Reading Material for

Medical Lab. Technician

(Parasitology & Mycology)

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PREFACE

A two years post matric teaching program of Medical Laboratory Technician for the students of Allied Health Sciences. The purpose of this reading material is to provide basic education to the paramedics about Parasitology and Mycology. This reading material attempts to cover almost all the basic theoretical knowledge required by students about Parasitology and Mycology .so that they can perform their work better in Pathology laboratory

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SECTION: 1

Parasitology

1. BASIC PARASIOTOLOGY

INTRODUCTION

Parasitologyisthesciencedealing withparasitesand their pathogenicity.

A **parasite** is a living organism that has adapted itself to exist in another animal called a**host**. Parasitic infestations in humans constitute the most common health problems,particularly in tropical and developing countries. Parasites infest man in more than onetissueandorgan.
A parasite is an organism that is entirely dependent on another organism, referred to as its host,for all or part of its life cycle and metabolic requirements.
Parasitism is therefore a relationship in which a parasite benefits and the host provides the benefit. The degree of dependence of a parasite on its host varies. An obligatory parasite is one that must always live in contact with its host. The term free-living describes the non-parasitic stages of existence which are lived independently of a host, e.g. hook worms have active free-living stages in the soil.

Terms used to describe parasite hosts

1.1.Definitive host*:* This is the host in which sexual reproduction takes place or in which themosthighlydevelopedformofaparasiteoccurs.Whenthemostmatureformisnotobvious,thedefinitivehostisthemammalianhost.
*1.2.****Intermediate host:***This is the host which alternates with the definitive host and in whichthelarvalorasexualstagesofaparasitearefound.Someparasitesrequiretwointermediatehostsinwhichtocompletetheirlifecycle.
*1.3.****Reservoir host:***This is an animal host serving as a source from which other animals canbecomeinfected.Epidemiologically,reservoirhostsareimportantinthecontrolofparasiticdiseases.Theycanmaintainanucleusofinfectioninanarea.

1.4 TYPES OF PARASITES

1.4.1 .**EXO-PARASITES**: These are the ones which live outside the host body, on body surfaces,e.glice.

1.4.2.**ENDOPARASITES**:These are the ones which live inside human body,e.g Entamoeba

HOST-PARASITESRELATIONSHIP

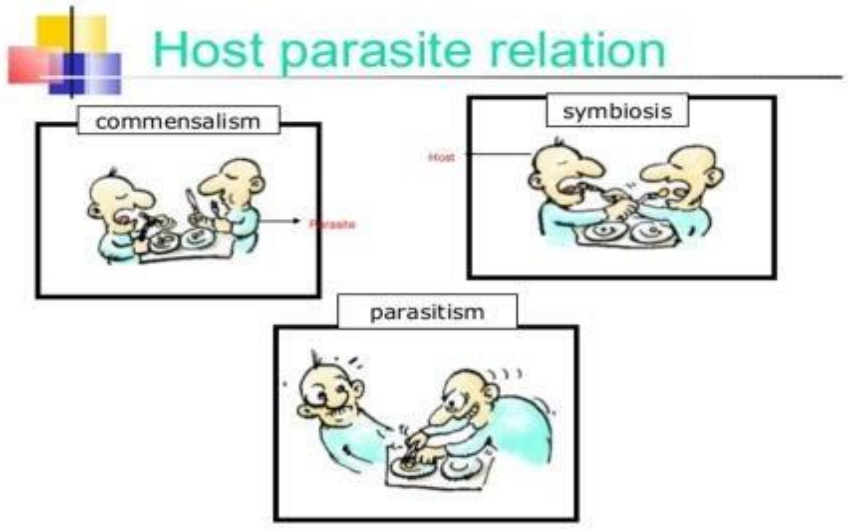


Figure 1.1 : Host Parasite Relation

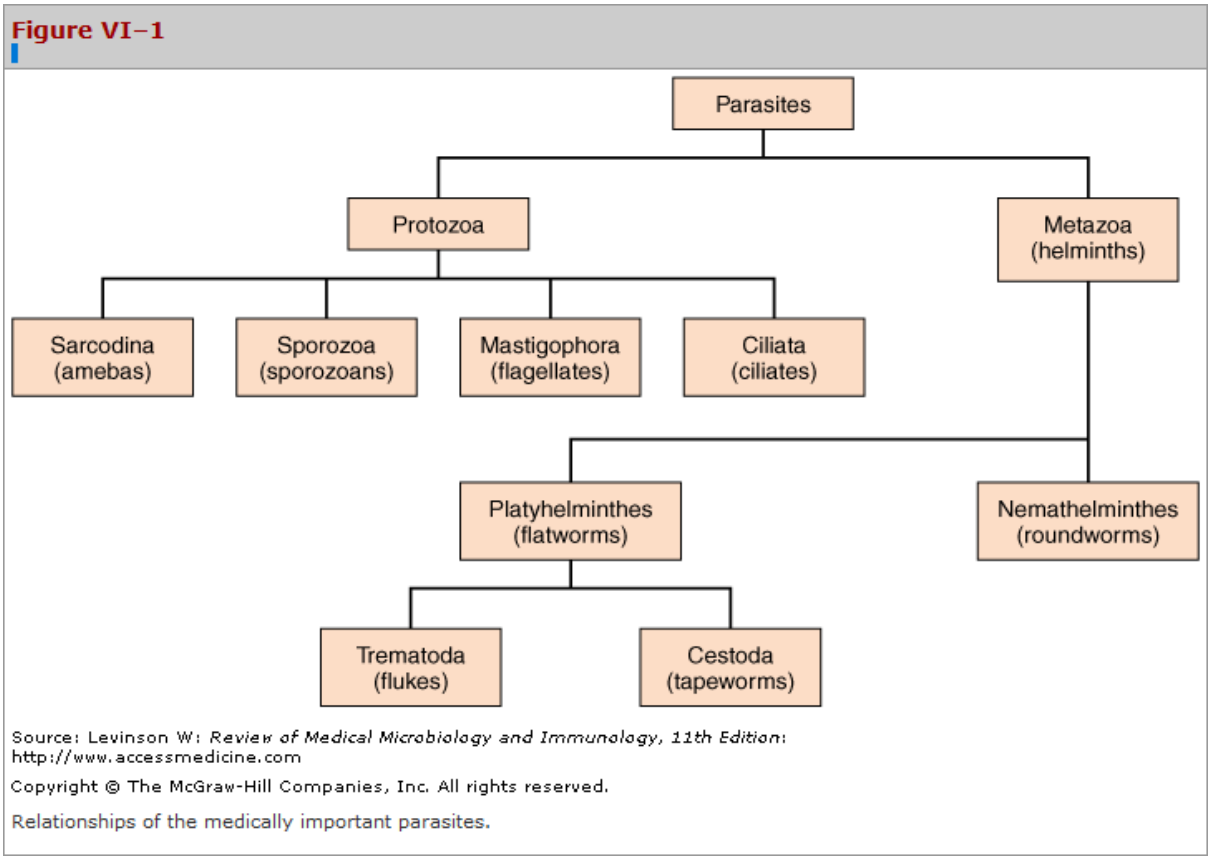


Figure: 1.2 CLASSIFICATION OF PARASITES

PROTOZOA

Protozoa can be defined as unicellular organisms that are independently complete.They can eat, respire, move and reproduce without help. They are divided into four classesasshown inTable

HELMINTHS

Helminths are multi-cellular organisms of varying sizes, elongated in shape and having are productive system. Other systems like a nervous system and a gut may be present in a rudimentary form. Only a few parasites occur in Pakistan and even fewer are important pathogens. They may infect man in their adult or larval forms. Although these diseases may prove fatal in certain cases, they are easy to treat and are curable provided these can be diagnosed. In the next few pages, the life cycles and methods of diagnosis of some important parasites will be discussed.

1.5 PROTOZOA:

1.5.1 Blood parasites

1.5.1.1 PLASMODIUM

Malaria is one of the most wide spread parasitic diseases of the world. It mainly occurs in tropical and subtropical are as but cases are found all over the world duetotravellingtoandfromtheseareas.Aprotozoanbelongingtotheclasssporozoaandthegenusplasmodiumcauses it. Five species are involved in it,namely, *P.vivax*,*P.ovale, P.malariae*, *P.falciparum and P. knowlesi*. All species differ in morphology, life cycle and the type of disease they cause. The parasite invadesanddestroysredbloodcells.Itistransmittedfromonepersontoanother throughbitesofamosquitoofthegenusanopheles.It can also be transmitted through blood transfusion of infected individual.

LIFECYCLE

The life cycle of a malarial parasite involves two hosts and consists of a **sexual cycle orsporogony** in the mosquito and an **asexual cycle or schizogony**in humans. Man isactually theintermediatehostwhilethemosquitoisthedefinitivehost(Fig1).

ASEXUAL CYCLE IN HUMANS(SCHIZOGONY)

During a blood meal, a malaria-infected female *Anopheles* mosquito inoculates sporozoites into the human host. Sporozoites infect liver cells and mature into schizonts,which rupture and release merozoites. This is the **pre-erythrocytic schizogony** or tissue phase. In *P.vivax* and *P. ovale* this is a dormant stage [hypnozoites] that can persist in the liver and cause relapses by invading the blood stream weeks or even years later;also called as **ex erythrocyticstage**) .After this initial replication in the liver (A),the parasites undergo asexual multiplication in the erythrocytes (**erythrocyticschizogony**(B). Merozoites infect red blood cells.The ring-stage trophozoites mature into schizonts which rupture,releasing merozoites.
When the infection is well established, some merozoites differentiate into sexual erythrocytic stages (**gametocytes**) after about 12 days. Blood-stage parasites are responsible for theclinical manifestations of the disease. The time taken to complete this cycle varies in different species of Plasmodium. In *P.vivax* it is 45 hours,in *P.ovale* 48 hours,in *P.malariae* 72 hours, P. knowlesi in 24 hours and in *P.falciparum* 48 hours.

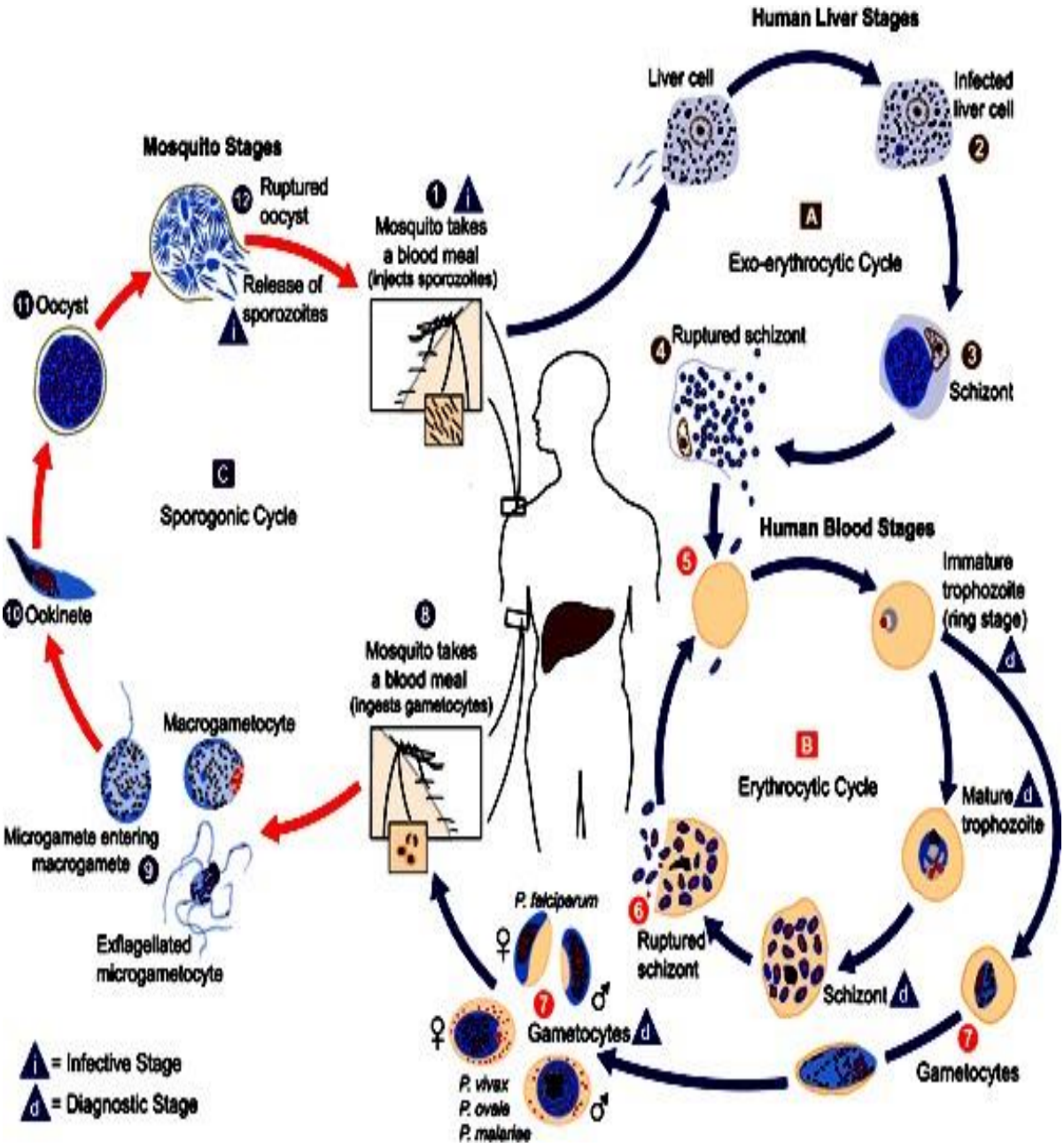


Fig1.3 :Sexual,asexual life cycles of Plasmodium species

SEXUAL CYCLE IN MOSQUITOE

Thesexualformsoftheparasitethegametocytes,male(microgametocytes)and female(macrogametocytes),areingestedbya female *Anopheles* mosquito during a blood meal .The parasites‘ multiplication in the mosquito is known as the **sporogony (C)**. While in the mosquito'sstomach, the microgametes penetrate the macrogametes, generating zygotes . The zygotes in turn become motile and elongated(ookinetes)and invade the mid-gutwall of the mosquito where they develop into oocysts . Theo ocysts

grow,rupture,and releases porozoites,which make their way to the mosquito's salivary glands.Inoculation of the sporozoites into a new human host perpetuates the malaria life cycle.

All sexual and asexual forms of the parasite described in the life cycle are seen in peripheralblood except in *P.falciparum* where most of maturation occurs in RBCs sequestered in small vessels. In this case only ring forms and gametocytes are seen in the blood.

1.5.1.2 LEISHMANIA

CUTANEOUSLEISHMANIASIS

Cutaneous leishmaniasis is prevalent in eastern Baluchistan and southern Punjab. A flagellate protozoan *Leishmania tropica* complex causes the disease. The parasite is transmitted from human to human by the sandfly of genus phlebotomus, which is the definitive host. Man is the intermediate host. The parasite exists in 2 different morphological forms in its life cycle. In man it occurs in the **Leishmanial (amastigote)** form.It is ovoid in shape,measuring1.5-5µm. It contains a nucleus and close to it a much smaller structure called the kinetoplast. In the body of the sandfly it is transformed into **leptomonad**(**promestigote**) formthat is large, elongated and has a polar flagellum in addition to a nucleus and a kinetoplast.Leishmaniasis is transmitted by the bite of the female phlebotomus. The sandflies inject the infective stage promastigotes,during blood meals.Promastigotes that reach the puncture wound are phagocytosed by macrophages and transform into amastigotes . A mastigotes multiply in infected cells and affect different tissues, depending in parton the *Leishmania*s pecies. The infected tissue presents the clinical manifestations of leishmaniasis.

Sand flies become infected during blood meals on an infected host when they ingest macrophages infected with amastigotes. In the sandfly's mid-gut, the parasites differentiate into promastigotes,which multiply and migrate to the proboscis.

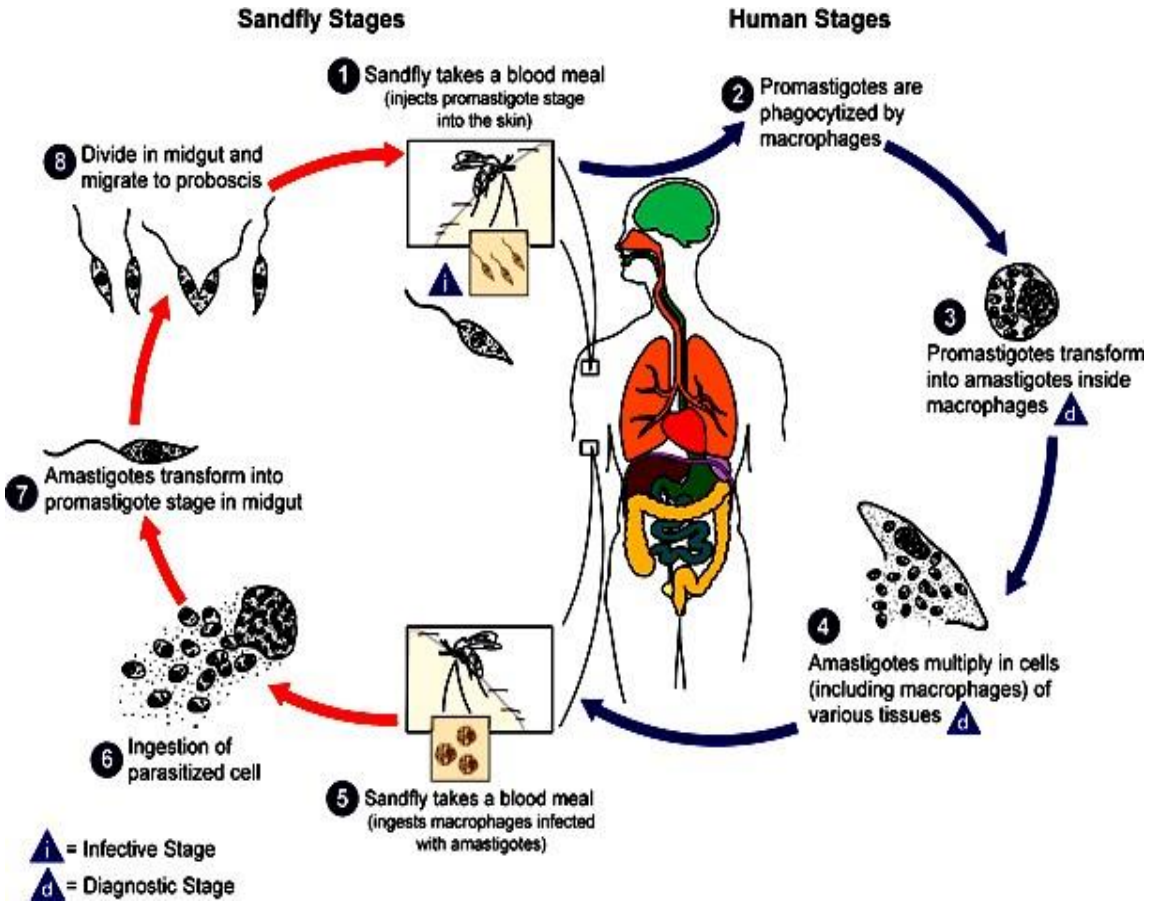


Fig1.4 Life cycle of Leishmania

VISCERAL LEISHMANIASIS

Commonly called Kala Azar, it is seen in Pakistan, particularly in Azad Kashmir and Baltistanareas. It is caused by at least three sub-species belonging to the *Leishmaniadonovani*complex,clinically and biochemically distinct having different geographic distribution.*Leishmaniadonovani*istransmittedthroughthebitesofa sandfly(phlebotomus).

The life cycle is similar to *Leishmaniatropica*except that, in this case the parasite attacks thereticuloendothelialsystemoftheliver,spleenandbonemarrow.

1.5.2. INTESTINAL PARASITES

1.5.2.1 ENTAMOEBA;

This disease is caused by the protozoan *Entamoeba histolytica*. Cysts are passed in faeces.Infection by *Entamoeba histolytica*occurs by ingestion of mature cysts in faecally -contaminatedfood, water or hands. Excystation occurs in the small intestine and trophozoites are released,whichmigratetothelargeintestine.Thetrophozoites multiplybybinaryfissionandproducecysts , which are passed in the faeces . (Trophozoites

can also be passed in diarrhoeal stools,but are rapidly destroyed once outside the body, and if ingested would not survive exposure tothegastric environment.) Inmany cases,thetrophozoites remainconfinedtotheintestinallumen (**noninvasive infection**) of individuals who are asymptomatic carriers,passing cysts intheir stool. In some patients the trophozoites invade the intestinal mucosa (**intestinal disease**),or,throughthebloodstream,extra-intestinal sitessuchastheliver,brainandlungs(**extra-intestinaldisease**),withresultantpathologicmanifestations.

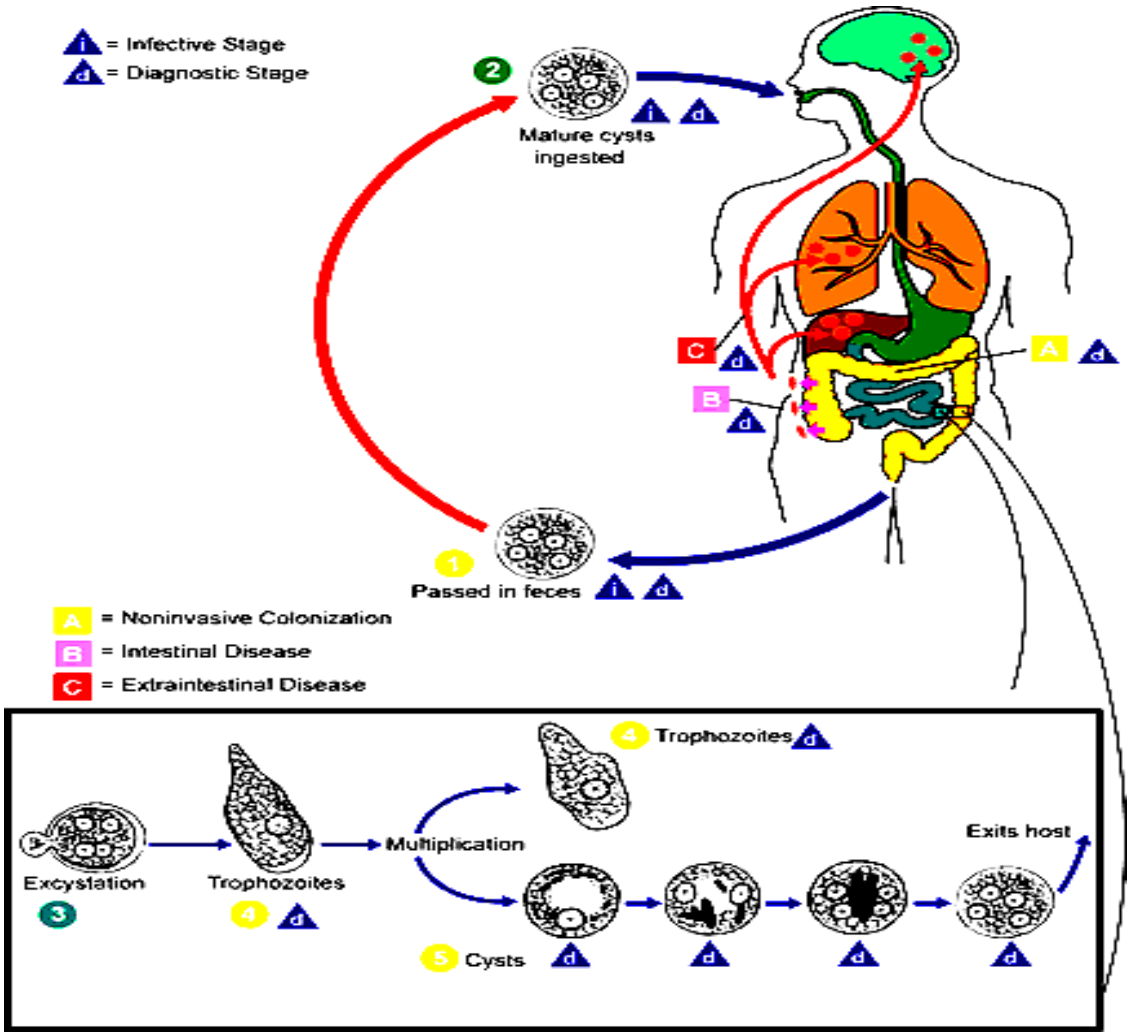


Fig 1.5;Life cycle of *Entamoeba histolytica*

Two developmental stages are;

1. **The Trophozoite Stage** or vegetative form is the invasive form. It invades the intestinal wall causing a typical flask-shaped ulcer in the caecum and ascending colon, but other parts of the large intestine may also be affected.From the intestine these may reach the liver via portal circulation. The trophozoites are 20-60 μmin diameter. They are motile due to explosive movements of pseudopodia. They ingest red blood cells,which is diagnostic. They have one nucleus and reproduce by binary fission
2. **Cystic Stage**: When the conditions are unfavourable, the trophozoites becomeimmobile,roundedandfinallyencyst.Theymayalsodividewithinthecyst.Amoebic cysts thus may contain multiple nuclei. Cysts contain rod-like structurescalled chromatoidbodies orbarsandaninconspicuousglycogenvacuole. Thecystsarepassedinstools andmay beingestedby anotherindividualthroughcontaminated food andwater.Onlythe four-cellstage cystisinfective.Then thecystwalldisappearsand8trophozoitesareliberated andthenattacktheintestinal mucosa.

1.5.2.2 GIARDIA

This disease is caused by a flagellate protozoan, *Giardia lamblia*. Infestation occurs in the upper small intestine and causes anaemia, weight loss and malabsorption. Diarrhoea and otherabdominal symptoms may or may not occur.The parasite is found in two forms. The **trophozoite** form is found in the intestine close to or on the microvillous border of theepithelium. Towards lumenand downintheintestinethe conditions becomeunfavourable fortrophozoites,which then encyst. **Cysts** are excreted in stools. Occasionally, trophozoite forms may be seen in faeces. If there is diarrhea, both cysts and trophozoites can be found in the faeces (**diagnostic stages**) .Infection occurs by the ingestion ofcysts incontaminatedwater,food, orby thefaecal-oral route(hands orfomites).In the small intestine, excystation releases trophozoites(each cyst produces twotrophozoites)whichremainin proximalsmallbowel.Encystationoccursastheparasites transit toward the colon.The cyst is the stage found most commonly innon-diarrhoealfaeces

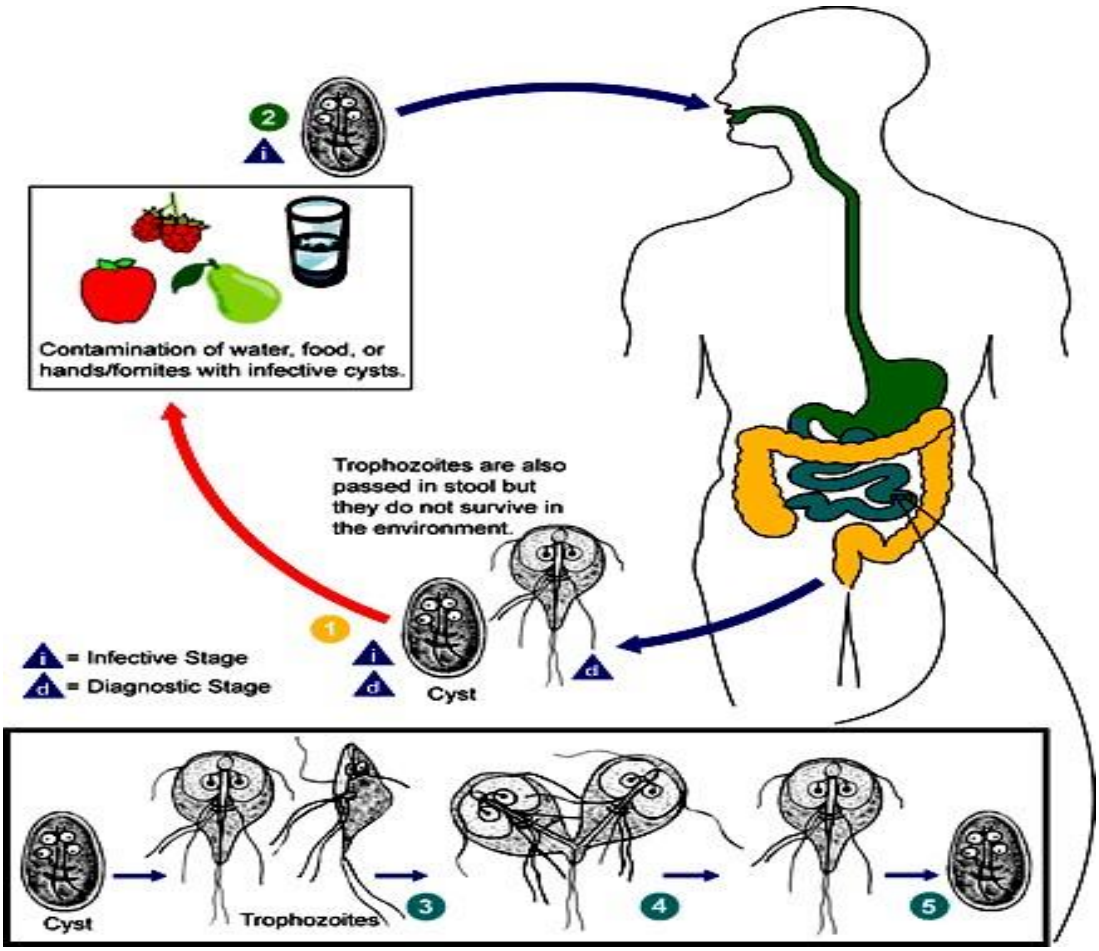


Figure 1.6 : Life cycle of *Giardia lamblia*

1.5.3. UROGENITAL PARASITES

1.5.3.1 TRICHOMONAS VAGINALIS

This protozoan is not an intestinal parasite. Normal body sites include the vagina and prostate. It is pathogenic in the genital system and sometimes the urinary tract. It is included in the list ofsexually transmitted diseases (STD). A living trophozoite is 5-15 µm in size but it may reach alength of 30µm. They have very jerky and non-directional movement. It has four anterior flagella plus a recurrent flagellum that arises anteriorly and parallels the body. The undulating membrane extends about half the distance to the posterior end of the body with no free flagellum.

Trichomonas vaginalis resides in the lower genital tract of females and the male urethra and prostate ,where it replicates by binary fission.The parasite does not appear to have a cyst form, and does not survive well in the external environment. *Trichomonas vaginalis* is transmitted among humans, its only known host, primarily by sexual

intercourse .

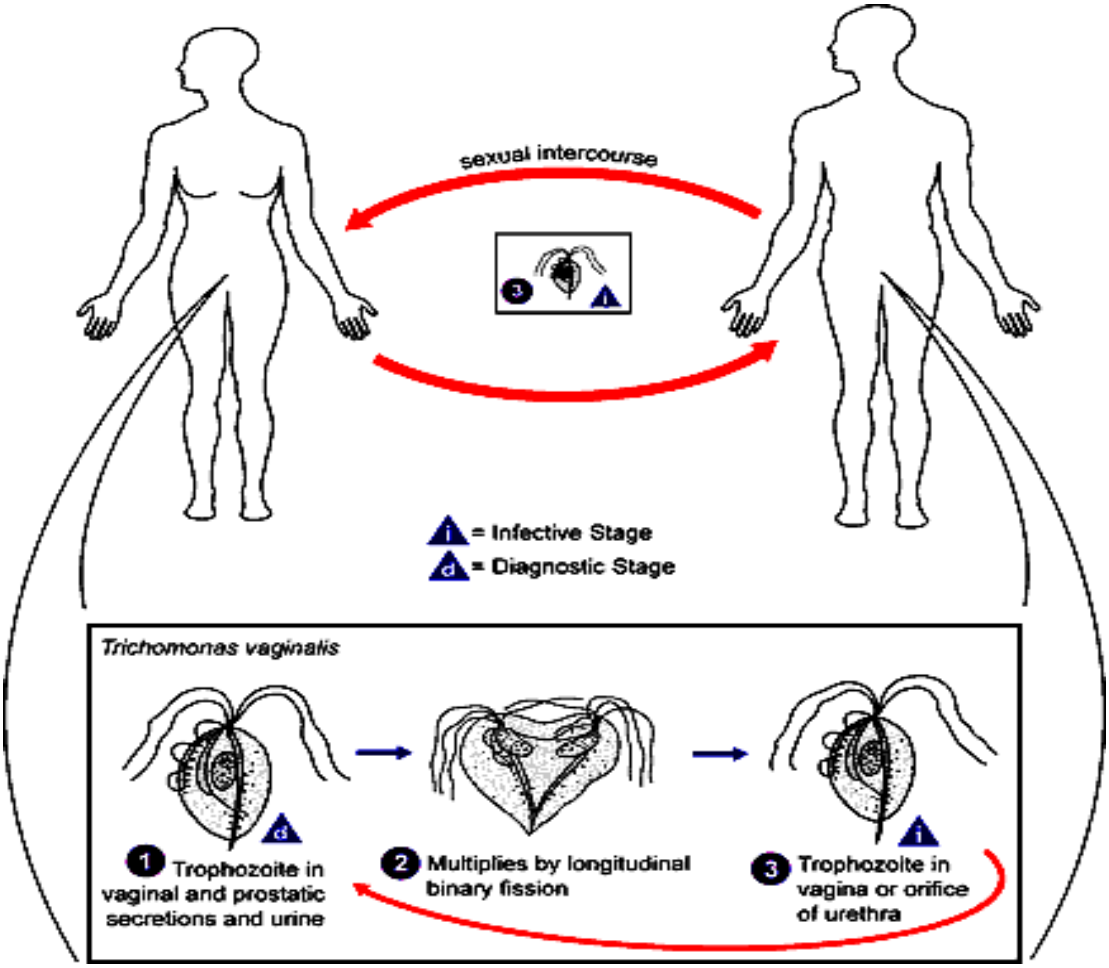


Fig 1.7 : Life cycle of Trichomonas

1.6 HELMINTHS

1.6.1 Nemathelminthes(Nematodes)

1.6.1.1 ASCARIS LUMBRICOIDES

Ascariasis is caused by a large round worm,*Ascaris lumbricoides* belonging to the nematode.It is the most common intestinal helminth in humans. Adult worms , live in the lumenof the small intestine. A female may produce approximately 200,000 eggs per day, which are passed in the faeces . Unfertilised eggs are not infective. Fertile eggs embryonate and become infective after 18 days to several weeks, depending on the environmental conditions(optimum:moist,warm,shaded soil).After infective eggs are swallowed, the larvae hatch, invade the intestinal mucosa,and are carried via the

portal,then systemic circulation to the lungs. The larvae mature further in the lungs(10-14days),penetratethealveolar walls,ascendthebronchial tree to the throat, and are swallowed . Upon reaching the small intestine, they developintoadultworms .Between2and3monthsarerequiredfromingestionoftheinfectiveeggs toeggproductionbythe adultfemale. Adultwormslivefor1-2years.

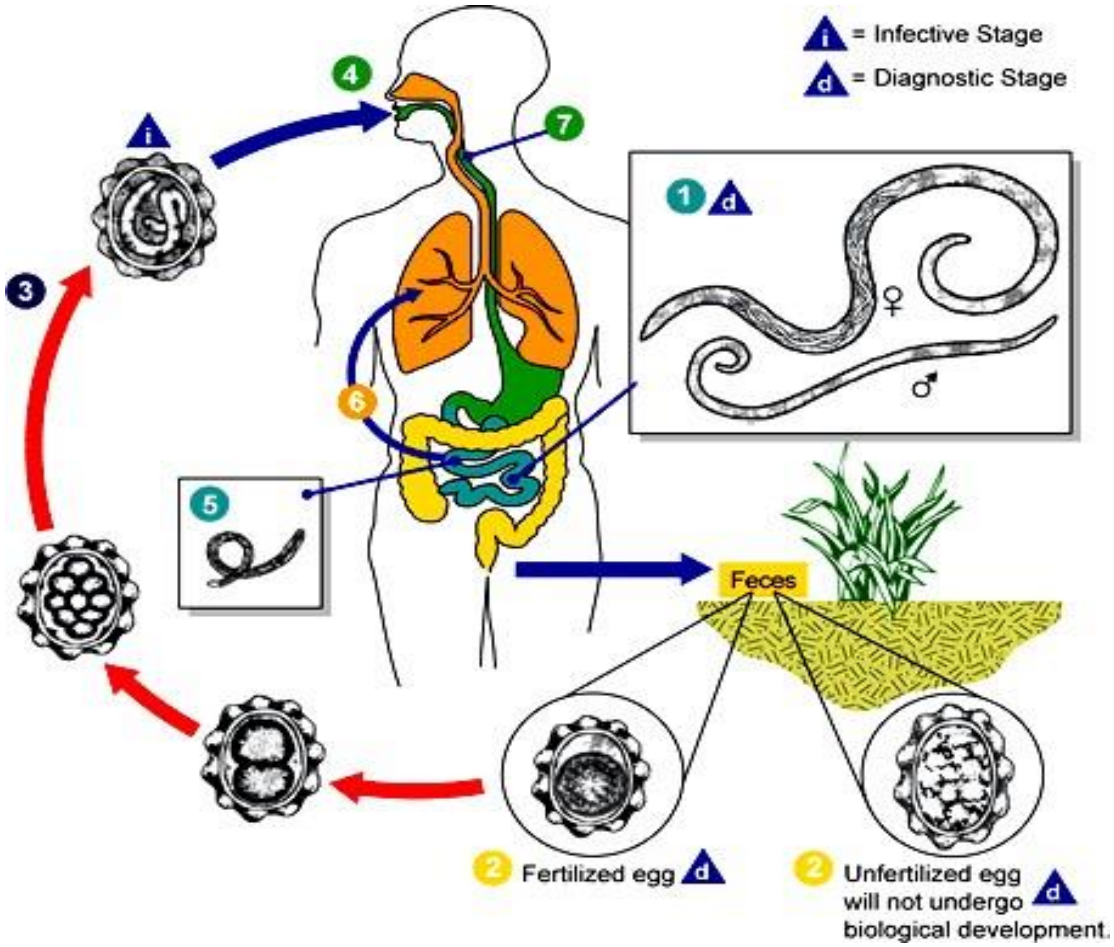


Figure 1.8 Life cycle of Ascaris lumbricoides

1.6.1.2 ANCYLOSTOMA DOUDENALE

Ancylostoma duodenale or Hook worm infection is one of the most common parasitic infections.The two Nematodes, *Ancylostoma duodenale* and *Necator americanus* cause it. Both are similar in shape and life cycle.Eggs are passed in the stool,and under favourable conditions (moisture, warmth, shade), larvae hatch in 1 to 2 days. The released rhabditiform larvae grow in the soil and after 5-10 days (and two months) later they become filariform (third-stage) larvae that are infective.These infective larvae can

survive 3-4 weeks in favourable environmental conditions. On contact with the human host for at least 5-10 minutes, the larvaepenetrate the skin and are carried through the veins to the heart and lungs.They penetrate intothe pulmonary alveoli,ascend the bronchial tree to the pharynx, and are swallowed . The larvae reach the small intestine, where they reside and mature into adults. Adult worms live in the lumen of the small intestine, where they attach to the intestinal wall with resultant blood loss by the host. Each parasite sucks about 0.1ml of blood per day and thousands may be present in one individual.They are the most common cause of iron-deficiency anaemia.Sexes are separate and both are required for producing the infective fertilized ova and larvae.

Ancylostoma duodenale has a dorsal hook that gives the parasite its name, hookworm. Both ova and larvae are passed in faeces and occasionally the adult worm may also be seen in stools. One female produces about 5000-10000 eggs /day.

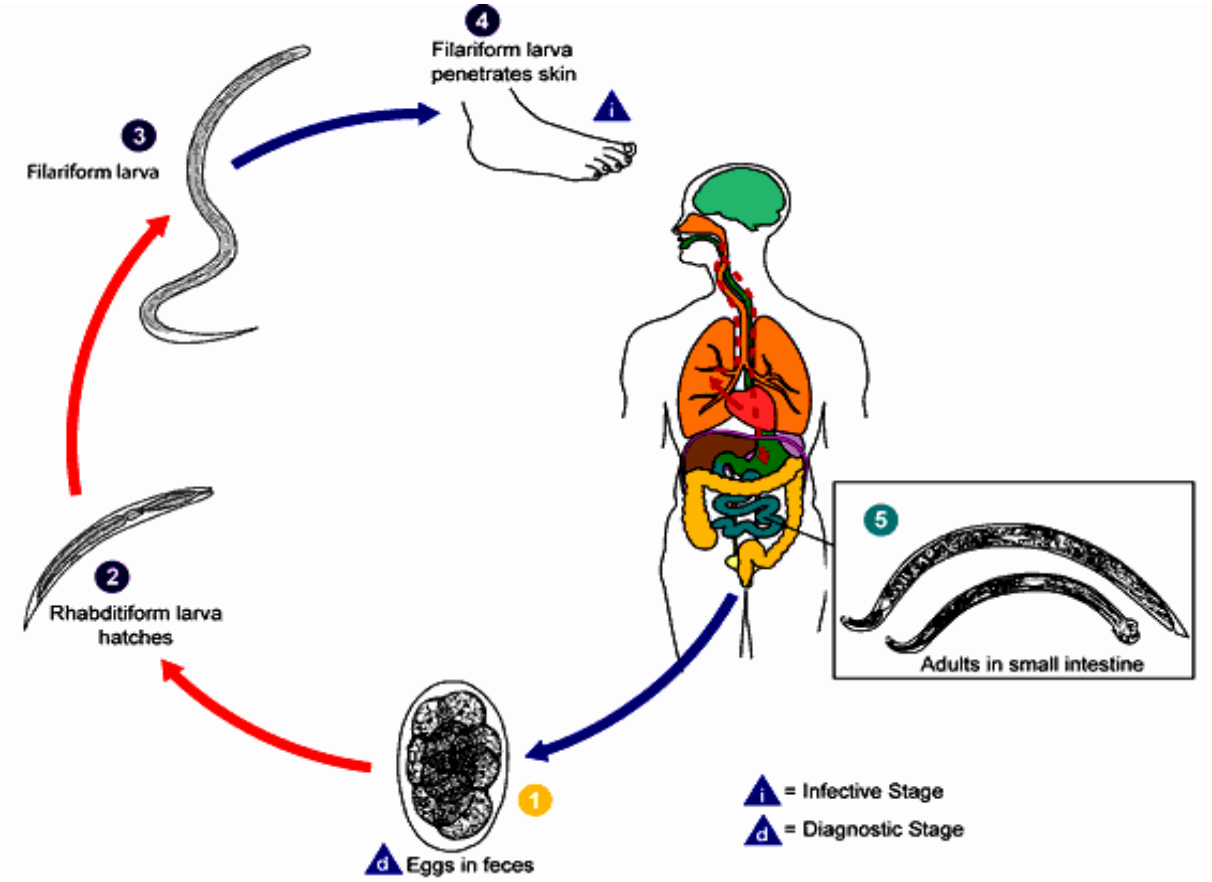


Figure 1.9: Life cycle of *Ancylostoma duodenale*

1.6.1.3.STRONGYLOIDES STERCORALIS

Strongyloides stercoralis causes strongyloidiasis. Important Properties *S. stercoralis*has **two distinct life cycles,** one within the human body and the other free living in the soil.The life cycle in the human body begins with the **penetration of the skin,** usually of the feet, by **infectious (filariform) larvae** and their migration to the lungs. They enter the alveoli, pass up the bronchi andtrachea, and then are swallowed. In the small intestine, the larvae molt into adults that enter the mucosa and produce eggs.The eggs usually hatch within the mucosa, forming rhabditiform larvae that are passed in the feces. Somelarvae molt to form filarial larvae, which penetrate the intestinal wall directly withoutleaving the host and migrate to the lungs (**autoinfection**). In immunocompetent patients,this is an infrequent, clinically unimportant event, but in immunocompromised patients,e.g., those who have AIDS or are taking high-dose corticosteroids, or patients who are severely malnourished,autoinfection can lead to **massive reinfection,** with larvae passing to man yorgans and with severe, some times fatal consequences. If larvaeare passed in the feces and enter warm, moist soil, they molt through successive stages to form adult male and female worms. After mating, the entire life cycle of egg, larva, and adult can occur in the soil. After several free-living cycles, filarial larvae are formed.
When they contact skin, they penetrate and again initiate the parasitic cycle with in humans

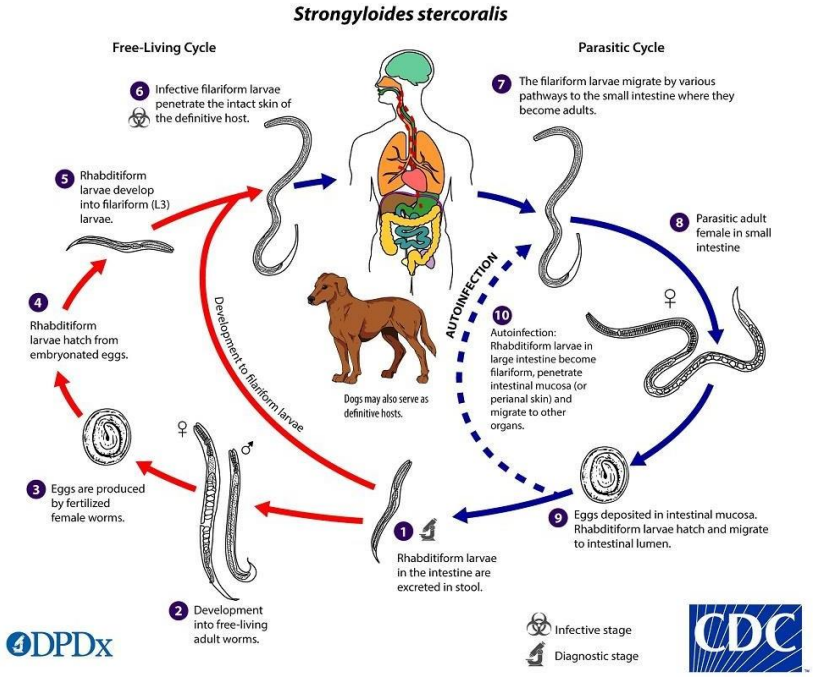


Figure 1.10: Life Cycle of Strongyloides Stercoralis

1.6.1.4 ENTEROBIUS VERMICULARIS

It is one of the commonest infestations caused by a nematode, *Enterobius vermicularis* commonly called pin worm due to perianal itching and it causes severe dermatitis of the perianal area. Eggs are deposited in perianal folds .Self-infection occurs by transferring infective eggs to the mouth with hands that have scratched the perianal area.Person-to person transmission can also occur through handling contaminated clothes or bed linens. Enterobiasis may also be acquired through surfaces in the environment that are contaminated with pin worm eggs (e.g., curtains, carpenting). The larvae hatch in the small intestine and the adults establish themselves in the colon.The time interval from ingestion of infective eggs to production of eggs by the adult females is about one month. The life span of the adult is about two months.Gravid females migrate nocturnally outside the anus and deposit eggs there, while crawling on the skin oftheperianalarea.Thelarvaecontainedinsidetheeggs develop(theeggsbecome infective) in 4-6 hours under optimal conditions .Retro infection, or the migration of newly hatched larvae from the anal skin back into the rectum,mayoccur.Parasites are found in the large intestine and appendix but may also migrate in to the urinary bladder and female genital tract from the perineum. The female is 5-10x0.5mm in size, while the male is only 2-5 mm long

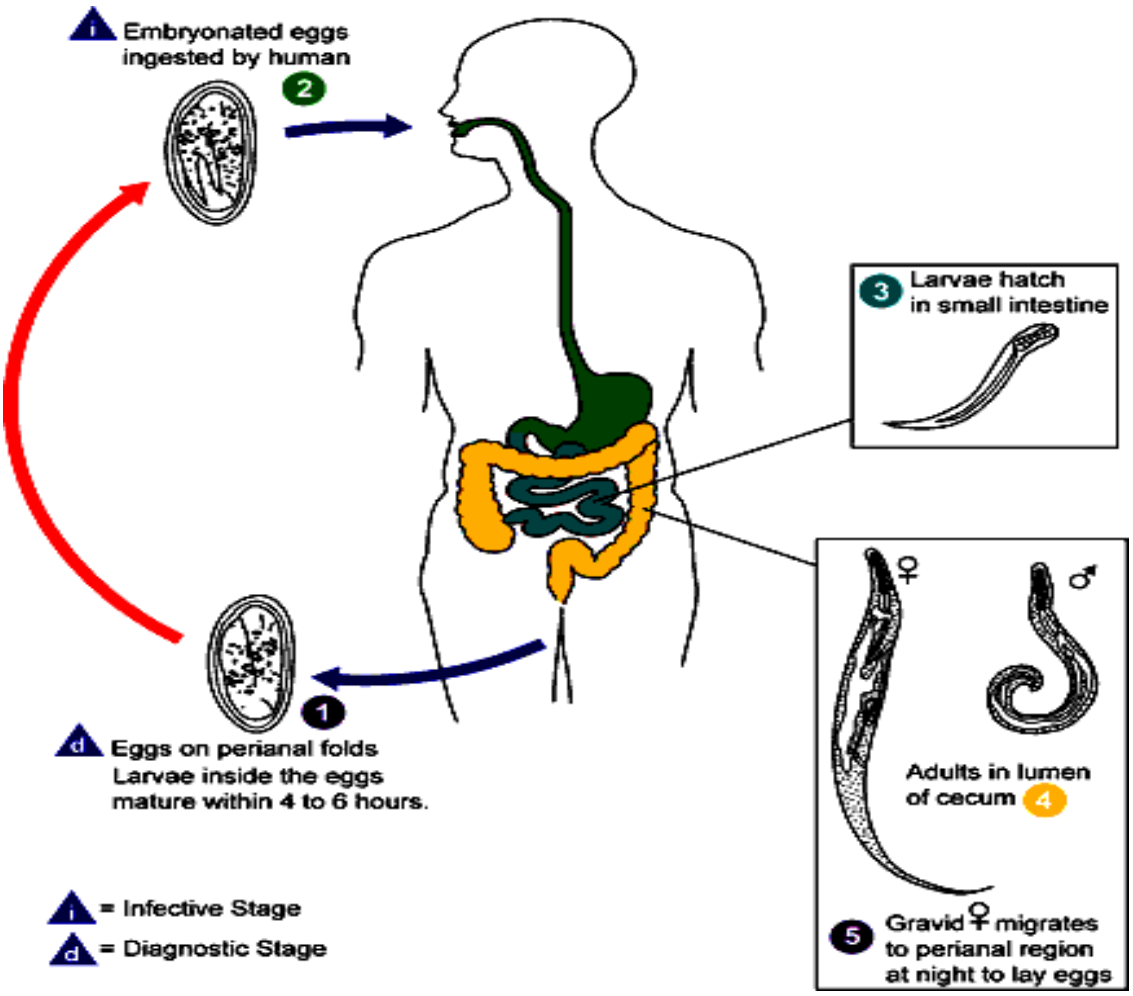


Figure 1.11 : Life cycle of Enterobius vermicularis

1.6.1.5 TRICHURIS TRICHURA

It is caused by a nematode;*Trichuris trichiura* commonly called a whip worm. The adult worm is 3-5 cm long with anterior3/5 slender, is embedded in mucosa and is thread-like. Posterior 2/5 is thick and bulbous and thus resembles a whip. Posterior end ofthe male is coiled like a watch spring. The parasites may cause ulcerative lesions in the largeintestine and appendix. The gravid female lays 3000-7000 eggs daily, which take 3 weeks in soil to mature and become infectious. The unembryonated eggs are passed in stools .In the soil,the eggs develop into a 2-cell stage,an advanced cleavage stage,and then the embryonated eggs become infective in 15 to 30 days. After ingestion (soil-contaminated hands or food), the eggs hatch in the small intestine, and release larvae that mature and establish themselves as adults in the colon.

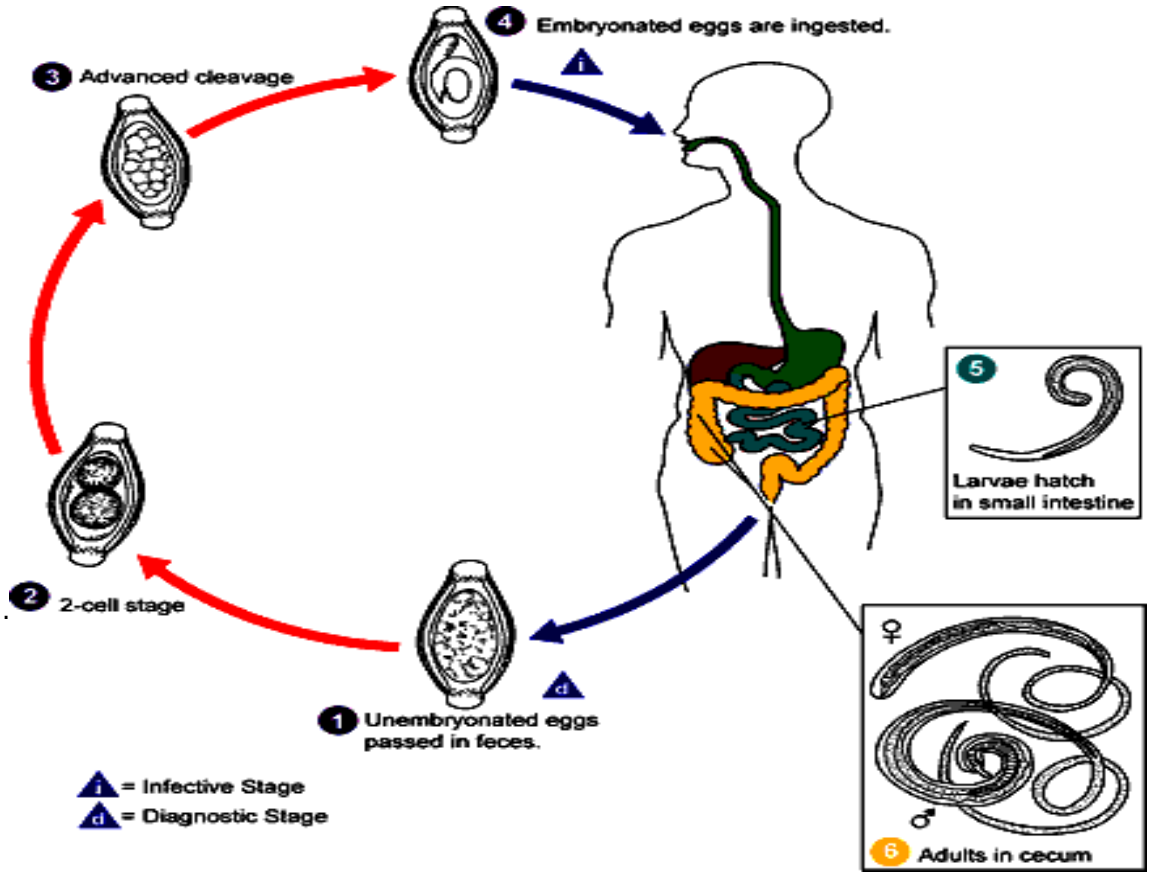


Figure 1.12 : Life Cycle of Trichuris Trichura

1.7 CESTODES

1.7.1 HYMENOLEPIS NANA

It is one of the most common cestode, *Hymenolepis nana* or dwarf tape worm. It causes abdominal pain,weight loss,diarrhoea,anorexia,weakness and malabsorption. Hypoproteinaemia with stunted growth may occur but allergic symptoms are more common. Anadult worm lives in the small intestine and measures 15-25x0.5 mm. It is segmented and has ascolex. A gravid segment becomes four times larger. Eggs are infective when passed in stools and cannot survive more than10days in the external environment.When an arthropod intermediate host ingests eggs, they develop into cysticercoids, which can infect humans orrodents upon ingestion and develop into adults in the small intestine. When eggs are ingested(in contaminated food or water or from hands contaminated with faeces),the oncospheres(hexacanth larvae) are released, penetrate the intestinal villus and develop into cysticercoidlarvae. Upon

rupture of the villus, the cysticercoids return to the intestinal lumen, evaginate their scoleces,attach to the intestinal mucosa and develop into adults that reside in the ileal portion of the small intestine, produc inggravid proglottids. Eggs are passed in the stool when released

from proglottids through its genital atrium or when proglottids disintegrate in the small intestine.

An alternate mode of infection consists of internal auto-infection, where the eggs release their hexacanth embryo, which penetrates the villus continuing the infective cycle without passing through the external environment. The life span of adult worms is 4 to 6 weeks.

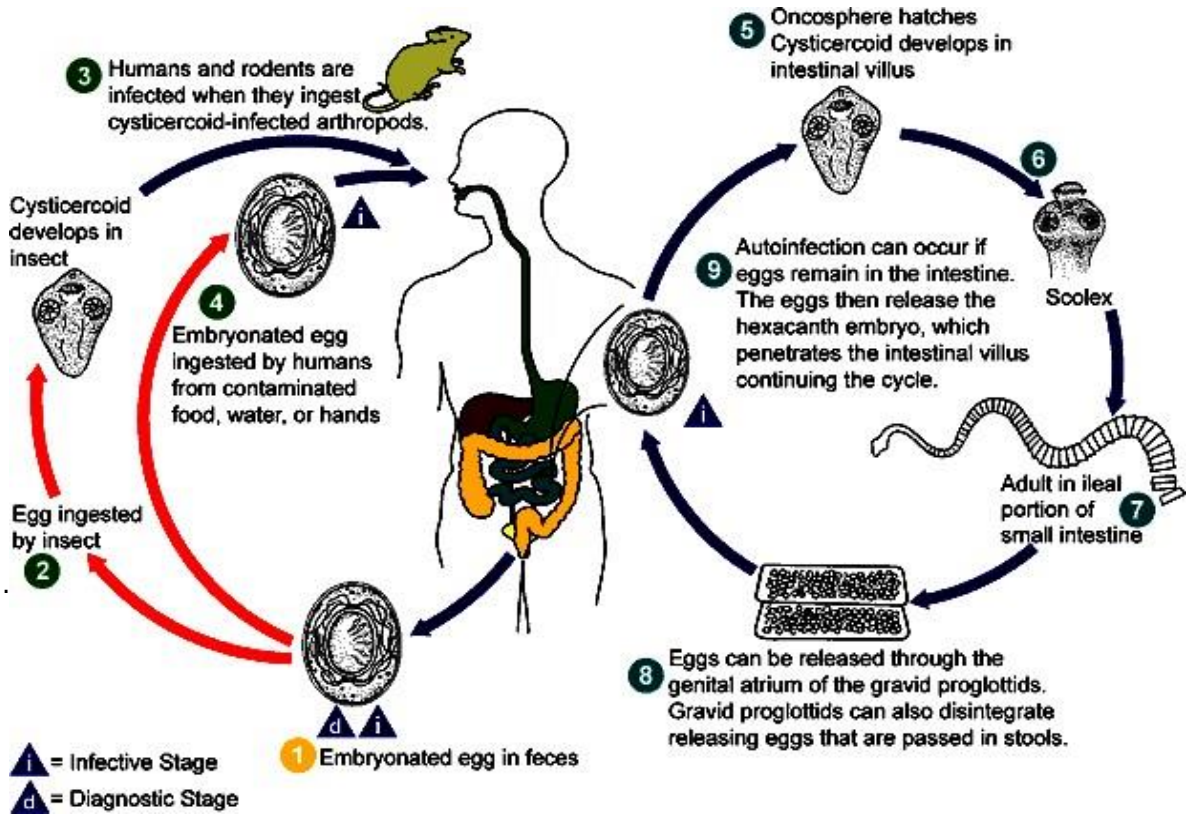


Figure 1.13 : Life cycle of Hymenolepis nana

1.7.2 TAENIA; One of the most common parasitic infections is caused by two cestodes,*Taenia saginata* and *Taenia solium*. Their type depends upon religious habits.In non-pork eating persons,*Taenia solium*does not occur, as a pig is the intermediate host for this. On the othe rhand,those who donote at beef(Hindus) donot have*Taenia saginata* as the intermediate host is cattle. The parasite is hermaphrodite.Humansarethe onlydefinitive hostsfor*Taeniasaginata*and *Taeniasolium*. Eggs or gravid proglottids are passed in faeces ; the eggs cansurvive for days to months in the environment. Cattle (*T.saginata*) and pigs (*T. solium*) becomeinfected by ingesting vegetation contaminated with eggs or gravid proglottids . In the animal's intestine, the oncospheres hatch , invade the intestinal wall, and migrate to the striated

muscles,where they develop into cysticerci.Acysticercuscansurviveforseveral yearsintheanimal.
Humansbecomeinfectedbyingestingraworundercookedinfectedmeat.Inthehumanintestine, thecysticercusdevelops over2months intoanadulttapeworm,which can survive for years. The adult tapeworms attach to the small intestine by their scolex and reside in the smallintestine . Length of adult worms is usually 5 m or less for *T. saginata*and 2-7 m for *T. solium*.The adults produce proglottids,which mature, become gravid, detach from the tapeworm, and migrate to the anus or are passed in the stool. *T.saginata* adults usuallyhave1,000to2,000proglottids, while *T.solium*adults have an average of 1,000 proglottids. The eggs are releasedafterthe proglottidsarepassed infaeces.

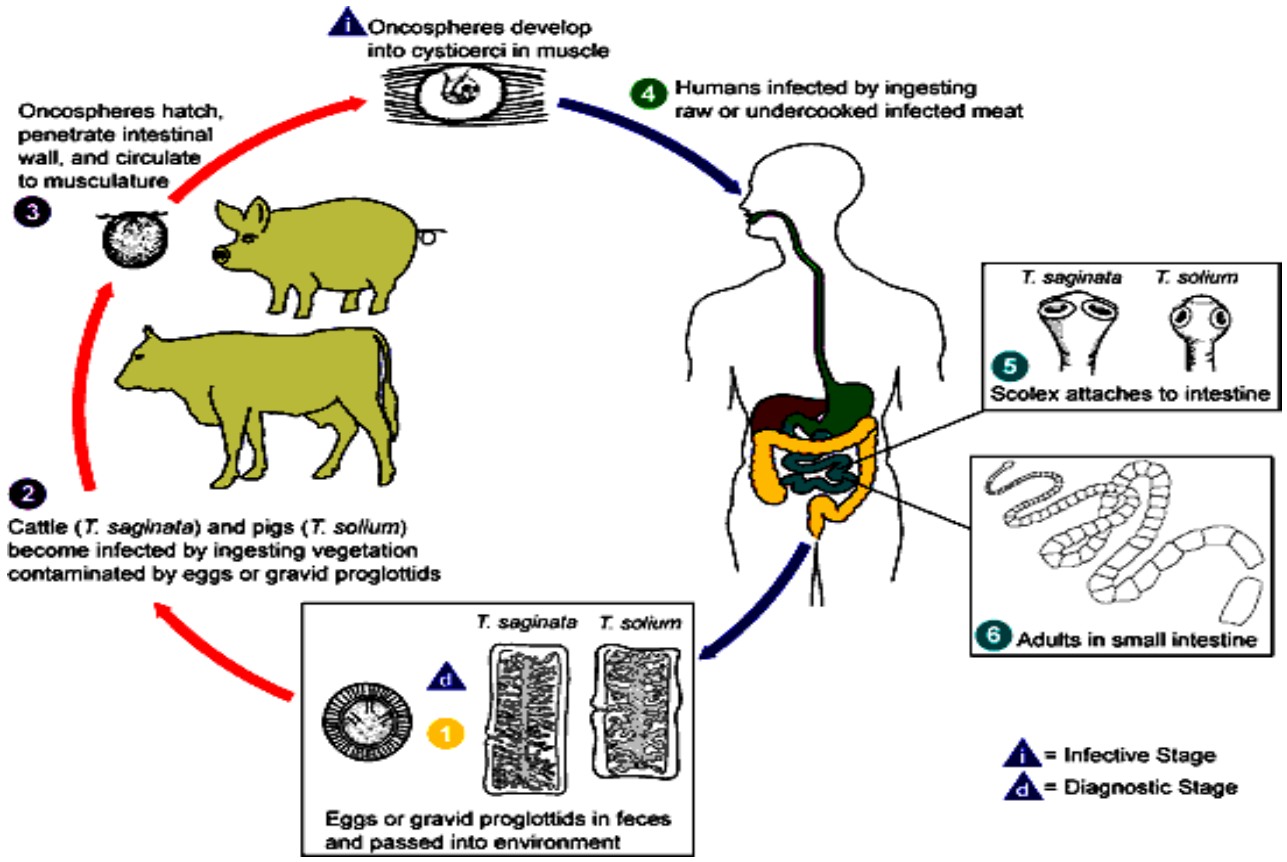


Figure 1. 14 : Life cycle of Taenia

1.7.3 ECHINOCOCCUS;

Itiscausedbyinfestationwithcysticerciofacestode*Echinococcusgranulosus*. Man is neither the definitive nor the intermediate host for this parasite but is infectedaccidentally. Theadult*Echinococcusgranulosus*(3-6mm) resides inthesmallbowelofthedefinitive

hosts,(dogs or other canines). Gravid proglottids release eggs that are passed in thefaeces.Afteringestionby asuitableintermediatehost(sheep,goat,swine,cattle,horses,camel),the egghatchesinthesmall bowelandreleases anoncospherethatpenetratestheintestinal wallandmigrates throughthecirculatorysystemintovariousorgans,especially the liver and lungs. In these organs, the oncosphere develops into a cyst that enlarges gradually,producing protoscolicesanddaughtercyststhatfill thecystinterior.Ingestingthecyst-containing organs of the infected intermediate host infects the definitive host. After ingestion, theprotoscolicesevaginate,attachto theintestinalmucosaanddevelopintoadult stagesin32-80days.Humans become infected by ingesting eggs, with resulting release of oncospheres in theintestineand thedevelopmentofcystsinvariousorgans.

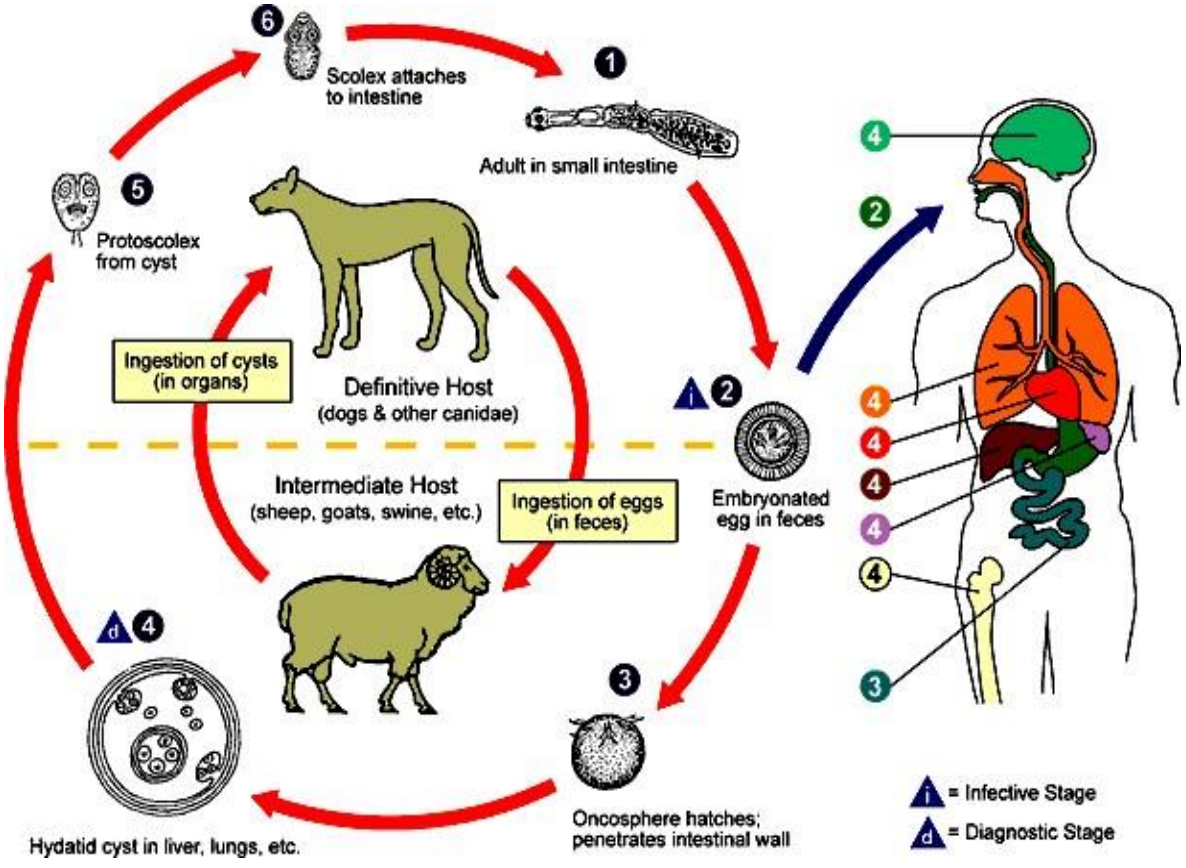


Figure 1.15; Life cycle of Echinococcus

1.8 TREMATODES

1.8.1 SCHISTOSOMES

Thesecausesschistosomiasis.*Schistosomamansoni*and*Schistosomajaponicum*affect thegastrointestinal tract, whereas *Schistosoma haematobium*affects the urinary tract.ImportantPropertiesIncontrasttotheothertrematodes,whicharehermaphrodites,adults chistosomesexistas **separate sexes** but live attached to each other. The female resides in a groove in the male, thegynecophoriccanal("schist"),wherehecontinuouslyfertilizesher eggs.Thethree species can be distinguished by the appearance of their eggs in the microscope: *S. mansoni*eggshavea **prominentlateralspine,**whereas*S.japonicum*eggshaveaverysmalllateralspineand *S. haematobium*eggs have a terminal spine. *S.mansoni*and *S. japonicum*adults live in the **mesenteric veins,** whereas *S. haematobium*lives intheveinsdraining theurinarybladder.Schistosomesare thereforeknownas**blood flukes.**

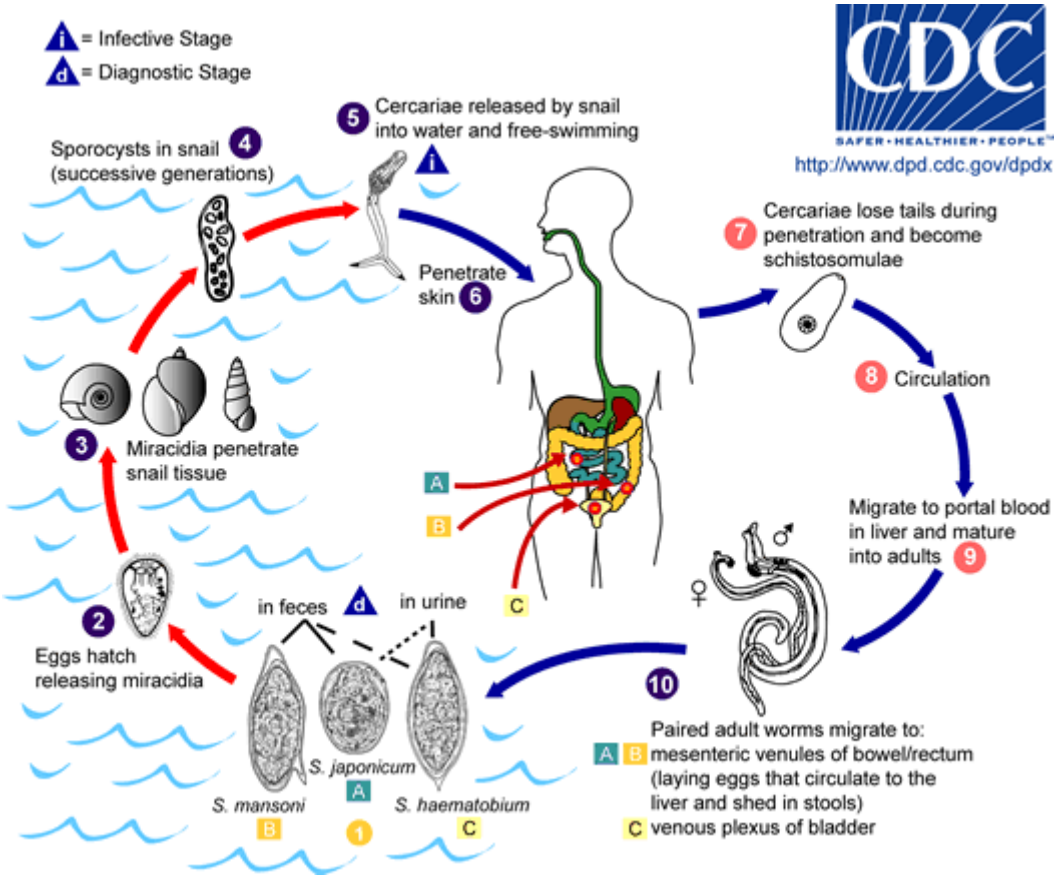


Figure 1. 16 :Life cycle of Schistosomes

2.PRACTICAL PARASITOLOGY

2.1.Introduction,generalrequirementforparasitologylab;

Chemicals:

1. Neutral red.
2. Iodine.
3. Potassium iodide.
4. Enthyl alcohol.

Specimens slides:

1. E.histolytica cyst.
2. E.coli cyst.
3. Giardia cyst.
4. Taenea saginata ova.
5. H.nana ova
6. Ancylostoma doudenale egg.
7. Trichuris trichura egg.
8. Ascaris lumbricoides egg.
9. S.mansoni egg.
10. S.japanicum egg.
11. S.haematobium egg.

Equipments:

1. Microscope.
2. Over head projector.
3. Slide projector.

Stains:

1. Field stain powder A.

2. Field Stain Powder B.
3. Lactophenol cotton blue.

Culture Medium:

1. Sabouraud's medium
2. N.N.N.Medium

Glass wares:

1. Glass slides
2. Cover slips.
3. Test tubes,beakers,funnels,flasks.etc.
4. Museum jars.
5. Reagent bottles.
6. Petri dishes.
7. Dropping bottles.
8. Graduated cylinders.
9. Ether.
10. Sodium chloride.
11. Sodium hydroxide.
12. Zinc sulphate.

2.2 Correct collection and transport of specimens;

The following are important:
● Use specimen containers that are leak-proof,clean, dry, and free fromtraces of antiseptics and disinfectants.
● If an anticoagulated blood specimen is required,use a suitableanticoagulant, e.g. sodium citrate. **Checking the specimen and request form** As well as checking thatthe specimenisclearly labelled andisaccompaniedbythe correctrequestformit isimportant tocheckthatthecontainerisnotleaking,
that thespecimenissuitableforthetestbeingrequestedandhasbeendelivered to the laboratory within the time specified for the particular investigation. Appropriate actionshouldbetakenifatestresult
isrequiredurgentlyortherearеclearproblemsinidentificationorcollection.

2.3 Microscopeintroductiondifferent parts and their functions

Microscope The light microscope is one of the most basic and essential equipment used in anylaboratory. It is used for visualising very small objects like cells, bacteria, parasites, theirova/cysts and crystals etc., that are otherwise not visible to the naked eye. It comprises a seriesof lenses, which magnify an illuminated small object several times to make it recognizable withthenakedeye.Suchamicroscopeiscalledcompoundlight microscope.

Introduction to the microscope's parts It hasthree basic components:Foot piece, BodyEyepiece

Foot piece;Itformsthebaseofthemicroscopeandprovides stability to the body andeyepieces.

Body; The body of the microscope is mounted on the foot piece. It holds a sub-stage condenser,astageandanosepiece.Sub-stage condenseriscomposedofa systemoflensesanddiaphragm. The intensity of light and the size of field illuminated by it are controlled by movingthe condenser up or down and adjusting the aperture of the diaphragm.The stage is a device forholding the objects for examination. It has a hole in the middle over which the object is placed.Exactlyunderneaththe holeis thesub-stagecondenser.Nosepieceisthe part ofthe body,which holds the objectives.An objective comprises a system of lenses, which magnify the
imageseveraltimes.Eachobjectiveismarkedwitha
colouredline,whichindicatesitsmagnification.
Following are the common objectives installed in an ordinary light microscope:
• Scanner: Redline, x4 magnification

• Low power: Yellow line, x10 magnification
• Dry high power: Blue line, x40magnification
•Oilimmersion:Whiteline,x100magnification
Eyepiece;The observer, to look at the object under examination uses this part of themicroscope.

Correct use of microscope

1. The microscope should be placed on a level bench, whichshould be free of vibrations.

2. The power socket, to which the microscope is plugged, shouldnot be loose and sparking.

3. The height of the microscope or chair should be adjusted in suchawaythattheeyesoftheuserarerightontheeyepieceswhilemaintainingthenormalcurvaturesofthebackbone.

4.Themicroscopeshouldthenbeadjustedfortheoptimumresolution and contrast to ensure maximum definition of specimen details. It can be done by using **Köehler technique**.

Köehler technique;Turn on the microscope at very low illumination and give 1-2 min to thefilament of the bulb to warm. Then adjust the light intensity. • Place the specimen on the stage,switchtox10objectiveandfocus.• Closetheirisdiaphragm ofthesub-stagecondenser andraise the sub-stage condenser to the top “stop”. • Close the field iris diaphragm of the lightassembly inthebody.• Movethesub-stagecondenser downuntiltheimageofthefieldirisdiaphragm is in sharp focus. • Now re-focus the specimen.• Centre field diaphragm image byusing adjustment screws in the condenser. • Enlarge field diaphragm image until it is just out ofthe field of view and the entire area under observation is illuminated. • Remove one eyepieceand look down the tube. • Adjust the aperture of diaphragm while observing the circular beam ofthelightsothelightbeamfills75%ofthefield.•Replacetheeyepiece.Adjustthedioptersetting and inter-pupillary distance.Place your forearms flat on the surface of the table whileusingmicroscope.

2.4 Dark-Field Microscope

It is also called a Dark-Field Illumination Microscope. There are certain micro-organismswhich areverydifficultto stain,e.g. spirochetes.To visualize themunderamicroscope,adarkfield illuminationisused.Themicro-organismsappearbrightagainstadarkbackground.Itissimilartodustparticlesseeninabeam oflightinadarkroomfromaventilator. Inthismicroscope,aspecial condenserwithacentralblack areaisplacedjust behind the objective. A dark-ground, phase-contrast microscope can be made fromanordinary microscope.Forthis,cutoutathicktalc sheetofthesizeofafilter.Colourthecentraltwothirds withblackink.Placeitalongthefilterintheholderbelowthecondenser.

Care of microscope; Microscope is very delicate equipment. Proper care not only enhancesprecision but also increases its life. Following points are helpful in the care of microscope:1.Protect from heat. 2. Clean it daily. When not in use, keep it covered with a plastic cover or apieceofclothbutnotwithmeshgauze.3.Cleantheobjectiveswithsofttissuepaper soakedinxylolandthenwithlintfreecloth.Becarefulasexcessofxylolmaydissolvethecement withwhich lens is fixed in the objective and may trickle into it. Do not clean with alcohol. 4. Removethe dust from the eyepieces with the help of soft tissue paper. 5. Always use soft tissue paper orlint free cloth for cleaning lenses and never rub but wipe gently. This protects lenses fromscratches.6. Switchoffthe poweratthe end ofmicroscopysession.

2.5 EXAMINATION OF STOOL:

2.5.1Collection of Faeces; Faeces can be collected in a bed-pan and care should be taken topreventanymixingwithurine.Fromthebed-pan,asuitableportionistransferredtoanappropriate container such as a waxed cardboard box, empty tin with a lid, alight plastic box orto a specially-designed glass jar for faeces collection with a spoon attached to the stopper. Thespecimenshouldatleastbe4ml(4cm3)inquantity.Thecollectionofasufficientquantity isnecessary in order to permit the detection of parasites in low concentration and to prevent therapiddryingofthefaeces. Care should be taken that the actual abnormal part(mucus and blood)is collected and sent to the laboratory immediately, preferably within one hour. It is important,especially when the vegetative form ofamoebaeistobeseen.Ifanumberofspecimensarereceivedatthesametime,liquidfaecesandt hosecontainingmucusor blood are examined first.

2.5.2 PHYSICAL EXAMINATION

Colour:

The normal colour of faeces is due to the presence of stercobilinogen produced by bacteria through the decomposition of bilirubin.On exposure to air it is converted to brownstercobilin. As breast-fed infants have no bacteria in their intestines, stercobilinogen is not produced and the colour of these faeces remains yellow. In diarrhea the movement of the intestine is so rapid that the bacteria do nothave time to decompose the bilirubin and green faeces may be passed. The colour of faeces depends upon various factors.The concentration of bile pigments gives a greenish colour to faeces particularly in diarrhea of infants (starvationfaeces). On the other hand,obstruction to the flow of bile into the intestine,gives rise to pale, tanor clay-

coloured faeces. Chlorophyll-rich foods produce green faeces. Bleeding into the upper gut gives rise to black faeces due to altered blood.If bleeding is in the lower part of the intestine, then the colour of the faeces is red. In addition, oral iron ingestion results in black faeces.Various drugs will change the colour of the faeces accordingly.

Odour;
A normal odour is because of indole and skatole.It varies with pH and is dependent on bacterial fermentation and putrefaction. Faeces are particularly offensive in amoebic dysentery.

Consistency;Normally, faeces are formed or semi-formed.Thefaeces can be liquid, semi-liquid,solid,semi-solidorfoamy.
Solidorhardfaecesarepassedinconstipationandloose

faecesindiarrhoea.Diarrhoealfaecesmixedwithmucusandbloodisseeninamoebicdysentery,carcinoma ofthelargebowelandtyphoid.Loosefaeces mixedwithpusandmucusoccur in bacillary dysentery, regional enteritis and ulcerative colitis. Paste-like and frothy, loosefaeces are seen in sprue, pancreatic insufficiency and other mal-absorption syndromes. Wateryfaeces(rice-waterfaeces)areseenincholera.

Parasites;Intact parasites like *Ascarislumbricoides*and *Enterobiusvermicularis*or segments of*Taeniasaginata*maybeseenwiththenakedeye.Evensmaller wormsandscolecescanbeseenwhenfaecesareliquefiedwithwaterandstrainedthroughawide-meshsieveandrestrainedthrough amedium-meshsieve.

Reaction of pH;The normal pH of faeces is either neutral or weakly alkaline. In general, onmixed or meat diets, the reaction tends to be alkaline and in a predominantly carbohydrate orfat-
richdiet,acidic.ThebreakdownofcarbohydrateschangesthepH
toacid(asinamoebicdysentery) and the breakdown of proteins changes it to alkaline (as in bacillarydysentery).In cases of lactose intolerance in infants(because of the excessive fermentation oflactose)the faecestend to be highlyacidic.

2.5.3 MICROSCOPIC EXAMINATION

2.5.3.1DIRECT WET PREPARATION

A small portion of freshly passed faeces is examined by making a thin suspension in a drop
ofnormalsalineandadropofLugol‘siodineonaglassslide.Thisiscoveredwithaglasscover.The faeces should be selected both from the exterior as well as the central portion of thefaecalmass.Faecal
matterselectedforexaminationshouldcontainbloodandmucus,inthecase of blood-stained faeces.Microscopically, one will see food residues(digested andundigested muscle fibres, fat globules and fatty acid crystals, starch granules and celluloseresidues), cells

(RBCs, WBCs and epithelial), crystals (triple phosphate, calcium oxalate,cholesterol and Charcot Leyden crystals), ova (*Ascarislumbricoides, Enterobiusvermicularis,Ankylostomadeudenale*etc.),parasitesortheircysts andmucusandforeignbodies(hair, wool,etc.). This method also demonstrates motile amoebae, which contain ingested RBC and showpurposeful,unidirectional movement by throwing out pseudopodia. Ova and cysts can be seenby moving the objective of the microscope up and down and keeping the light subdued. AdditionofadropofLugol'siodinefromtheedgeofthecover slipprovidesagoodcontrastandstainssomeinclusionsofprotozoancystslikeglycogensmass .Normal structuresshouldnotbeconfused with abnormal findings like ova and cysts. These include hair, vegetable fibres, starchcells,yeastsandspores,muscle fibres,fatglobulesandpollen grains.

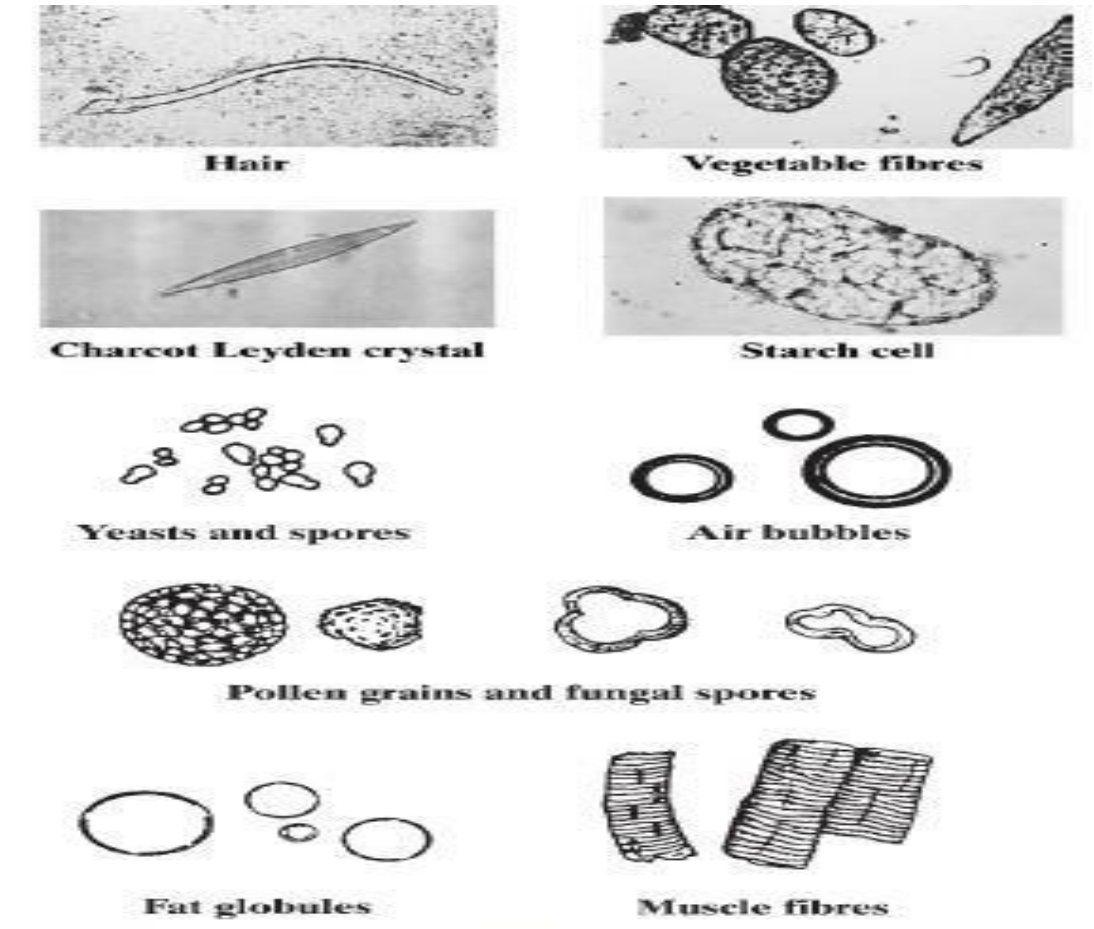


Plate 5.3 Structures found in faeces that required differentiation from parasites.

Figure 2.1 Structures Found in Stool

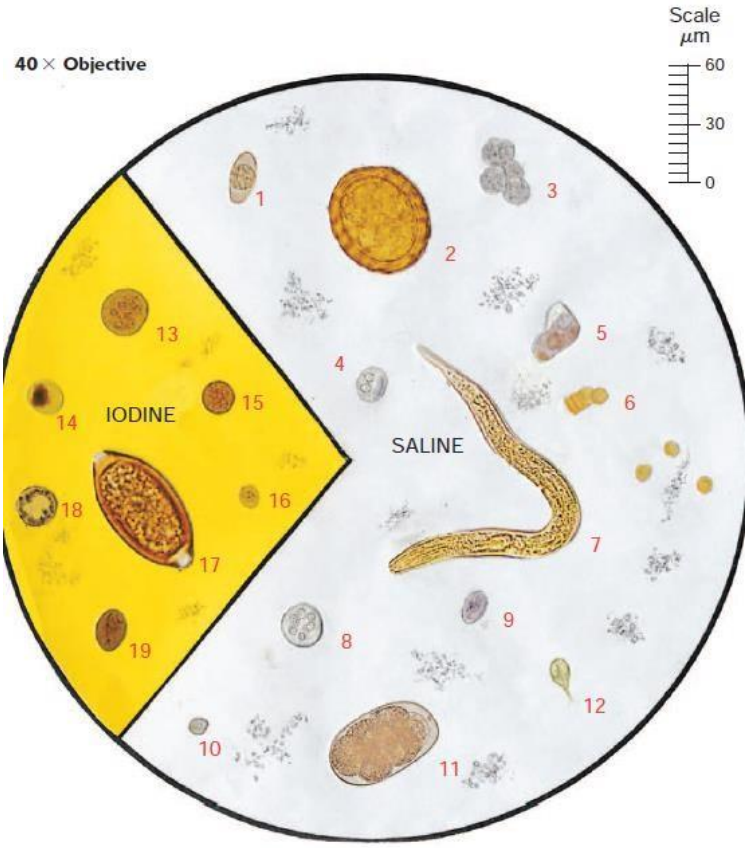


Figure 2.2 Relative sizes of trophozoites and cysts of intestinal protozoa, common nematode eggs and larva of *Strongyloides* as seen in microscope field using the 40 X objective (with10 X eyepieces). 1.*I.belli*oocyst,2. *A lumbricoides* egg, 3.Leucocytes, 4.*E.histolytica/E.dispar* cyst, 5.*E.histolytica* trophozoite(motile), 6. Red cells, 7. *S.stercoralis*larva (motile), 8. *E.coli* cyst (mature), 9. *G.lamblia* cyst, 10. *C.mesnili* cyst, 11. Hook worm egg, 12.*G.lamblia* trophozoite(motile).
Iodine preparation:13.*E.coli* cyst,14.*I.buetschlii*cyst,15.*E.histolytica/E.dispar*cyst,16. *V.nana*cyst,17.*T.trichiura*egg, 18.*Blastocystishominis*,19.*G.lamblia*cyst.
Note:Trophozoites,cystsandoocysts foundinfaecesaredescribedinsubunit5.4.

2.5.3.2 CONCENTRATION TECHNIQUES

These methods are used when ova or parasites are not found in direct saline preparation buttheir presence is highly suspected or symptoms persist. Ova of certain parasites are scantye.g.,*Schistosoma, Taenia*etc.somayrequireconcentrationmethodsfortheirdemonstration.Thesemethodsare:

A. Formalin Ether Sedimentation

Concentration techniques using formalin not only kill the parasites but also fix them preservingtheirmorphology,therefore,these areconsidered thebest.

Procedure: Emulsify about 2 ml of faeces in3 ml of saline in a 15 ml conical centrifugetube; addsalineto15mlmark.Centrifugeat1500rpmforonemin.Decantthesupernatantandre-suspendthedepositinanother15mlofsaline.Repeatuntilcleansedimentremains.Mixwith10 ml10%formalinandallowtostandfor5min.Add3mlether,stopperthetubeandshakevigorously .Removethestopper andcentrifugeat1500RPMfor 2min.Thefourlayersfromthebottom upwards will be:sediment containing parasites, formalin,faecal debris and, the upper-mostlayer,ether.Freethefaecaldebrisfrom thewallsandremovethetopthreelayers. Resuspend the deposit, prepare the saline and iodine wet films and examine under themicroscope.

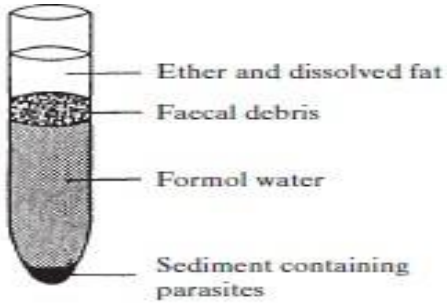


Fig. 5.1 Formal ether sedimentation concentration technique, after centrifugation.

B. **Sodium Chloride Floatation Technique;** The faeces are mixed with a saturated solution ofsodiumchloride.Theeggsarelighter inweight,sothesefloattothesurface.

Procedure:
Placeabout2ml offaecesinanemptycleansmall bottleor tube.Quarter-fillthebottlewithsaturated solution of sodium chloride (NaCl). Mix faeces with the help of an applicator and fillthebottletothetopwithNaCl.Placeacover slipover

themouthofthebottlesothatittouchesthe liquid without having air bubbles in between. Remove the cover slip; a drop of liquid shouldremainonit.Placethecoversliponaslideandexamineunder themicroscope.

Zinc Sulphate Floatation Procedure

ParasiticcystsandsomeHelmintheggs willrisetothesurfaceofaliquidhavinghighspecificgravity (zinc sulphate, specificgravity 1.180), due to their buoyant properties in that solution. Thesolutionofzincsulphatecanbepreparedbyadding330gofdry crystalsofzincsulphateto670mldistilledwater.

Procedure:Prepareafaecalsuspensionof¼to½ teaspoonin10-15mlofwater.Filter thismaterialthroughtwolayersofgauzeintoasmalltube.Fillthetubewithtapwatertowithin 2-3mm ofthetopandcentrifugefor1minat500Xg.Decantthesupernatantfluid,fillthetubewithwater, and re-suspendthe sediment by stirring with an applicator stick. Centrifuge for 1 min. at500xg. Decant the water, add 2-3 ml zinc sulphate solution,re-suspend the sediment, and fill thetube with zinc sulphate solution to within 0.5 cm of the top. Centrifuge for 1-2 min at 500xg,allowthe tube to come to a stop without interference or vibration. Without removing the tube from thecentrifuge,touchthesurfaceofthefilmofsuspensionwithawireloop,parallel tothesurface. Add the material in the loop to a slide containing a drop of dilute iodine or saline. (The slideshould be examined as soon as possible,because high specific gravity will distort the ova).Themorphologyofvariousprotozoa,cystsand ovafoundinstoolsissummarisedbelow.

1)EXAMINATION OF OVA AND CYSTS IN STOOL;

A) <u>PROTOZOA</u>

Entamoebahistolytica;Thefollowingcharacteristicsarevaluableintheidentificationof

*E.histolytica*in stoolsample;

Unstained Trophozoites: Progressive motility,hyaline pseudopodia, no ingested bacteria andinvisiblenucleiaresuggestive.Theingestionofredcellsisdiagnostic.

Stained Trophozoites: Clear differentiation of ectoplasm and endoplasm, no ingested bacteriaare suggestive, whereas fine, uniform granules of peripheral chromatin and small centralkaryosomeinthenucleus,ingestedredcellsandanaveragesizeofmorethan12µm isdiagnostic.

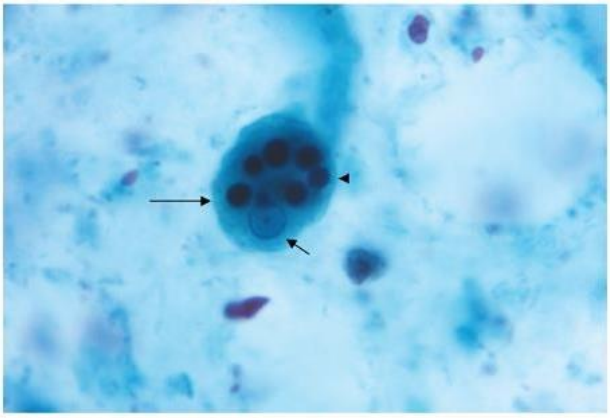


Figure 2.3 Entamoeba histolytica Cyst

Unstained Cysts: Four nuclei and rod-like chromatid bodies are suggestive.

Stained Cysts: A maximum of four nuclei having both karyosome and peripheral chromatin and a diameter of more than 10μ m is suggestive, where as a typical nuclear structure, chromatid bars with rounded or square dends and diameter greater than10μm is diagnostic.

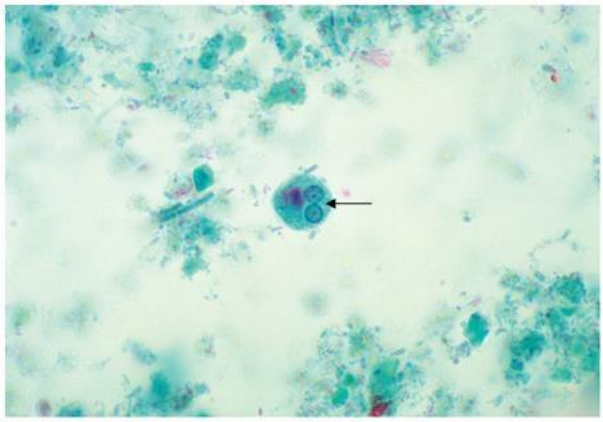


Figure 2.4 Giardia lamblia vegatititve Form

Giardia lamblia

The vegetative form is kite or pear shaped(front view) or spoon- shaped(side view), flagellated,motile organism (classically like a falling leaf). They are 10-18 μm in size. There are two nucleiand four pairs of flagella. It shows spinning or rapid jerky movements.Two large oval nuclei arefaintlyvisible.Cystsaresmall(8-12μm),ovalandrefractile,containing2-4nuclei usuallyatone

end with a small,faintly-coloured central karyosome.Two curved longitudinal axostyles are seenin the centre. The cytoplasm is shrunk away from the wall. The shell is double-walled and thick.The following characteristics are important for the identification of *Giardia lamblia* trophozoitesandcysts:

Unstained trophozoites:Progressive, **falling leaf** motility; pear shaped body with attenuatedposterior end is suggestive.

Stained trophozoites: the nuclei is in the area of a sucking disc:two median bodies, posterior to the sucking disk and a typical arrangement of axonemes arediagnostic.

Unstained cysts: Ovoid shape of the body and numerous refractile threads in thecytoplasm are suggestive.

Stained cysts: Four nuclei, four median bodies and a jumble ofaxonemesarediagnostic.

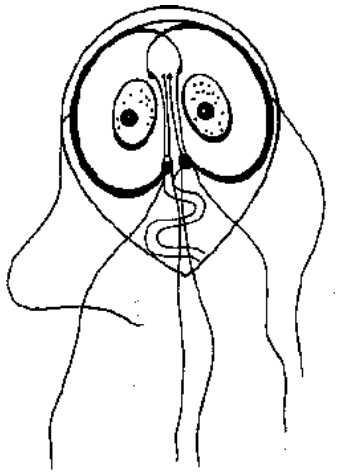


Figure 2.5 Trichomonas Vaginalis

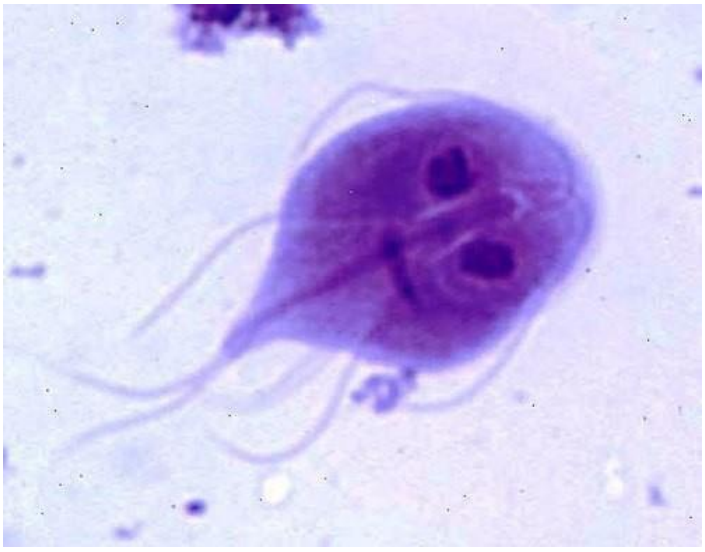


Figure 2.6 Trichomonas Vaginalis

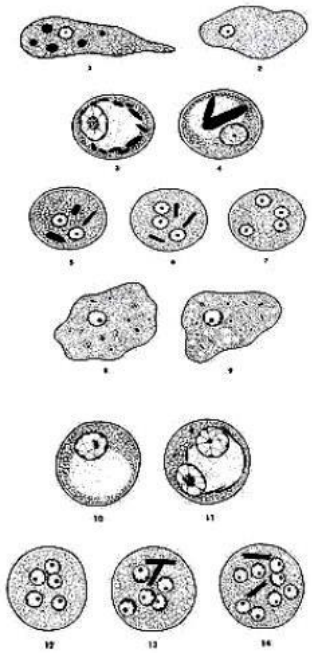


Figure 2.7: Protozoa in faeces.
1,2,TrophozoitesofEntamoebahistolytica.3,4,earlycystsofEntamoebahistolytica.5-7,Cystsof Entamoeba histolytica. 8,9,Trophozoites of Entamoeba coli. 10,11, Early cysts ofEntamoeba coli. 12-14, Cysts ofEntamoebacoli.

TRICHOMONAS; Diagnosis is by demonstration of trichomonas most commonly in wet-filmpreparation,althoughthey may readilyberecognisedinPapanicolaousmears.Themostcommonspecimenisvaginaldischargebutexaminationof urethraldischargeinthefemalemayyield positive results when no organism is found in the vaginal swab. Several specimens mayneed to be examined. It is absolutely necessary that the specimen is NOT contaminated withfaecal materialsincethemorphologyof*T.hominis*issimilar tothisorganism.

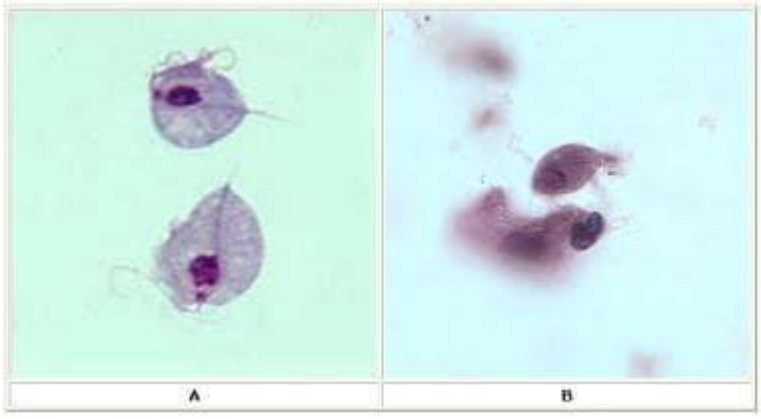


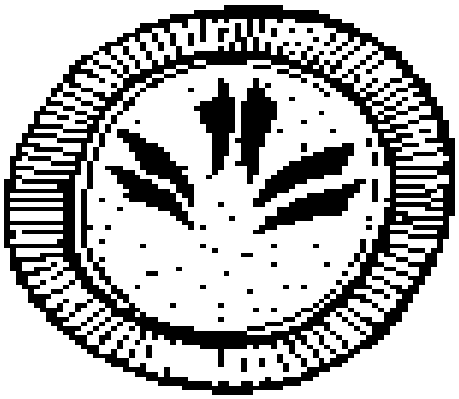
Figure 2.8 Trichomonas Vaginalis

B)HELMINTHS;

1)CESTODES;

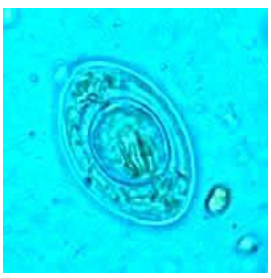
Taeniasaginata,Taeniasolium,Echinococcus;

Theeggsofthesetapewormsaresimilar.Eggsarespheroid,yellowtobrownincolourand30-40 µm in diameter (embryophore). The thick, radially-striated shell is dark yellowish brown incolour,covering a light yellowish grey material. Inside is a narrow clear space, lined by a thinmembrane in which lies a granular mass, the hexacanth embryo,with 3 pairs of refractile, lancet-shapedhooklets(oncosphere).



Hymenolepisnana

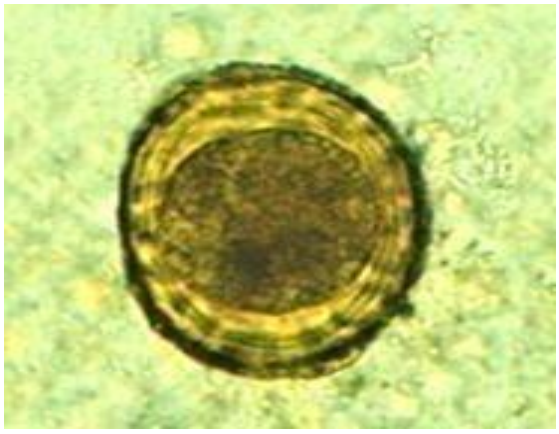
Ovum is nearly spherical, 45µm in diameter. It has two distinct walls; external membrane is thinand internal membrane is often thicker at poles with 4-8 hairlike filaments coming out from bothpoles. Some granules occupy the space between the two membranes. It contains a roundedmassofagelatinous substancewiththreepairs ofrefractilehookletsarrangedinafanshapeandoftensomewell-definedgranulesinthecentre(Hexacanthembryo).



2) NEMATHELMINTHS

Ascarislumbricoides

Fertilized ova with double shell: They are yellow-brown with a thick shell having an unevenrough, brown, albuminous outer coatand a thick, smooth,transparent inner shell.These measure50x70 µmand contain unsegmented fertilized ovum as a single,round, granular, central masswithclearcrescentricspacesat eitherpole.

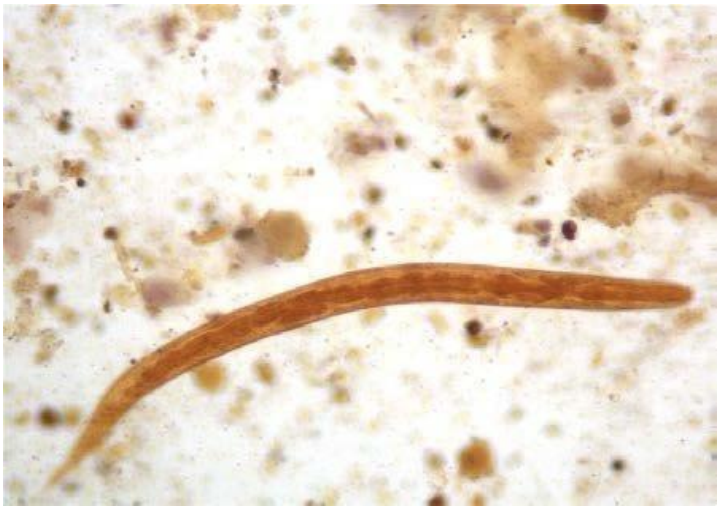


Enterobiusvermicularis

Ovum is asymmetrically ovoid with one side flattened. Thesize is 20x50 µm. It is transparentand colourless.There is a thin, double-lineshell, with a coiled larva inside or a small, granularmassin theshapeofanirregularovalfigure.

Strongyloidesstercoralis

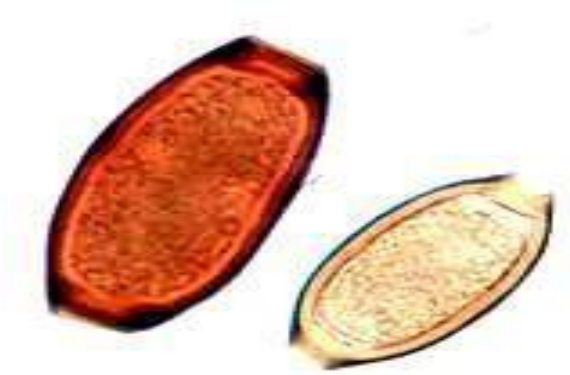
Rhabditiform larvae are demonstrated by concentration technique. Larva is 200-300µm and isun-sheathed.Thedigestivetubehasaswellingatoneend(oesophagus)andanother(analpore) at the other end. The tail is moderately tapered. The genital primordium is arounded,clearspacenearthemiddle.Theeggs areusuallynotfoundinfaecesbecausetheyhatch before evacuation, but liquid faeces may contain them. They are very similar to thatof*Ankylostomadeudenale*butare slightlysmaller(50 µm).



Rhabditiformlarvain stool

Trichuristrichiura

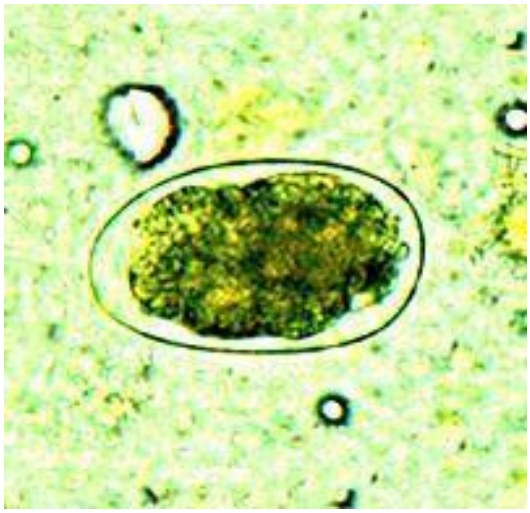
Ova are characteristically barrel-shaped and measure 50 µminlength. These are rounded andtransparent with plugs at both ends. These have a fairly thick, smooth shell with two layers.
Theshellisorangeincolourwhilethecontentsareyellow.Theycontainauniform,granularmass(un-segmentedovum).



Ancylostomadeudenale(Hookworm)

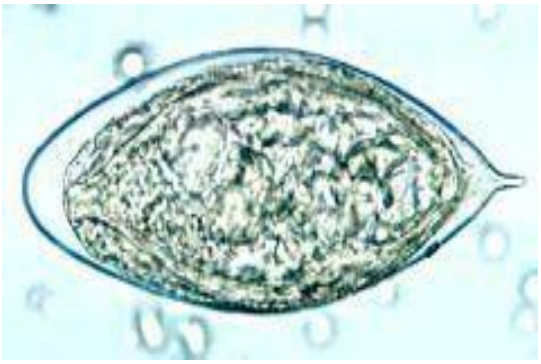
Ovum is oval with rounded slightly flattened poles, colourless with very thin shell that appearsasblackline.Itmeasures40x60μminsize.Itcontainsasegmentedembryoof4to16cel lsstagethatispalegreybutturns darkbrownwithiodinesolution.Thecontentsvary accordingto

the degree ofmaturity. Fresh faeces have grey granular, clear cell. Few hours‘ old faeces willhave a uniform mass of many small grey granular cells. 12-48 hours‘ old faeces will have smalllarvaein placeofcells.



TREMATODESSchistosomahaematobium

Ova are usually found in urine but sometimes in faeces also. They measure 50x150 μm,oval,elongated and dilated in the middle. The ovum is grey or pale yellow in colour with asmooth, very thin shell. It has a short terminal spine and contains fully developed ciliatedembryo(miracidium)surroundedbyamembrane.

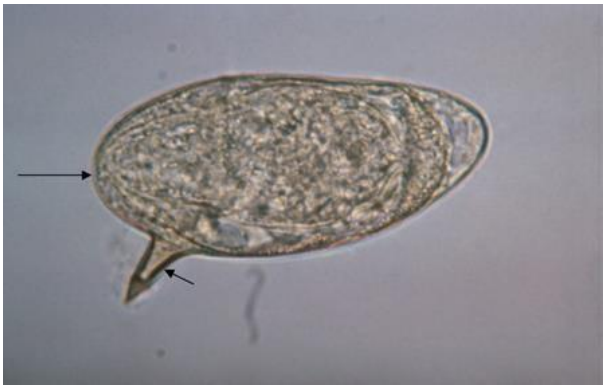


Schistosomamansoni

Ova are pale yellow, oval with a lateral (near theround pole), large, triangular spine.

Theeggmeasures50x150µmandithasaverythin,smoothshell.Itcontainsafully

Developedciliated embryo (miracidium), surrounded by amembrane. The calcified egg is usuallysmallerandblack, with aless-distinctspine.



2) TESTFORBLOODINFAECES

Bloodinfaecescanbedetectedby:

Benzidine Test

This test detects microscopic blood in faeces.More than 10 ml of blood will give a black colour

tothefaeces,whereas,lessthan10ml(occult)bloodfromthegastrointestinaltractwillbedetecte d by this test. Peroxidase in the haem of haemoglobin liberates oxygen from hydrogenperoxide

thatoxidisesbenzidineinanacidicmediumandchangesittobluecolouredcompound.A**false positive**testisgivenbymeat.Thepatientisaskedtoavoidmeatonedaybefore the examination. He/she should not take any iron-containing compound nor brush his/herteeth.

Procedure: Make a suspension of faeces in 10ml saline and boil to inactivate the oxidizingenzymes that are normally in faeces. Make 2 ml of a saturated solution of

benzidine in glacialaceticacidinanothertube.Add2ml ofH2O2andcheckwhetherablueorgreencolourdevelops.Ifso,discardthereagents.Addfaecalsuspension,dropbydrop,tothesolutionof

benzidine and H_2O_2 until there is a change of colour. The appearance of a deep blue colourindicatesthepresence ofblood.

The Orthotoluidine Test; Orthotoluidine is converted to a blue-coloured compound by blood.Two percent sodium perborate solution in water and 2%orthotoluidine solution in glacial aceticacidaremixedinequalvolumejustbeforeuse.Add6dropstoasmearoffaecesonafilter paper. A blue colour indicates the presence of occult blood. These tests also form the basis ofcommerciallyavailablestrips.

3) Examinationofbloodfor parasites

a) THICK FILM

Principle
A large amount of blood can be examined for parasitic forms by lysing the red cellsandstainingforparasite.Fixationisnotdonebymethanol.

Procedure

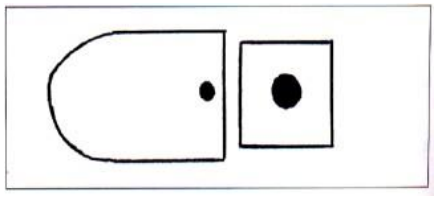
Touchalargedropofbloodfromthepulpofafinger withaglassslideandrotateittospreadbloodinanareaequal toatwo-rupeecoin.Thefilm shouldbesuchthatnewsprintcanbeseenthrough it. Alternatively, place a drop of blood in the centre of a glass slide and spread it with acornerofanotherglassslide.Drythebloodfilmfor30mins,at 37°Corleaveit ontopofamicroscopelampfor about7mins.DilutestockGiemsaStain20timesinbufferedwaterinastaining jar and immerse the slide in it for 20-30min. Take out and gently wash with bufferedwater and it stand upright to dry. The slide must not be blotted. Examine under an oil-immersionlens.

b) THINFILM

Principle
By spreading the blood cells in a thin layer, the size of the red cells, inclusions and extracellularforms can be more easily visualised. Leishman Stain is prepared in methanol, which also actsasafixative.

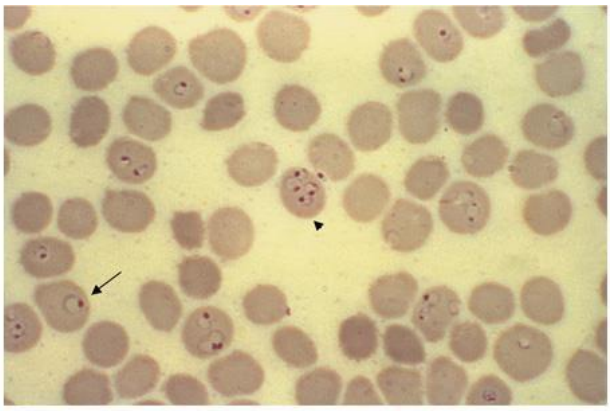
ProcedureSlidesarepreparedintheusualmanner andstainedinthesamewayasfordifferential leukocyte count and red blood cell morphology.More time should be spent on theexaminationoftheedgesand head-

endoftheslide.

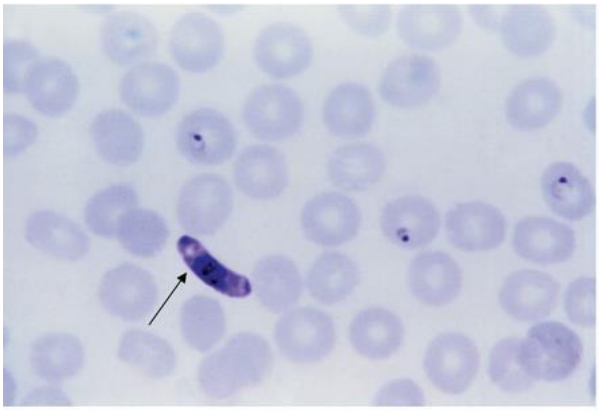


Fig; Sizeofblooddrop andareaof slideto coverformakingthickandthin bloodfilms

1) Plasmodium



Plasmodium falciparum—Ring-shaped trophozoite. Long arrow points to a red blood cell containing aring-shapedtrophozoite.Arrowheadpointstoared bloodcellcontainingfourring-shapedtrophozoites.Note the very high percentage of red cells containing ring forms. This high-level parasitemia is moreoftenseenin*Plasmodiumfalciparum*infectionthanininfectionbytheotherplasmodiaMorphologic characteristics of developmental stages of malarial parasites in the red blood cell. Notecytoplasmic Schüffner dots and enlarged host cells in *Plasmodium vivax* and *Plasmodium ovale*infections, the band-shaped trophozoite often seen in *Plasmodium malariae*infection, and the small,often multiply infected rings and the banana-shaped gametocytes in *Plasmodium falciparum* infections.Rings and gametocytes are typically seen in peripheral blood smears from patients with *Plasmodiumfalciparum*infections.



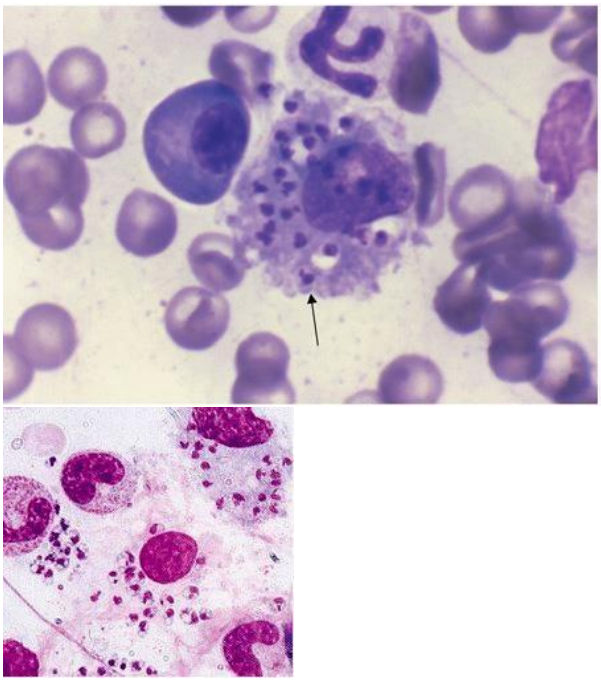
Plasmodium falciparum—Gametocyte. Arrow points to a "banana-shaped" gametocyte of *Plasmodiumfalciparum*

Stages	Parasites			
	Plasmodium vivax	*Plasmodium ovale*	*Plasmodium malariae*	*Plasmodium falciparum*
Ring stage				
Developing trophozoite				
Developing schizont				
Schizont				
Microgametocyte				
Macrogametocyte				

3)LEISHMANIA;

The diagnosis is made by an examination of a smear from the lesions, culture of material fromthelesionandbiopsy.TheeasiestwayistoexamineaGiemsaor Leishman-stainedsmearprepared from material obtained from the lesion.A smear can be prepared by any method givenbelow:Clean the edge of the ulcer and surrounding skin. Make a small, skin-deep incision with asharpblade,about5mm inlengthstartingfrom theulcermargin.Spreadthematerial ontoaclean glass slide.Take a corrugated dental needle and insert it into the skin at the margin of theulcer pointing towards the floor of the ulcer.Withdraw the needle without rotating.Spread thematerial sticking to the needle on a clean glass slide. Stain smears just like blood smears andexamine under high power objective (x40).Look for large macrophages with parasites and

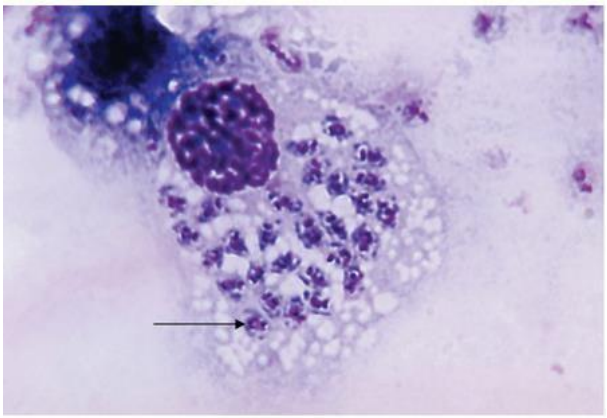
studythemorphologyoftheparasitesunderoil-immersionlens.



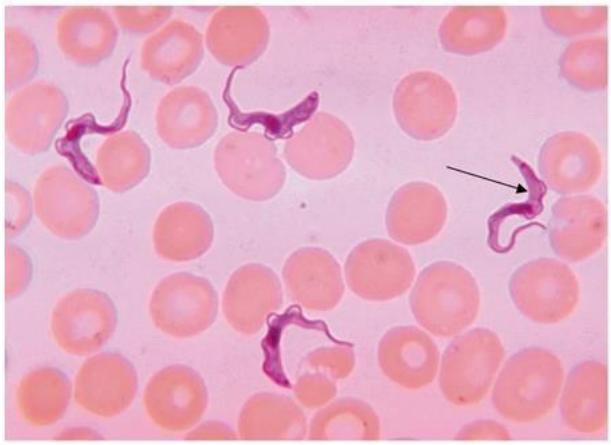
Fig;StainedslideshowingLt(*Leishmaniatropica*)**Bodies**

2)Trypanosomes

During the early stages, microscopic examination of the blood (either wet films or thick or thinsmears) reveals trypomastigotes. An aspirate of the chancre or enlarged lymph node can alsodemonstratethe parasites.



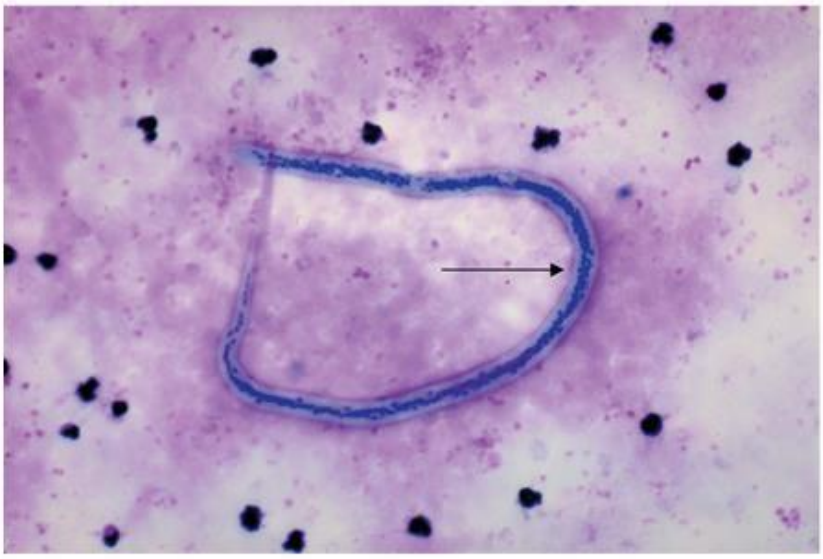
Trypanosomacruzi—
Amastigotes.Arrowpointstoanamastigote(nonflagellatedform)incytoplasm



Fig;Trypanosomabrucei—
Trypomastigotes.Arrowpointstoatrypomastigote(theflagellatedform)inthe blood

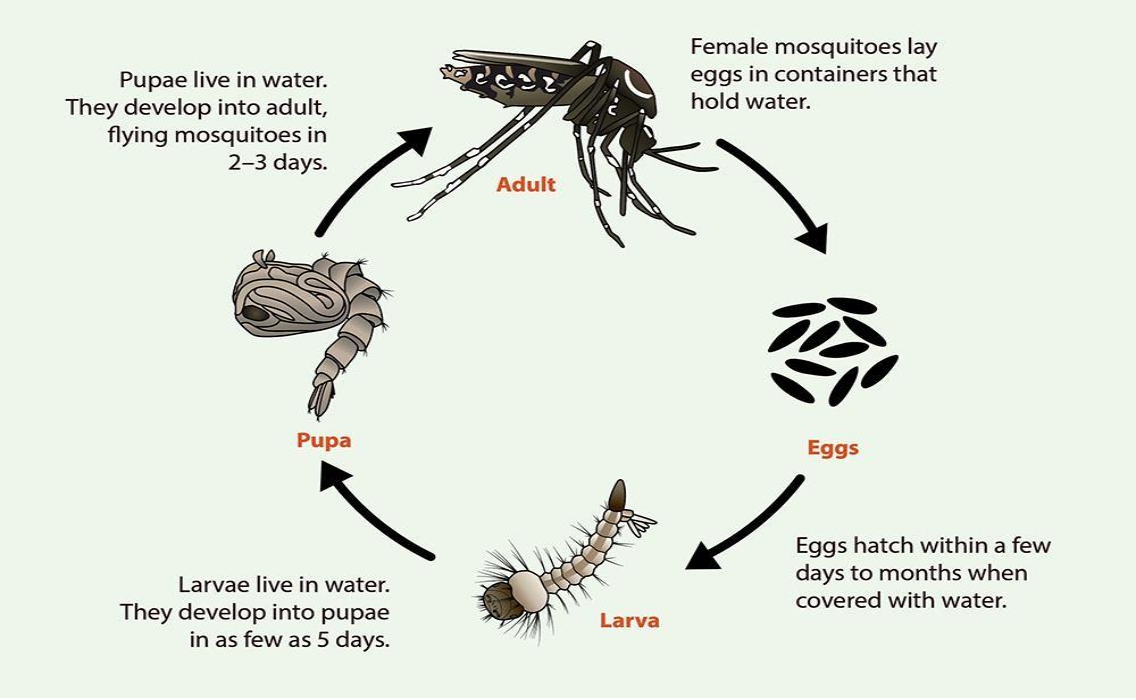
4)FilarialWorm

Blood should be collected around midnight, as this is the time when the parasite is present in theblood.Therearethreemethodsofexamination:
Prepare an ordinary thin-blood smear and stain in the usual manner.
Examine underlow-powerandthen,forfinerdetails, underhigh-power.
Make a thick-blood film stained with Giemsa Stain. Better results are obtained with haematoxylinand eosin staining. For this, the dried smear is first washed with water, dried in the air and fixedwith equal parts of ether and 95% alcohol for 10 min. It is dried and stained like histologicalsections.
In the concentration method, capillary blood is obtained in a centrifuge tube containing2%aceticacid.Itismixedthoroughly,centrifugedandthedepositisexamin
ed
underacoverslip.Actively-movingmicrofilariaecanbeobserved.

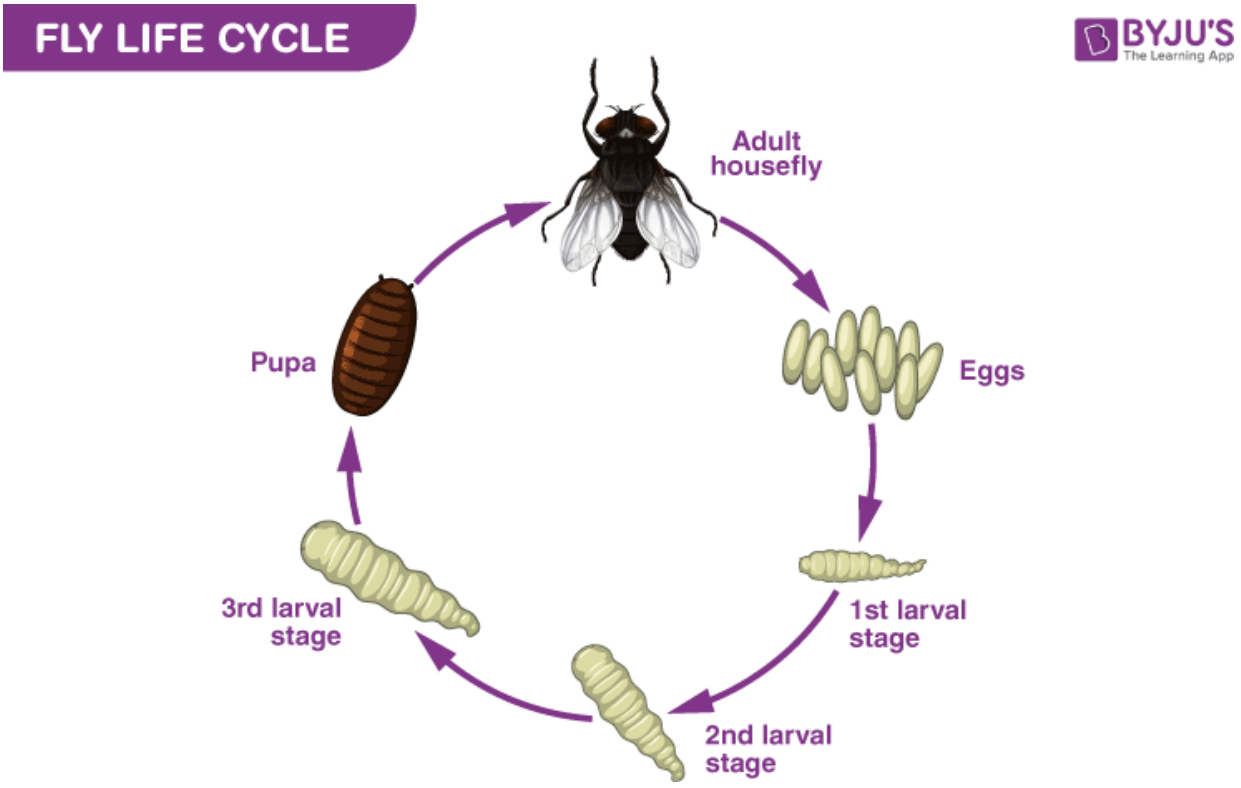


Wuchereriabancrofti—
Filarialworminblood.ArrowpointstofilarialworminbloodsmearENTOMOLOGY

A-LIFECYCLEOFMOSQUITOE



B-LIFECYCLEOFHOUSEFLY



Reading Material for

Applied Sciences – II

(Mycology)

Compiled By:

Punjab Medical Faculty

Specialized Healthcare & Medical Education Department

Government of the Punjab

CONTENTS

1)Introduction

Thestudyoffungiiscalled**Mycology**,andthediseasestheycausearecalled**mycoses**. Fungi exist as uni-cellular or multi-cellular, reproducing by the production ofspores.Yeasts are uni-cellular fungi, which reproduce by budding. The cytoplasm of theparent cell isextrudedthroughaholeinthecellwallandadaughter‘ cell is formed,which ultimately breaks away from its parent. This spore is called a **blastospore,** andthetypicalcolony formediscalledayeastcolony.Someyeasthowever,form**pseudohyphae**, which are elongated blastospores. Multi-cellular fungi (on a suitablemedium)formfilamentscalledhyphae.These**hyphae**may bedividedby transversewallsandarecalledseptate.Thesestructuresbranchandintertwineformingamesh work knownasmycelium.Apartofthis**mycelium**is inthemedium(**vegetativemycelium**)andapartremains onandabovethesurface(**aerialmycelium**).Hyphaemay be**septate**whenthereexistacross-wallinthefilamentsorthey maybenon-septate.Thereproductivestructureselevatedat theendsoftheaerialhyphaearecalledspores,eachofwhichcanbeidentifiedbydifferencesinth eappearanceofsporetypes.Someofthepathogenic fungi exhibitgrossvariationsintheirgrowthformsaccording toconditions suchastemperature.Suchfungiarecalled**dimorphic** fungi.

Asexual Spores: There are five types of imperfect (asexual)spores which are ofdiagnosticvalue:

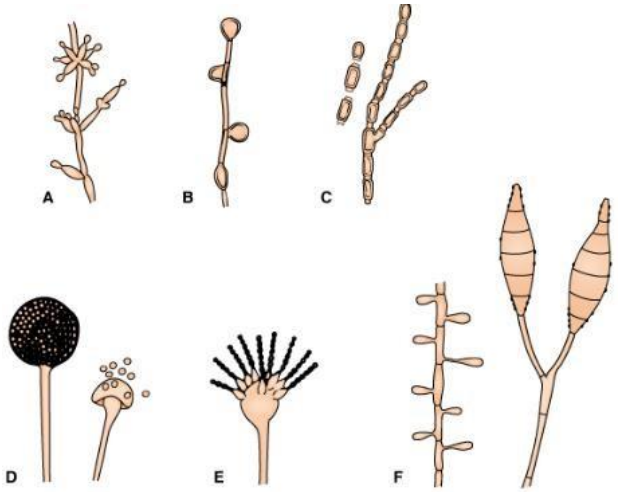
1. Blastospores:daughtercellsformed bybuddingoff froma parent cell

2. Arthrospores: formed by segmentation of a hypha into a series of separate cells,whichmaybecubicalorroundedinshape.

3. Conidia: formed on a specialized hypha(conidiophore) or borne directly on the sideofahyphawithnoapparentconidiophores.Theymay bemicroconidia(uni-cellular)ormacroconidia(multi-cellular)..

4. Chlamydospores:formed bythe rounding upof a cellwith athickeningofits wall

5. Sporangiospores: formed within a closed structure called a sporangium, the wall ofwhichrupturestoliberatethematuresporangiospores.
Sexual Spores: They are very rarely found in human disease.Basidiospores,ascospores,zygosporesaresome namesasexamples. Yeast cellsusuallygrowaslarge single cells, rarely forming filaments. Mostly they reproduce by the asexualprocessofbudding.



Asexual spores. **A:** Blastoconidia and pseudohyphae (*Candida*). **B:** Chlamydospores (*Candida*).**C:** Arthrospores (*Coccidioides*). **D:** Sporangia and sporangiospores (*Mucor*). **E:** Microconidia(*Aspergillus*).**F:**Microconidia andmacroconidia (*Microsporum)*

LABORATORYDIAGNOSISOF FUNGALINFECTIONS

Routinemycologicaltechniques

a-Direct microscopy; KOH

preparationb-Fungalstains

c-Fungalculture

d-Fungalserology

e-Moleculartechniques

1)TheCollectionofSpecimens:

Skin; Scrape the active periphery of the skin lesion using a sterile scalpel blade. Theyare collected on a piece of clean paper. Fold the paper and convey it to the lab benchforprocessing.

Nails;Using nail clippers, remove the affected nails.Remove debris beneath the nailwithabluntprobe.Collectanddispatch,asforskin.

Hair;Examine the scalp and other hair-bearing areas under the illumination of a Wood'sLamp(ultravioletlight)forfluorescence.Extractfluorescinghair(infectedwithMicrosporum)withforceps. Ifthereisno fluorescence,takespecimens of lusterlessorbrokenhair.Foldincleanpaperandsendittothelaboratory.Aplastic massagebrushmaybeusedtoobtainhairsamplesforculture.

Mucosa;Collectexudates andanythrush-likemembranethatis presentbyusing

cotton-woolswabs.

Sputum, Pus and Exudates;These specimens are taken into a sterile universalcontainerandexaminedwithoutdelay.

DIRECTMICROSCOPY **Skin Scrapings, Nails and Hair;** Direct microscopic examination is the best method ofdiagnosingringworm.Thespecimenisfirstsoftenedandcleanedwith20%KOH(potassium hydroxide).This will digest the keratin surrounding the fungi so that themorphology ofthefungicanbeseen.Adropofthis solutionisplacedonacleanglassslide. Asmallpiece ofthespecimen istransferred to this drop of KOHand covered withacoverslip.ThepreparationiskeptinaPetri Dishandkeptdampwithsomewetcottonwoolcontainedinit.Thetimetakentosoftenthemateria lwill dependonthetypeofspecimen.Hairwilltakeabout 10minutesandnails willtakeup to30 minutes.Gentleheatingoveraflamewillreducethetimerequiredtosoften/cleanthematerial.A ssoonasthespecimenissoftened,examineitmicroscopicallyusing10Xand40Xobjective.Loo k for branching hyphae, arthrospores and distinguish them from artifactslikeelasticfibres,strandsofcottonandcross-wallsarethecharacteristicsofpurehyphae.

Mucosae;ExamineunstainedwetpreparationsorinLactophenolCottonBlue

microscopically.Gramstainedsmearsmaybe prepared.

Sputum, Exudates and Body Fluids; Examine unstained wet preparations or inLactophenol Cotton Blue microscopically. If necessary (for opaque material), mount inKOH and heat gently.Further examine sputum after liquefaction with a mucolytic agentsuchasn acetylcysteine.Centrifugeand examinethe deposit.Prepareanother mount usingIndia Ink to demonstrate encapsulated yeasts(*Cryptococcus neoformans*).Examineexudates macroscopically forwhiteorcolouredgranules,crushanythatarepresent,betweentwoslides,stainby Gramandwithacid-faststains.Examinemicroscopically.

2)FUNGALSTAINING

Gram Stain: This can also be used to identify yeast, e.g. candida and cryptococcusaregram-positivewhile other fungidonot stainwithit.Thiswillalsodifferentiateinthe

case of mycetoma as to whether the causative organisms are fungi or actinomyces(grampositive rods).
MethenamineSilverStain:With thisstain,fungistain darkbrown.

Lactophenol Cotton Blue stain**;;**It stains Fungal hyphae with Blue Colour

t**India Ink Stain**; It stains the
back ground black and yeast
cells capsule appears as hollow.
CalcofluorWhiteStaining(fluorescentstaining)
Calcofluor white stains chitin-containing structures so that they fluoresce bright white under ultraviolet light in a fluorescent microscope. This stain in the microbiology laboratory has replaced the KOH (potassium hydroxide) wet mount because the contrast speeds examination.

Periodic Acid-Schiff (PAS): This stain has been used for various histopathologicalsmearsand sections for the identification of fungi in various tissues. The fungus willappearpinkincolour.

3)<u>THECULTIVATIONOFFUNGI;</u>

The followingmedia areroutinelyusedforculturesoffungus:

SabouraudDextroseAgar

SabouraudChloramphenicol/gentamicinAgar

Sabouraud Chloramphenicol/Gentamicin with Cyclohexamide (Actidione) Agar(fordermatophytes)

- Dermatophyte agar.
- RPMI (Roswell Park Memorial Institute)agar.

- Potato Dextrose Agar (PDA)
-

TrypticaseSoyaBroth(forbloodculture)

The general nutritional and cultural requirements of fungi differ from those of bacteria.Theygenerallygrowmoreslowlythanbacteria.Fungigrowbestatalowp Hi.e.betwee n5.0and6.0andcantoleratesugarconcentrationupto50%(sucrose).Theycan, therefore, grow on media that would exclude most bacteria. Sabouraud‘sAgar is amedium which

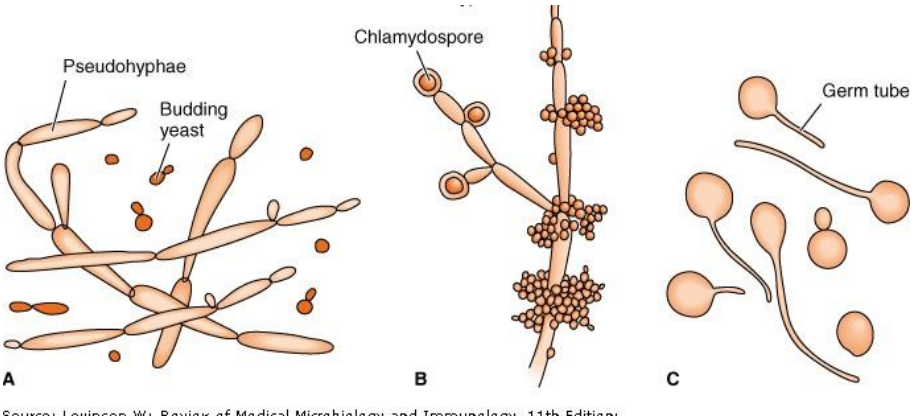
provides all of these conditions.Three plates or tubes are inoculated fordermatophytes; one plain Sabouraud,onewithout Cyclohexamide and thethird withChloramphenicol/Gentamicin.Cyclohexamide makes the medium selective fordermatophytes andinhibitsthegrowthofotherfungi.Themediumisincubatedaerobicallyfortwoweeksat22-28oCandisexamineddailyforgrowth

4)THEIDENTIFICATIONOF FUNGI

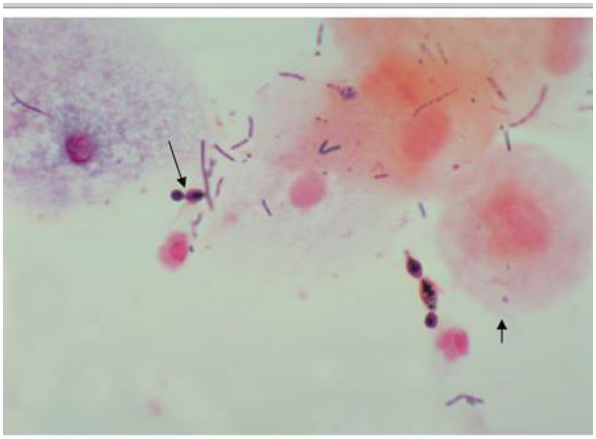
Once growthappearson the culturemedium, itscolonialmorphology, growthrate,colourandpresenceofpigmentationinthemediumisnoted.Fromthegrowth,takea part withastraightneedleorwireloopandemulsifyinLactophenolBlueon aslide,coverwithacoverslipandseeunderlowandhighpowerofthemicroscope.Mostoftheide ntification of fungi is based on their morphology. Alternatively, press a small piece ofclearvinyltape,e.g.Cello-tape,adhesivesidedown,ontothesurfaceofthecolony. Remove, and placethe tapeontoadropofLactophenolBlueon aslideandexaminedirectlyunderthemicroscope.

OTHERMETHODSOFIDENTIFYINGFUNGI

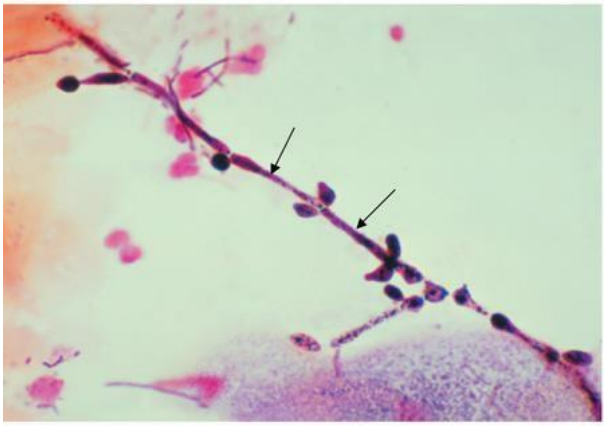
Germ Tube Test for *Candida albicans*: Place0.5 ml of serum (human or horse) in asmalltesttube.Emulsify asmallportionoftheyeastcolony obtainedafteran overnightgrowthofthespecimenonSabouraud's Agar.Incubatethetube at37oC for2hours. Place adropofthisserumon aslide,place acoverslip andexamine microscopically forgermtubeproductioni.ecylindrical filamentsoriginatingfromtheyeastcells.



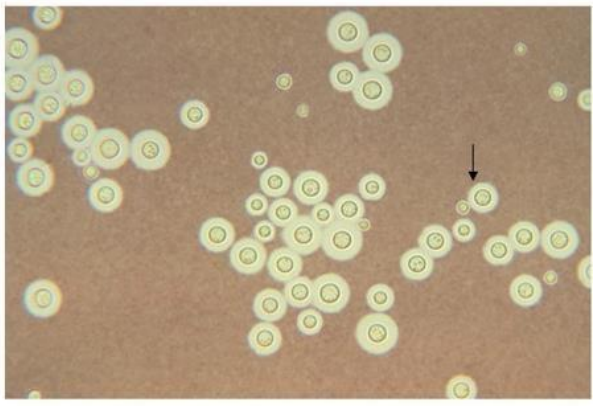
*Candidaalbicans.***A:**Buddingyeastsandpseudohyphaeintissuesorexudate.**B:**Pseudohypha eandchlamydospores inculture at20°C.**C:**Germtubes at37°C.



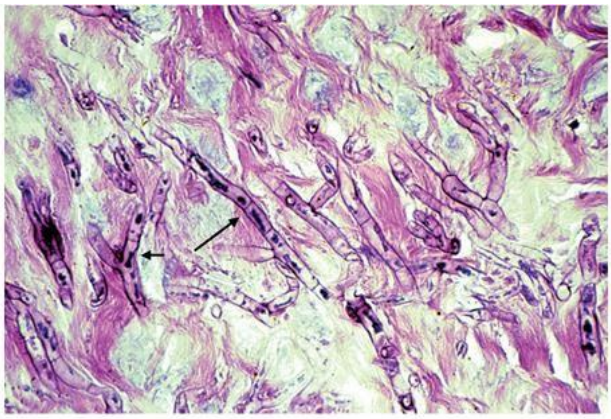
Candidaalbicans—Yeast. Longarrowpointstoabuddingyeast.



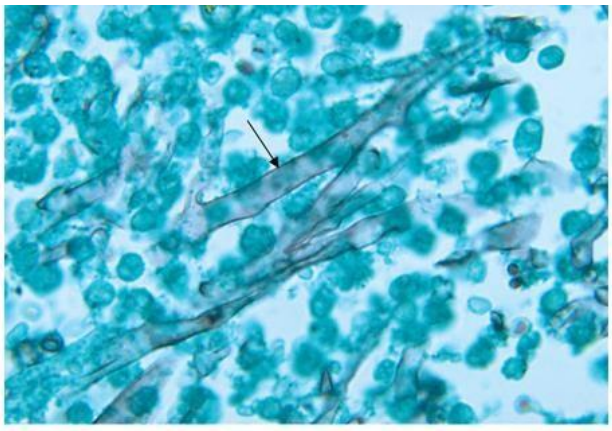
Candidaalbicans—Pseudohyphae. Twoarrowspointtopseudohyphaeof*Candidaalbicans*



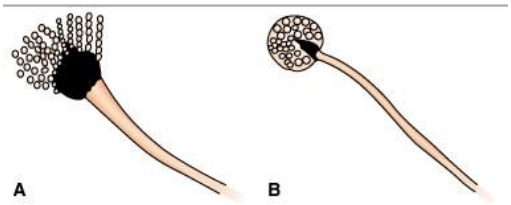
Cryptococcusneoformans—Indiaink preparation.Arrowpointsto abuddingyeast of*Cryptococcusneoformans*. Notethethick, translucentpolysaccharidecapsuleoutlinedbythedarkIndiainkparticles



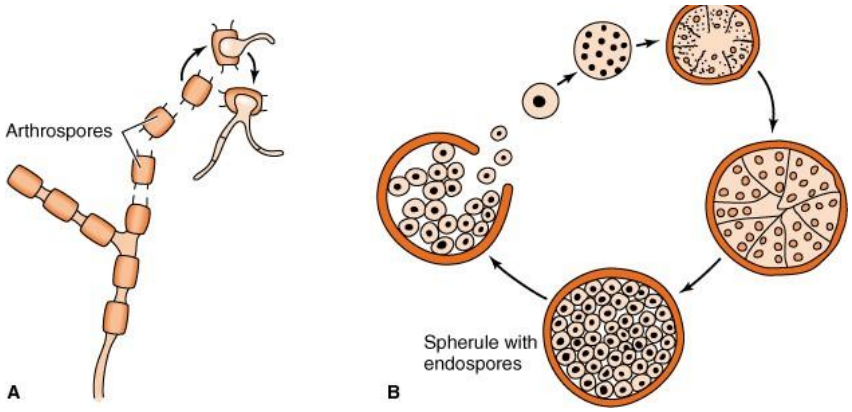
Aspergillusfumigatus—
Septatehyphae.Longarrowpointstotheseptatehyphaeof*Aspergillus.*Note the straight parallel cell walls of this mold. Short arrow points to the typical low-angle, Y-shapedbranching



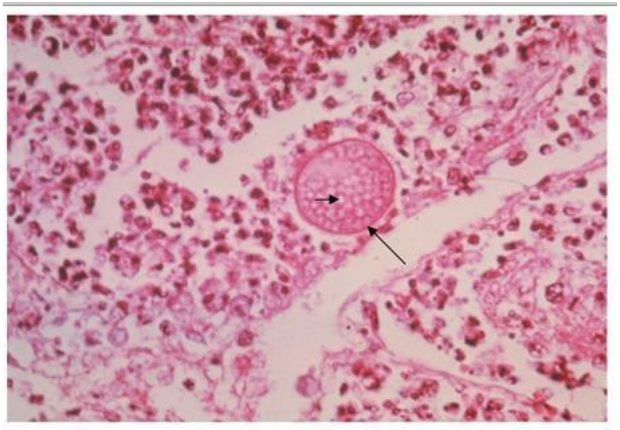
*Mucor*species—Non-septatehyphae. Arrowpointstoirregular-shaped, nonseptatehyphaeof *Mucor*



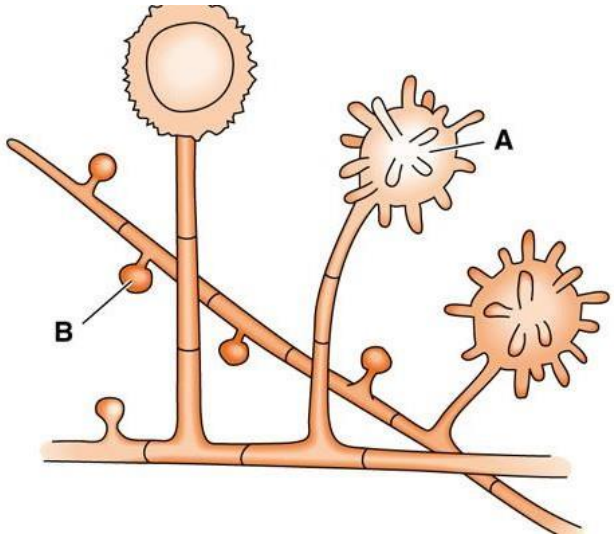
*Aspergillus*and*Mucor*inculture.**A:***Aspergillus*sporesforminradiatingcolumns.**B:***Mucor* sporesarecontainedwithinasporangium



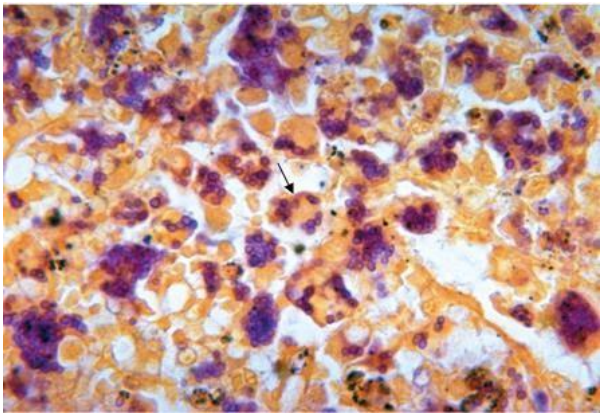
Stages of *Coccidioidesimmitis*. **A:** Arthrospores form at the ends of hyphae in the soil. Theygerminateinthesoiltoformnewhyphae.Ifinhaled,thearthrosporesdifferentiateintospher ules.**B:** Endospores form within spherules in tissue. When spherules rupture, endospores disseminateandformnewspherules.



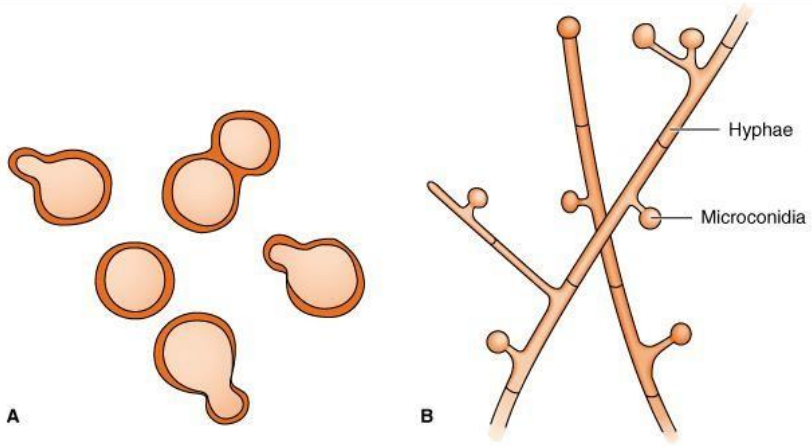
Coccidioidesimmitis—Spherule. Long arrow points to a spherule in lung tissue. Spherules arelargethick-walledstructurescontainingmanyendospores.Shortarrowpointstoanendospore.



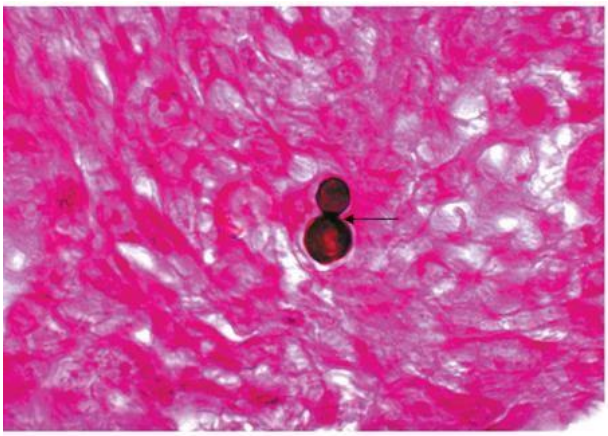
Asexualsporesof*Histoplasmacapsulatum*.**A:**Tuberculatemacroconidia.**B:**Microconidia



Histoplasma capsulatum—Yeasts within macrophages. Arrow points to a macrophagecontainingseveralpurple-stainedyeastsinthecytoplasm. Yeastswithinmacrophagescanbeseeninmanymacrophagesinthisspecimenofspleen.



Blastomycesdermatitidis.**A:**Yeastwithabroad-basedbudat37°C.**B:**Moldwithmicroconidiaat20°C



Blastomycesdermatitidis—Broad-basedbuddingyeast.Arrowpointstothebroadbaseofthebuddingyeast.

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