10-25 mm by 3-5 mm

Boat shaped, Smooth cuticle with out spine

Oral sucker is larger than ventral sucker

Simple unbranched caeca

Egg- *Size:* 25-30μm

Colour: shell; yellowish brown; contents pale yellow

Fine and smooth shell

Operculum: At the narrow end of the egg, fitting into thickened rim of the shell.

A small knob-like boss at the wide end of the egg

Contains a well organized ciliated embryo

Life cycle:

Egg→miracidium→ sporocyst→Redia→ercariae→ metacercariae→Adult

Definitive host: man Intermediate hosts: Primary intermediate host is *Bulimus* snail.

Secondary intermediate host is fresh water fish.

Man acquires infection from eating raw or inadequately cooked fresh water fish containing metacerariae. The metacercariae excysts in the intestine and migrates to the liver to become adult worm. The adult worm lays mature eggs and the eggs are excreted with the faeces. The miracidium hatches in water and develops into sporocyst. The sporocyst reproduces and further develops to rediae stage. The rediae develop to cercariae and shaded into the water. The cercariae swim resides under the scales of fresh water fish and become metacercariae.

Pathology: Causes clonorchiasis

Major symptoms are diarrhea, jaundice, cirrhosis, biliary obstruction, hepatomegally

Prevention and Control

1. Avoid eating raw fish
2. Sanitary disposal of faeces and not using faeces as a night soil
3. Destroy the snails
4. Inspection of fish
5. Treating infected person and giving health education

Laboratory Diagnosis

Finding:

1. Eggs in the faeces
2. Eggs in aspirates of duodenal fluids

3.2.2.3 Intestinal Flukes

General Characteristics

1. Adults live in the intestine
2. Eggs are large and contain undeveloped ovum when passed in the faeces.
3. They are hermaphrodite

Fasciolopsis buski

(The giant intestinal fluke)

Geographical Distribution

China, Taiwan, Thailand, Vietnam, Indonesia, etc.

Habitat:

Adults: small intestine of man , pig, dog,

Eggs: In the faeces of man Pig, dog,

Larval forms: Fresh water snails

Metacercariae: encysted on certain aquatic vegetation.

Morphology:

Adult:

Size: 20-75mm by 8-20mm

Large, fleshy, flat worm

Has no cephalic cone and shoulder

Oral sucker is smaller than the ventral sucker

Intestinal caeca is not branched

Tests are highly branched and in tandem position

Egg:

Size: 130 - 140 (m by 80 - 85(m pale yellow-brown in colour

Shape: oval

Small operculum

Unembroynate

Life Cycle: -

Egg→miracidium→ sporocyst→Redia→ercariae→ metacercariae→Adult

- Definitive host: Man
- Intermidate host: Segmentina species which are fresh water snails
- Man gets infection by feeding on infected water vegetation containing metacercariae.

Pathology: Diarrhae, Ulceration and inflammation of the intestine, malabsorption, eosinophilia.

Prevention and Control

1. Avoid eating uncooked water plants which may be infected
2. Construction of latrine

3. Avoid use of human faces as a fertilizer
4. Destroy snails and their habitat
5. Treating infected individuals and giving health education

Laboratory Diagnosis

Finnding

1. Eggs in the faeces, and
2. Adult worms in the faeces occasionally.

Heterophyes heterophyes

Geographical Distribution: China, Japan, Egypt, Korea, Taiwan

Habitat:-Adult: In small intestine of man, cat, dog, fox

Egg : In the faeces

Larval forms: In fresh water snails

Metacercariae: fresh water fish

Morphology:

Adult: *Size:* 1-*2mm*

Shape: elongated pyriform

Has three suckers; oral, ventral and genital suckers.

Tests are ova and side by side

Numerous integumantary scales / spines

Egg: Similar to the egg of *Clonorchis sinensis*

Size: 25-30μm

Shape: more oval, the operculum does not overlap

Yellow to dark brown in colour

Shell: Slightly thicker than that of *Clonorchis sinensis*

Contain developed miracidium

Life Cycle:

Egg→miracidium→ sporocyst→Redia→ercariae→ metacercariae→Adult

Requires three hosts to complete its life cycle.

Definitive host: Man

Intermediate host:

First intermediate host: Fresh water snail such as Pirenella

Second intermediate host: Brackish water fish such as Tilapia, mullet.

Man acquires infection from eating infected raw fish containing metacercariae.

Prevention and Control

1. Avoid eating raw or undercooked fish
2. Proper waste disposal of faeces in latrine
3. Avoid use of human faces as a fertilizer
4. Destroy snails and their habitat
5. Inspection of fish for metacercariae
6. Treating infected individuals and giving health education

Laboratory Diagnosis

Finding the characteristics eggs in the faeces

3.2.2.4. Lung Fluke

Paragonimus westermani

(Oriental lung fluke)

Geographical Distribution:-Extensively distributed in the Far East, and focally in West African countries such as Zaire, Nigeria, Cameroon and also in South America.

Habitat: Adults: In the lung of man

Eggs: In the sputum of man

Metacercariae: Fresh water crabs and crayfish

Morphology: Adult:

Size: 7.5mm-12mm by 4-6mm

Redish-brown in color and resembles one half of a pea

Integument covered with toothed spines

Tests are side by side

Egg:-Size: 70-100μm by 50-65μm

Yellow-brown or brow in colour

Shape: oval but asymmetrical

Has flattened operculum

Contain unsegmented ovum and mass of yolk cells

Life Cycle

Require three hosts to completely its life cycle

Definitive host: Man

Intermediate host ;

Primary Intermediate hosts are *Semisulcospira (Melania)* species of snails

Secondary intermediate hosts are fresh water crabs and crayfish

Egg→miracidium→ sporocyst→Redia→ercariae→metacercariae→Adult

Man gets infection by eating raw or undercooked crabs and crayfish containing metacercariae,

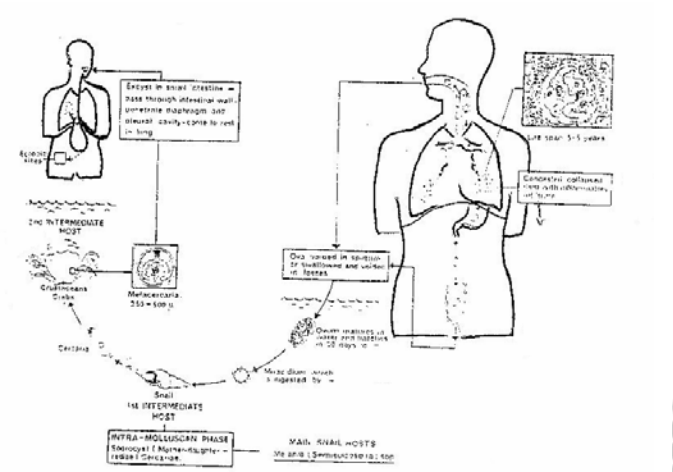


Figure 3.12. Life cycle of *P.westermani*. (From Jeffrey HC and Leach RM. Atlas of Medical Helminthology and Protozoology, 1975.)

Pathology: Causes paragonimiasis , pulmonary distomiasis or endemic haemoptisis

Major symptoms are fever, chronic coughing, haemoptysis, diarrhea and enlargement of liver

Prevention and Control

1. Avoid eating raw or uncooked crabs and crayfish
2. Avoid contamination of water with sputum or faeces
3. Destroy snails and their habitat
4. Inspecting crabs and crayfish for metacercariae
5. Treating infected individuals and giving health education

Laboratory Diagnosis

1. Eggs in the sputum. The sputum is usually bloody, mucoid and rusty brown
2. Eggs in aspirates of pleural fluid and occasionally in faeces

Review Questions

Trematoda

1. Explain the general mode of transmission of trematoda by giving example
2. Illustrate the classification of trematodes according to their habitat in human host.
3. Identify trematodes which have clinical significance in Ethiopia.
4. What are the main differences between blood flukes from other flukes?
5. How do you differentiate viable *Schistosoma* eggs from dead eggs?
6. What hosts are required to complete the life cycle of medically important lung flukes ?
7. While *P.westermani* is a lung fluke its eggs are occasionally found in stool.
   Comment.

3.3 Nemathelminths

3.3.1 Class Nematoda

Nematodes are the most common helminths parasitizing humans and includes intestinal nematodes as well as blood and tissue nematodes. The most common nematode 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 the characteristics eggs in feces.

The filaria are among the most important of blood and tissue nematodes. The filarai are long, slender round worms that parasitize the blood, lymph, subcutaneous and connective tissue of humans. All of the filaria are transmitted by insect vectors and most produce larva called microfilaria that may be demonstrated in the blood, lymph or connective tissue of the human host.

General Characteristics

1. Non segmented cylindrical or round worms
2. Possess a shiny cuticle which may be smooth, spined, or ridged
3. Mouth is surrounded by lips or papillae
4. Sexes are separate with the male worms being smaller than the female
5. In the male there is a testis at the distal end of a long tube which terminates in copulatory organs consisting of one or two projections called spicules
6. Copulatory bursa, caudal alae or genital papillae
7. Females are either viviparous (produce larvae) or oviparous (lay eggs)
8. Nematodes which infect humans live in the tissues or intestinal tract.

9. Tissue nematodes are transmitted mainly by insect vectors and most intestinal nematodes are feco-oral route and soil transmitted.

3.3.1.1. Intestinal Round Worms (Nematodes)

General Characteristics

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

Ascaris lumbricoides

(Round worm)

Geographical Distribution: Cosmopolitans. *A. lumbricoides* is one of the commonest and most wide spread of all human parasites.

Habitat: Adult: In the small intestine

Egg: In the faeces

Morphology:-*Adult:* colour: pinkish

***Male*:** size: about 15cm

curved tail and two copulatory spicules of unequal size

Female: size 2--25cm , with a straight tail.

Eggs:-There are five types of *Ascaris* eggs.

A. Fertilized Egg With Double Shell

Size: about 70μmShape: oval, or some times round

Shell: The two layer are distinct, rough , brown, covered with little lumps external shell and smooth, thick, colourless

internal shell.

Colour: 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 shell and thin internal shell.

content: full of large round very refractile granules

C. **Semi-decorticated Fertilized Egg Similar to Type A but With out the External Shell**

shell: single , smooth, thick and colourless or very pale yellow.

Content: a single rounded colourless granular central mass.

D. Semi-Decorticated Unfertilized Egg

Shell: a single smooth thin colourless shell (double line)

Content: large rounded colourless refractile granules.

E. Embryonated egg

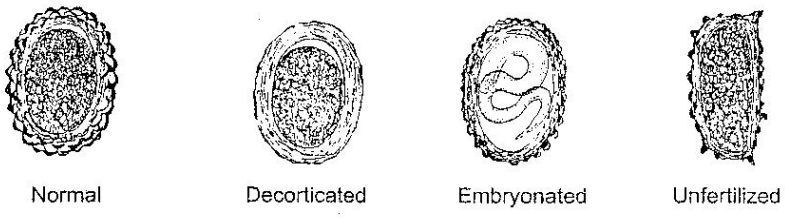


Figure 3.14. *Ascaris lumbricoides ova*. (From Jeffrey HC and Leach RM. Atlas of Medical Helminthology and Protozoology, 1975.)

Life cycle:

Egg→Larve→Adult

The infective stage is the egg containing second stage rhabditiform larva. Infection occurs by ingestion of the infective egg in contaminated food or drink, from contaminated hand. Following ingestion the larvae hatch in the small intestine and penetrate blood vessels in the small intestinal wall. The larvae follow a heart lung migrate from and develop. After migrating up the trachea, the larvae are swallowed. In the small intestine, they grow into mature worms.

After mating the female produces large number of eggs (200,000 eggs/day/ female) which are passed in the feces. In shaded soil. the egg develop and contain infective larva. The larva does not hatch until the egg is swallowed.

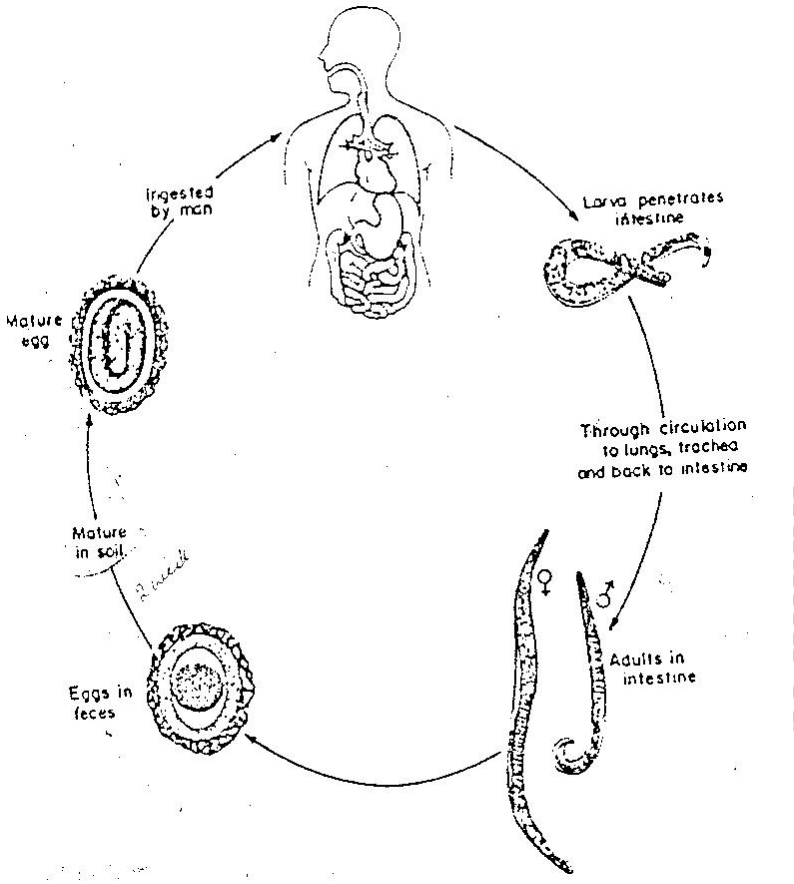


Figure 3.15. Lifecycle of *Ascaris lumbricoides*. (From Hegazi M. Applied Human Parasitology. 1st ed. 1994; Cairo, the Scientific Book Centers)

***Pathology*:** During their migration, Ascaris larva can cause inflammatory and hypersensitive reactions including pneumonia like symptoms, attacks of coughing, and bronchial asthma.

-Developing and mature worms in the intestine frequently cause pain, nausea, diarrhea and vomiting.

-Its infection in children is known to affect gastrointestinal function. Infected children are often Vitamin A deficient and have low serum albumin levels .Frequent exposure to infection may result in impairment of physical and intellectual development.

Prevention and Control:

1. Prevent soil contamination by sanitary disposal of faeces in latrines and avoid the use of night soil as a fertilizer and washing hands before eating
2. Around eating uncooked foods such as vegetables, green salads and fruits
3. Treatment and health education.

Laboratory Diagnosis

1. Finding the eggs in faeces
2. Identifying adult worms expelled through the anus or mouth.

Relevance to Ethiopia:

Ascaris lumbricoides is one of the commonest and most widespread human parasites in the world. This parasite, most common in the least developed countries, is estimated to infect a quarter of the world's population.

A. lumbricoides is the commonest nematode parasite of man in Ethiopia. Out of a total of 28,696 stool specimens obtained from all districts, 57.1% were found to be positive for this species. Highest rates of infection are recorded from children in the age group 5 to 9 years old. The distribution appears to be affected by altitude. being more common in higher than in lower altitudes.

Ascariasis is found in practically every Ethiopian community and is probably the most common communicable disease in the country, particularly in the malaria -free highlands. The most extensive survey of ascariasis in Ethiopia reported 44% of 32,276 persons , two thirds of them school children, infected. The highest rates were found in the highlands above 2,500 m elevation (59.2%) and the lowest rates in the lowlands below 1000 m (7.8%) apparently due to greater moisture availability in the former and thus greater survival rate of the ova and larvae in the soil. The overriding role of climate is also indicated by the distinct geographical distribution of the infection. Thus, between 50% and 75% of the children examined in Kefa, Gojam, Welega, and Gonder were infected; between 10% and 40% in Ilubabor, Sidamo, Wello, Tigray, Gamo Gofa, Shewa, Bale, and Arsi; and below 10% in the semiarid regions of Eritrea and Harerge. The highest prevalence rates were in children aged 15 to 18 (46.2%) but no significant sex differences in infection were observed (Kloos M, et al. 1993).

Prevalence rate of Ascaris lumbricoides in recent studies conducted in Ethiopia ranges from 17% to 77.7% (Yared M, 2001).

In one comparative study The prevalence of A. lumbricoides infection was 29% in highlands, 35% in the temperate areas and 38% in the lowlands (Leyekun J, 1998).

Entrobius vermicularis

(Pin Worm***)***

Geographical Distribution:-Cosmopolitant more common in temperate and cold climates than in warm climates more commonly infected than adults.

Habitat:

Adult: small intestine (terminal ileum)

Gravid female: Caecum and rectum

Eggs : In faeces or deposited on perianal skin

Morphology:

***Adults*:** Color: yellow white

Male: Size 2-5mm

Coiled tailed with a single spicule .

Female*:* 8-13mm, thin pointed tail

wing like expansion of cervical alae

Egg: Size: 50-60μm

Shape: oval but flattened on one side, rounded on the other side

Smooth and thin but with double shell

Content: either a small granular mass or a small curved up larvae.

- The egg is usually more easily found on the folds of skin round the anus.

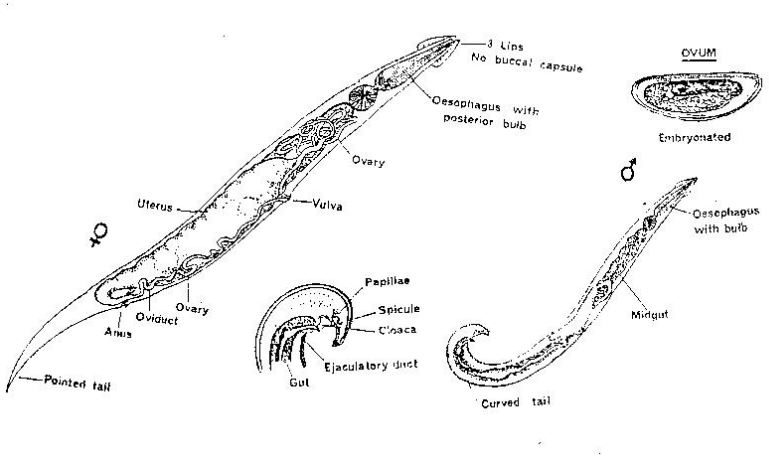


Figure 3.16. *E.vermicularis* adult and egg. (From Jeffrey HC and Leach RM. Atlas of Medical Helminthology and Protozoology, 1975.)

Life Cycle

Egg→Larve→Adult

Man gets infection with egg containing infective larva from contaminated hand food or drink. Following ingestion of infective eggs, the larvae hatch in the intestine and develop into adult worms in the large intestine. After mating , the female worms migrate to the rectum. the gravid females pass out of the anus and lay their eggs on the perianal skin, with in about six hours each egg contains infective larva . Man also acquires infection from clothing , bedding, air borne eggs autoinfection or retroinfection.

***Pathology*:** Its infection rarely causes serious symptoms. There is usually intense irritation around the anus. Worms in the appendix can cause appendicitis.

Prevention and Control:

1. Treating all members of a family in witch infection has occurred.
2. Washing of the anal skin each morning soon after waking.
3. Washing of clothing worn at night.

Laboratory Diagnosis

1. Finding eggs from perianal skin using cellulose adhesive tape
2. Finding eggs in the faeces
3. Finding adult worms in the faeces.

Relevance to Ethiopia

Most past surveys of intestinal parasitism have reported low *Enterobius vermicularis* infection rates largely because it is underreported due to failure of routine stool examination methods to detect the eggs in infected

persons. The finding that 5% of 569 school children in rural communities in Gonder region had *E. Vermicularis* eggs under their finger nails and that only 0.5% of them were found to shed eggs in the stool indicates that this parasite is much more common than reported in the literature (Kloos M et al,1993).

Recent studies done using routine stool examination method, a prevalence rate up to 1% were reported (Erko B, 1993 and Assefa T, 1998)

Trichuris trichiura

(The Whipworm)

Geographical Distribution:-Cosmopolitan: more common in moist warm climates. It is rarely found in arid areas and at high altitudes.

Habitat

Adult: large intestine (caecum) and vermiform appendix

Eggs : In the faeces, not infective when passed

Morphology

Adults: whip-like shape, anterior 3/5th of the warm resembles a whip & hence the name the posterior 2/5th are thick.

Male : Size 30-45 mm , coiled tail with a single spicule

Female*:* 35-50mm, straight thick tail.

Egg:

Size: 50-54μm

Shape: barrel-shaped with a colorless protruding mucoid plug at each end Shell: fairly thick and smooth, with two layers; & bile stained

Color: yellow brown

Content: a central granular mass which is unsegmented ovum

Life Cycle

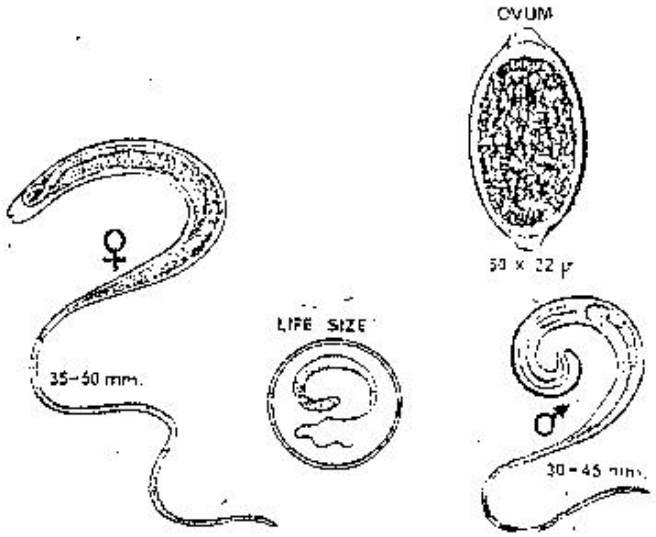


Figure 3.17 *T.trichuria* adult and egg. (From Jeffrey HC and Leach RM. Atlas of Medical Helminthology and Protozoology, 1975.)

Life Cycle

Egg→Larvae→Adult

A person becomes infected by ingesting eggs containing infective larvae from contaminated hands, food or drink. The infective eggs are ingested and the larva hatch and penetrate the villa of the small intestine. The larvae migrate to the large intestine and develop into adult worm. After mating the female worms lay eggs which are passed in the faeces. In a damp warm soil the larvae develop and each egg contains an infective larva.

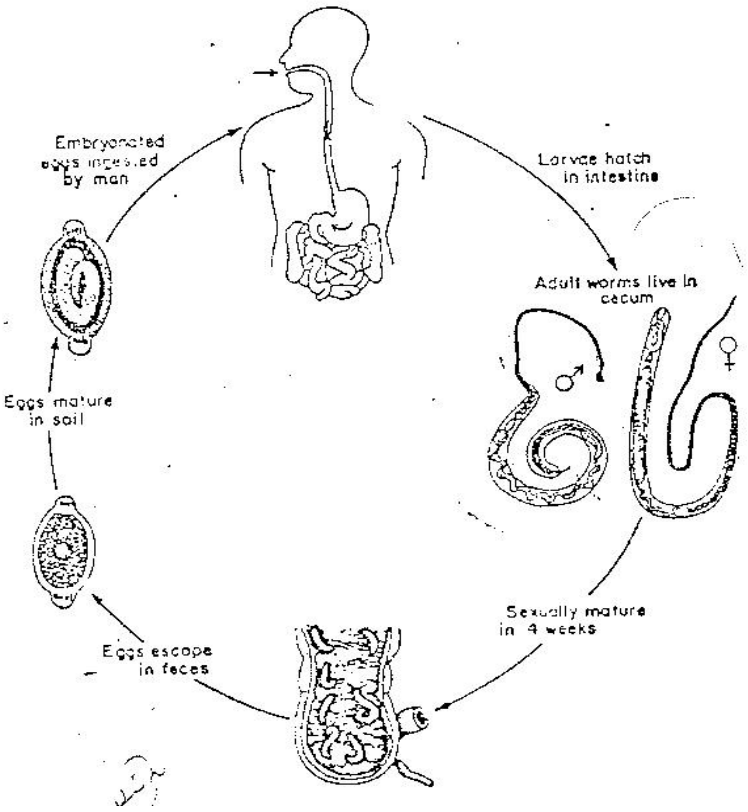


Figure 3.18. Life cycleof *T.trichuria*. (From Hegazi M. Applied Human Parasitology. 1st ed. 1994; Cairo, the Scientific Book Centers)

Clinical features and Pathology : Light infection produces few symptoms. In young children , severe infection can cause chronic diarrhea, intestinal ulceration with blood and mucus being passed in the feces, iron deficiency anemia, failure to develop at the normal rate, weight loss and prolapse of the rectum.

Prevention and Control

1. Sanitary disposal of faeces in latrine
2. Avoid the use of night soil as a fertilizer
3. Treatment of infected individuals and health education.

Laboratory Diagnosis:-Finding the characteristic eggs in the faeces.

Relevance to Ethiopia:-

- In a national survey in which 28, 696 stool specimens were examined, 36.1% were found positive for T. trichiura infection. This makes it a fairly common parasite of man in Ethiopia (Shibru T, 1986).
- As with the other intestinal helminths, pathology depends on worm burden , light infections being asympotomatic, This parasite commonly occurs together with *A.lumbricoides* and likewise mainly affects children. On the central and northern plateaus *T.trichuria* was found in more than 90% of the 50 communities surveyed for intestinal parasites , with a mean prevalence of 49% . Bure (Gojam) had a prevalence of 100% Whereas Mendida (shewa) was found to be free of trichuriasis (Kloos M et al. 1993).
- *T. trichuria* infection exhibited similar prevalences in lowlands, temprate and highlands regions 13% on average. The prevalence of infection due to *Ascaris lumbricoides and T.trichuria* was significantly correlated in the lowlands hinting the closely related distribution and co-occurrence of these parasites. The prevalence of infection due to hook worms and *T.trichuria* in the temprate area showed some pattern of association. A similar pattern also has also been noted between the prevalence of infection due to *A. lumbricoides and T.trichuria* in the highlands . The co-occurrence of *A. lumbricoides*

and T.trichuria infections in the lowlands and in the highlands suggest that a concurrent intervention against infection due to these two parasites using the same control methods would be advantageous(Jemane L, 1998).

Strongyloides stercoralis

(The dwarf thread worm)

Geographical Distribution:

world wide distribution in the warm moist climates of tropical and subtropical countries. Sporadic in the temperate and cool climates.

Habitat: Has both free living and parasitic generations

Parasitic Adults: buried in the mucosal epithelium of the small intestine of man.
Rhabditiform larvae: Passed in the faeces and external environments
Filariform larvae: soil and water the infective stage

Morphology:

Adult

Male (free living):-size = 1.7mm with rhabiditiform esophagus. It has no parasitic male.
Female (parasitic)

- 2.2mm in length cylinderical Oesophagus

Female (free living)

- 1mm in length and rhabiditiform oesophagus

First Stage Rhabditiform Larvae

Size: 200-300 μm long ; 15μm thick
Motility: very actively motile in the stool

Tail: Moderately tapered

Short buccal cavity and rhabiditiform esophagus

Filariforml Larave

- About 600-700μm
- Cylinderical esophagus
- Bifid tail end

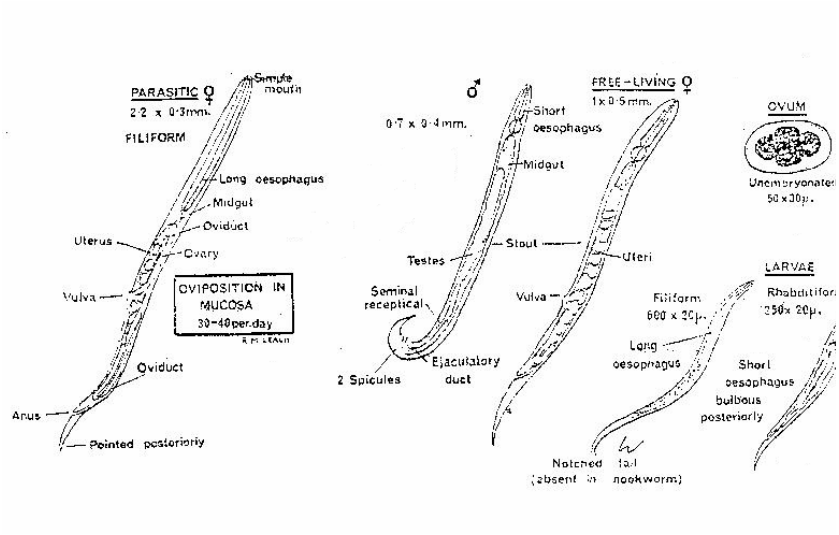


Figure 3.19. *S.stercoralis* adult, larrva and egg. (From Jeffrey HC and Leach RM. Atlas of Medical Helminthology and Protozoology, 1975.)

Life Cycle:

Egg→Larvae→Adult

The free living generation is the basic life cycle of the parasite and almost constantly present in the warm moist climates. It results considerable multiplication of potential infection.

In parasitic way of life, usually man acquires infection by filariform larva penetrating the skin. Following penetration, the larvae enter blood

vessels and undergo a heart lung migration to develop. After migrating up the trachea, the larvae are swallowed and they mature in the intestinal tract. Female worms become embedded in the wall of the small intestine and lay eggs. The rhabditiform larve hatch out in the intestine and either develop in the intestine in to infective larvae causing autoinfection or they are passed out in the faeces.

The rhabditiform larvae which are expelled in the faeces can follow free living way of life if the external environment is suitable or it develop in to the infective stage called filariform larvae.

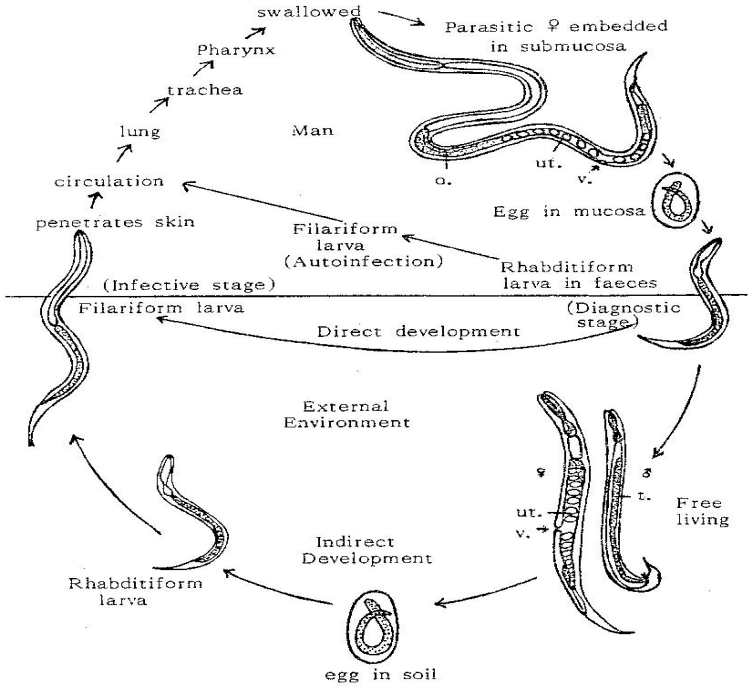


Figure 3.20. Life cycle of *S.stercoralis* **(**From Nasir NT. Review of Human Parasitology,2nd ed. Cairo:The Scientific Book Center.)

Clinical feature and Pathology*:* Causes strongyloidiasis, the disease is also known as cochin china diarnhea because in the feces of french cololnial troop who had been suffering in uncontrolled diarrhea in Cochin China.

The female adult worm by their invasion of the intestine cause inflammatory changes in the mucosa of small intestine leading to the development of gastrointestinal symptoms.

It is usually asymptomatic, in symptomatic cases shows the following phases:

1.Cutaneous phase: Infection caused by large number of larva produce itching and erythema at the site of infection within 24 hours of invasion.

2. Pulmonary phase: The migratory larva in the lung produces a considerable degree of host damage and injury to the alveoli and bronchial epithelium thereby producing bronchopneumonia and full blown pneumonitis.

3. Intestinal phase : Invasion by adult worms may produce abdominal pain and mucus diarrhea which alleviate constipation. Indigestion, nausea vomiting and anemia may also occur.

Heavy infection especially in children may result in malabsorbtion, steatorrhea and dehydration.

Auto- and hyper-infection syndromes occurs when the immune status of the host suppressed by either drugs, malnutrition or the concurrent diseases:

In these conditions, larva invade and are found in many of the tissues and serous cavities of the body producing a serious infection, which is fatal. In auto- and hyper-infection the mucosal inflammation is severe. The sigmoidal colon and rectum become frequently thickened and edematous .

Prevention and Control

1. Sanitary disposal of faeces in latrine
2. avoid use of night soil as a fertilizer
3. Wearing protective footwear
4. Treatment of infected individuals and Health education.

Laboratory Diagnosis

Finding the larvae in faeces or in duodenal aspirates using direct or concentration method.

In hyper-infection syndrome the larva may be found in sputum and in other specimens.

Note: The egg of *Strongloides steorcolaris* is seldom found in faeces. It is

- Color-transparent
- Oval in shape and measures 50µm long and 35 µm thick
- When passed, it contains a partially developed larva.

Relevance to Ethiopia:

Strongloides fulleborni: is a parasite of primates chimpanzees, baboons, and monkeys and it has been reported from man in Africa and southeast Asia. The parasite is endemic in rain forest regions, sporadic in secondary forest zones and savannah, with a very wide geographical distribution area involving tropical Africa from Sierra Leone to Ethiopia, from Central African Republic to Zimbabwe. In Africa, including Ethiopia, the parasite Co-exists with *S. stercoralis.*

Strongyloid infections are caused by the opportunistic nematode *Strongyloides stercoralis,* a fecally rather than soil-transmitted helminth. In Ethiopia strongyloidiasis is not highly prevalent in most areas and is

found in the same geographical areas and is found in the same geographical areas as hookworm infection: Nevertheless, rates up to 44% have been reported, *S. stercoralis* infection were reported from 41 of the 50 communities studied in central and northern Ethiopia . The infection is rare or absent in many arid lowland areas, including the Ogaden and pastoral areas in the Awash Valley.

Strongyloides fulliborni

Geographical Distribution:-Widely distributed in Zimbabwe, Zambia, Papua New Guinea, co-exists with *S.stercoralis* in Ethiopia. It is a common parasite of old world monkeys and apes. Infects dogs, cats, and especially young children and infants.

Habitat:-Has both free living and parasitic life.
It is natural parasites of monkey and dog but it can also infects humans.

Morphology:-Morphologically resembles *S.stercoralis* with certain exceptions

Adult:- Adult parasitic female does not have straight ovaries like *S.stercoralis* but it is spiral around the intestine.

-Free living female has marked constriction posterior to the vulva and the vulva lips are not prominent.

Egg:-Resembles eggs of hookworms but are shorter and smaller

-Colorless, Oval and 50 by 35µm in size

-Contain partially developed larvae

Life cycle

Egg→Larve→Adult

The life cycle is similar to the life cycle of *S.stercoralis* except it shed eggs in the feaces unlike *S.stercoralis* which sheds rhabiditiform larvae.

Mode of Transmission

1. Skin penetration by filariform larvae
2. Transmammary

Pathology:-In Papua New Guinea, the sub species s. fuelleborni kelly infections are associated with an acute often l infantile disease known as swollen belly illness.

Prevention and Control

1. Sanitary disposal of faeces in latrine
2. avoid use of night soil as a fertilizer
3. Wearing protective foot-ware
4. Treatment of infected individuals and Health education.

Laboratory Diagnosis

Finding eggs in stool fresh specimens. Many eggs may be present and typically appear embryonated.

N.B. If there is a delay in examining the feaces , the larva will hatch.

Ancylomstoma duodenale and Necator americanus

(old world hookworm) (New world hookworm)

Except some differences which described below both species are similar in many aspects.

Geographical Distribution: (N. americanus) **-**Wide spread in the western hemisphere. It is the predominant species in all parts of the tropics. For East, South Asia, pacific Islands, Tropical Africa, Central and South America.

Geographical Distribution: (A. duodenale)

World wide; It is essentially northern species occurring in Northern parts of China, Japan , Europe, North Africa, southern Europe, Middle East. It is less common in Ethiopia than *N.americanus.*

Habitat:

Adult: Jejunum and less often in the duodenum of man

Eggs: In the faeces; not infective to man

Infective larvae: free in soil and water

Morphology:

A.duodenale: **-**

Adult- longer & thicker than *N.americanus*

- Creamy white appearance
- Large mounth cavity (Buccal capsule); broader than long
- Two pairs of teeth, two plates & two subventral lancets
- Club shaped esopagus
- Female lays 20,000 eggs/day

Size - male and female measures 8 mmand 10-13mm respectively

N.americanus:-

Adult-short & thinner than *A. dudenale*

-Buccal capsule longer than broad

-2 cutting plates, 2 plates & 2- subdorsal lancets

-club shaped Oesophagus

Size -male and female measures 7-9mm and 9 to 11mm respectively

Rhabiditiform Larvae	**Filariform Larvae**
Size - 250-500μm	**- about 600- 700 μm**
- long buccm cavity	- short bucal cavity
- Riabiditoform Oesophages 1/3 body length)	- Cylinderical esophagus (1/4 body length)
- Pointed tail end	- Sharply pointed tail end

Egg:- Size : 65-40μm

Shape: oval

Shell: very thin and appears as black line

Colour: the cells inside are pale gray

Content: varies according to the degree of maturity. It contains an ovum which appears segmented usually 4-8 stage.

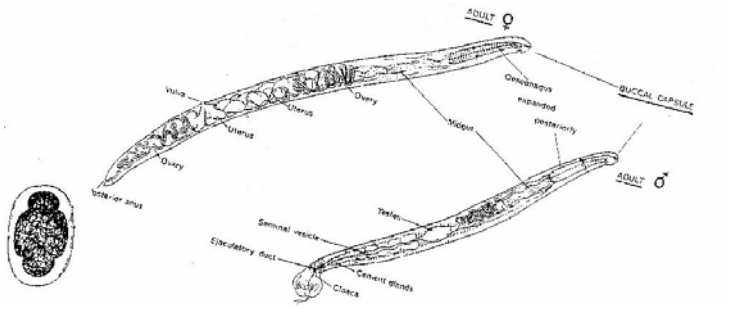


Figure 3.21. ***Hookworm*** **adult and egg**. (From Jeffrey HC and Leach RM. Atlas of Medical Helminthology and Protozoology, 1975.)

Life Cycle

Egg→Larve→Adult

The worms requires one host to complete their life cycle and the definitive host is man. Infection may occur in two ways: (1) through penetration of the skin and (2) ingesting filariform larvae. The larvae penetrate the skin and enter small blood vessels and following a heart-lung migration. After migrating up the trachea, the larvae are swallowed. In the small intestine they develop and mate.The worm attach to the wall of the small intestine by sucking part of the mucus and blood from the host. Female worms lay eggs which are passed in the faeces. In the external environment the egg develop and hatch the rhabditiform larvae. It feeds and moult twice to become infective larvae.

Pathology: causes hookworm infection. Major symptoms are severe itching at the site of skin penetration known as "ground itch", mild pneumonia with cough, sore throat, bloody sputum, headache, weakness, bloody diarrhea and anemia.

Adult hookworm cause chronic blood loss. It has been estimated that a single *A.duodenale* worm ingests about 0.15ml of blood/day and a *N.americanus* worm about 0.03ml.

Prevention and control:-

1. Sanitary disposal of faeces
2. Avoid the use of night as fertilizer
3. Wearing adequate protective foot ware
4. Treatment and health education.

Laboratory Diagnosis:-Finding eggs in faeces.

Relevance to Ethiopia:

The hookworms which most commonly infect humans are Ancylostoma duodenale and Necator americanus. Both parasites are found in Ethiopia, the latter being by far the more common than the former. *A. dudenale* is associated with areas of poor soil coverage and high rates of drainage, whereas *N. americanus* is found in red soil areas on flat plains. Altitude and moisture appear to be the major factors affecting the distribution of the two species. Hookworm infection is absent in the low, hot and dry areas of Ethiopia. Infection has not been recorded in places above 2500 m above sea level. The limiting factor in the former areas may be moisture whereas in the latter it is likely to be temperature.

Hookworm infections are most widespread in Ethiopia in the 800-1,200 altitudinal zone and in the humid western lowlands at even lower elevations, where moisture is assured through most of the year and where the average temperature of the coldest month is above 18NC.

Some of the highest infection rates, between 60% were found in lowlands of Ilubabor, Kefa and Welega and lower rates in both dry lowland areas of eastern, southern, and northern Ethiopia, and the highland areas. Of 95 communities surveyed in 12 administrative regions (excluding Eritrea and Tigray), 82 had hookworm infections, and in 20 communities both hookworm species were found. Twenty percent of the population was found to have *N.americanus* and 1% *A.duodenale* infections. The relatively wider distribution of *N.americanus* is in agreement with studies in other endemic areas , where this species was found to be more tolerant to a wider range of temperatures than *A. duodenale.* Soil type was also associated with the occurrence of the two hookworm species ; where as *N. americanus* was more common in flat areas with sandy clay

soil, *A.duodenale* occurred more frequently in well-drained sandy loam slopes.

Prevalence rate in A.A and other larger towns are low reflecting the influence of the urban physical environment and the greater use of shoes by the urban dwellers.

Trichostrongylus colubriformis

Are mainly parasites of ruminant equines and rodents

Geographical distribution*:* Cosmopolitan

Habitat

Adult: Jejunum and less often in the duodenum of man

Eggs: In the faeces; not infective to man

Infective larvae: free in soil and water

Morphology:

Adult: slender and hair-like, the head cutting plates or teeth

-Anterior extremity attenuated

-Yellowish or reddish in color and club-shaped esophagus

Male :-Size 5mm

Has copulatory bursa with two spicules

Long, slender, fused at the tip, barbed copulatory spicules

Female :-Size: 7mm

Egg:-The egg is almost identical with that of *A.duodenale*

Size 80 by 40 μm

Shape : one pole pointed and the other is blunt

Translucent Content: immature ovum (32 cell stage)

-When compared the egg of hook worm; hook worm has size of 60 x 40 μm oval in shape blunt pole and 4-cell stage in its contents

Rhabditiform and Filariform Larvae are similar to hookworm but have a minute knob at the tip of the tail.

Life cycle

Similar to hookworms except that transmission is due to ingestion of the filariform larvae in contaminated food or drink.

Egg→Larvae→Adult

Normally it is parasite of herbivore animals but man is accidentally infected by ingesting the filariform larvae in contaminated vegetables or water. The adult it the small intestine of man and other animals with the anterior end penetrating in the mucosa wall.

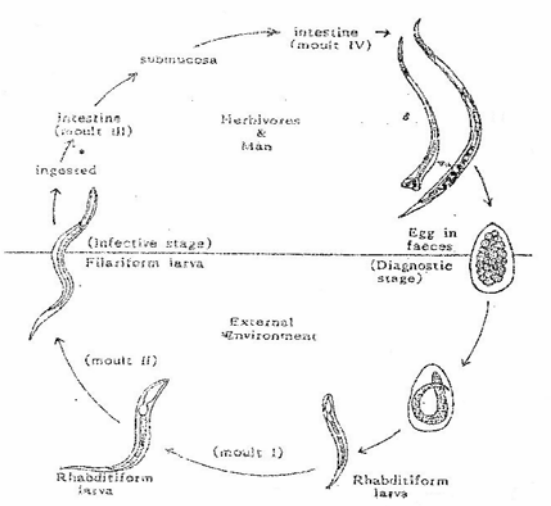


Figure 3.22. Life cycle of *T.colubriforms*. **(**From Nasir NT. Review of Human Parasitology,2nd ed. The Scientific Book Center, Cairo)

Pathology. Infection causes inflammation of the mucosa, abdominal pain, and anemia

Prevention and Control

1. Proper washing of vegetables or cooking before eating them.
2. Sanitary disposal of feces
3. Treatment and health education

Laboratory Diagnosis

Finding eggs in the feces

Review Questions

1. Identify the common name of the following parasite
   a) *Ascaris lumbricoides*
   b) *E. vermicularis*
   c) *S.stercoralis*
   d) *N.americanus*
   e) *T.trichuria*
2. Discuss the main differences between rhabiditiform and filariform larvae of hook worm and *S.stercoralis.*
3. Which intestinal nematodes do not have heart lung migration in their life cycle?
4. What are the difference and similarities between *N. americanus* and *A. duodenale* ?
5. List the possible sources of specimen and diagnostic stages of nematodes.

3.3.2 Tissue Nematodes

Tissue nematodes are those, adults of which live in the tissues of man. Adult worms of this group are filaria, guinea worm and Trichinella. They are parasites of the lymphatic system & subcutaneous tissues and muscle of man respectively.

The filaria nematodes are widely distributed in nature . the adults are long and slender measuring many centimeters in length, may inhabit virtually any tissue, blood and lymphatic vessels, pleural and peritoneal cavities, subcutaneous tissue, heart and brain. Some species migrate in the tissues others remains localized and may become encased in a fibrous tissue reaction. The adults mate in the tissue, producing many progeny called microfilaria. The microfilaria are small (200-300μm), slender, motile forms which may be found in the circulating blood or migrating in the subcutaneous tissue, depending on the species.

Microfilaria are classified by morphological characteristics, geographical location and type of clinical infection seen. The majority of divisions of microfilaria is by the presence or absence of sheath surrounding the parasite. Unsheathed microfilaria include *Onchocerca volvulus, Mansonella ozzardi, M.perstans and M. Streptocerca*. The sheathed microfilaria include Wuchreria bancrofti, Burgi malayi and Loa loa. Definitive identification to species is based on the presence and number of nuclei seen in the tail of the microfilaria.

Alternatively, these parasites can be divided as causes of cutaneous, lymphatic, or body cavity infections. Species identification of blood microfilaria is particularly important, because some may cause serious disease while others rarely do.

General Features

1. Female worms are viviparous, produce live larvae but does not lay egg
2. Long tread like worms
3. The immature first stage larva of filarial worms is called a microfilariae
4. In order to complete its life cycle the larva requires an intermediate host to develop to infective form, and there is no reproduction in the insect vector
5. Diagnosis of filariasis is based of finding the microfilaria with specific morphologic features such as

- Size
- Presence or absence of " sheath"
- Periodicity as Nocturnal, diurnal, sub-periodic, or aperiodic (non-periodic) forms
- Source of larvae (specimen) used for laboratory diagnosis such as blood, skin, nodule, urine, ulcer, etc.
- Appearance i.e. curvature, Kinks, coiling etc.
- The arrangement of the column of nuclei; and whether it reaches the very tip of the tail.
- Percentage distance of internal anatomical "land marks" from anterior end. These anatomical" land marks "are nerve ring, excretory pore, excretory cell, genital or rectal cells and anal pore.
- The arrangement of the column of nuclei and whether it retches the very tip of the tail.

Wuchereria bancrofti

Geographical Distribution:-The periodic form is the most widely distributed of the filarial worms in subtropics and tropics, Asia, Africa, America, Middle East, Far East, Including Ethiopia. The sub-periodic form is found in Eastern Pacific, Thailand and Vietnam

Habitat

Adults: Coiled in lymphatic glands, or lying in lymphatic vessels, superficial abscesses, or wondering in retroperitoneal tissues. Found usually in lymphatic of the lower limb.

Microfilariae: In lymphatic vessels, and in the peripheral blood normally at night but during day in lung and other internal organs.

Infective larvae: In the gut and muscles including mouth parts of certain species of mosquitoes

Morphology:

***Adults*: -**Creamy white with smooth surface

Male:-23-40mm by 0.1mm

` Tapered and rounded swollen head

Sharply curved tail with two spicules.

Unequal spicules

Female:-50-65mm by 0.16mm

Curved tail, tapered front end and Vulva at cervical area

Microfilaria:-Size: 275-300μm by 8-10μm

-Has a sheath which stains pink with Giemsa and palely with Haematoxylin stain

-Body nuclei are fewer and more distinct than in other species

-There are no nuclei in the end of the pointed tail

-Body curves are smooth and few with Smooth cuticle

-Bluntly rounded anteriorly and pointed caudally

Life Cycle:

Infective filariform larvae→Adult worm→microfilariae

It requires two hosts to complete its life cycle :

1) Definitive host: man
2) Intermediate hosts: species of female culex, Anopheles and Aedes mosquitoes

The infective filariform larvas are deposited on human skin when an infected female mosquito vector takes a blood meal. The larvae penetrate the skin through the bite and enter into the blood vessels and lymph nodes. Development takes place in the lymphatics and the adult worm mate to produce many microfilariae that enter the blood stream. The microfilariae are taken up by a mosquito vector when it takes a blood meal. In the stomach of the insect vector, the microfilariae lose their sheath and migrate from the mid-gut to the thorax of the vector where they develop into infective larvae and develop into infective form. The larvae are ready to be transmitted when the insect next takes a blood meal.

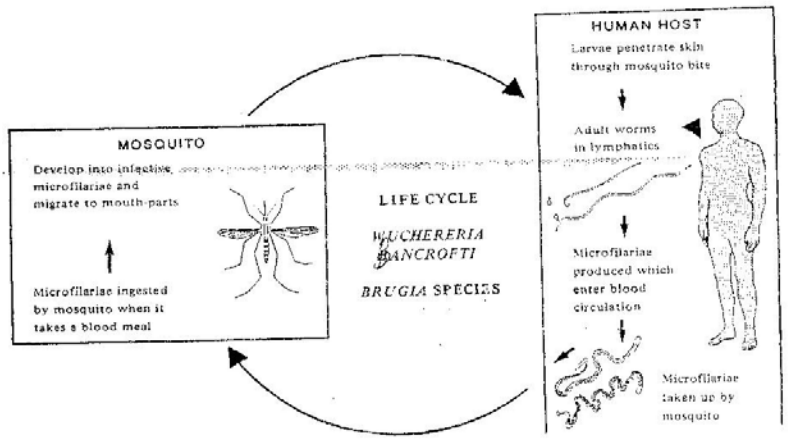


Figure 3.23. Life cycle of *W.bancrofti* and *Brugia* species(From Cheesbrough M. Medical laboratory Manual for tropical countries, Vol. I, 2nd ed. Britain:The Bath Press.**)**

Pathology: Causes lymphatic filariasis or elephantiasis of usually the upper limbs, genital organs and breasts.

Major symptoms are fever with painful inflamed lymphatics, thickening and blocking of lymphatic vessels, swelling, fibrosis, elephantiasis and hydrocoele of limbs, genital organs and breasts due to obstruction of the flow of lymph.

Prevention and Control:

1. Controlling mosquitoes vector
2. Avoid mosquitoes bite
3. Treating infected person
4. Giving health education.

Laboratory Diagnosis

1. Microfilariae in wet or stained blood films

2. Microfilariae in aspirates of hydrocele and lymph gland fluid.
3. Occasionally Microfilariae in chylous urine or hydrocoele fluid
   In chronic banchroftian filariasis, a condition called chyluria will occur, i.e., passing of chyle in urine. In such urine microfilaria can often b found.
4. Serological diagnosis

Collection Time of Blood for *W.bancrofti* microfilariae

Periodic *W.bancrofti* microfilariae: Collecting blood between 22.00-04.00 hours (time of peak density is 24.00 hour)
Nocturnal subperiodic *W.bancrofti* microfilariae: Collecting blood between 20.00-22.00 hours (time of peak density is 21.00 hour)

Diurnal subperiodic *W.bancrofti* microfilariae: Collecting blood between 14.00-18.00 hours (time of peak density is 16.00 hour)

The diurnal subperiodic variant *W.bancrofti* is found mainly in Eastern pacific. The nocturnal subperiodic variant is found especially in Thiland and Vietnam.

W. bamcrofti is found with *B.malayi* in parts of south East Asia & South India

Relevance to Ethiopia

In people living in two communities adjecent to the Baro river, near the town of Gambella in 1993. The overall microfilaria prevalence, using counter chamber technique, was 20.7% with males and females showing microfilaria rates 23.7% and 18.5%, respectively. In males, 20.3% had hydrocoele and this condition was noted above the age of 35. About 40% of those with hydroceole had microfilaremia (Jemaneh L et al, 1995).

Brugia malayi

Geographical Distribution:-It is distributed in countries such as Asia, Malaysia, India, Philippines, Vietnam, China, and Korea. The nocturnal periodic form is the most widely spread in swamps and rice growing areas whereas the nocturnal subperiodic form is found in fresh water swamps and forests along major rivers.

Habitat

Adult: In dilated lymphatic Vessels of man; usually in the upper limb.
Microfilariae: Lymph and peripheral blood at night, and in the lung and internal organs during day timeInfective filariform larvae: In the gut of mosquitoes.

Morphology

Adults :- Male: 13-23mm
Female**:** 43-55 mm

Microfilariae :- Size: 200-275μm by 5-6μm
-Body is usually coiled and kinked (has small angular curves)
-Has a sheath which stains dark pink with Giemsa and pink-mauve with haematoxylin.
-Body nuclei are dense and stain darkly.
-There are two discrete nuclei in the end of the tail which tapers irregularly.

Life cycle

The life cycle of *B.malayi* is similar to the life cycle of *W.bancrofti*. Man is the definitive host. Female anopheles and *Mansoni* mosquitoes are the intermediate hosts. Man gets infection by the bite of infected insect vector when it takes a blood meal.

Pathology: Causes malayan filariasis or elephantiasis of the lower limbs. Symptoms develop rapidly and children are more affected.
Prevention and Control :- similar method like *W.bancrofti.*

Laboratory Diagnosis

Finding the characteristic Microfilariae in wet or stained blood films

Collection Time for B.malayi Microfilariae

Periodic B.malayi Microfilariae:

Collect blood between 22.00-04.00 hours (time of peak density is 24.00 hour)

Subperiodic B.malayi Microfilariae:

Collect blood between 20.00-22.00 hours (time of peak density is 21.00 hour)

- Peroidic *B.malayi* is commonly found in open swamps and rice growing areas of coastal regions. The subperiodic variant is found mostly in fresh water swamp in forests along major rivers.
- *B. tumor*i shows nocturnal periodicity. It is found only in the lesser Sunda of Indonesia the species takes its name from the island of Timor which forms part of the group. it is found in low lying riverine and coastal areas.

Loa loa (Eye worm)

Geographical Distribution:

The Distribution of *L.loa* is restricted to the equatorial rain forest area of west and central Africa.

Habitat:

Adults: In connective tissues under the skin, in the mesentery and the parietal peritoneum. They commonly migrate rapidly in the body and may be seen in the subconjunctival tissue of the eye or in thin skinned areas.

Microfilariae: In peripheral blood of man during day time.

Infective larvae: In the gut, mouth parts and muscles of tabanide flies of the genus Chrysops.

Morphology

Adults:- Cylinderical and transparent

Male*:* 30-34mm

Female**:** 60mm

Microfilariae:

-Size: 250-300μm long and 8-10μm thick

-Has several curves and kinks

-Has a sheath which stains best with haematoxylin.

-Body nuclei are not distinct and appear more dense than those of *W.bancrofti*

-Nuclei extend to the end of the tail which is rounded.

Life cycle

Natural Definitive hosts are- Man & Monkeys. Reservoir host are simian hosts.

Similar to the life cycle of *W. bancrofti* but the habitat of the adult worms is in the subcutaneous tissues and they are freely moving in these tissues. The intermediate hosts are species of chrysops(horsefly).

Pathology: Characterized by the formation of swelling known as calabar swellings. The arms most frequently affected. Adult worms also migrate in sub-conjunctiva tissues. They can cause inflammation and irritation but not blindness.

Prevention and Control: Similar with the previous filaria worms.

Laboratory Diagnosis

1. Finding the characteristic microfilariae in stained blood films taken during the day time.
2. Occasionally the microfilariae can be found in joint fluid.

Onchocerca volvulus

Geographical Distribution:- occurs most widely along the courses of fast running rivers in the forests and Savannah areas of west and central Africa. It is endemic from Senegal in the west to Uganda and Ethiopia in the East and as far as south as Zambia. It also occurs in the Yemen Arab Republic. Saudi Arabia and in central America(Mexico and Guatimala). It is also found in South America.

Habitat:

Adults:- Subcutaneous nodules and in skin

Microfilariae:- Skin,eye and other organs of the body.

Infective larvae: In the gut, mouth parts and muscles of Simulium black fly.

Morphology:

Adults: *-**male**:* 25-40mm, curved and bulbous tail

*-**Female**;* 33-55cm in length

Microfilariae:-Size 240-360μm long and 5-9μm thick

-Has no sheath and head end is slightly enlarged

-Anterior nuclei are positioned side by side

-There are no nuclei in the end of the tail which is long and pointed.

Life Cycle

It requires two hosts man as definitive and *Simulum* as intermediate host. Infective larvae are deposited on the skin when an infected vector takes blood meal. It enters through the bite wound and develop into male and female worms in subcutaneous tissue. They mate and viviparous female produces many unsheathed microfilaria that can be found just below the surface of the skin in the lymph spaces and in the connective tissues. They can also be found in the fluid of nodules. The microfilaria also migrate to the eye. The microfilariae are ingested by its most commonest vector *Simulium damnosum* complex as it takes a blood meal and develop into infective larvae that migrate to the mouth parts of the blackfly ready to be transmitted when the fly next takes a blood meal.

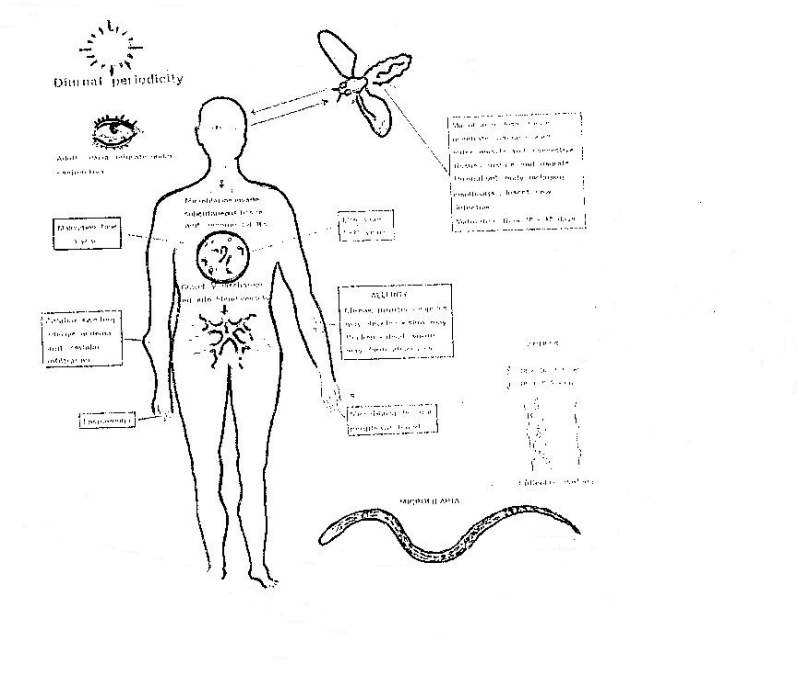


Figure 3.24. Life cycle of O.volvulus(From Cheesbrough M. Medical laboratory Manual for tropical countries, Vol. I, 2nd ed. Britain:The Bath Press.**)**

Pathogenicity:-It causes Onchocerciasis or river blindness

The clinical feature of onchocerciasis are caused mainly by the inflammatory reactions around damaged & dead microfilaria. In chronic onchocerciasis the skin loses its elasticity & becomes wrinkled which makes people more aged than they are (known as elephant skin). When the skin around the groin become affected hanging groin develops. The term Leopard skin refers to a spotted depigmentaion of the skin. The main clinical features are the formation of nodules, dermatitis & inflammatory reactions in the eye leading to blindness.

Prevention and Control

1. Destruction of simulim including
   - Selective use of insecticides

     Introducing other method to reduce the breeding of black files in rivers & streams .
2. Avoiding simulium bites by covering using as far as possible those parts of the body most at risk.
3. Identification of infected communities followed by treatment of communities by

***Laboratory Diagnosis*-**Finding the characteristic Microfilariae in skin snips.

***Note*:** In heavy Infection and following treatment, the microfilariae can also be found in urine, blood, and most body fluids. Unlike W.bancrofti which may also be found in urine the microfilaria of O.volvulus is unsheathed.

Differentiation of O.Volvulus from Mansonella species

- In west & central Africa O.volvulus requires differentation from streptocerca
- In West Indies & Central and South America.
- O.volvulus requires differentation from M.ozzardi.

Relevance to Ethiopia

It is endemic in a a large fertile Western, Southern and Northwestern part of Ethiopia. However, the ocular form which in other parts of the world causes serious lesion that leads to loss of vision and blindness is considered to be mild in Ethiopia. Although ocular manifestations are considered to be rare in Ethiopia, there is no sufficient explanation.

Onchocerchiasis is known to be more prevalent among older age groups and males and in this study the sex difference is seen to be the dominance of males in activities such as collection of firewoods, fishing and swimming as well as the habit of wearing light clothes and shorts by males and thus more exposure to the vectors.Bathing, swimming and fishing at the rivers were significantly associated with infection. This could be due to long stay and nakedness with these practices and thus more exposure to the vectors as compared to fetching water or crossing over the rivers(Habtamu A,1999).

The distribution of the disease covers savannah and forest ecology of the south, south western, north and north western Ethiopia, extending from Omo valley in the south to the Atbara and Teccaze drainage systems in the north bordering the Sudan. Although the prevalence of onchocenciasis and blindness due to the disease throughout the country is not well studied , it is now estimated that about one million people are suffering from the disease .

Presently, 29 Simulium species are recorded in the country . It is reported that two species viz . S.damnasum s.l. and S. ethiopiense are the vectors involved in transmission : the former playing a role at lower and higher altitudes while the later is mainly at higher altitudes .

A survey to determine the prevalence of onchocerciasis in a sample population aged 5 years and over was made in pawe western Ethiopia. A total of 986 persons (636 settlers and 350 indigene) were examined for parasitological (in skin snip) and clinical manifestations, of which 310 (27.8% settlers and 38.6% indgenious) were found infected with Onchocerca volvulus.

Males had significantly higher rate and density of infection but the difference by age was not significant. The prevalence of infection was

significantly higher among the students who had reported frequent bathing, swimming, fishing and collecting firewood at/or near the rivers identified as the probable breeding sites of the vector. The rate determined for different communities of Kaffa indicate that half of the studied areas have a prevalence of more than 20% in youngsters which indicates that this is a high prevalence area for Onchocechiasis.

Mansonella Perstans

Geographical Distribution

Widely distributed in South America and tropical Africa.

Habitat

Adults: In the serous cavities, mesentery, and connective tissue, especially the retroperitoneal.

Microfilariae: In peripheral blood at any time because non-periodic guts mouth parts .

Habitat:

Adults: In connective tissues under the skin, in the mesentery and the parietal peritoneum. They commonly migrate rapidly in the body and may be seen in the subconjunctival tissue of the eye or in thin skinned areas.

Microfilariae: In peripheral blood of man during day time.

Infective larvae: In the gut, mouth parts and muscles of chrysops fly.

Morphology:

Adults *:* ***Male****:* about 40mm

Female**:** 7 cm - 8 cm

Microfilariae

Size: 190-240 μm long by 4.5 μm thick

- Has no sheath, body nuclei are irregular Large nucleus in tip vectors

- Nuclei extend to the end of the tail which is rounded and there is a large nuclie at the tip.
- Nonperiodic lie found in day and night blood.

Life Cycle

Man is the definative host and female culicoides are the intermediate hosts. Man is infected by the bite of infected culicoides.

Pathology: non-pathogenic or of low pathogenicity.

Prevention and Control:-
1. Avoid the bite of vectors
2. Destruction of insect vectors
3. Treatment and health education.

Laboratory Diagnosis

Finding the characteristic microfilaria in the stained blood films at any time.

Differentiated from *L.loa* and *w.bancrofti* by its smaller in size, absence of sheath, tail features and non periodicity.

Mansonella Ozzardi

Geographical Distribution:-West Indies and South America.

Habitat

Adults: In the mesentery, retroperitoneal tissue, abdominal wall, and lymphatic vessels of man.

Microfilariae: In peripheral blood or skin of man any time.

Infective larvae: In the gut, and mouth parts of culicoides and simulium black flies

Morphology:

Adults*:-*Male: about 35mm

-***Female:*** 70mm

Microfilariae:-Size: 150-200 μm long and 4.5 μm thick

-Has no sheath and body nuclei are not distinct but the anterior nuclei are positioned side by side.

-There are no nuclei in the end of the tail which is long and pointed.

Life cycle

Man is the definitive host and the infective larvae are transmitted by the bite of culicoides and simulium species as an intermediate hosts.

Pathology: non-pathogenic or of low pathogenicity. Most infections are asymptomatic or cause chronic arthritis, skin rashes and other symptoms.

Prevention and control:- Similar method llike *M.perstance.*

Laboratory Diagnosis

Finding the microfilariae in stained blood film and occasionally in skin snip at any time since it is nonperiodic.

Mansonella Streptocerca

Geographical Distribution:- Found only in the rain forest of Africa especially in Ghana, Nigeria, Zaire and Cameroon.

Habitat

Adults: In cutaneous connective tissues of the chimpanzee,

Microfilariae: In the skin of man by day and by night, but are not found in the blood

Infective larvae: In the gut, muscle tissue and mouth parts of Culicoides midges.

Morphology

Adults*:* are recovered only in animal hosts and in man only microfilariae is known.

Microfilariae

Size: 180-240 μm long and 4.5 μm thick

Nuclei extends to tip of the tail

Has no sheath

When immobile, the tail usually appears hooked and its tip is rounded. In fresh preparations, the tip of the tail may appear forked.

Life cycle

Like other filarial worms It requires two hosts to complete its life cycle man as a definitive host and culicoides as its intermediate host.

Pathology: Non-Pathogenic or of low pathogenicity. Most infections are asymptomatic or sometimes cause an itching dermatitis, hypopigmented macules and thickening of the skin.

Prevention and Control:-Like other *mansonella* species.

Laboratory Diagnosis:-Finding the microfilariae in skin snip at any time. Differentiation from *O.volvulus* is by its smaller size, single file anterior nuclei and tail features. Less motile than *O.volvulus* in wet prepare

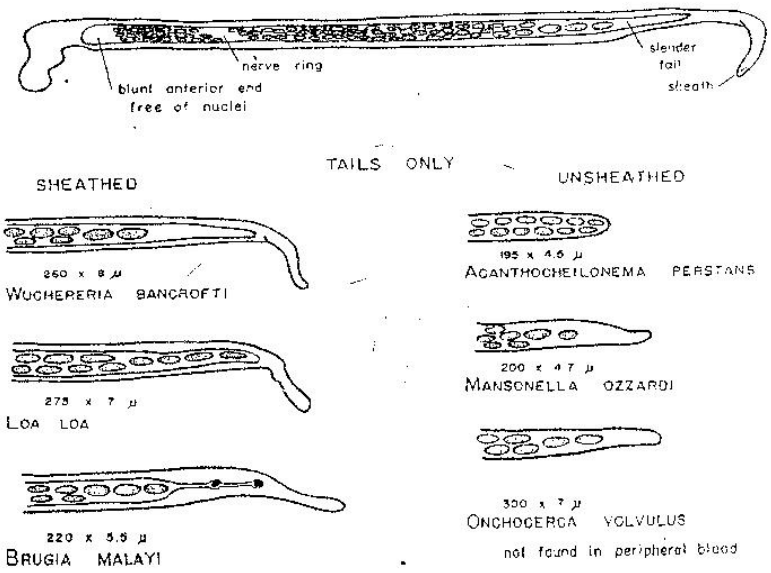


Figure 2.25. Microfilaria of humans: diagnostic characterstics (From Brown HW and Neva FA, Basic clinical parasitology, 5th ed. USA: Applenton-century Crofts,1983.)

Trichinella Spiralis

Geographical Distribution:-*T.*spiralls contain three subspecies:

1) T.S.Spiralis which is found in temprate regions,
2) T.S nativa which is found in the Arctic and
3) T.S nelsoni which is found in Africa & southern Europe.

Habitat

Adults: Embedded by its anterior part in mucosa of muscular epithelium of duodenum and Jejunum of Man, Dog, Rate, Cat, Pigs and many wild Carnivores. viviparous

Larvae: encysted in the straited muscle of the body of meat eating animals including man.

Egg: No eggs passed in the faeces , female gives birth to larvae.

Morphology:

Adults*:*-minute thread-like worms, white in color and attenuated anterior end

- cellular oesopagus.

Male: up to 1.5mm

Female*:* up to 4 mm, viviparous

Larva*:* Size: 0.5 mm long and 0.2 mm thick and lies along the muscle fibers Shape: Usually seen coiled inside a lemon shaped cyst. larvae appear black

Life cycle

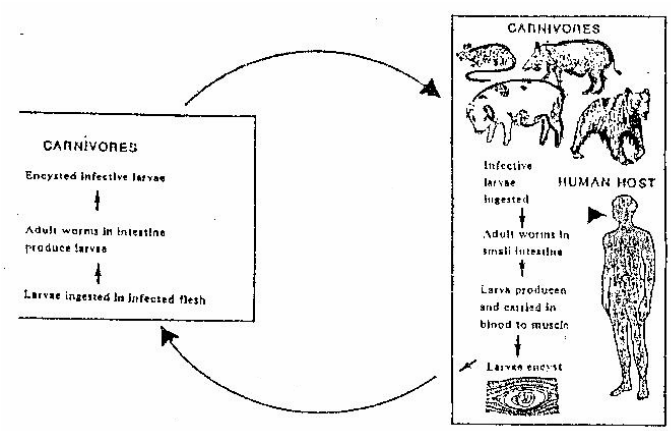


Figure 3.26. Life cycle of *T.spiralis*(From Cheesbrough M. Medical laboratory Manual for tropical countries, Vol. I, 2nd ed. Britain:The Bath Press,1987)

The same animal (and man) acts as final and intermediate host harbouring the adult parasite the larva.

When infected flesh of animals containing infective larvae is eaten by man, pig or other carnivore animals, the larvae are freed and become mature norms in the small intestine. Following fertilization, the viviparous females produce many larvae which are carried in the body circulation to striated muscles to form cyst. The natural cycle is completed when the flesh of an infected carnivore is eaten by another carnivore.

Pathology: causes trchinellosis. Major symptoms are nausea, vomiting, abdominal pain, diarrhea, headache, fever, blurred vision, edema of face and eye, cough, pleural pain, eosinophilia, acute local inflammation, with edema of the musculature.

Prevention and Control:-

1. Avoid eating of raw or undercooked pork
2. Inspecting meat for infective larvae
3. Not feeding raw garbage to pigs.

Laboratory Diagnosis:-

1) Finding the larvae in striated muscle.
2) Testing serum for Trichinella antibodies.

Relevance to Ethiopia

Like other parasite that are transmitted through eating of pork meat, it is uncommon. A case of Trichichinosis due to T.spirails and acquired by eating raw wartag meat was reported from Jimma.

Dracunculus Medinensis

(Guinea or Medina worm)

Geographical Distribution:- Since 1986, the global prevalence of drancunculiasis has been reduced by 97% and it is expected that the disease will be eradicate in the near feature. It is still endemic in 18 countries, 1.e., India, Yemen and 16 countries in sub-Sahara Asfricawith 77% of infections being found in Sudan.

Habitat:

Adults: thread like
Female in the subcutaneous tissues and intermusular connective tissues the of the lower extrimites; especially around the ankle.
Male resides in the retroperitoneal connective tissues and dies shortly after copulation
First stage larvae: In the ulcers or blisters.
Infective Filariform larvae : In the hemoceole of Cyclops .

Morphology

Adults*:* White with smooth surface

Male: 12-29mm , coiled posterior end.

Female : 70-120 cm (average 100 cm)

The longest nematode of man

Has cylinderical oesophagus

Viviparous

Larva*:* Size: 500-700 μm

Rounded anterior end

Long and pointed tail

Has Rhabiditiform Oesophagus

Life cycle

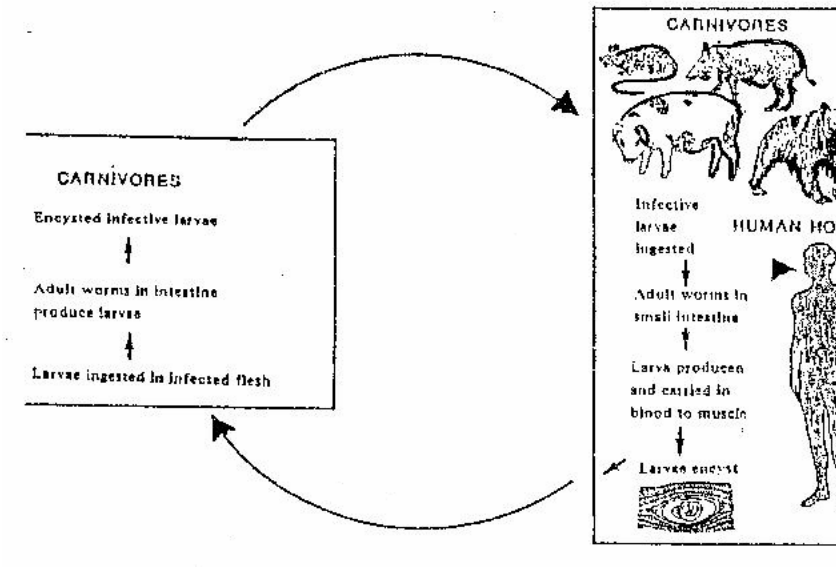


Figure 3.27 Life cycle of *D.medinensis*. (From Jeffrey HC and Leach RM. Atlas of Medical Helminthology and Protozoology, 1975.)

Man is the most important definitive host. It has also been found in dogs and cats. Cyclops or related crustaceans are the intermediate hosts. When Person swallows water containing cyclops that are infected with the larvae. The larvae are freed and penetrate through the duodenal wall. After development and fertilization in the connective tissues, the female worm migrates to the connective tissues of the lower limbs where within about a year it becomes fully mature.

The female worm buries its anterior end in the dermis forming a blister that ulcerates. when the blister (ulcer) is bathed in the water the uterus of the female protrudes through the ulcer and ruptures. large number of first stage larvae are released in to the water.

The larvae are unable to swim but are actively motile, coiling and uncoiling.

For the life cycle to be continued the larvae must be ingested by a Cyclops. In the body cavity of the Cyclops, the larvae develop in to infective larvae.

Pathogenicity in Man

- Disease Guinea worm ulcer disease
- toxic histamine like substances are liberated by the female guinea worm as soon as she starts migration, cousing profound ellergic symptoms
- This is followed by the appearance of worm under the skin associated with blister formation which bursts & the larvae are discharged after coming in contact with water.
- Premature liberation of embroys in the tissue cause irritation and inflammation this happens particularly when the its extraction prematurely

- Secondary infection may also occurs leading to cellulitis and occasionally septicaemia
- If a joint is involved, arthritis may develop

Prevention and Control:

The easiest parasite to be Prevented controlled

1. Prevent water contamination with the larvae
2. Avoid drinking infected water by boiling
3. Destroy the Cyclops by using chemicals example, organophosphorus or chlorine
4. Covering the blister with a water proof dressing

Laboratory Diagnosis:-

Finding the gravid worm in the blister.

A diagnosis is usually made when the blister has ruptured & the anterior end of the female worm can be seen. If required, laboratory confirmation of the diagnosis can be made as follows:

1. Place few drops of water on the ulcer to encourage discharge of larvae from the uterus of the worm.
2. After and left for a few minutes collect the water in a pipette and prepare a wet mount.
3. Transfer the water to a slide & examine microscopically for the motile larvae using the 10 x objective with the iris diaphram closed sufficiently to give good contrast.

Review Questions

1. Explain typical diagnostic characteristics which differentiate one filaria from the rest.
2. Describe. filaria worms according to their pathogenicity, habitat in human, presence or absence of sheath and their periodicity.
3. Why trichinella spiralis is not commonly found in our country ?
4. Identify the diagnostic and infective stages of tissue nematodes.
5. Enumerate the possible sources of specimen and thelaboratory diagnosis oftissue nematodes.

Appendix I

Laboratory Examination of Specimens

Learning Objectives

At the end, successful students be able to

1. Prepare necessary materials for the collection of parasitological specimens.
2. Select appropriate specimen for detection and identification of parasites.
3. Perform direct, concentration and staining techniques for stool specimens .
4. Differentiate between pathogenic and non-pathogenic parasitic organisms, observed under microscopic examination.
5. Apply the Scotch-tape technique for the laboratory diagnosis of E.varmicularis.
6. Apply the kato thick smear method for the diagnosis of ova of schistosomes and round worms.
7. Process and examine urine specimens for the diagnosis of urinary parasite.
8. Perform ,collect, process and examine blood specimens for the diagnosis of haemoparasites
9. Select the appropriate site of skin and perform examination for the laboratory diagnosis of Onchocerca volulus and Ieishmaniasis.
10. Prepare staining solutions and reagents (Giemsa, Wright, iodine, slaine, formalin and others).
11. Record and report all parasitological results in the appropriate formats; including all neccesary information

Introduction

In the field of diagnostic Medical Parasitology, proper specimen collection is critical since the final laboratory results are based on parasite recovery and identification will depend on the initial quality of the samples taken. Unless the appropriate specimens is properly collected, preserved and processed, these infections may not be detected; therefore, as a part of any overall continuous programme for the laboratory, the generation of test results must begin with stringent criteria for specimens acceptance or rejection.

Laboratory procedures detects organisms within clinical specimens using morphological criteria, rather culture or biochemical tests and/or physical growth characterstics. Many clinical specimens, such as those from intestinal tract, contain multiple artifacts that complicate differentiation of parasites from surrounding derbis.

The main ways in which laboratory diagnosis of parasitic infections include:

1) ***Microscopy***:- the majority of intestinal, blood, urinary and skin parasites are usually detected by microscopically in stained or unstained; either directly or following concentations.

2) ***Culture***:- only minority of parasitic infections are diagnosed routinely by culture techniques. Relatively few of protozoa and none of the helminth parasites, can be cultured in a manner that is useful for laboratory identification. Other than strictly for research purpose , the only culture methods in general use are for the isolation of such as *E.histolytica, T.vaginalis, T.cruzi* and *Leishmania* species. are identified by this method.

3) ***Immunodiagnosis****:*-it is based on the detection of :

A. Antibody in a person's serum, produced in response to a particular parasitic infection. The antibody may persist for a long period of time in the serum after an infection has ended and therefore antibody tests are unable to distinguish between past or present infection. When used to assist in diagnosing parasitic disease , antibody tests need to be interpreted with care.

B. Antigen, which is excreted by parasites and can be found in the serum, urine, CSF, feces or other specimens. Antigen tests provide evidence of present infection and are therefore greater value than antibody tests in the clinical diagnosis of parasitic infections.
Immunodiagnostic techniques are required when:

a) Parasites live in the tissue of internal organ and can not therefore easily remove for examination.
b) Parasites can be found in specimens only in certain stages of infection, e.g., in the acute stage not in the chronic stage.
c) Parasites are present intermittently or in too few numbers to be easily detected in the specimens.
d) The techniques used to detect parasiteds are complex or time consuming.

Those parasitic disease for which immunodiagnosis is of particular value include:

- South American trypanosomiasis , Chronic stage
- African trypanosomiasis, when parasitaemia is low
- Leishmaniasis

- Filariasis
- Amoebic liver abscess
- Toxoplasmosis
- Hydatid disease
- Trichinolosis
- Toxocarisis
- Schistosomiasis

The principal type of immunodiagnostic tests are intradermal and serological.

With the introduction of immunoassays, there are now many more options avilable to the diagnostic laboratory, different laboratories will select different approaches. In this appendix only the microscopy method will be dealt in detail.

A. Stool Examination

The most frequently performed parasitological procedure is the stool examination. The detection and identification of parasites; such as adult worms, larvae, eggs, trophozoites and cysts depends on its proper collection.

Procedure

1. Provide the patients with specimen containers with tight-fitting lids.

Note:- Three specimens are usually required at a three alternate days to detect all intestinal parasitic infections. The stool should be collected before radiological examination is carried out using barium. Stool specimens containing barium are unacceptable for examination, and intestinal protozoa may be undetectable for 5-10 days after barium is administered to the patient.

- Some substances and medications also interfer with the detection of intestinal protozoa, including mineral oil, bismuth, antibiotics, antimalarial agents and nonabsorbable antidiarrhoeal preparations. After adminstration of any of these compounds to the patient ,

parasite may not be recovered for a week or more.

2. Collect sufficient quantity of stool. It should contain at least 4ml ($4cm^3$**)** of Stool.
3. Examine the stool as soon as possible.

   As it is not possible to predict what organisms will be present in the specimen, however, the most conservative time frames should be used for parasite recovery. The examination of liquid specimens should occur within 30 minutes of passages, not 30 min. the time they reach the laboratory. Soft specimens may have a mixture of protozoan trophozoties and cysts and should be examined within 1 hour of passage. Formed specimens can be examined at any time within 24hrs.
4. If specimens cannot be examined in the above time frame, put them in available preservatives.

I. Macroscopic Examination

Stool specimen is examined with the naked eye for :

1. Presence of worms:- may have adult helminthes or segments
   Example: *Ascaris, Taenia species, E.vermicularis and gravid Taenia* species.
2. Consistency (degree of moisture)- It varies in diet but certain clinical conditions associated with parasite presence may be suggested by particular consistencies.

   - It will be described as hard, formed, semi-formed and diarrhoeic(watery).
3. Colour:- any abnormal colour

E.g., pale yellowish passed in steatorrhoeac conditions such as gardiasis,dark or black-stools occur when

iron or bismuth is taken or when there is intestinal hemorrage

4. Pathologic odour Offensive, non-offensive
5. Abnormal features seen (composition): mucus, blood or fat globules.

II. Microscopic Examination

The detection and identification of species of parasites require microscopic examination of specimens

1. Direct Microscopic

Routine microscopic examination of stool specimen with physiological saline and Dobell's iodine solution helps to detect and identify the stages of some parasitic organisms.

1.1. Direct Microscopic Examination of Stool Specimen with Physiological Saline and Dobell's Iodine Solutions

Material and Methods

Wooden applicator sticks
Microscopic slides
Cover slips
Dropping bottles containing physiological saline(0.85%w/v) and Dobell's Iodine solutions
Microscope
Pasture pipette

Procedure

1. Place a drop of physiological saline (0.85%w/v) in the center of the left half of the slide and place a drop of Dodell's Iodine solution in the center of the right half of the slide.

2. With an applicator stick, pick up a small portion of the feces (Approximately 2mg which is about the size of a match head) and put on the drop of saline. Add a similar portion of stool sample to the drop of iodine.
3. Mix the feces with the drops to form homogeneous suspensions.
4. Cover each drop with a cover slip by holding the cover slip at an angle of 30^0, touching the edge of the drop, and gently lowering the cover slip onto the slide so that air bubbles are not produced.
5. Examine the saline preparations using the 10X objective for motile forms, cyst and oocyst of intestinal protozoa and for any ova or larva of helminths.
6. Examine the iodine solution preparation using 40X objective to identify the cyst stages of protozoa. The iodine will stain the nuclei and the glycogen mass of the cyst.

1.2 Modified Ziehl-Neelsen technique (Acid-Fast Stain)

Modified Ziehl-Neelsen staining of fecal smear helps to detect oocysts of *Cryptosporidium, Cyclospora* species and *I.belli*.

Material and Methods:

- Carbol fuchsin stain
- 0.25% Malachite green (Methylene blue)
- 1% acid alcohol
- Slides
- Cover slip
- Microscope

Procedure

1. Prepare a thin fecal smear on a slide then air dry.

2. Fix the smear with methanol for 2-3 minutes.
3. Stain the smear with cold carbol fuchsin for 5-10 minutes.
4. Wash off then stain with clean tap water.
5. Decolorize with 1% acid alcohol for 10-15' until color ceases to flow out of the smear.
6. Rinse in tap water and counter stain with 0.5% malachite green (or methylene blue) for 30'
7. Wash off the stain with tap water.
8. Stand the slide in a draining rack for the smear to dry.
9. Examine the smear microscopically using100X objective to detect and identify oocyst.

3. Concentration Methods for Fecal Specimens

The concentration and the separation of protozoa cysts and helminths egg from other elements of the fecal specimen can be of great advantage in diagnosis. This can be accomplished by sedimentation, flotation and combination of the two. Feces normally contain a great variety of materials, most of which are either lighter or denser, smaller or larger than the cysts, eggs and larva of parasites.

The concentration of parasites in parasitological specimens is some times called the '' enrichment technique '' because it enables to examine greater quantity of stools in less volume.The purpose in using a concentration technique is to separate as completely as possible parasites from all other elements of the Stool.

In general the concentration technique may be necessary:

1. To detect parasites when they are not found in a direct saline wet mount examination but the symptoms of intestinal parasitic infection continue.
2. To detect the eggs of parasites which are often few in number

such as those of Schistosome or Taenia species.

3. To check whether treatment has been successful.
4. To investigate the prevalence and incidence of a parasitic infection as part of epidemiological survey .

The choice of concentration technique depends on

1. The species of parasite
2. The number of specimen to be examined
3. The equipment and time available

A direct microscopical examination of stool must always be done before preparing a concentration because motile forms of flagellates, ciliates , and amoebae die during the concentration procedure.

Concentration Technique of Fecal Specimen is Divided into two :-

1. Floatation Techniques

Floatation technique concentrates the cysts and eggs of parasites at the top because their density is less than that of the suspending medium. The waste products, crystals, body cells etc. have a higher specific gravity therefore these substances will sink to the bottom. The top layer can be removed and placed on a slide to be examined under the microscope.

Zinc Sulfate Floatation Technique

Zinc sulfate floatation technique is one of the most widely used method of concentration. It has a special merit of being suitable for routine examination of both cyst of protozoa and eggs of most helminths. Operculated eggs of Trematodes and Cestodes, infertile Ascaris ova and larva of nematodes are not concentrated because they have greater specific gravity than the suspending medium. However; the eggs of

Clonorchis, Opisthorchis and some small trematodes are satisfactorily concentrated. The technique is not also suitable for concentrating eggs or cysts in fatty faeces.

A Zinc sulfate solution which is used for the concentration of parasite 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 where they are collected on a cover glass or can be collected by pasteur pipettes.

Materials and Reagents

1. Lipless test tube about 10ml capacity.
2. $ZnSO_4$ solution 33 W/V (about 33 grams of crystals in 100 ml solution) specific gravity of 1,180-1,200 . use a hydrometer to check the specific gravity (relative density) of the solution is corrected. Adjust with distilled water or more chemical if required.
3. Cover glass
4. Microscope slide
5. Applicator stick

Procedure: -

1. About one quarter fill the tube with the zinc sulfate solution.
2. Add an estimated 0.5 gram of faeces and using a rod or stick, emulsify the specimen in the solution.
3. Fill the tube with the zinc sulfate solution and mix well.
4. Stand the tube in a completely vertical position in a rack.
5. Using a plastic bulb pipette or pasteur pipette, add further solution to ensure that the tube is filled to the brim.
6. Carefully place a completely clean (grease free) cover glass on top of the tube, avoiding trapping any air bubbles.

7. Leave undisturbed for 30-45 minutes to give time for the cysts and eggs to float.

Note: -After 60 minutes, the eggs will begin to sink.

8. Carefully lift the cover glass from the tube by a straight pull upward. place the cover glass face downwards on a slide.The eggs and cysts will be found adhering to the cover glass.
9. Examine microscopically the entire preparation using the 10x objective use the 40x objective and run a drop of iodine solution under the cover glass to identify the cysts.

Other flotation methods includes:

a) Brine (Saturated NaCl) flotation
b) Saturated sugar flotation
c) $ZnSo_4$ centrifugal flotation

2. Sedimentation Techniques

In this technique cysts and eggs of parasites settle and are concentrated at the bottom because they have greater density than the suspending medium. The cysts and eggs can be sedimented by natural gravity or by accelerating the process by centrifugation.

Formalin-Ether Centrifugal Sedimentation Technique

This method is recommended as the best technique for concentrating the eggs and larvae of helminths and moderately satisfactory for cysts of protozoa. It is most useful for detecting the eggs of Schistosome in feces. The formalin is used for fixation and preservation of the morphology of

parasites. The fecal debris absorbs ether and becomes lighter than water.

Advantages of this technique includes:

A) It is rapid and suitable for fresh or preserved stool

B) It is also used for concentrating parasites on which Zinc sulfate floatation has given poor results due to excessive amounts of fats and fatty acids, and for operculated ova of some trematodes and cestodes.

C) The morphology of most parasite is retained for easy identification

D) It will cover most intestinal parasite

Materials and Reagents:-

- Electric centrifuge
- 1.5 ml Conical centrifuge tubes
- Stoppers
- Funnel
- Gauze /Sieve/
- Graduated cylinder /Pipette/
- Beaker
- Applicator stick
- Slide
- Coverslip
- 10% formalin solution
- Ether
- Normal Saline
- Lugol iodine solution

Procedure

1. Take about 2g or 2ml of stool and mix it in about 10ml of normal saline solution.
2. Filter through two layers of gauze into a centrifuge test tube
3. Centrifuge for one minute at medium speed (2000-5000 rpm) . If the supernatant fluid is very cloudy, wash the deposit again i.e. mix it with 10ml of normal saline solution ,

4. Centrifuge for one minute at medium speed and pour of the supernatant fluid.
5. Add 10ml of formaldehyde solution to the sediment.
6. Stir or mix a suspension well and let it stand for five minute.
7. Add 3ml of ether.

Note :- Ether is highly flammable, therefore make sure there is no open flame in the laboratory and it is well ventilated because ether vapor is anesthetic.

8. Stopper the tube, turn it on its side and shake vigorously for 30 seconds or one minute.
9. Remove the stopper carefully and centrifuge for one minute at low speed (1500 rpm).

There will be four layers in the tube:-

1st layer : Ether

2nd layer : Debris

3rd layer : Formaldehyde solution.

4th layer : The deposit containing stages of parasites (cyst, egg and/or larvae)

10. Free the layer of debris by rotating the tip of a wooden applicator stick between it and the sides of the tube. Tilt the tube and pour of all the supernatant fluid. use a cotton swab to remove any debris adhering to the side of the tube.
11. Mix the remaining fluid well with the deposit by tapping the tube gently.
12. Place two drops of the deposit on a slide. Add a some drop of iodine solution to the second drop of deposit only.
13. Place cover slips over both drops .

14. Examine microscopically the entire preparation using the 10x objective for eggs and larvae of helminths, and 40x objective for cysts of protozoa .
15. Identify the stages and species of parasites and count the number of each type of parasites in the entire preparation and report the result.

Note:- For formalin preserved specimen follow the same steps but in step one use distilled water instead of normal saline solution.

Other sedimentation techniques are:

a) Sedimentation in water, either by gravity or centrifugation
b) Acid-ether sedimentation

4. Kato-thick Smear Egg Count

Materals and Reagents

1. Applicator sticks, wooden
2. Screen , stainless steel, plastic: 60- 105 mesh.
3. Template, Stainless steel, plastic, or cardboard templates of different sizes have been produced in different contries. A hole of 9mm on a 1 mm thick template will deliver 50 mg of faeces; a hole of 6 mm on a 1.5 mm thick template, 41. 7 mg; and a hole of 6.5 mm on a 0.5 mm thick template, 20 mg. The templates should be standardized in the country and the same size of templates should always be used to ensure reputability and comparability of prevalence and intensity data.
4. Spatula, plastic.
5. Microscope slides (75x25mm).
6. Hydrophilic cellophane, 40-50um thick, strps 25x30 or 25x35 mm in size

7. Flat- bottom jar with lid
8. Forceps.
9. Toilet paper or absorbent tissue.
10. Newspaper.
11. Glycerol- maiachite green or glycerol- methylene blue solution (1ml or 3% aqueous maiachite green or 3% methylene blue is added to 100 ml of glycerol and 100 ml of distilled water and mixed well).This solution is poured onto the cellophane strips in a jar and left for at least 24 h prior to use.

Procedure

1. Place a small amount of faecal material on newspaper or scrap paper and press the small screen on top so that some of the faeces are sieved through the screen and accumulate on top
2. Scalp the flat- sided spatula across the upper surface of the screen to collect the sieved faeces
3. Place template with hole on the center of a microscope slide and faeces from the spatula so that the hole is completely filled. Using the side of the spatula pass remove excess faeces from the edge of the hole
4. Remove the tempate carefully so that the cylinder of faeces is left on the slide Cover the fecal material with the pre- soaked cellophane strip. The strip must be very wet if the faeces are dry and less so if the faeces are soft (if excess glycerol solution is present on upper surface of cellophane wipe with toilet paper) in dry climates excess glycerol will retard but not prevent drying.
5. Invert microscope slide and filmy press the faecal sample against the hydcellophane strip in another microscope slide or on a smooth hard surface such as a piece of tile or on a flat table. The faecal material will be spread evenly between the microscope

slide and the cellophane strip. It should be possible to read newspaper print through the smear after clarification

6. Carefully remove slide by gently sliding it sideways to avoid separating the cellophane strip or lifting it off. Place the slide on the bench with the cellophane upwards. Water evaporate while glycerol clears the faeces.
7. For all ova except hookworm eggs, keep slide for one or more hours at ambient temperature to clear the faecal material prior to examination under the microscope. To speed up clearing and examination, the slide can be placed in a 40 degree centigrade incubator or kept in direct sunlight for several minutes.
8. Ascaris and Trichuris eggs wiil remain visible and recognizable for many months in these preparations Hookworm eggs clear rapidly and will no longer be vessel after 30-60 minutes Schistosomiasis endemic area to examine the slide preparations within 24 hours.
9. The smear should be examined in a systematic manner and the number of eggs of each species reported. Later multiply by the appropriate number to give the number of eggs per gram of faeces (by 20 if using a 50 mg template: by 50 for a 20 mg template: and by 24 for 3 41.7 mg template) with high egg counts. To maintain a rigorous approach while reducing reading time, the stoll quantitative dilution technique with 0.1 mol/liter NaOH may be recommended.

Key to Identification of Eggs

1. Size- their size are generally within specific range.
2. Shape -each species has its own particular shape

3. Stage of development when passed- in some species the eggs consists of a single cell; in some there may be several cells; some species are usually embryonated.
4. Thickness of egg shell- some species like Ascaris have thick egg shell,others like hookworm , have thin shell
5. Color- Some eggs are colorless(e.g., Hookworm, Entrobius) others are bile stained (yellow-brown)
   e.g., Ascaris, Trichuris.
6. Presence of characterstics like opercula(lids), spines .plugs, hooklets, or mammilated outer coats.

Reporting Results of Stool Examination

The following details should be given when recording the result of a stool examination:

1. Macroscopic characterstics
2. Parasites fond by microscopic examination specifying:
   Species Example. Giardia lamblia
   Stage of development:- Cyst, trophozoite, ova, larva.
   Quantity:- count the eggs and larvae,if specifically requested.

Otherwise it will not have any effecct on treatment.

Describe the quantity of parasites as follow:-

Scanty /Occasional.....1.3 per slide
A few.....4-10 Per slide
Moderate number.....11-20 Per slide
Many.....21-40 Per slide
Very many.....More than 40 per slide

Pseudoparasites

Pseudoparasites (artifacts):- are elements found in stool which structurally resemble parasites.

Examples:-

- Vegetable cells resembles cyst but differentiated by their thick cellulose walls and striation
- Plant hair and Muscle fiber resembles larva.
- Starch granule, Yeast/spore, Pollen grain, Air bubble and Fat globule resembles cyst/egg
- Soap, epithelial cell, WBC, pus cell.....cyst/egg

B. Collection of urine specimen

When urine sample is received with request to find parasites, the best method to use is the one descibed below for the diagnosis of schistosomes. This will concentrate most of the parasite to be found.

1. Collect random urine sample, preferably at midday, into a sterile container.
   The number of ova in the urine varies throughout the day, being highest in urine obtained between 10:00h and 14:00h.

N.B. It may be necessary to examine several specimens collected on different days. Even when persons are heavily infected, eggs may not present in the urine at all time. Neither exercising before passing the urine nor collecting terminal urine (last few drops), increase the number of eggs present in the specimen (as once was thought).

2. Centrifuge 10ml of urine at 1500RPM for 2 min. to deposite the ova. Decant the supernatant.
3. Place a drop of deposit onto a microscope slide and cover with a cover slip.

Examine the whole preparation for ova using 10X objective .

- In the earlt stages of urinary schistosomiasis , the egg count is an indicator of the severity of the disease. So count the number o f eggs in the preparation and report the number /10mlof urine. If more than 50 eggs are present , there is no need to continue counting. Report the countas 'more than 50eggs/10ml of urine'. Such count indicate heavy infection.
- Ova of *E.vemicularis* may occasionally be seen in a centrifuged deposit of urine particulrly in female children. This is as a result of the adult female crawling from the anal area and depositing eggs in the proximity of the urethra.
- Trophozoites of T. vaginalis may also be observed if it is present.

  This parasite is usually found either in - Vaginal and urethral discharge, or
- Fresh urine sediments.

  To make a permanent preparation, make a smear of the the urine sediment on a slide, allow it to dry and fix it in methanol. Stain with Giemsa diluted 1:25 buffered water pH 6.8 for 25 min.

Collection of Urine for Diagnosis of Microfilariae

Microfilaria are occasionally found in the urine when the lymphatic system is severly obstructed. Obstruction is severly caused by the following species: *Wuchereria bancrofti,B. malayi and Onchocerca volvulus.* The urine appears milky due to the presence of that fat globules from the lymph. This appearance is known as chyluria.

1. Place approximately 9ml of urine in capped bottle,add 3ml of ether and shake well (to dissolve the fat globules). Transfer to a centrifuge tube.
2. Centrifuge the urine at 2500RPMfor 2min.
3. Place a drop of the deposit onto a microscope slide cover with a cover slip and examine using 10x objective for microfilaria.
4. For species diagnosis the microfilaria can be stained in a smear from the deposit using hematoxylin or Giemsa stain.

If urine is contaminated with stool, parasites, which can be found in stool specimens, may also be found in urine deposit.

C. Viginal and Urethral Discharge

Vaginal and urethral material are exmined for the presence of Trichomonas vaginalis, a flagellate parasite of urogential system. It parasitize both men and women, put men are usually asymptomatic. T. vaginalis is usually identified in wet mounts of vaginal and urethral material.(In stained preparations these organisms are badly distorted and may not be recognizable)

Technique

1. With a sterile cotton swab, collect the vaginal or urethral disehage.

2. Put the swab immediately into a sterile tube containing 3 ml of sterile saline, the top of the stick can be broken off it is too long for the tube.
3. Smears for staining can be made if desired – for these, collect more material with a second sterile swab and smear in the slide. Allow to dry.
4. Label tubes and slides with patients name or number, and the date of collection.

N.B If the patient can come to the laboratory, wet mounts can be examined directly: tubes are not needed.

Direct Examination of Viginal and Urethral Smear

1. If the patient can come to the laboratory, obtain some of the vaginal or urethral discharge with a steril swab and put into a drop of saline on a microscope slide.
2. Cover with a cover slip and examine with X10 and X40 objective for motile flagellates.

D. Collection of Blood Specimen

Next to feces, the blood provides the most common medium for recovery various stages of animal parasites. From blood specimen a diagnosis is routinely made for malaria, African trypanosmiasis, Visceral leishmanisis and most types of filariasis less frequently of chagas disease; and rarely of toxoplasmosis.

Blood examination of malaria parasites needs to be collected and when ever possible reported before treatment is started. Careful attention is necessary in the collection and preparation of blood films.

The following care should be taken while collecting blood.

1. Collect sufficient quantity of blood.
    a. Capillary blood from finger prick , toes, or ear lobes
    b. Venous blood.

The collected blood should be enough to make wet unstained film, stained thin and/or thick films, or to be used for concentrating the parasites.

2. Time of collection

Collect the blood specimen at the appropriate time based on the clinical investigations. E.g. For microfilariae, malarial parasites, etc. Usually most malaria parasites are found in the blood towards the end of an attack of fever. Always collect blood for malaria parasite investigation before anti-malaria drugs are given to the patients. Blood should be collected in accordance with the periodicity of microfilariae of filarial worms.

3. If anti-coagulated blood specimen is to be used.

Use a suitable anticoagulant. E.g. Acid citrate dextrose (ACD) or sodium citrate for microfilariae, EDTA for malaria parasites and Trypanosomes.

4. After collection, protect specimens adequately.

The following types of blood examination can be carried out for the laboratory diagnosis of haemoparasites:

1. ***Wet Blood Films:-***For microfilaria:

Tube centrifugation lyzed blood technique

10ml of venous blood is lyzed in saponin-saline. The microfilaria are concentrated by centrifugation . the addition of blue nuclear stain helps to identify the species. The number of microfilaria (mf) counted divided by 10 gives the number of mf/ml.

Microhaematocrit tube technique

Capillary blood (preferably ear lobe blood) is collected into heparinized capillary tubes or about 100µl is first collected into EDTA anticoagulant and transferred to plain capillary tubes . The blood is centrifuged in a microhaematocrit centrifuge and the buffy coat is examined for motile microfilaria. In areas where the species is known and *Mansonella* mf are not found , this is the rapid technique for detecting microfilaria.

Wet slide preparation

Collect 0.02ml of blood and mix with 2 drops of water (to lyze the RBCs) on a slide. Cover with cover glass and examine for motile mf using 10x objective, preferably by dark field microscopy. The technique is sometimes used as a screening test but it is not as sensitive as the above procedures.

2. Thick Blood Film

Disinfect the tip of the finger and puncture with a quick jub of a needle or lancet, using and gauze sponge after ward to wipe away traces of the disinfectant and blood.

Touch a clean slide to a drop of blood and using the corner of another slide, spread the blood in a rectangular pattern so that the blood slowly flows down and does not immediately form a drop at the lower edge when the slide is tilted side ways. A good thick film is the size of the postage stamp and so thick that you can just see the hands of a watch or news prints through it. The slide should be allowed to dry in a flat position such as table top, and do not use hear to dry the film. Then stain with Giemsa.

3. Thin Blood Film

Collect a drop of blood on a clean slide usually from the finger tip. Touch a clean slide to a small drop of blood so the blood is near one end of the slide. Place the slide blood side up on the table. Quickly take a second new clean slid. And holding it at an angle of 30 degrees to the first slide: draw it back until lt touches the drop of blood and then push forward so that blood spreads out behind. The amount of blood should be small enough so that it is used before the spreader slide reaches the end of the first slid. In this manner a smooth film. One-cell in thickness can be prepared. Such a film is best for the study of blood cells as well as parasites. Then air dry and stain either with Geimsa or wright stain.

Significance of Thick and thin Blood film

- About twenty times more blood can be examined in a thick film than in a thin film in the same period of time. A thick film is therefore the most suitable for the rapid detection of malaria parasites. In area where P.malariae exists, unless a thick film is examined, infection is likely to be missed because parasitaemia is normally low with this species .
- More blood can be examined in a thick film because the film is not fixed and therefore the red cells are lyzed during staining. The parasites are not destroyed and after being stained they can be detected among the white cells, against a background of lightly stained hemoglobin.
- A thin blood film is required to confirm the Plasmodium species if this is not clear from the thick film. A thin film is fixed and therefore the parasites can be seen in the red cells. Depending on the species, parasitized red cells may become enlarged, oval

in shape and show stippling. These features, together with the parasitic forms present can greatly assist in confirming a mixed infection and in identifying P.ovale and P.malariae which are more difficult to differentiate in thick film.

Making of Thick and Thin Blood film:

A thick and thin film can be made on the same which has the advantage that fewer slides are used. The thin film can be used for labeling the specimen when slides with frosted ends (for labeling) are not used.

To ensure good staining and standardization of reporting, the amount of blood used, particularly y to make thick films should be kept as constant as possible and the blood should be spread evenly over a given area of the slide.

Blood from a finger prick is usually used for malaria smears. The thin and thick smear may be stained with wrights or Giemsa stain. A thin smear is best stained with wrights and thick best stained with Giemsa. If both are to be stained with Giemsa. The thick smear must be fixed in methyl alcohol. Giemsa stain removes the heamog;obin.

Staining of thin Blood Film With

A. Wright Stain:

1. Dry the smear in air
2. Put the slide on staining rack
3. Cover the slide with wright stain and wait for 2-3 minute
4. Add equal amount of distilled water
5. Mix the distilled water with weight stain by blowing until metallic scum appears.
6. Wait for 8 minutes.
7. With out disturbing the slide, flood with distilled water and wash

until thinner parts of the film become pinkish.

8. Stand the glass slide on end to dry.

Staining thick and films with Giemsa stain:

1. Allow the thick and thin blood smear to dry
2. Place the smear on staining rack (film side up)
3. First fix 1-3 minute. (Take care that methanol must not touch thick smear)
4. Cover the thick and thin blood smear with diluted Giemsa stain Leave the diluted stain for 30 minutes
5. Wash the stain with distilled water
6. Allow the smear to dry and
7. Examine with the oil immersion objective

Result of Staining of Blood Films

Malaria Parasites

Cytoplasm of parasite.....blue
Nuclear chromatin dot.....dark red
Malaria pigment.....brown- black
Red cells..... grey to pale mauve
Leukocytes.....stain darkly
James, Schuffener's and Maurer's dots..... purplish red

Trypanosomes Parasites

Nucleus..... mauve red
Kinstoplast..... dark mauve -red
Cytoplasm..... Pale mauve

Leishmania Parasites

Nucleus..... Mauve red
Kinetoplast..... Dark-mauve red
Cytoplasm..... Pale-mauve

Reporting L. Donovani amastigote Number in Spleen aspirate Smears

The following grading system is recommended using 10 x eye piece and 100x oil immersion objective

Grade	Average parasite density
0	0/1000 fields
1+	1-10/1000 fields
2+	1-10/100 fields
3+	1-10/10 fields
4+	1-10/ field
5+	10-100/ Field
6+	>100/ field

Microfilariae

Nuclei.....dark purple
Sheath of W.bancrofti..... pink
Sheath of B. malayi.....dark pink
Sheath of L.loa.....pale grey or unstained

Reporting Results.

Reporting results of blood examination for haemoparasites parasite
Specify:-

A. The species of parasite found
B. The stage of development

C. The parasite density

Techniques to Estimate Malaria Parasite Density

A. Counting the Percentage of Parasitized Red Cells in a thin film

To estimate the parasitized cells, count a total of at least 500 red cells making a note of the number that contain parasites excluding gametocytes and at the end report in percentage.

For this procedure the best method is to insert in the eye piece of the microscope a disc with a central square to reduce the size of the field. This will make counting easier by reducing the number of red cells soon in the field.

Note:- A serious P.falciparum infection is indicated when 5% of more of the red cells are infected. E.g. When 10-20% of the cells are parasitized the prognosis is serious, with 20-30% it is grave and over 30% it is exceptionally grave.

B. Counting of Malaria Parasites Against with cells in a thick mm.

Absolute number of parasites can be estimated in thick film by counting the parasites against white cells and using a fixed white cell count values or patients known WBC count (More accurate) to calculate number of parasites per microliter of blood. Counting of the parasites is made easier if a grid is placed in the eye piece.

1. Select part of the thick film where the white cells are evenly distributed and the parasites are well stained.
2. Using the immersion objective systematically count 100 white blood cells (WBC) estimating at the same time the numbers of parasites (asexual) in each field covered. Repeat this in two other areas of the film and take an average of the three counts,
3. Count the number or parasites per micro litre or blood as follows.

Parasite Count: WBC count x Parasites counted against 100 WBC

100

Example:- Patient WBC count = 5, 000/microliter

Parasite counted against 100 WBC - 500

Parasite count = 500 x 500 = 25,00/micro-litre

100

C. Reporting the parasites and approximate number of parasites (trophozoites, schizonts, and gametocytes) found in thick film using plus signs.

1- 10 per 100 high power fields..... +

11-100 per 100 high power fields..... ++

1- 10 in every high power field..... +++

More than 10 in every high power field..... ++++

D. Collection of Skin Specimen

Human cutaneous (skin) and subcutaneous tissues may contain parasites such as:

Microfilriae of O.volvulus and M.streptocerca.

Amastigote stage of Leishmania,

First larval stage of D.medinensis

A very small pieces of the patient's skin is collected to see the highly motile microfilariae in a normal saline mount preparation of the skin snip. Scalpel, and needles or punches can be used to take the skin specimen. For microfilariae, the skin snip is collected from;

I. Patients With Nodules

- On the chest (over the ribs)
- On the hips
- On the legs (tibia, calves)
- On the back (shoulder blades)

The nodules are round and hard, 1-5cm in diameter; when pushed with the fingertips they slide about under the skin. Take the specimen from the skin in the center of the nodule.

II. Patients without nodules

Take the skin snip from:

- The top of the buttocks (the upper outer part where intra -muscular injections are given)
- The upper outer part of the calf
- The back (center of shoulder-blade)

Method

1. Cleanse the skin (or the nodulated area) with swab. Allow the area to dry.
2. Quickly flame a needle and let it cool. Do not let it red hot, or it will get blunt.
3. Push the needle into the skin where you want to take the snip. Hold it flat & only just push the point in.
4. Lift up the needle, & this will raise a fold of skin.
5. Cut off skin just under the needle with a sterile scalpel or sterile razor blade. The skin snip will stick tot he needle.
6. Put the skin snip on the slide, put a drop of saline on it cover it with cover slip.
7. Wait 2 – 3 minutes, examine microscopically using the low. Power dry objective. The micro filarial are seen coming out of the piece of the skin & start moving actively in the saline.

E. The Grham Scotch Cellophane Tape Method Technique for Pin-Worm (E. Vermicularis) eggs collection.

Perianal fecal debris exam:-

The highest number of eggs can usually be recovered in the morning soon after waking and before bathing. Eggs can be collected from skin around the anus or from clothing by applying clear adhesive tape or saline swab. The eggs are detected microscopically by sticking the tape directly on a slide. They rarely appear in the stool.The fecal material from around the anus is usually examined for diagnosis of *Enterobius* infections. Often *Taenia* eggs, undetected by direct saline exam. are also found in perianal specimen.

The fecal swab should be collected either late at night or early in the morning before bathing or defection 3 or 4 samples collected on alternate nights, should be examined before are diagnosis for pinworm is made.

Scotch tape Method

1. Take a strip of cellophane (''Scotch ''tape) & fold it around the bottom of a test tube or Tongue depressor or glass slide with its sticky side out – words.
2. Press the stickly surface of the ''Scotch '' tape firmly on the skin round the anus (peri-anal –feld). Flatten the felds of the skin as your process the eggs on the skin will stick to the cellophane tape.
3. Cut off the middle part of the strip of cellophane tape which has been pressed on the skin.
4. Put 3 or 4 drops of Xylene or toluene on the slide & stick the middle part of the tape stickly side down on top of it.

5. Look at he strip with the low power and dry objective & you will easily see the ova of pin worm.

Swab Method

1. Moisten a cotton wool swab in fresh physiological saline. Swab around the perianal area .
2. Agitate the swab in a container of 4-5ml of saline to wash off the eggs. Cap the container and safely discard the swab
3. Transfer the saline to aconical tube and centrifuge to sediment eggs. Discard the supernatant fluid and without delay , transfer the sediment to a slide , cover with a cover glass.
4. Examine microscopicaly using 10x objective to detect the eggs and 40x objectiveto identify them.

Appendix II

Preservation of Specimens

To preserve protozoan morphology and to prevent the continued development of some helimenth eggs and larva e, stool specimen can be placed in preservative s either immediately after passage (by the patient using collection kit) or once the specimen is received by the laboratory.

There are sevseral fixatives available the more commen ones being : feormalin , Merthiolet -iodine-formalin(MIF) Sodium acetate -acetic acid - formalin (SAF), Shaudinn's fluid and polyvinyl alcohol (PVA). When using any of this fixatives , adequate mixing of the specimen, and preservatives is essential.

When possible, examination of faeces should be carried out immediately. Fixatives are required for preserving parasites in faeces if:-

1. Specimens need to be sent to a reference laboratory for identification.
2. Microscopical examinations are not available locally.
3. Specimens are sent from reference laboratory to peripheral laboratories as part of a parasitology qualify assessment program.
4. Large number of faecal specimens are collected in the field for epidemiological survey.
5. Individual laboratory wants to preserve the sample for teaching purpose

Methods of Preservation:-

1. Refrigeration

Refrigeration at 3-5°C can preserve trophozoite for several days in dysenteric stools and cysts in normal feces may remain viable for more

than a month. Freezing should be avoided.

2. Formalin

Formalin is an all purpose fixative appropriate for helminth eggs and larva, protozoan cyst, coccidian oocysts and microsporidian spores. Two concentrations are commonly used: 5% which is recommended for preservation of protozoan cysts; and 10%, which is recommended for helminth egg and larvae. Although either concentration can be used, most commercial manufacturers provide 10%, which is more likely to kill all helminth egg. To help maintain organism morphology, the formalin can be buffered with sodium phosphate buffers, i.e., neutral formalin. Hot (60°C) formalin can be used for specimens containing helminth eggs since in cold formalin some thick -shelled eggs may continue to develop, become infective, and remain viable for long periods(Ascaris lumbricods).

3. 10%v/v Formol saline:-

This solution preserves cysts and eggs for some months. It also preserves the larvae and adult worms.

4. Merthiolate-Iodine-Formaldehyde (MIF):-

This fixative contains iodine and eosin, therefore it is used both as a fixative and a stain.

It is a good stain for most parasites found in faeces. It is especially used for field surveys. It is used with all common types of stools and aspirates; protozoa, eggs and larva can be diagnosed without further staining in temporary wet mounts, either made immediately after fixation or prepared several weeks later.. the MIF preservative is prepared in two stock solutions, stored separately and mixed immediately before use. They are well preserved for a year or more.

5. Sodium Acetic Acid Formaldehyde (SAF)

SAF preserved faecal specimens can be examined using both the concentration and the permanent stained smear and the fixative has the advantage of not containing mercuric chloride , as is found in Schudinn's fluid and PVA.

SAF has a long shelf life and is easy to prepare, the smear preparation technique may be slightly more difficult for less experienced laboratory personnel. Helminths eggs and larvae, protozoan trophozoites and cysts, and coccidian oocyts and microsporidian spores can be preserved using this method. If specialist stains are to be used, e.g. Iron haematoxylin , a suitable fixative is SAF. Emulsify approximately 1g of faeces in about 10 ml of SAF solution.

6. Schaudinns Fluid

This fixative is used to preserve trophozoite stages of protozoa up to one year.

It is designed to be used with fresh stool specimens or samples from the intestinal mucosal surface. Permanent stained smears are then prepared from fixed material.

7. Polyvinyl Alcohol (Pva)

PVA is as plastic resin that can be incorporated into schaudinn's fixative. The PVA powder serve as an adhesive for the stool material: when the stool PVA mixture is spread onto the glass slide and allowed to dry, the stool material adheres because of the PVA component. Fixation still accomplished by the Schauudin's fluid itself. Perhaps the greatest advantage in the use of PVA is the fact that a permanent stained smear can be prepared. PVA fixation solution is highly recommended as a means of preserving cysts and trophozoites for examination at a later time.

Three parts of fixative are used to emulsify on part of faeces (1:4).

8. Beyer's Solution:-

It is recommended for preserving cysts and eggs in faeces. It maintains their morphology for long periods and allows the specimen to be examined as a direct preparation or after concentration by the formol ether technique.

9. Domestic Bleach Solution

S.haematobium eggs can be preserved in urine by adding

- 1 ml of 1% v/v domestic bleach solution to every 10 ml of urine or
- 1ml of undiluted formalin (37%) to each 100ml of urine or
- 1ml of hydrochloric acid (20 drops) and 2ml of commercial bleach
  (40 drops) for every 100ml of urine.

The urine can be examined either by sedimentation or filtration technique.

Table 5: Summary of advantages and disadvantages of commonly used fixatives

Fixative	Advantages	Disadvantages
Formation	Good overall fixative for stool concentrate; easy to prepare, long shelf life Concentrated sediment (FA)/ unconcentrated material (EIA) can be used with immunoassays	Does not preserve trophozoites well nor adequately preserve organism morphology for good permanent stained smear
MIF	Components both fix and provide stain color easy to prepare, long shelf life Contains no mercury compounds Useful for field surveys	Morphology of organisms on permanent stained smears generally not as good as that with Schaudinn's fluid /PVA (mercuric chloride base)
SAF	Can be used for concentration and permanent stained smears Easy to prepare, long shelf life Contain no mercury compounds Concentrated sediment (FA) unconcentrated material (EIA) can be used with immunoassays	Poor adhesive properties, albumin-coated slides recommended Ptotozoan morphology better if iron hematoxylin stain used for permanent stained smears Slightly more difficult to use but really a limiting factor
Schaudinn's fluid	Fixative for smears prepared from fresh faecal specimens or samples from intestinal mucosal surfaces Excellent preservation of protozoan trophozoites and cysts	Not generally recommended for use in concentration procedures Contains mercuric chloride Poor adeshive-qualities with liquid or mucoid specimens

Continuation of table 5

PVA	Can prepare permanent stained smears and perform concentration techniques Excellent preservation of protozoan trophozoites and cysts Long shelf life (months to years in tightly sealed contains at room temperature) Allows specimens to be shipped to laboratory for subsequent examination	*Trichuris trichiura* eggs and *Glaridia lamblia* cysts not concentrated as easily as from formaline-based fixatives *Strongyloides stercoralis* larval morphology poor (formalin-based preservation better) *Isospora belli* oocysts may not be visible from PVA – preserved material (formalin-fixed specimens better) Contains mercury compounds (Schaudinn's fluid) May turn white and gelatinous when dehydrates or refrigerated Difficult to prepare in laboratory Preserved specimens cannot be used with immunoassay diagnostic reagents

N.B: PVA, SAF and Schaudinn's fixatives are used especially for making stained preparation of the preserved specimen for the diagnosis of protozoa.

Chemical Tests

Occult Blood Test

Bleeding into the gastrointestinal tract may be easily seen with the naked eye. When the bleeding is chronic with only small amounts of blood being passed in the faeces. It is not recognized in the faeces. Bleeding in the gut that cannot be seen is called occult Bleeding. occult means hidden. Occult blood testes are usually made to investigate the cause of iron deficiency anaemia or to assist in the diagnosis of hookworm infection, peptic ulcer; etc.

Principle :-

Haemoglobin and its derivatives react in a similar way to peroxidase enzyme, that is, they catalyze the transfer of an oxygen atom from a peroxide such as hydrogen peroxide, to a chromogen such as benzidine, o-tolidine, guaiacum, 2,6-dichlorophenolindophenol or aminophenazone. Oxidation of the chromogen is shown by the production of a blue, blue-green, or pink colour.

$H_gb + H_2O_2 \rightarrow O2$

O_2 + colorless chromogen $\rightarrow$ colored chromogen

Occult Blood Test with Aminophenazone.

Reagents :

1. 10%v/v acetic acid
2. 95% v/v alcohol (ethanol)
3. Hydrogen peroxide (fresh 10-vol. solution)
4. Aminophenazone crysals.
5. Working Aminophenazone reagent.

95%v/v ethyl alcohol 15ml
10%v/v Acetic acid 1ml
4-Aminophenazone 0.4g

The amounts given are sufficient for one test with positive and negative controls. Always prepare fresh working reagents.

Dissolve the aminophenazone in the alcohol solution and immediately before use add the acetic acid. Mix well.

Procedure

1. Dispense about 7ml of distilled water into a wide bore test tube.
2. Add a sample of faeces about 10-15mm in diameter taken from various parts of the specimen. Using a glass or plastic rod, emulsify the faeces in the water.
3. Allow the faecal particles to settle or centrifuge the emulsified specimen.
4. Take three completely clean tubes and lable them :
   T ..... Patient's test
   Neg. ..... Negative control
   Pos. ..... Positive control.
5. Add into each tube as follows.
   Tube
   T- 5ml supernatant fluid from emulsified faeces
   Neg. ..... 5ml distilled water.
   Pos. ..... 5ml distilled water in which 0.05ml of whole blood has been mixed.
6. Layer 5ml of working aminophenazone reagent on top of the fluid in each tube. Do not mix.

7. Add 10 drops of the 10 vols. hydrogen peroxide solution. Do not mix. Allow to stand for one minute.
8. Look for the appearance of a blue colour where the aminophenazone reagent meets the sample or control solutions. Report the results as follow

No colour change ..... Negative
Pale blue (positive reaction) ..... +
Dark blue (strong positive reaction) ..... ++
Blue black(very strong positive reaction .. +++

Negative control : This should show no colour change
Positive control : This should show a colour change

False Reactions:-

A false positive reaction may occur if the faeces contains peroxidase like substances particularly meat (myoglobin), fish, green vegetables. To avoid this it is best if the patient does not eat these foods, take any drink containing iron compounds and brush his teeth vigorously for at least one day before the test specimen is obtained.

A false negative reaction may be obtained if the faeces contains a high concentration of ascorbic acid.

Preparation and storage of reagents that are commonly used in parasitology laboratory

When you prepare reagents try to apply the following things.

1. Weigh as accurately as possible chemicals that are to be used to make solutions and reagents.

2. Always label clearly all reagents. Include the full name of a reagent and where appropriate, its concentration and date of preparation.
3. If a reagent is, harmful, toxic, corrosive or flammable, indicate on the bottle label.
4. Store reagent in completely clean bottles that have leak proof screw – caps or stoppers, use brown bottles for storing light sensitive reagents.
5. Protect all reagents from sun light and excessive heat. Store light sensitive reagents and stock solutions of stains in the dark and unstable reagents at 2-8^{o}C.
6. Filter stains as required, into stain dispensing containers.

Glossary

Accidental (or incidental): infection of a host than the normal host species, a parasite may or may not continue full development in an accidental host.

Accloe: On the outer edge.

Acetabula (sing. acetabulum): Muscular suckers found on the ventral surface of the trematodes.

Anaphylaxis (Anaphylactic shock): An exaggerated reaction by the host's body to foreign protein or other substances

***Anorexia*:** Loss of appetite.

Anthroponosis: Parasites that are normally maintained by man alone and animals are accidental hosts

Aperiodic (non-periodic): The microfilariae present in peipheral blood throughout the 24 hours constantly.

Arthropod: A phylum of animals having a chitinous exoskeleton and jointed leg. Includes insects and arachnids among others.

Atria (sing. atrium)*:* Refers to the mouth, vagina and urethra.

Autoinfection: When the host himself is the direct sources of reinfection to himself. E.g. H.nana, Strongyloides.

***Axoneme*.** The intracellular portion of the flagellum.

Axostyle: The axial rod functioning as a support in flagellates.

Biological Incubation Period /prepatent period*:* The time interval between infection and detection of parasites in the specimens.

Brood Capsule: a structure within the daughter cyst in E. granulosus in

which many scolices grow.

Buccal Capsule (cavity)*:* Oral cavity or roundworms (in the case of hookworm the cavity contains either cutting plates or cutting teeth.)

Bursa (Pl. busae)*:* Fan- shaped cartilage expansion at the posterior end of some

Carrier: A host harboring and disseminating a parasite but exhibiting no clinical signs or symptoms.

Ceostoda: A subclass of Cestoidea within the phylum platyhelminthes, which includes the tapeworms. These helminths have elongated, ribbnlike segment, has no alimentary canal with a scolex at one end. bodies.

Cercaia (pl. cercariae): The stage of the fluke life cycle that develops from germ cells in a daughter sporocyst or redia. This is the final developmental stage in the snail host, consisting of a body and a tail that aids in swimming.

Chromatin: Basophilic nuclear DNA.

Chromatoidal Body (or bar): A rod-shaped structure of condensed RNA material within the cytoplasm of some protozoa cysts.

Cilia: Hair like processes attached to a free surface of a cell, functions for motility of fluids at the surface of the cell.

Ciliata: A class of the phylum protozoa containing animals, which move by means of cilia.

Cutaneous Larval Migrans: A disease caused by the migration of larvae or Ancylostoma braziliense or Ancylostoma cainum (dog or cat hookworm) under the skin of humans. Larval migration is marked by thin red. Papular lines of eruption also timed creeping eruption

Commensal: The association of two different species of organisms in which one partner is benefited and the other is neither benefited nor injured.

Commonsalism: The association of two different species of organisms in which one partner is benefited and the other is neither benefited nor injured.

Copularory spicules: needlelike bodies possessed by some male nematodes: these lie in pouches rear ejaculatory ducts and may be inserted in the vagina of the female worm during copulation;

Coracidium: A ciliated hexacanth embryo; D. latum eggs develop to this stage and then hatch in fresh water.

Corticated: possession an outer, mammillated albumious coating, as on the eggs of Ascaris lumbrcoides.

Crithidia Form: A flattened, spindle-shaped, flagellated form seen primarily in the gut (e.g. reduviid bug) or salivary glands (e.g. tsetse fly) of the vectors in the life cycle of trypanosomes. It has an undulating membrane extending form the flagellum (attached at the anterior end of the organism) to the small kinetoplast located just anterior to the large nucleus located at the midpoint of the organism.

Cryptozoite*:* The stage of plasmodium spp., which develops in liver cells from the inoculated sporozoites also, called the exoerthrocytic stage or tissue stage.

Cutaneous**:** pertaining to the skin.

Cuticle: The surface of roundworms; a rough protective covering that is resistant to digestion.

Cyst: The immotile stage protected by a cyst wall, in this stage the protozoan is readily transmitted to a new host.

Cysticercoid: A larval stage of some tapeworms; a small bladder like structure, containing little or no fluid, in which the scoles is enclosed (e.g. H. nana)

Cysticercus: A thin- walled, fluid -filled bladder-like cyst, which encloses a scolex and also termed a "bladder worm" (e. g. Taenia spp.)

Cytosome**:** The rudimentary mouth.

Dermatitis: inflammation of the skin.

Diagnostic stage: A developmental stage of a pathogenic organism that can be detected in human body secretions, discharges, feces, blood or tissue by chemical means or microscopic observation to aid in diagnosis.

Differential Diagnosis: The comparison of symptoms of similar diseases in a manner designed to determine from which of these the patient is suffering.

Digensis: Successive reproduction by sexual and sexual generations.

Disease*:* A definite morbid process having a characteristic train of symptoms.

Distomiasis: A tumor or growth of lymphoid or other cells.

Diurnal Periodicity**:** Microfilariae are present in greatest numbers in the peripheral blood during day hours.

Diurnal: Occurring during the daytime.

Dysentery: A disorder marked by bloody diarrhea and mucus in feces.

Ectoparasites: A parasite established on or in the exterior surface of a host

Ectoplasm: The gelatinous material beneath the cell membrane.

Edema*:* Unusual excess fluid in tissue causing swelling.

Elephantiasis: Overgrowth of the skin and subcutaneous tissue due to obstructed circulation in the lymphatic vessels; occurs in the presence of some long-term filarial infections.

Embryination: The development of a fertilized helminthes embryo in to a larva

Embryophore: the shell of Taenia eggs as these are found in feces.

Endoparasites*:* A parasite established within the body of its host.

Endoplasm**:** The fluid inner material of a cell.

Endosome: The dot or mass of chromatin within the nucleus, comparable to a nucleolus of metazoan cells. (Also termed karyosome.)

Enteritis: inflammation of the intestine.

Epidemlology*:* A field or science dealing with the relationships of the various factors, which determine the frequency and distribution of an infectious process or disease in a human community.

Excystation: Transformation from a cyst to a trophozoite after the cystic form

Exflagellation: The processes whereby a sporozoan microgametocyte releases haploid flagellated microgametes that can fertilize the macrogamete and thus form a dipoid zygote (ookinete.)

Facultative Parasite: An organism capable of living an independent or a parasitic existence. Not an obligatory parasite, but potentially parasitic.

Filaria (Pl. Filaria): A nematode worms of the superfamily Filarioidae: requires an arthropod intermediate host for transmission.

Filariform Lava: infective, non-feeding, sheathed, third-staged larva having a long, slender esophagus.

Flagellum (pl. flagella.): An extension of ectoplasm, which provides locomotion, resembles a tail, which moves with a whip-like motion.

Fomite: An object, which can absorb and harbor organisms and can cause from man and vise versa. (Parasitic infections that are normally maintained by animals and man as accidental host.)

Gamete: A mature sex cell.

Gametocyte: The malaria sexual in human blood that can produce gametes after entering the mosquito.

Gametogony*:* The phase or the develpment cycle of the malarial parasite in humans in which male and female gametocytes are formed.

Generic name: The name given to an organism consisting of its appropriate genus and species title

Genus (pl. genera): A taxonomic category subordinate to family (and tribe) and superior to species, grouping those organisms which are alike in broad features but different in detail.

Gravid: pregnant, containing developing eggs or young.
has been swallowed by the host.

Hermaphroditic: Having both male and female reproductive organs within the same individual

Hexacanth: A tapeworm larva having six hook-lets (see onchosphere)

Host: The species of animal or plant, which harbors a parasite and provides some metabolic resources to the parasitic species.

Hydatid cyst: A vesicular structure formed by E. granulosus larvae in the

Hydatid Sand: Granular material consisting of free scolices; hook-lets daughter cysts, and amorphous material. Found in the fluid of older cysts of E. granulosus.

Incubation Period: The time from initial infection until the onset of clinical symptoms of a disease.

Infection: invasion of the body by any pathogenic organism (except arthropods) and the reaction of the host tissues to the presence of the parasite or to released toxins.

Infective Stage: The stage of a parasite at which it is capable of entering the host and continuing development within the host.

Infestation**:** The establishment of arthropods upon or within a host (including insects, ticks. mites).

Intermediate Host: A host for only the larval or sexually immature stages of parasite development.

Karyosome**:** see endosome.

Kinetoplast: This form is seen in the blood of humans with trypanosomiasis and inside the insect vectors.

Kinetoplast**:** An accessory body found in many protozoa, especially in the family

L D. body (Leishman - Donovan body): Each of the small ovoid leishmanial forms found in tissue macrophages of the liver and spleen in patients with leishmanial donovani infection.

Larva (pl. larvae): An immature stage in the development of a worm before it becomes a mature adult. Nematodes molt several times during development, and each subsequent larval stage is increasingly mature.

Leishmania Form: A small, ovoid non-flagellated form. Notable structures include a kinetoplast and large nucleus. Also called L D. body

Leptomonas Form: A body similar to the crithidial form except that the kinetoplast is located at the anterior end of the organism; therefore it has

on undulating membrane this form is seen in the mid-gut and pharynx of vectors in the life cycle of the leishmania parasites. male nematodes, e.g. hookworm)

Mastigophora: A superclass of the phylum protozoa containing forms, which move by means of one or more flagella.

Merozoite: One of the trophozoites released from human red blood cells or liver cells at maturation of the asexual cycle of malaria.

Metacercaria (pl. . meracercariae): The stage of the fluke life cycle occurring when a cercaia has shed its tail, secreted a protective wall and encysted as a resting stage on water plants or in a second intermediate: host infective stage for humans.

Metazoa: A subkingdom of animals consisting of all multicellular animal organisms which cells are differentiated to form tissue, includes all animals except protozoa.

***Microfilaria*:** A term used for the embryo of a filaria, Usually in the blood or tissues of humans ingested by the arthropod intermediate host.

Miracidium (pl. miracidia): Ciliated first -swimming lava of a trematode, which emerges from the egg and must penetrate the appropriate species of snail in order to continue its life cycle development.

***Molt*.** A process or replacement of the old cuticle with an inner new one and subsequent shedding of the old cuticle is termed ecdysis

Nematode: roundworms.

Nocturnal Periodicity*:* microfilariae are present in greatest numbers in the peripheral blood during the night hours.

Obligatory Parasite: A parasite, which cannot live apart from its host.

Onchosphere: The motile first- stage larva of certain cestodes armed

with six hook-lets.

Oocyst: The encysted form of the ookinete, which occurs on the stomach wall of Anopheles spp. mosquito infected with malaria.

Ookinete: The motile zygote of plasmodium spp. formed by microgamete (male) fertilization of a macrogamete (female). The ookinete encysts (see oocyst).

Operculum: The lid or cap-like cover on certain helminth eggs.

Oviparous: Reproducing by laying eggs

Ovoviviparous: Reproducing by laying eggs and larvae as well

***Parasitemia*:** The presence of parasites in the blood (e.g. malaria forms)

Parasitism: The association of two different species of organisms in which the smaller species lives upon or within the other, and has a metabolic dependence on the larger host species.

Parasitosis: The state of infection or infestation with an animal parasites.

***Paratenic Host*:** A host in which parasites do not develop to final stage (adult) but develop to the larva stage.

Paroxysm: The fever-chills syndrome in malaria. Spiking fever corresponds to the release of merozites and toxic materials from the parasitzed red blood cell (RBE) and shaking chills occur during schizont development. Occurs in malaria cyclically every 36 to 72 hours, depending on the species.

Partheongenic: Capable of unisexual reproduction; no fertilization is required e.g. Strongyloides stercoralis.

Patent: Apparent or evident.

Pathogenic: production of tissue changes or disease.

Pathogenicity: The ability to produce pathogenic changes.

Periodicity: Recurring at a regular time period.

Phorentic Vectors: Intermediate hosts that mechanically transmit parasites to man

Pleurocercoid: The larval stage in the development of D. latum that develops after the proercoid stage is ingested by a freshwater fish, this form has the scolex at one end and is infective if eaten by humans.

***Pre-patent period*:** The time elapsing between initial infection with the parasite and reproduction by the parasite.

Proercoid: The larval stage developing from the coracidium of D. latum. It develops in the body of a freshwater crustacean

Proglottid: one of the segments of a tapeworm. Each contains male and female reproductive organs when mature.

Protozoa: a phylum of the animal kingdom consisting of unicellular animals.

***Pruitus*:** intense itching.

Pseudocyst: An abnormal or dilated space resembling a cyst.

Pseudopod: A protoplasmic extension on the trophozoites of amoebae allowing them to move and engulf food.

Racemose: Clusters with branching nodular terminations resembling a bunch of grapes. Used in reference to larval cysticercosis caused by the migration and development of T. solium larvae en the brain tissue of humans.

Rectal Prolapse: weakening of the rectal musculature resulting in a "falling down" of the rectum; occasionally seen in heavy whip-worm infections, particularly in children.

Redia: The second or third larval stage of a trematode, which develops within a spocyst. Elongated saclike organisms with a mouth and gut many rediae develop

Reservoir host: An animal which harbors a species of parasite that is also parasitic for man, and from which man may become infected.

Rhizopodea: Asexual multiplication of sporozoa multiple nuclear division precedes cytoplasmic division.

Rostellum: The fleshy anterior protuberance of the scolex of some tapeworms (genus specific); may bear a circular row (or rows) of hooks.

Sarcodina: a superclass of phylum protozoa containing amoebae, which move by means of pseudopodia

Schizogony: Asexual multiplication of sporozoa, multiple nuclear division precedes cytoplasmic division.

Schizont: The oocyst in which the sporozoites of plasmodium have developed.

Scolex (pl. scolices): Anterior end of a tapeworm by which attachment occurs to the wall of the intestine of the host.

Sporogony: sexual cycle of sporozoa.

Sporozoa: A subphylum (or class) of the phylum protozoa containing animals whose life cycle alternates between sexual and asexual generations.

Sporzoite: The form of plasmodium, which develops inside the sporocyst and infects the salivary glands of the mosquito.

Subperiodic (Nocturnal or diurnal): microfilariae can be found in the peripheral blood throughout the 24 hours with only a slight increase in numbers during day or night hours.

***Symbiosis*:** The association of two different species of organisms exhibiting metabolic dependence by their relationship.

Tegument (integument)*:* The absorptive body surface of platyhelminth.

Trematode: Are flukes, which are flattened, leaf - shaped bodies bearing muscular suckers. Many species are hermaphroditic.

Trophozoite: The motile stage of protozoan, which feeds, multiplies and maintains the colony within the host.

Trypanosome Form: A body similar to the crithidial form except that the kinetoplast is located at the posterior end of the organism. and the undulating membrane extends from the flagellum (anterior end) to the posterior end attached .

***Undulating Membrane*:** A protoplasmic membrane, running like a fin along the outer edge of the body of certain protozoa, which moves, in a wavelike pattern.

Vector: Any arthropod or other living carrier, which transports a pathogenic microorganism from an infected to a non-infected host. A vector may transmit a disease passively (mechanical; vector) or may be an essential host in the life cycle or the pathogenic organism (biologic vector)

Viviparous: Bearing young instead of laying eggs

Xenodiagnosis: infections with Trypanosoma cruzi may be diagnosed by allowing an uninfected reduviid bug to feed no the patient (the bite is painless); the insect feces are examined for parasites (trypanosome forms)

Zoonosis: The process of those infectious diseases that are transmissible

Zygote: The cell resulting from the union of male and female gametes.

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