Textbook of
MEDICAL
PARASITOLOGY

ANTONI VAN LEEUWENHOEK
Born: 24.10.1632 - Died: 30.8.1723
Delft-Holland

This man, born poor, with little education, a draper in his hometown of Delft had surprising visitors! They included great men of science as well as the Royalty like the Tsar Peter the Great, Frederick the Great of Prussia and King James II of England. This was due to his hobby of grinding fine lenses through which he looked at various objects and brought forth the wonder world of small things that none had seen before. He kept clear descriptions and accurate drawings of what he saw and communicated them to the Royal Society in London. A strict check convinced the Society of their authenticity. The unlettered Antoni was elected a Fellow of the Royal Society! The papers sent by him over decades can still be seen in the Philosophical Transactions of the Royal Society.

The discoveries he made are legion. He described the first protozoan pathogen Giardia. He also discovered many types of bacteria, human and animal spermatozoa and eggs of various animals realizing their importance in reproduction. He could not recognize the significance of the different types of bacteria and to him, they were just 'little animalcules'. His fault was in being much before the time, for it took two centuries more for people to accept the microbial origin of infectious diseases. But that should not deter us from acknowledging the great contributions made by Leeuwenhoek to Biology and many other branches of Science. He was truly the **Founder of Microbiology.**

Textbook of MEDICAL PARASITOLOGY

SIXTH EDITION

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Preface to the Sixth Edition

This, the 6th edition ot the Textbook of Medical Parasitology comes after 18 years of its birth, which is a milestone! In India, an 18 year-old can vote and choose who is to rule the land. By the same analogy, this book has come of age and can decide its own fate.

Till now the Author was guided largely by the views of the students and teachers using the book. While they were generally happy with the narrative style, many had asked for improvements in pictures, both in their numbers and quality. So the emphasis this time has been here. Several pictures have been added, many in pretty colours, hoping they may attract and arrest the readers' attention.

The ultimate judges of a textbook are the students and the teachers. As in the past, we solicit their opinion and suggestions for improving the quality of the book.

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Preface to the First Edition

Parasitic infections continue to account for a large part of human illness. Antimicrobial drugs and vaccines that have made possible the effective control of most bacterial and viral diseases have not been as successful against parasitic infections. The numbers of persons afflicted by parasites run into many millions. Malaria still affects over 500 millions, pinworm and whipworm 500 millions each, hookworm 800 millions and roundworm a billion persons. Filariasis, leishmaniasis and schistosomiasis remain serious public health problems. Infections due to opportunist parasites are becoming increasingly evident in the affluent countries.

In recent years there has been a resurgence in the study of parasitic infections. Much new knowledge has been gained making possible precise diagnosis and more effective control of parasites and the diseases they cause.

This textbook attempts to present the essential information on parasites and parasitic diseases, with emphasis on pathogenesis, epidemiology, diagnosis and control. Every effort has been made to incorporate recent advances in the subject.

It is hoped that medical students, teachers and physicians will find this book useful. Their comments and suggestions for improvement of the book will be most welcome.

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CHAPTER 1

General Introduction

The earliest agents of human infection to have been observed were helminthic parasites. The common roundworm, often passed live and wriggling in stools, or emerging from the nostril of an infected child, would surely have caught the attention of ancient humans and could have been associated with illness. However, in some cultures the worms were considered as even useful, helping in the digestion of food. According to an old Chinese belief, a person had to have at least three worms to be in good health!

Intestinal worms and their empirical remedies were apparently known from early antiquity in different parts of the world. The well-preserved body of a young man who died on the snow-clad Alps mountain some 5300 years ago was discovered in 1991. Whipworm eggs were identified in the colonic contents. A pouch tied to the body contained plant materials with anthelmintic properties. This finding takes the history of human helminthic infection back to over five millennia.

In more recent times, parasites have figured in various milestones along the story of infectious disease. The first description of a human pathogenic microbe was given by the pioneer microscopist Leeuwenhoek in 1681, when he observed Giardia in his own stools and communicated to the Royal Society of London, unmistakably accurate diagrams of the protozoan parasite. In the 19th century, when the silkworm disease Pebrine caused devastating epidemics in Southern Europe, Louis Pasteur was requested to investigate it. Pasteur's results published in 1870 served to control the disease, which was caused by a microsporidian parasite. This was the first instance of a scientific study on a protozoal disease, leading to its control and prevention. This also was Pasteur's first introduction to applied microbiology.

With the coming of colonialism, interest in parasitic diseases suddenly soared as many of the tropical countries could be penetrated only after controlling parasitic infections like malaria, kala-azar, amoebiasis, trypanosomiasis and schistosomiasis. Their aetiological agents were identified and control measures introduced. A seminal discovery was made in 1878 by Patrick Manson about the role of mosquitoes in filariasis. This was the first evidence of vector transmission. Soon afterwards, Laveran in Algeria discovered the malarial parasite (1880) and Ronald Ross in Secunderabad,

India showed its transmission by mosquitoes (1897). A large number of vector borne diseases have since been identified. This provided a new approach to disease control, by targeting the vectors.

Many parasitic infections are associated with overcrowding, poor sanitation, contaminated food and water, undernutrition and other poverty-related factors. They were considered the concern of the developing countries only. While this is generally true, the rich nations are not exempt, and infact there are some parasites like the pinworm which are more prevalent in the West.

A major drawback in the fight against parasitic diseases is the inability to prevent them by immunisation. No effective vaccine is currently available against any parasitic disease. However, host immunity is decisive in determining the course of many parasitic infections. Increased susceptibility to many parasitic infections is a consequence of immunodeficiency, as in the HIV infected. Many new parasitic infections have been identified in AIDS patients in the developed countries.

Control and eradication programmes had been carried out against some important parasitic diseases, such as malaria and filariasis, with varying degrees of success. But in many cases the benefits gained could not be maintained and the situation has reverted to the original level or worse.This has been due to slackening of control measures or due to drug resistance in the parasite or its vector.

By mid-twentieth century, with dramatic advances in antibiotics and chemotherapy, insecticides and antiparasitic drugs, and increased affluence and improved lifestyles, all infectious diseases seemed amenable to control. Great dreams of eradicating infectious diseases were entertained and when global eradication of the great scourge smallpox became a reality, euphoria prevailed. Then came nemesis, with microbes rebounding. Antibiotics and antipesticides lost their efficacy, faced with microbial and vector resistance. New emerging diseases became a serious threat. The HIV pandemic provided a fertile field for old and new pathogens to spread. This applies equally to parasitic infections as to bacterial, viral or mycotic infections. In this context a new enhanced interest attaches to the study of human parasites.

PARASITISM

Medical parasitology deals with the parasites which cause human infections and the diseases they produce. Parasites are organisms that infect other living beings. They live in or on the body of another living being, the *host* and obtain shelter and nourishment from it. They multiply or undergo development in the host. Parasitism arose early in the course of biological evolution. Some organisms, instead of remaining as free-living forms deriving nourishment from raw materials in the environment, learned to use the bodies of other organisms as readymade food. One manner of achieving this is by *predation*, where larger animals prey on smaller ones which they kill and eat. Another is *saprophytism* (from Sapros, Greek for decayed), in which organisms feed on the dead and decaying bodies of animals, plants and other organic matter and help to decompose them. *Parasitism* is a more durable and intimate association in which the parasite establishes itself in or on the living body of the

host, being physically and physiologically dependent on it for at least part of its life cycle. This may or may not lead to disease in the host. Parasites which live in complete harmony with the host, without causing any damage to it are called *commensals*, while those which cause disease are called *pathogens*. This distinction is however not absolute, as many commensals can act as facultative or opportunist pathogens when the host resistance is lowered. Rarely, even free-living organisms may become pathogenic under special circumstances.

The discipline of parasitology, by tradition deals only with parasites belonging to the animal kingdom. Though bacteria, fungi and viruses are also parasitic, they are excluded from the purview of 'parasitology.' Human parasites may be either unicellular microbes (protozoa), or larger organisms (metazoa), some of which may be many metres in size.

Parasites may be classified as ectoparasites or endoparasites. *Ectoparasites* inhabit the body surface only, without penetrating into the tissues. Lice, ticks, mites and other haematophagous arthropods are examples of ectoparasites. They are important as vectors transmitting pathogenic microbes. The term *infestation* is often employed for parasitisation with ectoparasites in place of the term infection used with reference to endoparasites. *Endoparasites* live within the body of the host. All protozoan and helminthic parasites of humans are endoparasites.

Parasites may pass their life cycles in more than one host. The host in which the adult stage lives or the sexual mode of reproduction takes place is called the *definitive* host. The species in which the larval stage of the parasite lives or the asexual multiplication takes place is called the *intermediate* host. Man is the definitive host for most human parasitic infections (e.g. filaria, roundworm, hookworm), but is the intermediate host in some instances (e.g. malaria, hydatid disease). A vertebrate host in which a parasite merely remains viable without development or multiplication is called a *paratenic* host. Such a host may serve to pass on the infection to another and so is sometimes called a *transport* host.

Parasites infecting humans may be proliferous or nonproliferous. *Proliferous* parasites are those that proliferate in the human body so that the parasite originally introduced multiplies many fold to cause high intensity of infection. Protozoan parasites are proliferous. On the other hand, most adult helminths do not multiply in the human body. They are *nonproliferous*. High intensity of infection results from repeated infection as in roundworm, or from high multiplicity of initial infection as in trichinosis. A few helminths, such as *Strongyloides stercoralis* and *Hymenolepis nana* multiply in the human host.

Parasitic infections which humans acquire from animals are known as zoonotic infections or *zoonoses*. In most of these, the parasite lives normally in cycles involving domestic or wild animals, *domestic zoonoses* and *feral* or *sylvatic zoonoses* respectively without affecting humans. Human infections are only accidental events and may not profit the parasite because the chain of transmission is usually broken with human infection. The vertebrate species in which the parasite passes its life cycle and which may act as the source of human infection is called the *reservoir host*. Intermediate hosts in which metazoan parasites undergo multiplication are called *amplifier hosts*.

The term *anthroponoses* has been applied for infections with parasitic species that are maintained in humans alone. Malaria and filariasis are exampIes. The term *zooanthroponoses* refers to infections in which human is not merely an incidental host, but an essential link in the life cycle of the parasite. Beef and pork tapeworms are examples of zooanthroponoses.

Sources of Infection

Parasitic infections originate from various sources and are transmitted by various routes. The major sources of infection are listed below:

Soil

a. Embryonated eggs which are present in soil may be ingested, e.g. roundworm, whipworm.
b. Infective larvae present in soil may enter by penetrating exposed skin, e.g. hookworm, strongyloides.

Water

a. Infective forms present in water may be swallowed, e.g. cysts of amoeba and giardia.
b. Water containing the intermediate host may be swallowed, e.g. infection with guinea worm occurs when the water that is drunk contains its intermediate host cyclops.
c. Infective larvae in water may enter by penetrating exposed skin, e.g. cercariae of schistosomes.
d. Free-living parasites in water may enter through vulnerable sites, e.g. Naegleria may enter through nasopharynx and cause meningoencephalitis.

Food

a. Contamination with human or animal feces, e.g. amoebic cysts. pinworm eggs, echinococcus eggs. toxoplasma oocysts.
b. Meat containing infective larvae, e.g. measly pork. *Trichinella spiralis*.

Insect Vectors

1. Biological vectors
   a. Mosquito—malaria, filariasis
   b. Sandflies—kala-azar
   c. Tsetseflies—sleeping sickness
   d. Reduviid bugs—Chagas' disease
   e. Ticks—Babesiosis.
2. Mechanical vectors
   a. Housefly—amoebiasis.

Animals

1. Domestic
   a. Cow, e.g. beef tapeworm, sarcocystis.
   b. Pig, e.g. pork tapeworm, *Trichinella spiralis*
   c. Dog, e.g. hydatid disease, leishmaniasis
   d. Cat, e.g. toxoplasmosis, opisthorchis.
2. Wild
   a. Wild game animals, e.g. trypanosomiasis.
   b. Wild felines, e.g. *Paragonimus westermani*
3. Fish, e.g. fish tapeworm
4. Molluscs, e.g. liver flukes
5. Copepods, e.g. guinea worm.

Other Persons

Carriers and patients, e.g. all anthroponotic infections, vertical transmission of congenital infections.

Self (autoinfection)

a. Finger to mouth transmission, e.g. pinworm.
b. Internal reinfection, e.g. strongyloides.

Modes of Infection

The major modes of transmission are the following:

Oral Transmission

The most common method of transmission is oral, through contaminated food, water, soiled fingers or fomites. Many intestinal parasites enter the body in this manner, the infective stages being cysts, embryonated eggs or larval forms. Infection with *Entamoeba histolytica* and other intestinal protozoa occurs when the infective cysts are swallowed. In most intestinal nematodes, such as the roundworm. whipworm or pinworm, the embryonated egg which is the infective form is swallowed. In trichinellosis and in beef, pork and fish tapeworm, infection occurs by ingestion of flesh containing the mature larval stages. Infection with the tissue nematode guinea worm follows consumption of water containing its arthropod host cyclops carrying infective larvae.

Skin Transmission

Entry through skin is another important mode of transmission. Hookworm infection is acquired when the larvae enter the skin of persons walking barefooted on contaminated soil. Schistosomiasis is acquired when the cercarial larvae in water penetrate

the skin. Many parasitic diseases, including malaria and filariasis are transmitted by blood sucking arthropods. Arthropods which transmit infection are called *vectors*.

Vector Transmission

Parasites undergo development or multiplication in the body of true vectors, which are called *biological vectors*. Some arthropods may transmit infective parasites mechanically or passively without the parasites multiplying or undergoing development in them. For example, the housefly may passively carry amoebic cysts from faeces to food. Such vectors which act only as passive transmitters are called *mechanical vectors*. In the case of a mechanical vector there need be no delay between picking up a parasite and transferring it to a host. A housefly picking up amoebic cysts from feces can within seconds transfer the cysts by landing on food being eaten by a person, who may thereby get infected. But in the case of biological vectors. A certain period has to elapse after the parasite enters the vector before it becomes infective. This is necessary because the vector can transmit the infection only after the parasite multiplies to a certain level or undergoes a developmental process in its body. This interval between the entry of the parasite into the vector arthropod and the time it becomes capable of transmitting the infection is called the *extrinsic incubation period*. For example, an Anopheles mosquito picking up *Plasmodium vivax* gametocytes from a person in its blood meal becomes capable of transmitting the infective stage of the malaria parasite only some ten days later, i.e. the extrinsic incubation period is ten days.

Direct Transmission

Parasitic infection may be transmitted by person-to-person contact in some cases; by kissing in the case of gingival amoebae and by sexual intercourse in trichomoniasis. Inhalation of air-borne eggs may be one of the methods of transmission of pinworm infection. Congenital infection (vertical transmission) may take place in malaria and toxoplasmosis. Iatrogenic infection may occur as in transfusion malaria and toxoplasmosis after organ transplantation.

Course of Infection

Following its establishment in the host, the parasite has to multiply or undergo development before the infection is manifested either biologically or clinically. The interval of time between the initial infection and the earliest appearance of the parasite or its products in the blood or secretions is called the *biological incubation period* or *prepatent period*. The prepatent period in malaria is about a week; in filariasis it is a year or more. When the parasite becomes demonstrable and the host is potentially infectious to others, the infection is said to be *patent*. *Clinical incubation period*,which is the interval between the initial infection and the onset of the first evidence of clinical disease is usually longer than the biological incubation period.

PATHOGENESIS

Parasitic infections may remain inapparent or give rise to clinical disease. A few, such as *Entamoeba histolytica* may live as surface commensals, multiplying in the 1umen of the gut for long periods without invading the tissues. Some parasites may lead to completely asymptomatic infection even though they live inside tissues. Many persons with filarial infection may not develop any clinical illness though microfilariae are demonstrable in their blood. Clinical infection produced by parasites may take many forms—acute, subacute, chronic, latent or recurrent. Some of the pathogenic mechanisms in parasitic infections are as follows:

Intracellular protozoa can damage and destroy the cells in which they multiply. Malarial parasites rupture the infected erythrocytes causing anaemia as a long-term effect and fever as the immediate response.

Enzymes produced by some parasites can induce lytic necrosis. *E. histolytica* lyses intestinal cells, enabling it to penetrate the gut wall and produce abscesses and ulcers.

Damage may be due to physical obstruction. Masses of roundworms cause intestinal obstruction. Even a single worm can cause damage when it blocks the appendix or bile duct. Hydatid cysts cause illness due to pressure on surrounding tissues. Parasites in vulnerable sites such as brain and eyes may produce serious damage by pressure. Physical obstruction may sometimes cause severe secondary effects. Falciparum malaria may produce blockage of brain capillaries leading to fatal cerebral malaria.

Clinical disease may sometimes be due to trauma inflicted by parasites. Hookworms feeding on jejunal mucosa leave numerous bleeding points which ultimately lead to anaemia. Migration of helminth larvae through the lungs may rupture many pulmonary capillaries and cause considerable extravasation of blood. Schistosome eggs with their hooks tear vesical blood vessels and produce haematuria. Roundworms may perforate the intestine and cause peritonitis.

Clinical illness may be caused by host response to parasitic infection. This may be due to inflammatory changes and consequent fibrosis, as in the case of filariasis in which it leads ultimately to lymphatic obstruction and oedema. Host response may also be hypersensitive or allergic. Fatal anaphylactic shock may occasionally be caused by escape of hydatid fluid from the cyst.

A few parasitic infections have been shown to lead to malignancy. The liver flukes Clonorchis and Opisthorchis may induce bile duct carcinoma and *Schistosoma haematobium* may pave the way for bladder cancer.

Migrating parasites may seed bacteria and viruses in ectopic foci, leading to disease. Strongyloidiasis, particularly in the immunodeficient person may result in gram-negative bacillary septicaemia as the migrating helminth transports intestinal bacteria to the circulation.

IMMUNITY IN PARASITIC INFECTIONS

Like other infectious agents, parasites also elicit immune responses in the host, both humoral as well as cellular. But immunological protection against parasitic infections

is much less efficient than it is against bacterial and viral infections. Several factors may contribute to this.

Compared to bacteria and viruses, parasites are enormously larger and more complex structurally and antigenically so that the immune system may not be able to focus attack on the protective antigens. Many protozoan parasites are intracellular in location and this protects them from immunological attack. Several parasites, both protozoa and helminths live inside body cavities as in the intestines. This location limits the efficiency of immunological attack and also facilitates dispersal of the infective forms. Secretory IgA which is so effective against luminal virus infections does not appear to play an important role in defence against parasites. Some parasites live within cysts whose capsules are partly composed of host tissues. In this location they are safe from immunological attack.

Trypanosomes causing sleeping sickness exhibit antigenic variation within the host. When antibody response to one antigenic form reaches high levels, a genetic switch causes a new set of antigens to appear, which are unaffected by the antibodies present. This enables the prolonged persistence of the parasites in the host. A similar mechanism may be operative in the recrudescences in human malaria.

Some parasites adopt antigenic disguise. Their surface antigens are so closely similar to some host components that they are not recognised as foreign by the immune system. Many nematodes have a cuticle which is antigenically inert and evokes little immune response. Immunological tolerance is established in some parasitic infections. Some infections may produce immunodeficiency due to extensive damage to the reticuloendothelial system, as for example in visceral leishmaniasis.

Unlike in other microbial infections, complete elimination of the infecting agent followed by immunity to reinfection is seldom seen in parasitic infections. A possible exception is cutaneous leishmaniasis in which the initial infection heals, leaving behind good protection against reinfection. However, the general situation in parasitic infections is that immunity to reinfection lasts only so long as the original infection persists at least in a small degree. Once the parasitic infection is completely eliminated, by natural means or by therapy, the host becomes again susceptible to reinfection. This type of immunity to reinfection dependent on the continued presence of a residual parasite population is known as *premunition*. A similar phenomenon is seen in syphilis.

In most parasitic infections a balance is established, the parasite being kept in check by the host without being completely eliminated. This may be achieved by the immune response controlling the numbers of the parasite (*numerical restraint*) or by limiting the space it occupies (*topical restraint*). The fact that immunity normally plays an important role in the containment of parasitic infections is illustrated by the florid manifestations caused by opportunistic parasites such as *Pneumocystis carinii* and *Toxoplasma gondii* when the immune response is inadequate as in AIDS and other immunodeficiencies.

Immune response to parasitic infections has been employed for diagnostic purposes. Antibodies to the infecting parasites may be demonstrated by various serological techniques, but serodiagnosis in parasitic infections is vitiated by numerous cross reactions and so does not have the precision and specificity present in bacterial and

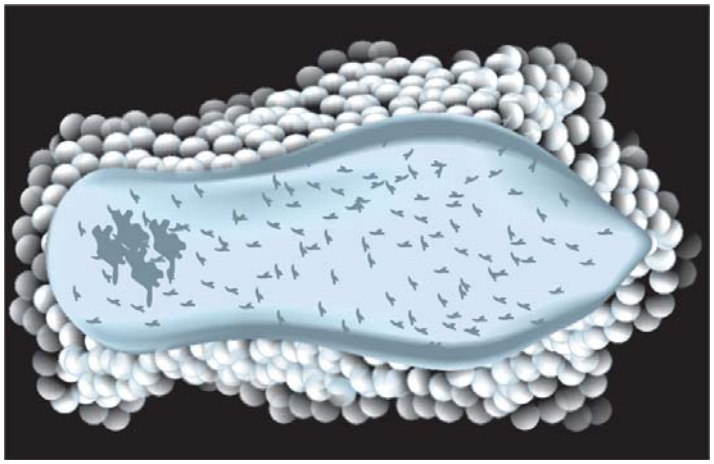


FIGURE 1.1: Eosinophils surrounding schistosomulum
(An example of immune attack in bloodstream)

viral infections. Antibodies belonging to the different immunoglobulin classes are produced in response to parasitic infections. Selective tests for IgM antibodies are helpful in differentiating current from old infections. However, IgA antibodies are not very prominent. Instead there occurs an excessive IgE response, particularly in intestinal helminthiases. Polyclonal activation of B lymphocytes with excessive production of irrelevant immunoglobulins is seen in some parasitic disease, as in kala-azar. Cell-mediated response to parasitic antigens also has been employed in diagnostic tests, but here again cross reactions are frequent. A characteristic cellular response in parasitic infection is eosinophilia, both local and systemic (Fig. 1.1).

Immunoprophylaxis and immunotherapy have not been significantly successful in parasitic infections. Though no vaccine is as yet available for any parasitic disease, great progress has been made in identifying protective antigens in malaria and some other infections, with a view to eventual development of prophylactic vaccines.

CHAPTER 2

Protozoa: General Features

Single-celled microorganisms belonging to the animal kingdom are classified as *Protozoa* (Greek *protos*—first; *zoon*—animal). Within its single cell, the protozoon contains all structures required for performing its various functions. Some free-living protozoa resemble plants in containing green plastids that enable them to perform photosynthesis. It is believed that these represent the earliest forms of animal life. Numerous varieties of protozoa have evolved to suit all manner of environmental conditions. Free-living protozoa are found in all habitats—in deep ocean or in shallow fresh waters, in hot springs or in ice, under the soil or in snow on mountain tops. Parasitic protozoa have, however adapted to different host species, with more restricted physicochemical requirements.

Protozoa exhibit a wide range of size, shape and structure, yet all possess certain essential common features. The typical protozoan cell is bounded by a trilaminar unit membrane, supported by a sheet of contractile fibrils which enable the cell to change its shape and to move. The cytoplasm can often be differentiated into an outer rim of relatively homogeneous ectoplasm and a more granular inner endoplasm. The ectoplasm serves as the organ of locomotion and for engulfment of food materials by putting forth pseudopodial processes. It also functions in respiration, in discharging waste materials and also as a protective covering for the cell.

Within the endoplasm is the nucleus within a tough nuclear membrane. The nucleus is usually single, but may be double or multiple, some species having as many as a hundred nuclei in one cell. The nucleus contains one or more nucleoli or a central endosome or karyosome. The chromatin may be distributed along the inner surface of the nuclear membrane (peripheral chromatin) or as condensed masses around the karyosome. The endoplasm shows a number of structures—the endoplasmic reticulum, mitochondria and Golgi bodies. Contractile vacuoles may be present which serve to regulate the osmotic pressure. Several food vacuoles also may be seen.

The active feeding and growing stage of the protozoa is called the *trophozoite* (G.trophos-nourishment). The cell may obtain nourishment from the environment by diffusion or by active transport across the plasma membrane. Larger food particles are taken in by phagocytosis through pseudopodia. Some species ingest food through

special mouth-like structures or cytostomes. Minute droplets of food may also enter by pinocytosis. Several species possess a resting or resistant cystic stage which enables prolonged survival under unfavourable conditions. The cystic stage may also involve reproduction by the nucleus dividing once or more to give rise to daughter trophozoites on excystation. The cyst is usually the infective stage for the vertebrate host.

Reproduction is usually asexual. The most common method is binary fission by mitotic division of the nucleus, followed by division of the cytoplasm. In amoebae, division occurs along any plane, but in flagellates division is along the longitudinal axis and in ciliates in the transverse plane. Some protozoa, as for instance the malaria parasites exhibit schizogony in which the nucleus undergoes several successive divisions within the schizont to produce a large number of merozoites. Sexual stages are seen in ciliates and sporozoa. In ciliates the sexual process is conjugation in which two organisms join together and reciprocally exchange nuclear material. In sporozoa, male and female gametocytes are produced, which after fertilisation form the zygote giving rise to numerous sporozoites by sporogony.

CLASSIFICATION OF PROTOZOA

Protozoan parasites of medical importance have been classified into the following groups or Phyla: Sarcomastigophora, Apicomplexa, Microspora and Ciliophora.

A. Phylum Sarcomastigophora

Phylum Sarcomastigophora has been subdivided into two subphyla based on their modes of locomotion—Amoebae which have no permanent locomotory organs, but move about with the aid of temporary prolongations of the body called pseudopodia are grouped under subphylum *Sarcodina* (Sarcos, meaning flesh or body); and protozoa possessing whip-like flagella are grouped under subphylum *Mastigophora* (Mastix, meaning whip or flagellum).

Amoebae

These protean animalcules assume any shape and crawl along surfaces by means of foot-like projections called *pseudopodia* (literally meaning false feet).They are structurally very simple and are believed to have evolved from the flagellates by the loss of the flagella. Two groups of amoebae are of medical importance.

(a) *Amoebae of the alimentary canal:* The most important of these is *Entamoeba histolytica* which causes intestinal and extraintestinal amoebiasis. Amoebae are also present in the mouth.

(b) *Potentially pathogenic free-Iiving amoebae:* Several species of saprophytic amoebae are found in soil and water. Two of these, Naegleria and Acanthamoeba are of clinical interest because they can cause eye infections and fatal meningoencephalitis.

Flagellates

These protozoa have whip-like appendages called flagella as the organs of locomotion. The fibrillar structure of flagella is identical with that of spirochaetes and it has been suggested that they may have been derived from symbiotic spirochaetes which have become endoparasitic. In some species the flagellum runs parallel to the body surface, to which it is connected by a membrane called the undulating membrane. Flagellates parasitic for man are divided into two groups:

(a) *Kinetoplastida:* These possess a kinetoplast from which arises a single flagellum. They are the *haemoflagellates* comprising the trypanosomes and leishmania which are transmitted by blood sucking insects and cause systemic or local infections.

(b) *Flagellates without kinetoplast:* These bear multiple flagella. Giardia, trichomonas and other *luminal flagellates* belong to this group. Because most of them live in the intestine, they are generally called *intestinal flagellates*.

B. Phylum Apicomplexa

Phylum Apicomplexa formerly known as Sporozoa, members of this group possess at some stage in their life cycle, a structure called the *apical complex* serving as the organ of attachment to host cells. They are tissue parasites. They have a complex life cycle with alternating sexual and asexual generations. To this group belong the malaria parasites (Suborder Haemosporina, Family Plasmodiidae); toxoplasma, sarcocystis, isospora and cryptosporidum (under the Suborder Eimeriina); babesia (under the Subclass Piroplasma); and the unclassified *Pneumocystis carinii*.

C. Phylum Microspora

Phylum microspora contains many minute intracellular protozoan parasites which frequently cause disease in immunodeficient subjects. They may rarely also cause illness in the immunocompetent.

D. Phylum Ciliophora

These protozoa are motile by means of cilia which cover their entire body surface. The only human parasite in this group is *Balantidium coli* which rarely causes dysentery.

The zoological classification of protozoa is complex and subject to frequent revisions. The following is an abridged version of the classification proposed in 1980 by the Committee on Systematics and Evolution of the Society of Protozoologists, as applied to protozoa of medical importance.

Kingdom ANIMALIA

Subkingdom PROTOZOA

PHYLUM	SARCOMASTIGOPHORA (having flagella or pseudopodia)
Subphylum	MASTIGOPHORA (having one or more flagella)
Class	ZOOMASTIGOPHORA

Order	KINETOPLASTIDA
Suborder	TRYPANOSOMATINA
Genus	*Trypanosoma*
	Leishmania
Order	RETORTAMONADIDA (two or four flagella; cysts present)
Genus	*Retortamonas*
	Chilomastix
Order	DIPLOMONADIDA
Suborder	ENTEROMONADINA
Genus	*Enteromonas*
Suborder	DIPLOMONADINA
Genus	*Giardia*
Order	TRICHOMONADIDA
Genus	*Trichomonas*
	Dientamoeba
Subphylum	SARCODINA (pseudopodia present)
Superclass	RHIZOPODA
Class	LOBOSEA
Order	AMOEBIDA
Suborder	TUBULINA
Genus	*Entamoeba*
	Endolimax
	Iodamoeba
Suborder	ACANTHOPODINA
Genus	*Acanthamoeba*
Order	SCHIZOPYRENIDA
Genus	*Naegleria*
PHYLUM	APICOMPLEXA (possessing apical complex)
Class	SPOROZOEA
Subclass	COCCIDIA
Order	EUCOCCIDIA
Suborder	EIMERIINA
Genus	*Cryptosporidium*
	Isospora
	Sarcocystis
	Toxoplasma
Suborder	HAEMOSPORINA
Genus	*Plasmodium*
Subclass	PIROPLASMIA
Order	PIROPLASMIDA
Genus	*Babesia*
PHYLUM	CILIOPHORA (possessing cilia)
Order	TRICHOMASTIDA
Genus	*Balantidium*

CHAPTER 3

Amoebae

Amoebae are structurally simple protozoa which have no fixed shape. They are classified under the Phylum-Sarcomastigophora, Subphylum-Sarcodina, Superclass-Rhizopoda, Order-Amoebida. The cytoplasm is bounded by a unit membrane and can be differentiated into an outer ectoplasm and an inner endoplasm. Pseudopodia are formed by the ectoplasm thrusting out, being followed by the endoplasm flowing in, to produce blunt projections. Pseudopodial processes appear and disappear, producing quick changes in the shape of the cell. These are employed for locomotion and engulfment of food by phagocytosis. Amoebae may be free-Iiving or parasitic. A few of the free-living amoebae can, on occasion act as human pathogens, producing meningoencephalitis and other infections. Some of them can act as carriers of pathogenic bacteria. The parasitic amoebae inhabit the alimentary canal.

PARASITIC AMOEBAE

Parasitic amoebae belong to the following genera:

	Genus	*Species*
1.	*Entamoeba*	*E.histolytica, E.hartmanni, E.coli, E.polecki*
2.	*Endolimax*	*E.nana*
3.	*Iodamoeba*	*I.butschlii*
4.	*Dientamoeba*	*D.fragilis* (now classified as Amoeboflagellate)

Entamoeba histolytica is an important human pathogen, causing amoebic dysentery as well as hepatic amoebiasis and other extraintestinal lesions. *E.hartmanni* is non-pathogenic, though it resembles *E. histolytica* very closely except for its smaller size and was therefore known as the 'small race' of *E. histolytica*. *E.polecki* a natural parasite of pigs and monkeys may sometimes infect humans causing diarrhoea. *E. coli* is a common commensal in the colon and its importance is that it may be mistaken for *E.histolytica*. *E.gingivalis* is present in the mouth, being found in large numbers when the oral hygiene is poor. It has no cystic stage and so the trophozoites depend for transmission on direct oral contact as in kissing, air-borne spread through salivary droplets and fomites such as shared drinking and eating utensils. It is

generally nonpathogenic, though it has been claimed that it contributes to periodontal disease.

All the genera of intestinal amoebae other than *Entamoeba* are nonpathogenic commensals, except *D.fragilis*, which may occasionally cause chronic, but mild intestinal symptoms. Intestinal amoebae can be differentiated based on their morphological features.

ENTAMOEBA HISTOLYTICA

History

Entamoeba histolytica was discovered in 1875 by Losch in the dysenteric feces of a patient in St Petersburg, Russia. He also observed it in colonic ulcers at autopsy and produced dysentery in a dog by inoculation through the rectum. In 1890, William OsIer reported the case of a young man with dysentery who later died of liver abscess. Councilman and Lafleur in 1891 established the pathogenesis of intestinal and hepatic amoebiasis and introduced the terms 'amoebic dysentery' and 'amoebic liver abscess.'

Geographical Distribution

E. histolytica is world-wide in prevalence. It is much more common in the tropics than elsewhere, but it has been found wherever sanitation is poor, in all climatic zones, from Alaska (61° N) to the Straits of Magellan (52°S). It has been reported that about 10 per cent of the world's population and 50 per cent of the inhabitants of some developing countries may be infected with the parasite. The infection is not uncommon even in affluent countries, about 1 per cent of Americans being reported to be infected. While the large majority of the infected are asymptomatic, invasive amoebiasis causes disabling illness in an estimated 50 million persons and death in 50,000 annually, mostly in the tropical belt of Asia. Africa and Latin America. It is the third leading parasitic cause of mortality, after malaria and schistosomiasis.

E. histolytica is found in the human colon. Natural infection also occurs in monkeys, dogs and probably in pigs also but these animals do not appear to be relevant as sources of human infection. Infection is mostly asymptomatic. It commonly occurs in the lumen of the colon as a commensal, but sometimes invades the intestinal tissues to become a pathogen.

Morphology

E. histolytica occurs in three forms—the trophozoite, precystic and cystic stages (Fig. 3.1).

Trophozoite

The trophozoite or the vegetative form is the growing or feeding stage of the parasite. It is irregular in shape and varies in size from about 12 to 60 µm. It is large and actively motile in freshly passed dysenteric stools, while in convalescents

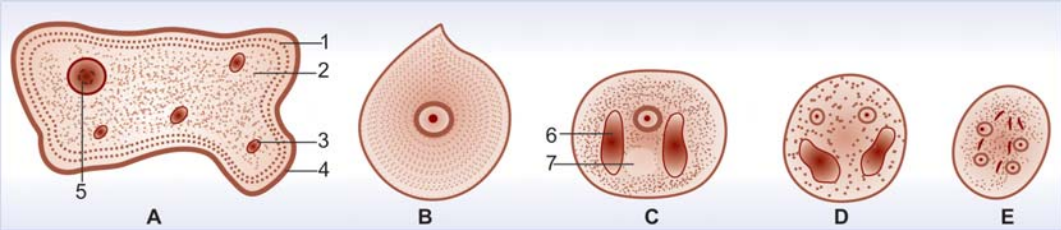


FIGURE 3.1: *Entamoeba histolytica.* (A) Trophozoite; (B) Precystic stage; (C) Uninucleate cyst; (D) Binucleate cyst; (E) Mature quadrinucleate cyst; 1—Ectoplasm; 2—Endoplasm; 3—Ingested erythrocytes; 4—Pseudopodium; 5—Nucleus; 6—Chromidial bar; 7—Glycogen mass

and carriers, it is much smaller. The parasite as it occurs free in the lumen as a commensal is generally smaller in size, about 15 to 20 µm and has been called the *minuta* form.

The protoplasm is differentiated into a thin outer layer of clear, transparent, refractive ectoplasm and an inner finely granular endoplasm having a ground glass appearance. Pseudopodia are formed by a sudden thrusting movement of the ectoplasm in one direction, followed by the streaming in of the whole endoplasm. The direction of movement may be changed suddenly, with another pseudopodium being formed at a different site, when the whole cytoplasm flows in the direction of the new pseudopodium. Typical amoeboid motility is a crawling or gliding movement and not a free-swimming one. The cell has to be attached to some surface or particle for it to move. In culture tubes, the trophozoites may be seen crawling up the side of the glass tube. Pseudopodium formation and motility are inhibited at low temperatures.

The endoplasm contains the nucleus, food vacuoles and granules. The nucleus is not clearly seen in the living trophozoite, but can be distinctly demonstrated in preparations stained with iron-haematoxylin or Gomorri's trichrome stains. The nucleus is spherical, 4 to 6 µm in size and contains a small central karyosome surrounded by a clear halo. The karyosome is anchored to the inner surface of the nuclear membrane by fine radiating fibrils called the linin network giving a 'cartwheel appearance.' The delicate nuclear membrane is lined by a rim of chromatin distributed evenly as small granules.

The trophozoites from acute dysenteric stools often contain phagocytosed erythrocytes. This feature is diagnostic as phagocytosed red cells are not found in any other commensal intestinal amoebae.

The trophozoite divides by binary fission once in about 8 hours. Trophozoites are delicate organisms and are killed by drying, heat and chemical disinfectants, They do not survive for any length of time in stools outside the body. Therefore, the infection is not transmitted by trophozoites. Even if live trophozoites from freshly passed stools are ingested, they are rapidly destroyed in the stomach and cannot initiate infection.

Precystic Stage

Some trophozoites undergo encystment in the intestinal lumen. Encystment does not occur in the tissues nor in feces outside the body. Before encystment the trophozoite extrudes its food vacuoles and becomes round or ovoid about 10 to 20 µm in size. This is the precystic stage of the parasite. It secretes a highly refractile cyst wall around it and becomes the cyst.

Cystic Stage

The cyst is spherical, about 10 to 20 µm in size. The early cyst contains a single nucleus and two other structures—a mass of *glycogen* and one to four *chromatoid bodies* or *chromidial bars*, which are cigar-shaped or oblong refractile rods with rounded ends. The chromatoid bodies are so called because they stain with haematoxylin like chromatin. As the cyst matures, the glycogen mass and chromidial bars disappear. The nucleus undergoes two successive mitotic divisions to form two and then four nuclei. The mature cyst is quadrinucleate. The nuclei and chromidial bodies can be made out in unstained films, but they appear more prominently in stained preparations. With iron-haematoxylin stain the nuclear chromatin and the chromatoid bodies appear deep blue-black, while the glycogen mass appears unstained. When stained with iodine the glycogen mass appears golden brown, the nuclear chromatin and karyosome bright yellow and the chromidial bars appear as clear spaces, being unstained.

Life Cycle

The infective form of the parasite is the mature cyst passed in the feces of convalescents and carriers. The cysts can remain viable under moist conditions for about ten days. The cysts ingested in contaminated food or water pass through the stomach undamaged and enter the small intestine. When the surrounding medium becomes alkaline. The cyst wall is damaged by trypsin in the intestine, leading to excystation. The cytoplasm gets detached from the cyst wall and amoeboid movements appear causing a tear in the cyst wall through which the quadrinucleate amoeba emerges. This stage is called the *metacyst.* The nuclei in the metacyst immediately undergo division to form eight nuclei, each of which gets surrounded by its own cytoplasm to become eight small amoebulae or metacystic trophozoites. If excystation takes place in the small intestine, the metacystic trophozoites do not colonise there, but are carried to the caecum.

The optimum habitat for the metacystic trophozoites is the caecal mucosa where they lodge in the glandular crypts and undergo binary fission. Some develop into precystic forms and cysts, which are passed in feces to repeat the cycle (Figs 3.2 and 3.3).

The entire life cycle is thus completed in one host.

Infection with *E. histolytica* does not necessarily lead to disease. Infact, in most cases it remains within the lumen of the large intestine, feeding on the colonic contents

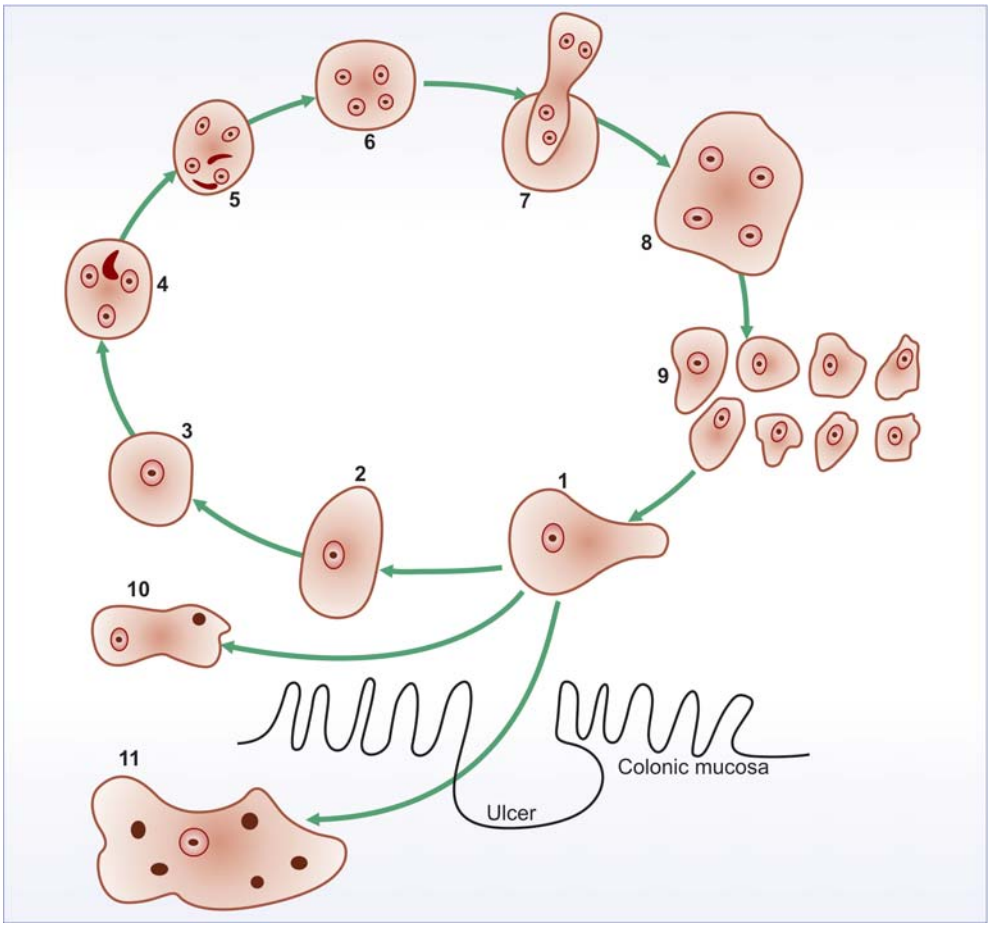


FIGURE 3.2: Life cycle of *E. histolytica.* (1) Trophozoite in gut lumen, (2) Precystic form, (3) Uninucleate cyst, (4) Binucleate cyst, (5) Quadrinucleate cyst, passed in faeces, (6) Mature cyst—infective when ingested, (7) Excystation in small intestine, (8) Metacystic form, (9) Eight daughter amoebulae, (10) Trophozoite shed in faeces—cannot encyst, (11) Tissue form of trophozoite in colonic ulcer—shows ingested erythrocytes

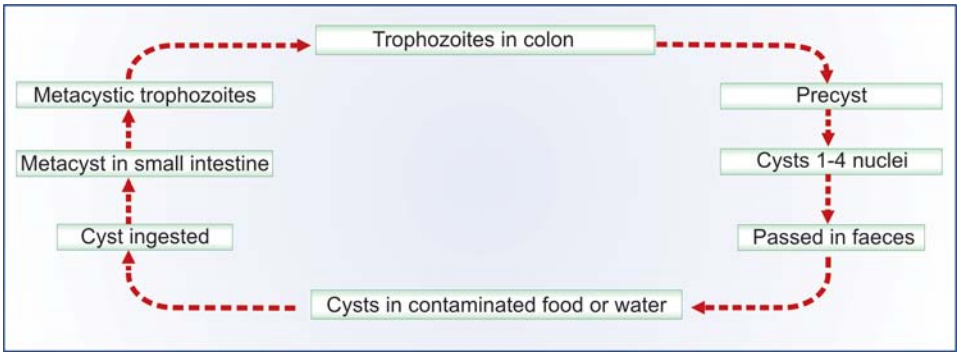


FIGURE 3.3: Life cycle of *E. histolytica* (Schematic)

and mucus as a commensal without causing any ill effects. Such persons become carriers or asymptomatic cyst passers, as their stool contains cysts. They are responsible for the maintenance and spread of infection in the community. The infection may get spontaneously eliminated in many of them. Sometimes, the infection may be activated and clinical disease ensues. Such latency and reactivation are characteristic of amoebiasis.

Culture

Boeck and Drbohlav reported the successful cultivation of *E.histolytica* in 1925 using an egg slant-Locke's solution diphasic medium. A monophasic liquid medium was described by Balamuth in 1946. Robinson's medium has been widely used for cultivation of amoebae. In these media and their modifications, amoebae grow only in presence of enteric bacteria or other protozoa and starch or other particles. Axenic cultivation which does not require the presence of other microorganisms or particles, was first developed by Diamond in 1961. This yields pure growth of the amoeba and has been very useful for physiological, immunological and pathogenicity studies of amoebae.

Pathogenicity

The lumen dwelling amoebae do not cause any illness. Only when they invade the intestinal tissues do they cause disease. This happens only in about 10 per cent of cases of infection, the remaining 90 per cent being asymptomatic. The factors that determine tissue invasion are not fully understood.

Not all strains of *E.histolytica* are pathogenic or invasive. All strains can adhere to host cells and induce proteolysis of host cell contents *in vitro* but only pathogenic strains can do so *in vivo*. Differentiation between pathogenic (P) and nonpathogenic (NP) strains can be made by several methods including susceptibility to complement mediated lysis and phagocytic activity or by the use of genetic markers or monoclonal antibodies. Amoebic cysteine proteinase which inactivates the complement factor C3 is an important virulence factor of P strains. Based on electrophoretic mobility of 6 isoenzymes (acetylglucosaminidase, aldolase, hexokinase, NAD-diaphorase, peptidase, phosphoglucomutase), *E.histolytica* strains can be classified into at least 22 zymodemes. Of these only 9 are invasive (P) and the rest are noninvasive (NP) commensals. The zymodemes show a geographical distribution. Even in endemic areas, NP zyodemes are far more common than P ones, which account only about 10 per cent of the total population.

It has been proposed that P and NP strains though morphologically identical may represent two distinct species—the P strains being *E.histolytica*, and the NP strains reclassified as *E.dispar*. Trophozoites of *E.dispar* contain bacteria, but no RBC.

Host factors such as stress, malnutrition, alcoholism, corticosteroid therapy and immunodeficiency may influence the outcome of infection. Some glycoproteins in colonic mucus bind avidly to surface receptors of the amoeba trophozoites, blocking their attachment to epithelial cells. Alteration in the nature and quantity of colonic

mucus may, therefore, influence virulence. Virulence may also be conditioned by the bacterial flora in the colon.

The metacystic trophozoites penetrate the columnar epithelial cells in the crypts of Lieberkuhn in the colon. Penetration is facilitated by the tissue lytic substances released by the amoebae which damage the mucosal epithelium and by the motility of the trophozoite. Mucosal penetration by the amoeba produces discrete ulcers with pinhead centre and raised edges. Sometimes the invasion remains superficial and confined to the mucosal epithelium leading to erosion which may spread laterally. These heal spontaneously without any ill effects. More often, the amoebae make their way to the submucosal layer where they multiply rapidly and form colonies, destroying the tissues around by lytic necrosis and forming an abscess. The abscess breaks down to form an ulcer. Amoebic ulcer is the typical lesion seen in intestinal amoebiasis. The ulcers are multiple and confined to the colon, being most numerous in the caecum and next in the sigmoido-rectal region. The intervening mucous membrane between the ulcers remains healthy.

Ulcers appear initially on the mucosa as raised nodules with pouting edges. They later break down discharging brownish necrotic material containing large numbers of trophozoites. The typical amoebic ulcer is flask-shaped in cross section, with mouth and neck being narrow and the base large and rounded. Multiple ulcers may coalesce to form large necrotic lesions with ragged or undermined edges and covered with brownish slough. The ulcers generally do not extend deeper than the submucous layer, but amoebae spread laterally in the submucosa causing extensive undermining and patchy mucosal loss. Amoebae are seen at the periphery of the lesions and extending into the surrounding healthy tissues. Occasionally, the ulcers may involve the muscular and serous coats of the colon, causing perforation and peritonitis. Blood vessel erosion may cause haemorrhage.

The superficial lesions generally heal without scarring, but the deep ulcers form scars which may lead to strictures, partial obstruction and thickening of the gut wall. Occasionally, a granulomatous growth may develop on the intestinal wall from a chronic ulcer. This amoebic granuloma or *amoeboma* may be mistaken for a malignant tumour.

During its invasion of the intestinal wall, amoebae often penetrate radicles of the portal vein and are transported through the portal circulation to the liver. Most of them fail to lodge, but some manage to get established in the hepatic lobules, where they multiply and initiate lytic necrosis with little inflammatory reaction. Hepatic invasion is multifocal, the right lobe being more affected. With increasing size of the lesions and continuing necrosis, there occurs considerable leucocytic infiltration. There is also an enlargement of the liver. This stage is known as *amoebic hepatitis*.

One or more of the lesions in the liver may extend peripherally to develop into *amoebic abscesses*. Which may vary in size from a few millimeters to several centimeters. The centre of the abscess contains thick chocolate brown pus ('anchovy sauce pus') which is liquefied necrotic liver tissue. It is bacteriologically sterile and free of amoebae. Immediately surrounding the central necrotic area is a median zone consisting only of coarse stroma. At the periphery is almost normal liver tissue, which contains invading amoebae. If the abscess has developed rapidly, there may be no limiting

capsule other than liver tissue, but more chronic lesions are surrounded by a fibrous wall. Liver abscess may be multiple or more often solitary,usually located in the upper right lobe of the liver. Jaundice develops only when lesions are multiple or when they press on the biliary tract. Untreated abscesses tend to rupture into the adjacent tissues and organs, through the diaphragm into the lung or pleural cavity, into the pericardium, peritoneal cavity, stomach, intestine or inferior vena cava, or externally through the abdominal wall and skin.

Very rarely, amoebiasis of the lung may occur by direct haematogenous spread from the colon, without hepatic involvement, but it is most often due to direct extension from the liver by an abscess rupturing through the diaphragm. It is, therefore, most common in the lower part of the right lung. A hepatobronchial fistula usually results, with expectoration of chocolate brown sputum. Less often, an amoebic empyema develops.

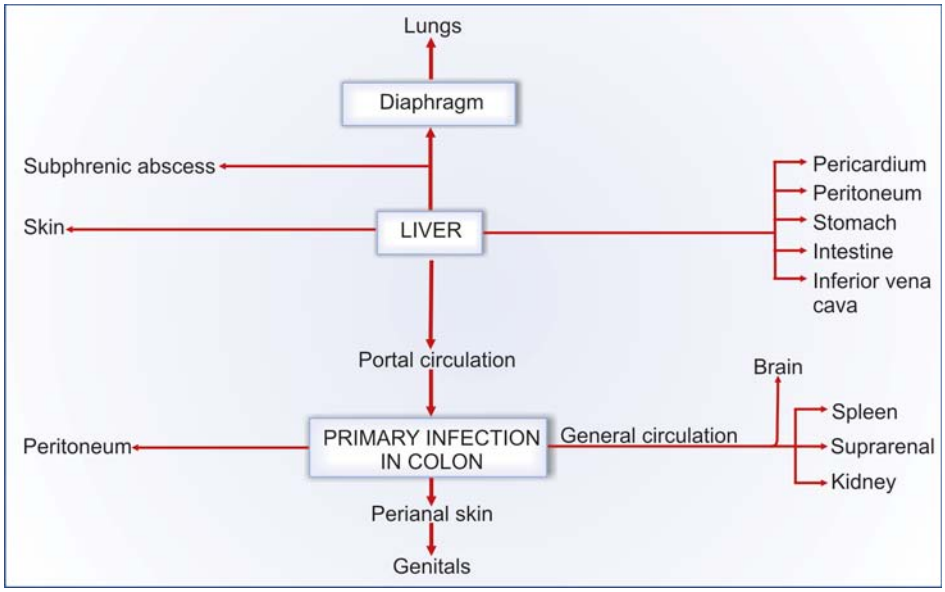


FIGURE 3.4: Sites affected in amoebiasis

Involvement of distant organs is by haematogenous spread. Instances are abscesses in the brain, spleen, adrenals and kidneys.

Cutaneous amoebiasis is by direct spread, from the rectum perianally and from colostomy openings and sinuses draining liver abscesses. Extensive necrosis and sloughing occur. Trophozoites can be demonstrated in the lesions. It can also occur as a venereal infection of the penis following anal intercourse (Fig. 3.4).

Clinical Features

The incubation period is highly variable, from 4 days to a year or longer. On an average it is from 1 to 4 months. The clinical course is characterised by prolonged latency, relapses and intermissions.

Amoebiasis can present in different forms and degrees of severity depending on the organ affected and the extent of damage caused. It can be classified as intestinal and extraintestinal amoebiasis.

Intestinal Amoebiasis

The clinical picture covers a wide spectrum from noninvasive carrier state to fulminant colitis.

The typical manifestation of intestinal amoebiasis is amoebic dysentery. This may resemble bacillary dysentery, but can be differentiated on clinical and laboratory grounds. Compared to bacillary dysentery, it is usually insidious in onset and the abdominal tenderness less and localised.

The stools are large, foul smelling and brownish black, often with bloodstreaked mucus intermingled with faeces. The red blood cells in stools are clumped and reddish brown in colour. Cellular exudate is scanty . Charcot-Leyden crystals are often present. *E.histolytica* trophozoites can be seen containing ingested erythrocytes. The patient is usually afebrile and nontoxic. In fulminant colitis there is confluent ulceration and necrosis of colon. The patient is febrile and toxic.

Intestinal amoebiasis does not always result in dysentery. Quite often there may be only diarrhoea or vague abdominal symptoms popularly called 'uncomfortable belly' or 'growling abdomen.' Chronic involvement of the caecum causes a condition simulating appendicitis.

Extraintestinal Amoebiasis

Hepatic involvement is the most common extraintestinal complication of amoebiasis. Though trophozoites reach the liver in most cases of amoebic dysenterys, only in a small proportion do they manage to lodge and multiply there. Several patients with amoebic colitis develop an enlarged tender liver without detectable impairment of liver function or fever. This acute hepatic involvement (amoebic hepatitis) may be due to repeated invasion by amoebae from an active colonic infection or to toxic substances from the colon reaching the liver. It is probable that liver damage may be caused not directly by the amoebae, but by lysosomal enzymes and cytokines from the inflammatory cells surrounding the trophozoites. In about 5 to 10 per cent of persons with intestinal amoebiasis, liver abscess may ensue. It is more common in adult males. The patient feels heaviness and pain in the liver area and referred pain around the right shoulder. Fever with chills is common, as also weight loss. Jaundice is not common.

Pleuropulmonary amoebiasis usually follows extension of hepatic abscess through the diaphragm and therefore, the lower part of the right lung is the usual area affected. Very rarely, abscess formation may occur at any site on either lung due to haematogenous spread. The abscess draining into a bronchus leads to reddish brown pus being coughed out.

Amoebic abscess of the brain may occasionally result from haematogenous spread from amoebic lesions in the colon or other sites. It causes severe destruction of brain

tissue and is fatal. Abscesses in other organs such as spleen, kidney and suprarenal gland are rare and follow blood spread.

Cutaneous amoebiasis occurs by direct extension around the anus, colostomy site or discharging sinuses from amoebic abscesses. Extensive gangrenous destruction of the skin occurs. The lesion may be mistaken for condylomata or epithelioma.

The prepuce and glans are affected in penile amoebiasis which is acquired through anal intercourse. Similar lesions in females may occur on vulva, vaginal wall or cervix by spread from perineum. The destructive ulcerative lesions resemble carcinoma.

Laboratory Diagnosis

Definitive diagnosis of amoebiasis depends on the demonstration of *E.histolytica* trophozoites or its cysts in stools, tissues or discharges from the lesions. Cultures are not employed for routine diagnosis. Immunological tests are not helpful for diagnosis of intestinal infection but may be of use in extraintestinal amoebiasis.

Intestinal Amoebiasis

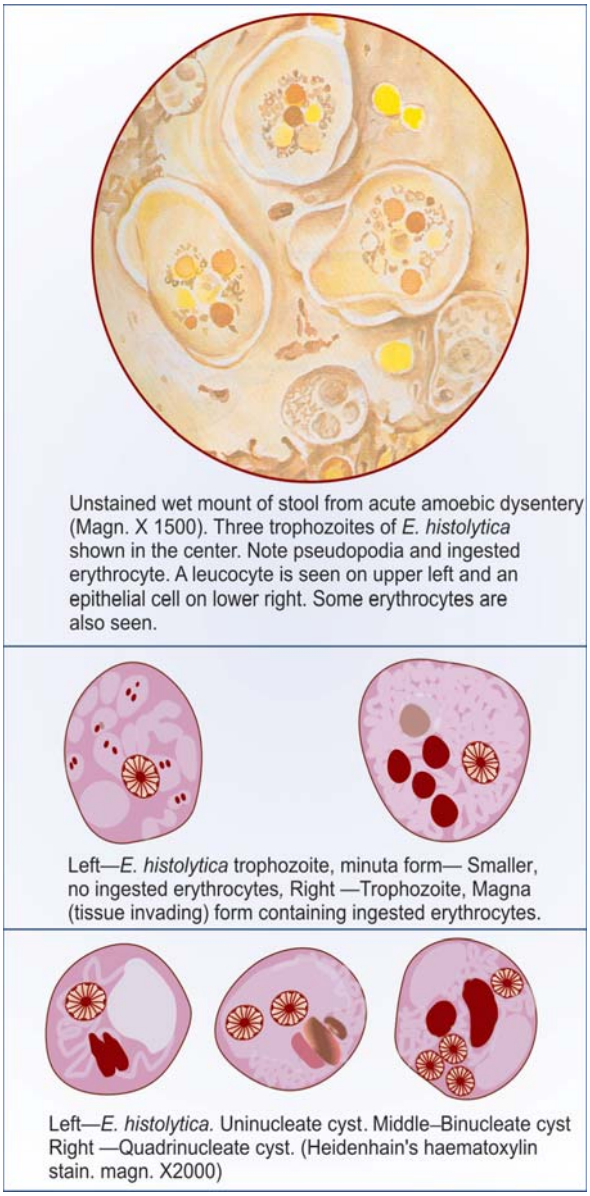
Acute amoebic dysentery: The disease has to be differentiated from bacillary dysentery (Table 3.1). The stool sample has to be collected directly into a wide mouthed container and examined without delay. Prior administration of antiamoebic drugs, bismuth, kaolin or mineral oil may interfere with demonstration of the trophozoite. It should be inspected for macroscopic and microscopic features, as well as routine examination for other parasites also. Examination of three separate samples is recommended.

a. *Macroscopic appearance:* The stool is copious, semiliquid, brownish black in colour and contains foul smelling faecal material intermingled with blood and mucus. It is acid in reaction. It does not adhere to the container.
b. *Microscopic appearance:* The cellular exudate is scanty and consists of a few pus cells, epithelial cells and macrophages. The red cells are aggregated and yellowish or brownish red in colour. Charcot-Leyden crystals are often present. But this finding is only suggestive, because they may also occur in some other bowe1 disorders such as ulcerative colitis and malignancy. In freshly passed motion unmixed with urine or antiseptics, actively motile trophozoites of *E.histolytica* can be demonstrated in unstained saline mounts.The presence of ingested erythrocytes clinches the identity of *E.histolytica*, as they are not found in any other intestinal amoeba. Stained films may not be necessary as a routine for diagnosis in acute cases, but trichrome or iron-haematoxylin stained films provide the most dependable identification and differentiation (Fig. 3.5).

   Culture and serology are not routinely employed. Serology is usually negative in early cases and in the absence of deep invasion.

Chronic Amoebiasis and Carriers

Sigmoidoscopy may show amoebic ulcers in the colon, from which biopsy tissue may be taken for direct microscopy and histopathology. Identification of asymptomatic



Unstained wet mount of stool from acute amoebic dysentery (Magn. X 1500). Three trophozoites of *E. histolytica* shown in the center. Note pseudopodia and ingested erythrocyte. A leucocyte is seen on upper left and an epithelial cell on lower right. Some erythrocytes are also seen.

Left—*E. histolytica* trophozoite, minuta form— Smaller, no ingested erythrocytes, Right —Trophozoite, Magna (tissue invading) form containing ingested erythrocytes.

Left—*E. histolytica*. Uninucleate cyst. Middle–Binucleate cyst Right —Quadrinucleate cyst. (Heidenhain's haematoxylin stain. magn. X2000)

FIGURE 3.5

carriers is important in epidemiological survey and in screening persons employed in food handling occupations.

In chronic patients, convalescents and carriers, besides naturally passed stools, it may be necessary to examine stools obtained after a saline purge for trophozoites and cysts. Excretion of amoeba is irregular and repeated stool examination is therefore necessary. The demonstration of cysts is facilitated by the use of a suitable concen-

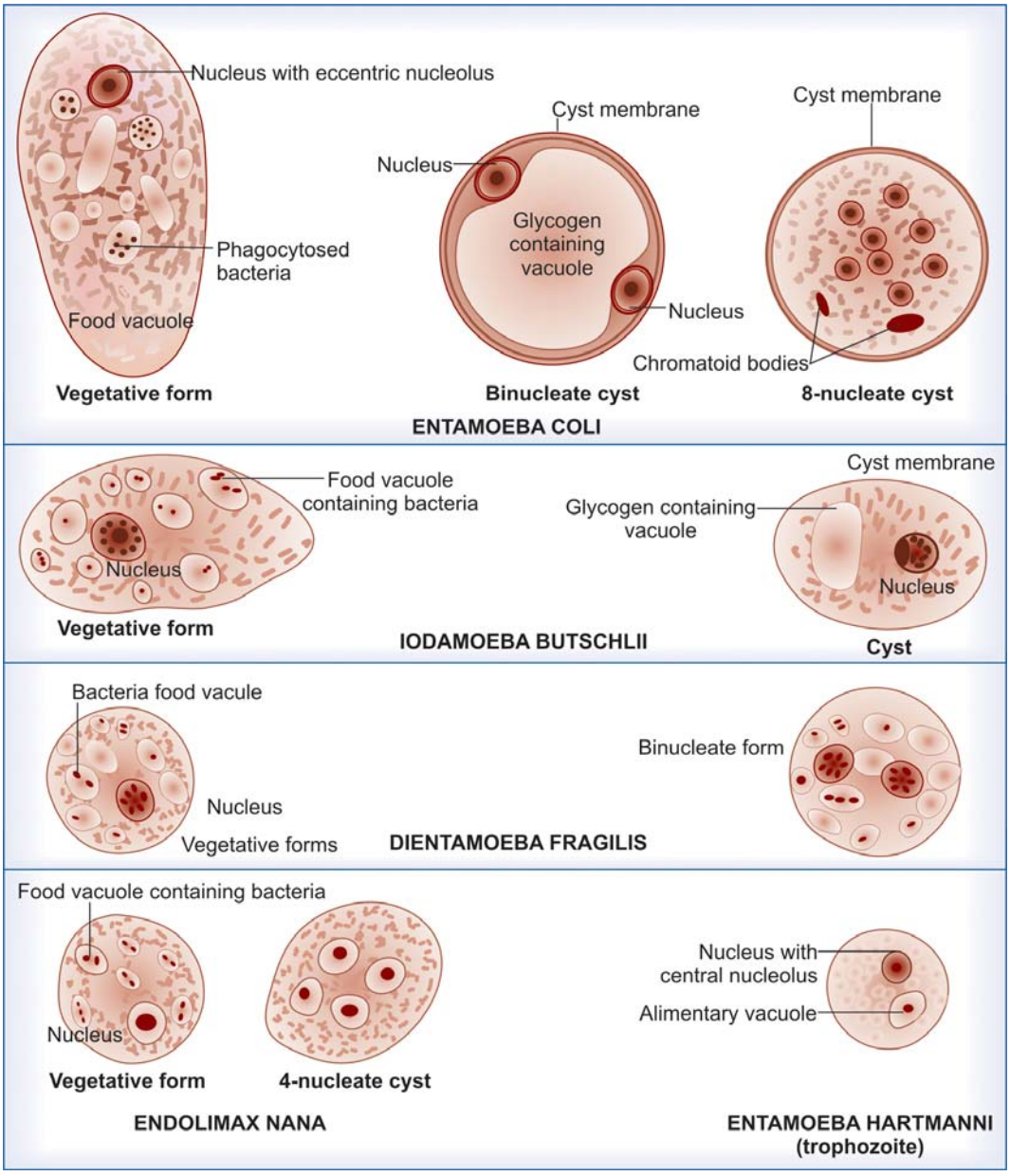


FIGURE 3.6: Note the characteristic nuclear structure of the different amoebae parasitic to man. (Heidenhain's haematoxylin Magn. x 2000)

Table 3.1: Differential features of amoebic and bacillary dysentery

Features	*Amoebic dysentery*	*Bacillary dysentery*
Clinical		
Onset	Slow	Acute
Fever	Absent	Present
Toxicity	Absent	Present
Abdominal tenderness	Localised	Generalised
Tenesmus	Absent	Present
Stool		
Frequency	6-8 per day	Over 10 per day
Odour	Offensive	Nil
Colour	Dark red	Bright red
Nature	Faeces mixed with blood and mucus	Blood and mucus with little or no faeces
Consistency	Not adherent	Adherent to container
Reaction	Acid	Alkaline
Microscopy		
Cellular exudate	Scanty	Abundant
Red blood cells	Clumped yellowish brown	Discrete or in rouleaux, bright red
Macrophage	Few	Several, some with ingested red blood cells
Eosinophils	Present	Absent
Charcot-Leyden crystals	Present	Absent
Motile bacteria	Present	Absent
Amoeba	Motile trophozoites with ingested red blood cells	Absent

tration method such as the zinc sulphate centrifugal floatation technique. Examination of iodine and iron-haematoxylin stained preparations is helpful. Trophozoites, when present may be in the minuta form and may not have ingested erythrocytes. Differentiation from other amoebae may require the study of nuclear morphology after staining. Samples may be fixed with 10 per cent formalin, Schaudin's fixative or polyvinyl alcohol and stained with Gomorri trichrome or periodic acid Schiff stains. The differential characters of intestinal entamoebae are shown in Table 3.2.

Cultures are not used routinely but may on occasion prove positive in cases found negative by microscopy. Cultures permit the determination of zymodeme patterns for differentiation between pathogenic and nonpathogenic strains. Serological tests may not be positive except in cases of invasive amoebiasis.

Immunodetection tests for identifying *E.histolytica* antigens in clinical samples are available. Highly specific ELISA reagents can differentiate between *E.histolytica* and *E.dispar* antigens. Polyvalent immunochromatographic strip tests can detect amoeba, giardia and cryptosporidium antigens in stool samples.

Extraintestinal (Invasive) Amoebiasis

Hepatic amoebiasis:In diffuse hepatic amoebiasis (amoebic hepatitis) without localised abscess formation, laboratory diagnosis may be difficult. Often stool examination

Table 3.2: Differential features of intestinal entamoebae

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

is negative for amoebae and a history of dysentery may be absent. In such cases serological tests can be helpful.

Craig (1928) was the first to report a complement fixation test in amoebiasis. Subsequently a number of different serological tests have been developed including indirect haemagglutination (IHA), latex agglutination (LA), gel diffusion precipitation (GDP), cellulose acetate membrane precipitation (CAP) test, counter current immunoelectrophoresis (CIE) and enzyme linked immunosorbent assay (ELISA). While lHA and LA are highly sensitive, they often give false-positive results. They remain positive for several years even after successful treatment. Gel precipitation tests are less sensitive, but more specific. ELISAs are both sensitive and specific and like GDP and CIE become negative within six months of successful treatment. Highly sensitive radioimmunoassay (RIA) and DNA probes have been introduced for detection of amoeba antigens in blood pus and faeces but these are too complex for routine use.

In case of liver abscess when diagnostic aspiration is done the pus obtained from the centre of the abscess may not contain amoebae as they are confined to the periphery. The fluid draining after a day or two is more likely to contain the trophozoite. Aspirates from the margins of the abscess also would show the trophozoites. Cysts are never seen in extraintestinal lesions.

Other Extraintestinal Amoebiasis

In pulmonary amoebiasis the trophozoite may be seen in the expectorated anchovy sauce sputum. Cutaneous amoebiasis, and whenever accessible materials from other invasive lesions also show the trophozoites.

Immunity

Infection with invasive strains induces both humoral and cellular immune responses. Local and systemic antibodies can be demonstrated within a week of invasive infection.

Infection confers some degree of protection as evidenced by the very low frequency of recurrence of invasive colitis and liver abscess in endemic areas. The course and severity of amoebiasis do not seem to be affected by HIV infection. Serological response is hardly ever seen in infection with noninvasive zymodemes.

Epidemiology

Amoebiasis is worldwide in prevalence though it is more common in the tropical areas where sanitation is poor. Prevalence rates vary from as low as 1 per cent in affluent countries to more than 50 per cent in some developing countries. Some 500 million new infections occur each year worldwide. Infection occurs at all ages and in both sexes, though it is more common in adults than in children, and in males than in females.

The source of infection is a carrier or asymptomatic cyst passer. The patient with acute dysentery is of no importance in transmission as the stools then contain only trophozoites which are not infective. Carriers may shed the infective cysts for years. When cooks and other food handlers happen to be carriers they can transmit the infection readily. Amoebiasis in animals does not appear to be of any importance as a source of human infection.

Amoebiasis is essentially an endemic disease though it can occasionally occur in epidemic form due to contamination of water sources. Contaminated food and water constitute the most important vehicles of infection.

The cysts are relatively resistant. They can survive for several months in water at 0°C, 3 days at 30°C, 30 minutes at 40°C and 5 minutes at 50°C. In grossly contaminated water and sewage they may survive longer. They remain viable in moist soil for upto 10 days. They can resist 1/2500 mercury bichloride, 5 per cent HCl or 0.5 per cent formalin for 30 minutes and 1/500 potassium permanganate for 1 to 2 days. They are killed by boiling, desiccation, freezing to below—5°C, 1/20 cresol in 30 minutes and 5 per cent acetic acid in 15 minutes. Ordinary residual chlorination of water may not destroy them, though super-chlorination does.

Flies and cockroaches may act as mechanical vectors. Viable cysts have been found in their droppings for a day or two after exposure.

Increased and varied male homosexual practices, particularly in the West, have enhanced the incidence of amoebiasis, which has been recognised as a 'gay bowel disease.'

Prophylaxis

General prophylaxis is as for all faecal-oral infections. Food and water have to be protected from contamination with human excreta. Detection and treatment of carriers and their exclusion from food handling occupations limit the spread of infection. Health education and inculcation of healthy personal habits help in control.

Treatment

Two classes of drugs are used in the treatment of amoebiasis—the luminal amoebicides (e.g. diloxanide furoate, iodoquinol, paromomycin, tetracycline) acting in the intestinal lumen, but not in tissues, and the tissue amoebicides (e.g. emetine, chloroquine) effective in systemic infection, but less so in the intestine. Metronidazole and related compounds act at both sites. Emetine, for long the sheet anchor in treatment of amoebiasis has largely been given up because of its toxicity.

Opinion is divided about the need for treating asymptomatic cysts passers in endemic areas. It may perhaps be futile in view of the high rate of reinfection.

ENTAMOEBA HARTMANNI

Entamoeba hartmanni occurs wherever *E.histolytica* is found and was till recently considered to be a "small race" of the latter. It was quite often mistaken for *E.histolytica* and reported as such. It is now considered to be a separate species of nonpathogenic commensal intestinal amoeba. It is much smaller than *E.histolytica*, the trophozoite measuring 4 to 12 µm and cyst 5 to 10 µm in size. Trophozoites do not ingest red cells and their motility is less vigorous. The cyst resembles that of *E.nana*.

ENTAMOEBA COLI

Entamoeba coli was first described by Lewis (1870) and Cunningham (1871) in Calcutta and its presence in healthy persons was reported by Grassi (1878). It is worldwide in distribution. It is a nonpathogenic commensal intestinal amoeba. Its medical importance is that it has to be differentiated from *E.histolytica*. It is larger, about 20 to 50 µm with sluggish motility and contains ingested bacteria but not red cells.

The nucleus is clearly visible in unstained films and has a large eccentric karyosome and thick nuclear membrane lined with coarse granules of chromatin. Cysts are large, 10 to 30 µm in size, with a prominent glycogen mass in the early stage. The chromatoid bodies are splinter like and irregular. The mature cyst has eight nuclei. The life cycle is the same as in *E.histolytica* except that it remains a luminal commensal without tissue invasion and is nonpathogenic.

ENTAMOEBA POLECKI

Originally described as an intestinal parasite of pigs and monkeys, *E.polecki* has been detected in the human intestine in some parts of South East Asia, particularly in Papua-New Guinea, where it is a common intestinal commensal. However, it does not appear to be a significant human pathogen.

The trophozoite resembles that of *E.coli*. The cyst is uninuclear, with many prominent pointed chromidial bars and one or more large nonglycogen inclusions.

ENTAMOEBA GINGIVALIS

Entamoeba gingivalis was discovered by Gros in 1849 and so was the first amoeba of humans to have been described. It is global in distribution. Only the trophozoite

is found, the cystic stage being apparently absent. The trophozoite is about 10 to 20 µm, actively motile with multiple pseudopodia. The cytoplasm contains food vacuoles with ingested bacteria, leucocytes and epithelial cells. The presence of ingested leucocytes and their nuclear fragments is diagnostic as no other amoeba ingests these cells. The nucleus is round, with a delicate central karyosome and nuclear membrane lined with coarse chromatin granules.

The amoeba lives in the gingival tissues and is abundant in unhygienic mouths. It is a commensal and is not considered to cause any disease. It is transmitted by direct oral contact, through droplets of saliva or fomites.

E.gingivalis has been found in bronchial washings from cases of pulmonary suppuration and in sputum, where it can be mistaken for *E.histolytica* from lung abscess.

The amoeba has been reported in vaginal and cervical smears of women using intrauterine devices and they disappear spontaneously with the removal of these devices. Similar amoebae have been observed in the mouth of dogs, cats and monkeys.

ENDOLIMAX NANA

This common commensal amoeba is widely distributed. It lives in the human intestine. The trophozoite is small (nana, meaning small), less than 10 µm in size, with a slow slug like motility.The nucleus has a conspicuous eccentric karyosome connected to the nuclear membrane by one or more coarse stands. The cyst is small, oval and tetranucleate with the glycogen mass and chromidial bars inconspicuous or absent. It is nonpathogenic.

IODAMOEBA BUTSCHLII

This is widely distributed, though less common than *E.coli* and *E.nana*. The trophozoite is small, 6 to 12 µm, with a conspicuous nucleus. The prominent karyosome is half the size of the nucleus and surrounded by refractile globules. The cyst is oval, uninucleate and has a prominent iodine-staining glycogen mass (iodophilic body). Hence the name 'Iodamoeba.' It is nonpathogenic.

DIENTAMOEBA FRAGILIS

Dientamoeba fragilis , long considered an amoeba has been reclassified as an aberrant trichomonad flagellate (*amoeboflagellate*) based on electron microscopic features.

The trophozoite is 7 to 12 µm in diameter. It is motile with broad hyaline leaf-like pseudopodia. They have 1 to 4 nuclei, the binucleate form being the most common. The name *Dientamoeba fragilis* refers to the binucleate feature and the fragile nature of its cytoplasm. The nuclear chromatin is present as 3 to 5 granules in the centre, with no peripheral chromatin on the nuclear membrane. *D. fragilis* has no cyst stage.

It is seen worldwide and is reported to be the most common intestinal protozoan parasite in Canada. It lives in colonic mucosal crypts, feeding on bacteria. It does not invade tissues, but may rarely ingest RBCs. Formerly believed to be nonpathogenic, it has now been associated with a variety of symptoms like intermittent diarrhoea,

abdominal pain, flatulence and fatigue. Metronidazole, iodoquinol, paromomycin and tetracycline have been used for treatment. In the absence of a cyst stage, its mode of transmission is not clear. It has been proposed that trophozoites shed in feces may survive in enterobius, ascaris or other nematode eggs and be transmitted through them.

Definitive diagnosis depends on demonstration of the characteristic nuclear structure in permanently stained films. Examination of unstained fecal smears is not satisfactory.

The comparative morphologies of amoebae infecting man is shown in (Figs 3.6 and 3.7).

PATHOGENIC FREE-LIVING AMOEBAE

Among the numerous types of free-living amoebae found in water and soil, a few are potentially pathogenic and can cause human infections. The first of these to have been recognised was *primary amoebic meningoencephalitis* (PAM) caused by the amoeboflagellate *Naegleria*. *Acanthamoebae* have been found to cause two diseases, *granulomatous amoebic encephalitis* (GAE) and *chronic amoebic keratitis* (CAK). A few instances of GAE caused by *leptomyxid* amoebae have also been reported. While PAM and CAK occur in previously healthy individuals, GAE has been associated with immunodeficient states.

The term *amphizoic* has been used for organisms such as these, which can multiply both in the body of a host (*endozoic*) and in free-living (*exozoic*) conditions (Fig. 3.8).

NAEGLERIA

Naegleria fowleri, the only pathogenic species of naegleria is named after Fowler who, with Carter described it first from Australia in 1965. It is found worldwide in warm fresh waters.

N. fowleri has 3 stages in its life cycle—a dormant cyst form, an amoeboid trophozoite form and a flagellate form (hence classified as an amoeboflagellate). The amoeboid form is about 10 to 20 µm, showing rounded pseudopodia (*lobopodia*), a spherical nucleus with a big endosome, and pulsating vacuoles. This is the feeding, growing and replicating form, seen on the surface of vegetation, mud and water. In water, some of them get transformed into a 'pear-shaped form' with 2 flagella. This rapidly motile flagellate form is the main infective stage, more so than the trophozoite. The flagellate can revert to the amoeboid form. Cysts develop from the trophozoites and are seen in the same locations as trophozoites. The cysts are spherical. They are the resting dormant form and can resist unfavourable conditions such as drying and chlorine up to 50 ppm. The trophozoites can withstand moderate heat (45°C), but die at chlorine levels of 2 ppm and salinity of 0.7 per cent.

Human infection comes from water containing the amoebae and usually follows swimming or diving in ponds. Patients are mostly previously healthy young adults or children. The amoebae invade the nasal mucosa, pass through the olfactory nerve branches in the cribriform plate into the meninges and brain to initiate an acute

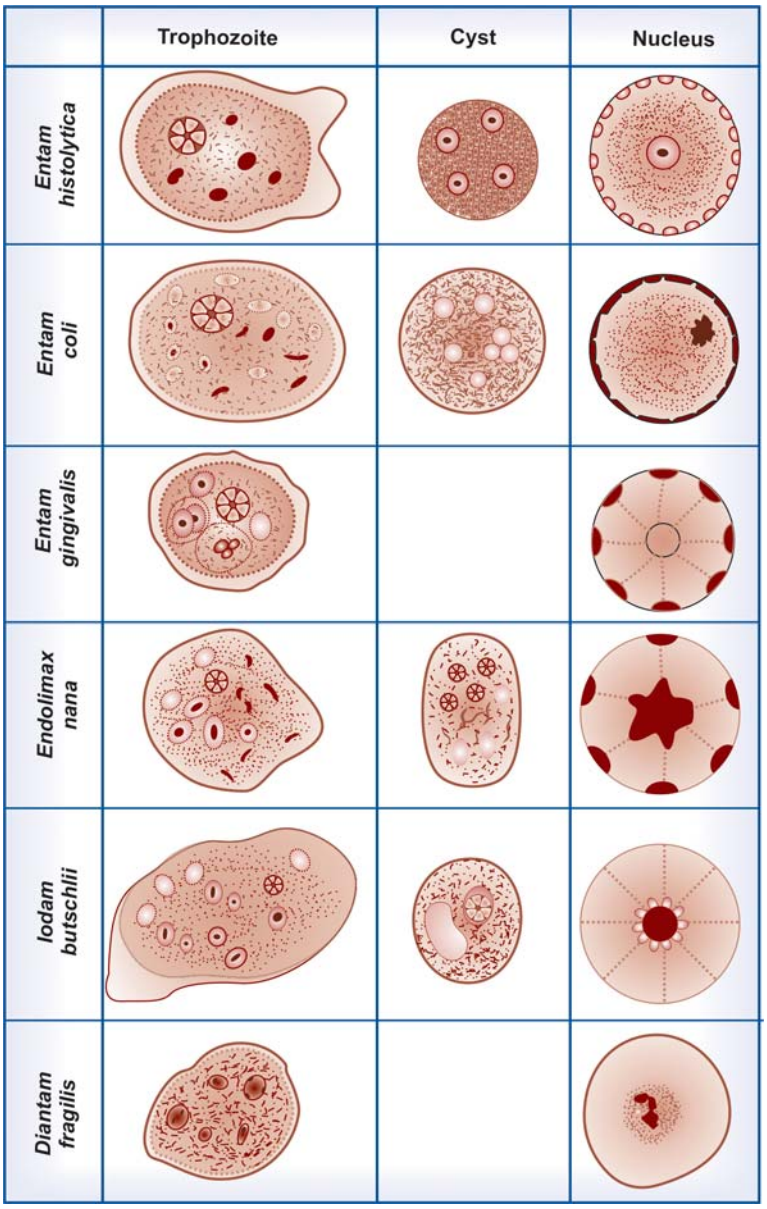


FIGURE 3.7: Comparative morphology of amoebae infecting humans, showing trophozoite and cyst stages, as well as enlarged representation of their nuclear structure

purulent meningitis and encephalitis (primary amoebic meningoencephalitis). The incubation period is 2 days to 2 weeks. The disease almost always ends fatally within a week. Over 200 cases of PAM have been reported from many countries, including India. Most cases have been from the developed countries.

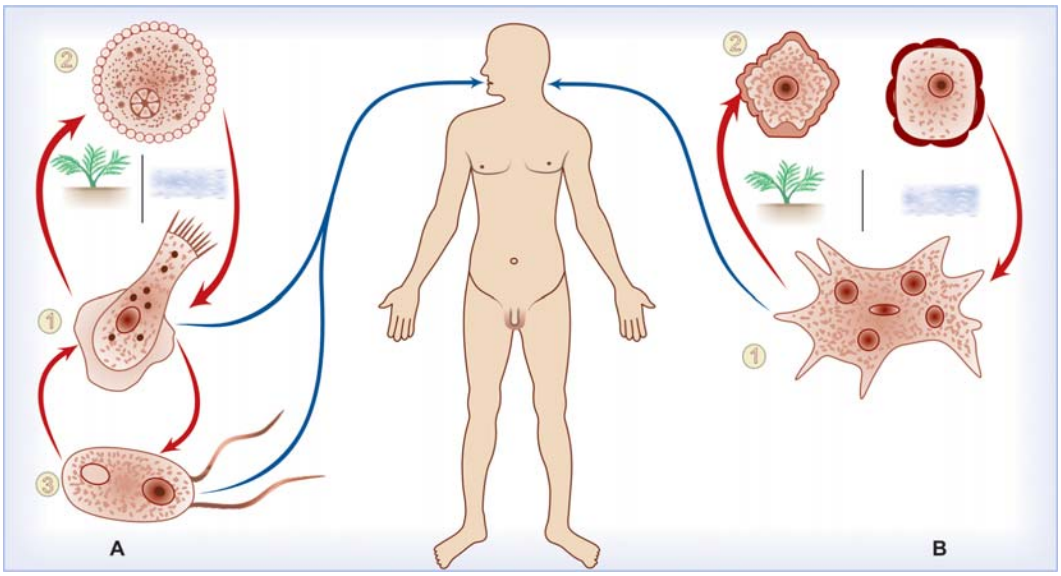


FIGURE 3.8: Pathogenic free-living amoebae. (A) *Naegleria fowleri:* 1—Amoeboid trophozoite showing lobopodia, nucleus with large endosome, and vacuoles. 2—Cyst. 3—Pear-shaped flagellate form showing flagella. (B) *Acanthamoeba culbertsoni:* 1—Trophozoite, showing spinous acanthopodia. 2—Cyst

Diagnosis can be made by CSF examination. The fluid is cloudy to purulent, with prominent neutrophil leucocytosis, elevated proteins and low glucose, resembling pyogenic meningitis. Failure to find bacteria in such specimens should raise the possibility of PAM. Wet film examination of CSF may show the trophozoites. Cysts are never seen CSF or brain. At autopsy, trophozoites can be demonstrated in brain histologically.Culture can be obtained in agar seeded with *Escherichia coli* or in the usual cell cultures used for virus isolation. Both trophozoites and cysts occur in culture. Amphotericin B has been used in treatment with limited success.

ACANTHAMOEBA SP

Acanthamoeba culbertsoni (formerly Hartmanella culbertsoni) is the species most often responsible for human infection, but other species such as *A. polyphaga*. *A.castellani* and *A.astromyxis* have also been reported.

The trophozoite is large, 20 to 50 µm in size and characterised by spine-like pseudopodia (*acanthopodia*). It differs from *Naegleria* in not having a flagellate stage and in forming cysts in tissues. The polygonal double walled cysts are highly resistant. The cysts are present in all types of environment all over the world.

Infection can be acquired by inhalation, ingestion or through traumatised skin or eyes, from contaminated water. It presents chiefly as two chronic conditions—keratitis and encephalitis. Granulomatous lesions in other sites such as skin, lungs, middle ear have also been reported.

Chronic amoebic keratitis or keratouveitis, of which over a thousand cases have been reported, develops from the entry of the amoebic cyst through abrasions on the cornea. The large majority of such cases have been associated with the use of contact lenses. The picture resembles that of severe herpetic keratitis with a slow relapsing course, but the eye is severely painful in the amoebic infection.

Diagnosis is by demonstration of the cyst in corneal scrapings by wet mount, histology and by culture. Growth can be obtained from corneal scarpings inoculated on nutrient agar, overlaid with live or dead *Escherichia coli* and incubated at 30°C.

Treatment with drugs such as propamidine, polyhexamethylene biguanide, chlorhexidine and ketoconazole, along with surgical procedures has been found useful.

Granulomatous amoebic encephalitis is believed to follow inhalation of the dried cysts. In persons with predisposing factors such as steroid use, alcoholism, diabetes and immunodeficiencies, including AIDS. The incubation period is long and the evolution of the illness slow. The picture is that of an intracraneal space occupying lesion with pareses, seizures and mental deterioration. CSF shows lymphocytic pleocytosis. Acanthamoeba trophozoites and cysts can be demonstrated in brain biopsy by microscopy, culture and immunofluorescence using monoclonal antibodies. No effective treatment is available. Over a hundred cases have been reported, the majority of them in the HIV infected.

The aetiological agent in a few cases of granulomatous amoebic encephalitis has been identified as a leptomyxid amoeba *Balamuthia mandrillaris*.

FREE-LIVING AMOEBAE AS ALLERGENS

Naegleria and *Acanthamoeba* have been claimed to be responsible for allergic pneumonitis. This is believed to be due to inhalation of amoebic antigens derived from amoebae growing in the humidifiers of air conditioning plants.

FREE-LIVING AMOEBAE AS CARRIERS

Some free-Iiving water amoebae may sometimes harbour pathogenic bacteria such as legionella, pseudomonas and cholera vibrios. The bacteria can grow and multiply in the amoebae and survive in the cysts, resisting adverse environments, for example in chlorinated water. This may be significant in hospital infection if the water is contaminated with free-living amoebae. These water amoebae have been shown to be acceptable hosts for *Chlamydia pneumoniae, Legionella pneumophila* and some enteroviruses.

BLASTOCYSTIS HOMINIS

Blastocystis hominis, a common inhabitant of the human intestine was first identified in 1912. Its taxonomical status and clinical significance still remain unclear. After various suggestions, it is now classified as a protozoon assigned to a new suborder of Amoebida.

B. hominis is a round cell, 6 to 40 µm in size, characterised by a large membrane—bound central body, surrounded by a layer of cytoplasm containing nuclei (Fig. 3.9). It can put forth pseudopodia and ingest bacterial and other debris. Reproduction is by binary fission and sporulation.

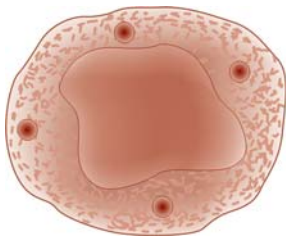


FIGURE 3.9: *Blastocystis hominis*

Infection is generally asymptomatic, though unconfirmed claims relate it to various illnesses from diarrhoea to arthritis. Long-term carriers are common. Infection leads to production of circulating antibodies detectable by ELISA or immunofluorescence. The mode of spread is believed to be faecal-oral. Infection is reported to be more common in those in close contact with animals.

CHAPTER 4

Flagellates

Parasitic protozoa which possess whip-like flagella as their organs of locomotion are classified under the Phylum-Sarcomastigophora, Subphylum-Mastigophora, Class-Zoomastigophorea (from *mastix*-whip, *phoros*-bearing). Depending on their habitat, they can be considered under two headings:

1. *Lumen-dwelling flagellates:* Flagellates found in the alimentary and urogenital tracts.
2. *Haemoflagellates:* Flagellates found in blood and tissues.

INTESTINAL FLAGELLATES

Most luminal flagellates are nonpathogenic commensals. Two of them cause clinical disease, *Giardia lamblia* which can cause diarrhoea and *Trichomonas vaginalis* which can produce vaginitis and urethritis. Intestinal flagellates found in humans are listed below, with the sites affected by them shown in parenthesis.

1. *Giardia lamblia* (duodenum, jejunum)
2. (a)*Trichomonas vaginalis* (vagina, urethra); (b) *T. tenax* (mouth); (c) *T. hominis* (caecum)
3. *Chilomastix mesnili* (caecum)
4. *Enteromonas hominis* (colon)
5. *Retortamonas intestinalis* (colon)
6. *Dientamoeba fragilis* (See Chapter 3).

GIARDIA LAMBLIA

History and Distribution

This flagellate was observed by Leeuwenhoek (1681) in his own stools and was thus one of the earliest of protozoan parasites to have been recorded. It is named Giardia after Professor Giard of Paris and lamblia after Professor Lambl of Prague who gave a detailed description of the parasite. Worldwide in distribution, it is the most common intestinal protozoan pathogen. Infection may be asymptomatic or cause diarrhoea.

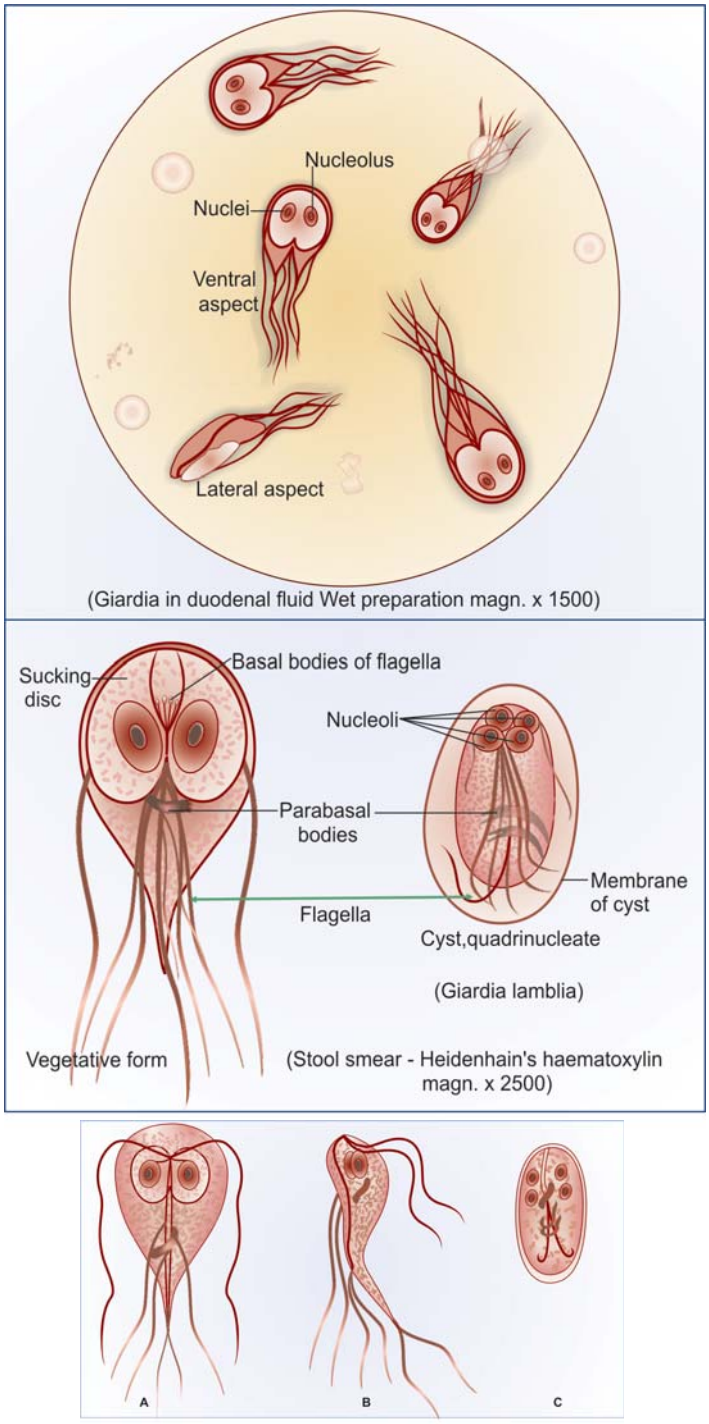


FIGURE 4.1: *Giardia lamblia.* (A) Trophozoite—ventral view (B) Trophozoite—lateral view (C) Cyst

Morphology and Life Cycle

G. lamblia lives in the duodenum and upper jejunum and is the only protozoan parasite found in the lumen of the human small intestine. It occurs in the vegetative and cystic forms.

The vegetative form or *trophozoite* is rounded anteriorly and pointed posteriorly, about 15 µm long, 9 µm wide and 4 µm thick. It has been described variously as pyriform, heart-shaped or racket-shaped. Dorsally it is convex and ventrally it has a concave *sucking disc* which occupies almost the entire anterior half of the body. It is bilaterally symmetrical and possesses 2 nuclei, one on either side of the midline, two *axostyles* running along the midline, 4 pairs of flagella and 2 sausage shaped *parabasal* or *median bodies* lying transversely posterior to the sucking disc (Fig. 4.1).

The trophozoite is motile, with a slow oscillation about its long axis, which has been likened to the motion of a 'falling leaf.' It divides by longitudinal binary fission. It lives in the duodenum and upper part of the jejunum attached by means of the sucking disc to the epithelial cells of the villi and crypts feeding by pinocytosis.

Encystation occurs in the colon. The trophozoite retracts its flagella into the axonemes which remain as curved bristles in the cyst. The cyst is ovoid about 12 µm by 8 µm in size and surrounded by a tough hyaline cyst wall. The young cyst contains two and the mature cyst four nuclei situated at one end. Cysts are passed in stools and remain viable in soil and water for several weeks. There may be up to 2,00,000 cysts present per gram of faeces. In diarrhoeic stools trophozoites also may be present, but they die outside and are not infectious.

Infection is acquired by the ingestion of cysts in contaminated food and water. Infectivity is high, as few as 10 cysts being capable of initiating infection. Within half an hour of ingestion, the cyst hatches out into two trophozoites which multiply successively by binary fission and colonise the duodenum. The trophozoites as they pass down the colon develop into cysts.

Pathogenesis and Clinical Features

G. lamblia is seen typically within the crypts in the duodenum. It does not invade tissues, but remains tightly attached by means of the sucking disc to the epithelial surface in the duodenum and jejunum. This may cause abnormalities of villous architecture. Often no clinical illness results, but in some it may lead to mucus diarrhoea, dull epigastric pain and flatulence. The diarrhoea in some cases may be steatorrhoeic with excess mucus and fat, but no blood. Children may develop chronic diarrhoea, malabsorption, weight loss and a sprue-like syndrome. It has been suggested that enormous numbers of the parasite adhering to the mucosal surface of the small intestine may interfere with absorption. Increased bacterial colonisation of the small intestine has been observed in subjects with giardiasis and steatorrhoea. Occasionally giardia may colonise the gallbladder, causing biliary colic and jaundice. The incubation period is variable, but is usually about 2 weeks.

Diagnosis

The cysts and trophozoites can be found in diarrhoeal stools. Only the cysts are seen in asymptomatic carriers. Concentration by formalin ethyl acetate or zinc sulphate centrifugal floatation is useful when the cysts are sparse. Duodenal aspiration may sometimes be necessary to demonstrate the parasite in cases in which biliary symptoms predominate. A useful method for obtaining duodenal specimens is the *enterotest*, which uses a coiled thread inside a small weighted gelatin capsule. This is swallowed after attaching the free end of the thread to the cheek. The capsule passes through the stomach to the duodenum. After 2 hours, the thread is withdrawn, placed in saline and mechanically shaken. The centrifuged deposit of the saline is examined for giardia.

ELISA and immunochromatographic strip tests have been developed for detection, of giardia antigens in faeces, but are not in routine use. Antibody demonstration is not useful in diagnosis.

Epidemiology

The infection is worldwide, especially in children. Endemicity is very high in some areas. Visitors to such places, frequently develop traveller's diarrhoea caused by giardiasis, through contaminated water. Epidemics of giardiasis have been reported on a number of occasions. While ingestion of food and water contaminated with the cysts is the most common mode of infection, direct person-to-person transmission may also occur in children, male homosexuals and the mentally-ill. Enhanced susceptibility to giardiasis is associated with blood group A, achlorhydria, use of cannabis, chronic pancreatitis, malnutrition and immune defects such as 19A deficiency and hypogammaglobulinaemia. HIV infection has not apparently been associated with increased risk of giardiasis.

Cats, dogs, cattle, sheep and many wild animals have been found naturally infected. While they are not considered to be responsible for human infection ordinarily, instances of giardiasis observed in some remote areas have been claimed to be due to water sources contaminated by such animals. *Giardia* species infecting birds, amphibians and mice can be differentiated from *G. lamblia* by morphological features.

Prophylaxis

Prevention is as for other faecal-oral infections by better personal hygiene and prevention of food and water contamination. Iodine is effective in disinfecting drinking water.

Treatment

Metronidazole and tinidazole are the drugs of choice. Furazolidone is slower in action, but is preferred in children as it has fewer adverse effects. Only symptomatic cases need treatment.

TRICHOMONAS VAGINALIS

History

Trichomonas vaginalis was first observed by Donne (1836) in vaginal secretion.

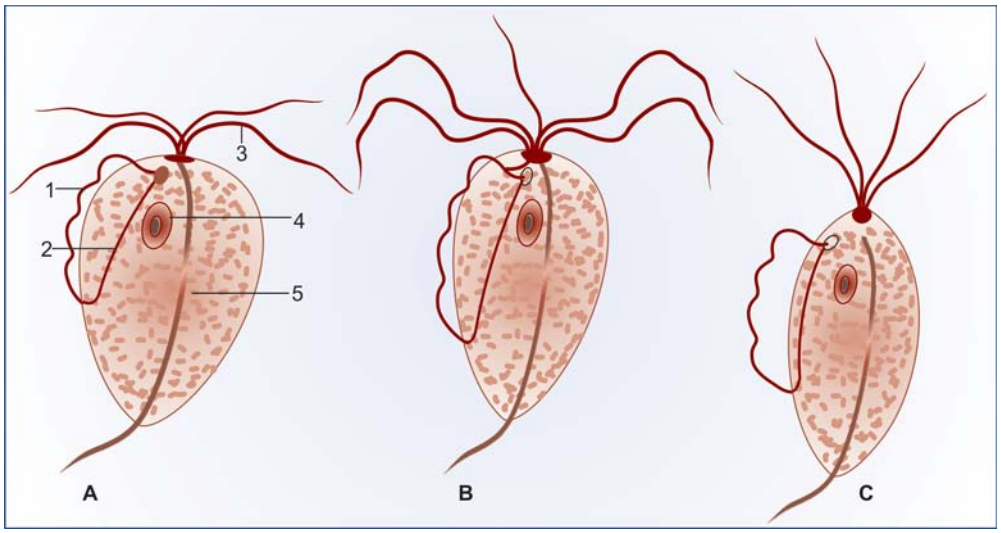


FIGURE 4.2: *Trichomonas* species. (A) *T. vaginalis*, (B) *T. hominis*, (C) *T. tenax.*
1—Undulating membrane, 2—Costa, 3—Flagella, 4—Nucleus, 5—Axostyle

Morphology and Life Cycle

T. vaginalis occurs only as the trophozoite, there being no cystic form in trichomonas. The trophozoite is ovoid or pear-shaped, about 10 to 30 µm long and 5 to 10 µm broad, with a short undulating membrane reaching up to the middle of the body. It has 4 anterior flagella and a fifth running along the outer margin of the undulating membrane, which is supported at its base by a flexible rod, the *costa*. A prominent *axostyle* runs throughout the length of the body and projects posteriorly. The cytoplasm shows prominent granules which are most numerous alongside the axostyle and costa (Fig. 4.2).

It lives in the vagina and cervix in the female, and may also be found in the Bartholin's glands, urethra and even the urinary bladder. In males, it occurs mainly in the anterior urethra, but it may also be found in the prostate and preputial sac.

It is motile, with a jerky rapid movement. It divides by binary fission. As cysts are not formed, the trophozoite itself is the infective form.

Culture

It can be grown in a variety of solid and liquid media, in tissue culture and in eggs. CPLM (cysteine, peptone, liver, maltose) medium is often used.

Pathogenesis

T.vaginalis infects selectively squamous and not columnar epithelium. Infection is often asymptomatic, particularly in the male. In females, it may produce severe pruritic vaginitis with an offensive, yellowish, often frothy discharge. Cervical erosion is common, with endometritis and pyosalpinx as infrequent complications. Dysuria in women is often due to trichomoniasis. Rarely neonatal pneumonia and conjunctivitis have been reported in infants born to infected mothers. In males it may produce urethritis (one type of nongonococcal urethritis). The incubation period is 4 days to 4 weeks.

Diagnosis

The trichomonad may be found in sedimented urine and vaginal secretions, in wet films or Papanicolaou smears. Specimens collected on cotton swabs through a vaginal speculum and left for some time in a tube containing 5 per cent glucose saline show better shape and motility on examination. Prostatic massage may sometimes be necessary for detection of the parasite in males. Serological tests like indirect haemagglutination and gel diffusion are available for antibody detection.

Epidemiology

T.vaginalis infection occurs worldwide, with an annual estimated incidence of 170 million. The trophozoite cannot survive outside the body and so infection has to be transmitted directly from person-to-person. Sexual transmission is the usual mode of infection. Trichomoniasis often coexists with other STDs—candidiasis, gonorrhoea, syphilis or HIV infection. Babies may get infected during birth. Fomites such as towels have been implicated in transmission. Prevention is as for other sexually transmitted diseases.

Treatment

Metronidazole is the drug of choice. Simultaneous treatment of the sexual partner is necessary for cure.

TRICHOMONAS TENAX

Also known as *T.buccalis* , this is smaller (5 to 10 µm) than *T.vaginalis*. It is a harmless commensal which lives in the mouth. in the periodontal pockets, carious tooth cavities and less often in tonsillar crypts. It is transmitted by kissing, salivary droplets and fomites.

TRICHOMONAS HOMINIS

This measures 8 to 12 µm and carries 5 anterior flagella and an undulating membrane that extends the full length of the body. It is a very common harmless commensal of the caecum.

CHILOMASTIX MESNILI

This occurs as trophozoites and cysts. The trophozoite is pear-shaped and asymmetric due to a spiral groove running through the middle of the body. The cysts are lemon shaped. It is a harmless commensal of the caecum.

ENTEROMONAS HOMINIS

The trophozoites are small (4 to 10 µm) ovoid bodies with 3 anterior and one posterior flagella. The cyst contains 2 to 4 nuclei. This commensal lives in the large intestine, mainly in the caecum.

RETORTAMONAS INTESTINALIS

This rare commensal of the caecum occurs as an elongated pyriform trophozoite and ovoid or pyriform cyst.

HAEMOFLAGELLATES

Medically important Haemoflagellates require two hosts to complete their life cycle and are therefore called *digenetic* or *heteroxenous*. They live in the blood and tissues of human and other vertebrate hosts, and in the gut of insect vectors.

Haemoflagellates infecting human belong to two genera, in the family, Trypanosomatidae—*Trypanosoma* and *Leishmania*. Members of this family have a nucleus, a kinetoplast and a single flagellum. The *kinetoplast* (sometimes referred to incorrectly as the micronucleus) consists of a deeply staining *parabasal body* and a adjacent dot-like *blepharoplast*. The blepharoplast and parabasal body are connected by one or more delicate fibrils. The *flagellum* arises from the blepharoplast. The portion of the flagellum which is inside the body of the parasite is called the *axoneme* or *axial filament* (Fig. 4.3).

Multiplication in vertebrate and in vertebrate hosts is by binary fission. No sexual cycle is known.

Haemoflagellates exist in two or more of four morphological stages. These were formerly called the *leishmanial, leptomonad, crithidial* and *trypanosomal* stages. But as the above names have also been given to different genera within the family, it led to confusion. The names of the morphological forms have, therefore, been changed as described below (Fig. 4.4).

These names are formed by the suffix 'mastigote' (derived from the Greek word *Mastix* for whip) combined with various prefixes referring to the origin, course and arrangement of the flagellum in relation to the position of the nucleus, and its point of emergence from the cell.

i. *Amastigote* (formerly *leishmanial*) stage is rounded or ovoid without any external flagellum. The nucleus. kinetoplast and axial filament can be seen. This is the stage in which *T. cruzi* and Leishmania are found intracellularly in vertebrate hosts.
ii. *Promastigote* (formerly called *leptomonad*) stage is lanceolate. The kinetoplast is anterior to the nucleus (*antenuclear kinetoplast*), near the anterior end of the cell,

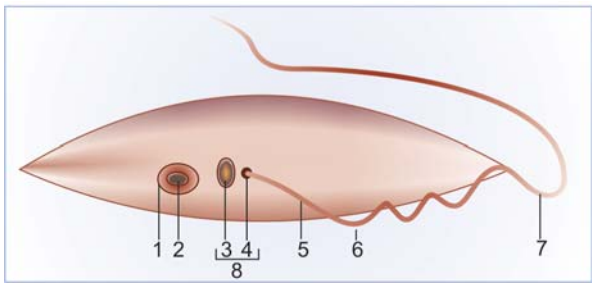


FIGURE 4.3: Basic morphology of haemoflagellates. (1) Nucleus, (2) Karyosome, (3) Parabasal body, (4) Blepharoplast, (5) Axoneme, (6) Undulating membrane, (7) Flagellum, (8) Parabasal body and blepharoplast together constitute the kinetoplast

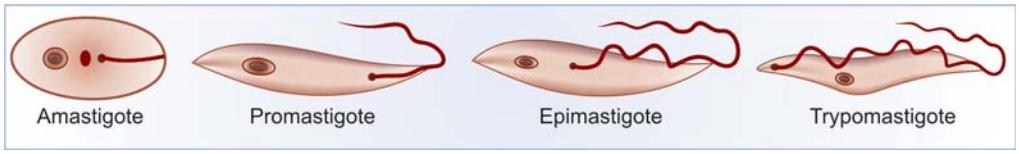


FIGURE 4.4: Morphological stages of haemoflagellates

from which emerges the flagellum. There is no undulating membrane. This is the infective stage of Leishmania found in the midgut and proboscis of the insect vector. This is also the form in which Leishmania occur in cultures *in vitro.*

iii. *Epimastigote* (formerly called *crithidial*) stage is more elongated, with the kinetoplast placed more posteriorly though close to and in front of the nucleus (*juxtanuclear kinetoplast*). The flagellum runs alongside the body as a short undulating membrane before emerging from the anterior end. This is the stage in which *T.gambiense* and *T. rhodesiense* occur in the salivary glands of the vector testes fly, and *T.cruzi* in the midgut of the vector reduviid bug. This stage is lacking in leishmania.

iv. *Trypomastigote* (formerly called *trypanosomal*) stage is elongated, spindle shaped with a central nucleus and the kinetoplast posterior to the nucleus (*postnuclear kinetoplast*) situated at the posterior end of the body. The flagellum runs alongside the entire length of the cell to form a long undulating membrane before emerging from the anterior end. This is the infective stage of trypanosomes found in the vector arthropod and the stage found in the blood of the infected vertebrate. This stage is lacking in *Leishmania.*

Some transitional stages have been recognised. These include the *sphaeromastigote*, a motile round form with free flagellum, which is a transitional stage from amastigote to promastigote, seen in the genus Trypanosoma and the *paramastigote*, a transitional form leading to the infective promastigote in leishmania.

TRYPANOSOMES

All members of the genus *Trypanosoma* (*trypanes*-to bore, *soma*-body) exist at sometime in their life cycle, as the trypomastigote (trypanosomal) stage with an elongated spindle-shaped body, a central nucleus, a posterior kinetoplast and a long undulating

membrane. Volutin granules are found in the cytoplasm. In addition to the typical forms, cells with atypical features are frequently found, a condition known as *polymorphism*.

A blood sucking insect constitutes the intermediate host and vector. The vector becomes infective to the vertebrae host only after an extrinsic incubation period during which the parasite undergoes development and multiplication. In the vector, the trypanosomes follow one of two modes of development and are accordingly classified into 2 groups—*Salivaria* and *Stercoraria*. In salivaria, the trypanosomes migrate to the mouth parts of the vectors (anterior station) so that infection is transmitted by their bite (inoculative transmission). Examples are *T. gambiense* and *T. rhodesiense* causing African trypanosomiasis, which are transmitted by the bite of tsetse flies. In stercoraria, the trypanosomes migrate to the hindgut (posterior station) and are passed in faeces (stercorarian transmission). Examples are *T.cruzi* causing Chagas' disease which is acquired by rubbing the feces of the vector bug into the wound caused by its bite, and *T.lewisi*, the rat trypanosome which is transmitted by ingestion of the faeces of infected rat fleas.

Classification

The trypanosomes infecting humans are classified into the following groups.

i. *T. brucei subspecies* (human strains) causing African trypanosomiasis or sleeping sickness.
   T. brucei gambiense
   T. brucei rhodesiense
   (The third subspecies *T. brucei brucei* is not infective for humans, but causes *nagana*, an important disease of animals in Africa. This is believed to be the ancestral type of trypanosome from which the other two subspecies have been derived by adaptation to the human host. *T.b.gambiense* appears to be better adapted to human and produces a milder chronic infection while the adaptation of *T.b.rhodesiense* to human is more recent so that it causes a more acute infection).
ii. *T. cruzi* causing South American trypanosomiasis or Chagas' disease.
iii. *T.rangeli* a nonpathogenic trypanosome causing harmless human infection in South America.

Trypanosomes infect several animal species, sometimes causing important diseases. Some examples are:

i. *T.brucei* (animal strains) causing the economically important disease nagana in African cattle.
ii. *T. evansi* causing the disease 'surra' in horses, mules, camels and also in elephants. lt is transmitted mechanically by biting flies (*Tabanidae, Stomoxys*) and also by vampire bats. The infection is found in India.
iii. *T. equiperdum* causing 'stallion's disease' in horses and mules. It is transmitted by sexual contact, without the need for an insect vector.
iv. *T. lewisi* causing a common harmless infection in rats all over the world. The vector is the rat flea, which passes the infective metacyclic trypomastigotes in feces, which when ingested by a rat, infect it.

A trypanosome resembling *T. lewisi* was reported from Madhya Pradesh, India in the peripheral blood of two persons with short-term fever. Three human trypanosome infections have been reported from Malaysia, but their identity and vectors are not known.

Human trypanosomiasis is strictly restricted to certain geographical regions—the African and South American trypanosomiases being seen only in the respective continents. This is due to the vector species being confined to these places alone.

AFRICAN TRYPANOSOMIASIS (SLEEPING SICKNESS)

Trypanosomiasis is believed to have been extant in tropical Africa from antiquity. Tsetse flies and trypanosomes had kept man and cattle away effectively from a quarter of the area of the African continent. In the tsetse belt of tropical Central Africa, where several species of tsetse flies (*Glossina* species) breed, most of them feed on the blood of wild game animals, in which they transmit enzootic trypanosomiasis which causes mild or harmless infection. However, in domestic cattle they cause nagana.

Causative Agents

Trypanosomes which cause nagana in cattle and sleeping sickness in humans are morphologically indistinguishable. Based on host specificity, clinical manifestations, geographic distribution and epidemiological features they were originally classified into three species:

T. brucei, infecting cattle and wild game animals, causing nagana;

T.gambiense causing the West African sleeping sickness in humans; and

T.rhodesiense causing the East African sleeping sickness in humans.

Subsequently it was accepted that all the above should be considered as belonging to a single species called *T. brucei*, consisting of three subspecies designated *T. brucei brucei, T. b.gambiense* and *T.b.rhodesiense*.

The suffix '-deme' has been employed to refer to populations of trypanosomes that differ from others belonging to the same species or subspecies in regard to specified properties. For example, the term nosodeme refers to trypanosome populations causing similar clinical patterns of disease, serodemes to those possessing similar antigens, zymodemes to those showing similar isoenzyme pattern, etc.

For differentiation between the 'human strains' and 'animal strains' of *T. brucei.* the blood incubation infectivity test (BIIT) had been widely used. The strain is incubated with oxalated human blood and then inoculated into the multimammate rat or other susceptible rodents. The infectivity of 'animal strains' will be neutralised by human blood, while 'human strains' retain infectivity after incubation with human blood. *In vitro* culture systems are now employed instead of rodents for testing infectivity.More recently their differentiation is based on isoenzymes, DNA and RNA characteristics.

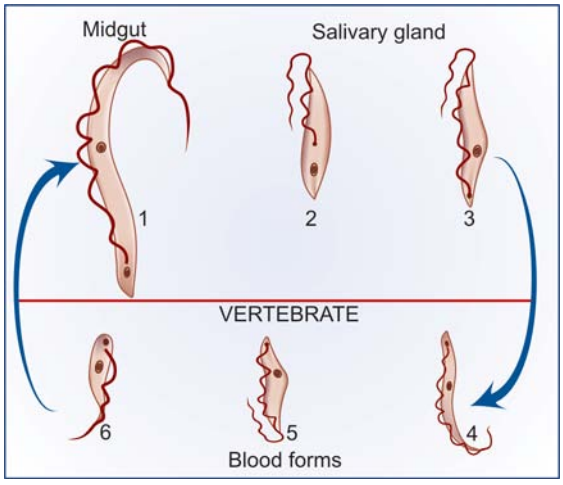


FIGURE 4.5 Life cycle of *T. brucei*. (1) Elongated trypomastigote form in midgut of tsetse fly (2) Epimastigote, in salivary gland which develops into (3) Metacyclic trypomastigote, the infective form for vertebrates. (4,5 and 6) are trypomastigote forms in vertebrate blood, — the slender form (4) the intermediate form (5) and the short stumpy form (6)

Morphology and Life Cycle

The morphology and life cycle of the human strains of *T. brucei* (*T. gambiense* and *T. rhodesiense*) are identical (Fig. 4.5).

Human infection is acquired by the bite of the vector tsetse fly. The infective form of the parasite is the metacyclic trypomastigote. On introduction into the dermis, this proliferates initially at. the site of inoculation and then through the lymphatics, enters the bloodstream.

In the blood, three forms of trypanosomes are found, the long slender trypomastigote, a short broad form with the flagellum attenuated or absent and an intermediate form. The trypomastigotes are about 15 to 40 µm long and 1.5 to 3.5 µm broad. In fresh blood films, they may be seen as colourless spindle-shaped bodies that move rapidly, spinning the red cells around. In smears stained with Giemsa or other Romanowsky stains, the cytoplasm appears pale blue and the nucleus red. The kinetoplast appears as a deep red dot and volutin granules stain deep blue. The undulating membrane appears pale blue and the flagellum red (Fig. 4.6).

When a vector tsetse fly feeds on a person with parasitaemia, it takes in the trypomastigotes along with its blood meal, particularly the short broad forms. These become long slender forms in the midgut and hindgut of the fly, where they proliferate and ultimately reach the salivary glands. Here they become broad epimastigotes which multiply and fill the cavity of the gland. The fly becomes infective when the epimastigotes become transformed into metacyclic trypomastigotes. It takes about 3 weeks from the time of the blood meal for the fly to become infective

(extrinsic incubation period). Thereafter, the fly remains infective for life, about 6 months.

The trypanosome remains extracellularly throughout its life cycle, both in the vertebrate and in the vector. It was believed that the trypomastigote is the only form present in vertebrates, but recently amastigote forms of the parasite have been found in the choroid plexus blocking blood vessels and obstructing CSF circulation. It is possible that this may have a role in the pathogenesis of cerebral manifestations of the condition.

Trypanosomes exhibit antigenic variation of their surface glycoproteins. There is a cyclical fluctuation in the trypanosomes in the blood of infected vertebrates. Each successive wave represents a variant antigenic type (VAT) of trypomastigote possessing variant surface specific antigens (VSSA) or variant surface glycoproteins (VSG). Besides this, trypanosomes have other mechanisms also helping them to evade host immune responses.

West African (Gambian) Sleeping Sickness

This infection caused by *T.b.gambiense* is endemic in scattered foci in West and Central Africa between 15°N and 18°S latitudes.(The trypanosome was first isolated from the blood of a steamboat captain on the Gambia river—hence the name gambiense.) The principal vectors are the riverine tsetse flies *Glossina palpalis* and *G. tachinoides*. Humans are the reservoir host and source of infection, though pigs and other domestic animals can act as chronic asymptomatic carriers of the parasite. The disease may sometimes occur as epidemics. During epidemics, the vector fly has been found to transmit the infection mechanically through its soiled proboscis when it bites a susceptible person soon after biting an infected person. Congenital transmission also has been recorded.

The incubation period is about 1 to 2 weeks. The illness is chronic and can persist for many years. There is an initial period of parasitaemia, following which they are localised predominantly in the lymph nodes. Intermittent fever, chills and headache mark this stage. There is hepatosplenomegaly with lymphadenopathy particularly in the posterior cervical region. With the invasion of the central nervous system, which occurs after several months, the sleeping sickness stage starts. This is marked by increasing headache, mental dullness, apathy and sleepiness. The patient falls into profound coma followed by death from asthenia.

Histopathology shows chronic meningoencephalitis. The meninges are heavily infiltrated with lymphocytes, plasma cells and morula cells which are atypical plasma cells containing mulberry shaped masses of IgA. Brain vessels show perivascular cuffing. This is followed by infiltration of the brain and spinal cord, neuronal degeneration and microglial proliferation.

East African (Rhodesian) Sleeping Sickness

This form of African trypanosomiasis is caused by *T.b. rhodesiense*. It is found in foci situated to the east of the area affected by *T.b. gambiense*. The principal vector is

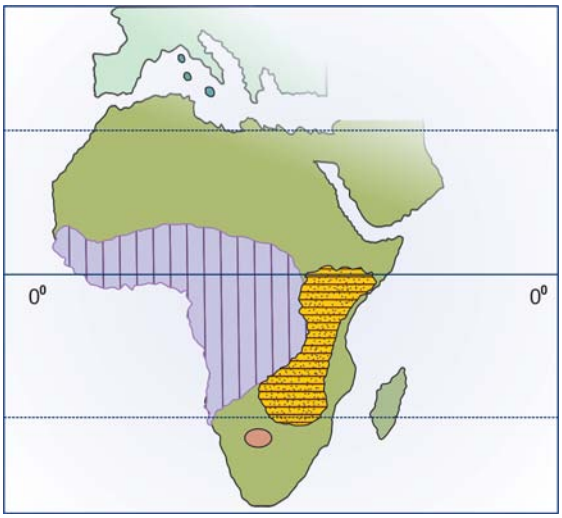


FIGURE 4.6: Geographical distribution of trypanosomiasis in Africa. Lines indicate areas endemic for *T.gambiense* and dots *T. rhodesiense*

G. morsitans, G. palpalis and *G. swynnertoni* which live in the open savannah country. Though the infection is usually transmitted by the vector from man-to-man, the disease is actually a zoonosis, with the reservoir being game animals such as the bush buck (Fig. 4.6).

East African trypanosomiasis is more acute than the Gambian form and may end fatally within a year of onset, before involvement of the central nervous system develops. Fever, weakness, rapid loss of weight and myocarditis are the usual manifestations. Mania and delusion may occur, but the typical sleeping sickness picture is seldom seen.

Diagnosis

Diagnosis is established by the demonstration of the trypanosomes in peripheral blood, bone marrow, lymph nodes or cerebrospinal fluid. The methods available are direct microscopy of stained or unstained preparations, cultivation in Weinman's or Tobie's medium and inoculation into rats. Several serological tests have been developed for detecting antibodies. These include direct agglutination, indirect haemagglutination, gel precipitation immunofluorescence and ELISA.

Prophylaxis

Preventive measures depend mainly on control of the vector.

Treatment

Suramin and pentamidine are used for early cases. Melarsoprol is the only drug effective in late cases with neurological involvement.

SOUTH AMERICAN TRYPANOSOMIASIS (CHAGAS' DISEASE)

History

This condition, caused by *Trypanosoma cruzi*, is limited to South and Central America. Carlos Chagas, investigating malaria in Brazil in 1909, accidentally found this trypanosome in the intestine of a triatomid bug and in the blood of a monkey bitten by the infected bugs. It was only later that Chagas found the trypanosome in the blood of a sick child and showed that it was responsible for an endemic disease, which came to be named after him. In this instance, therefore, the parasite and the vector were discovered before the disease was identified. Chagas named the parasite *T. cruzi* after his mentor Oswaldo Cruz.

Vectors and Life Cycle

T. cruzi passes its life cycle in two hosts—vertebrate hosts including humans and the insect vector—the reduviid bug. The parasite occurs in three different but overlapping infection cycles, a sylvatic zoonosis in wild animals such as armadillos and opossums, a peridomestic cycle in dogs, cats and other domestic animals, and a domestic cycle in humans. Different vector species are active in these infection cycles. The vectors important in human infection are the reduviid bugs adapted to living in human habitations, mainly *Triatoma infestans, Rhodnius prolixus* and *Panstrongylus megistus*. These are large (up to 3 cm long) night biting bugs which typically defecate while feeding. The faeces of infected bugs contains the metacyclic trypomastigotes which are the infective forms. Infection is acquired when they are rubbed into the bite wound or enter through mucosal surfaces, particularly the conjunctiva, being transferred there by the person's fingers. The trypomastigotes may induce a local inflammatory reaction and swelling at the site of entry in the skin called 'Chagoma.' When they enter through the conjunctiva a unilateral oedematous swelling of the eyelids results (Romana's sign).

The parasite spreads through the lymphatic system involving various tissues and cells throughout the reticuloendothelial system. Inside these cells, they get transformed into amastigote forms which divide by binary fission. After passing through promastigote and epimastigote forms, they again become tyrpomastigotes which are released into the blood stream. No multiplication occurs in the trypomastigote stage. Multiplication takes place only intracellularly in the amastigote form and to some extent as promastigotes or epimastigotes when about to be released from the cell (Fig. 4.7).

When a reduviid bug bites a person with trypanosomes in peripheral blood they get into the midgut of the insect. Here, the trypomastigotes are transformed into epimastigotes which migrate to the hindgut and proliferate.

These in turn develop into metacyclic trypomastigotes which are excreted in faeces (stercorarian transmission). The extrinsic incubation period is 8 to 10 days.

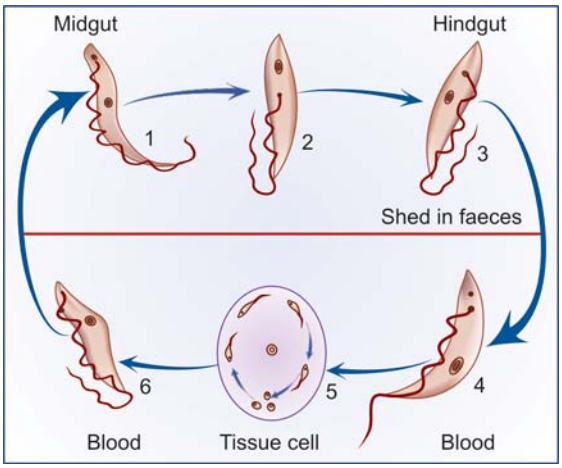


FIGURE 4.7: Life cycle of *T. cruzi*.
1. Trypomastigote form enters midgut of reduviid bug and transforms into 2. Epimastigote form which migrates to hindgut, multiplies and becomes 3. Metacyclic trypomastigote which is shed in faeces and infects vertebrates. 4. Trypomastigote in blood enters reticuloendothelial and other tissue cells 5. In which it passes through epimastigote and promastigote stages to become amastigotes which replicate and again through promastigote and epimastigote stages become 6. Trypomastigotes released into bloodstream. These are the infective forms for the vector bug

Pathogenicity and Clinical Features

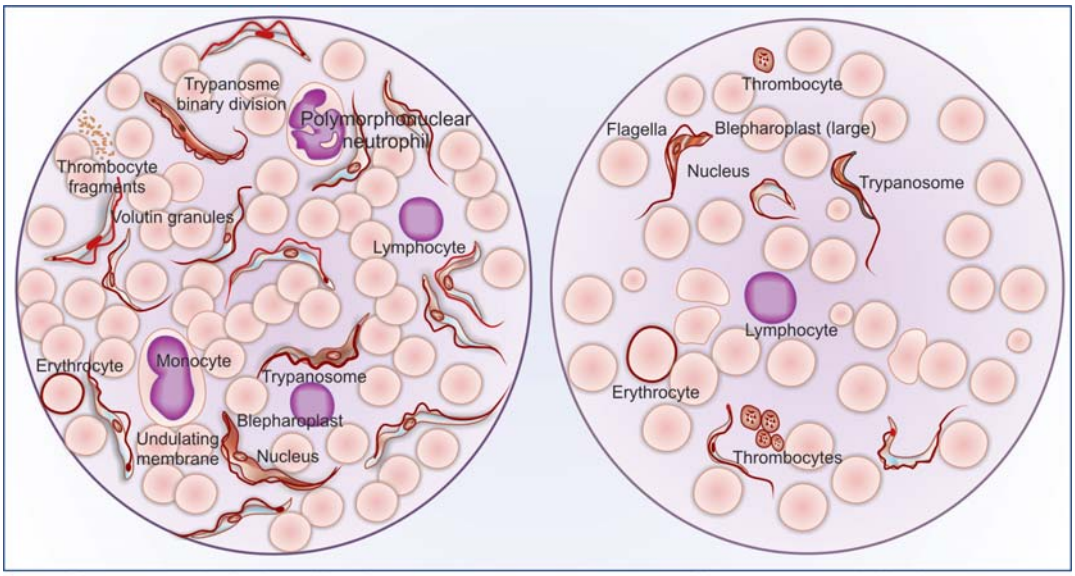
The incubation period in man is 1 to 2 weeks. The disease manifests in two forms, acute and chronic. In the acute form, usually found in children, it presents with fever and generalised nonpitting oedema of the body. The disease lasts for 3 to 4 weeks and sometimes ends fatally with myocarditis or meningoencephalitis. The chronic form found in adults presents as neurotropic, cardiotropic or viscerotropic forms and may last for several years.

The pathogenesis depends on the intracellular multiplication of the amastigote form in various locations causing damage to the cells and tissues. The sites commonly affected are myocardium, skeletal muscles, neuroglial cells and cells of the reticulo-endothelial system. Damage to myocardium is often associated with conduction defects. Damage to autonomic nerve cells often leads to the so called 'megadisease' consisting of megaoesophagus, megacolon and megaureter.

Diagnosis

Diagnosis is by demonstration of *T.cruzi* in blood or tissues, or by serology. In stained peripheral blood smears, the trypomastigote often appears in a C-shaped form (Fig. 4.8). *T.cruzi* can be grown in NNN medium or its modifications. Guinea pig inoculation may be done with blood, CSF, lymph node aspirate or other tissue materials and the trypomastigote looked for in its blood smears. Xenodiagnosis may be attempted by allowing a parasite—free reduviid bug to bite the pateint and by demonstrating the parasite in its intestinal contents.

Serological tests employed for detection of antibodies include complement fixation (Machado-Guerreiro test), indirect haemagglutination, immunofluorescence and ELISA. Specific tests have been developed for demonstration of the parasite antigen in blood and urine.An intradermal test has been described for demonstration of hypersensitivity. The antigen 'cruzin' is prepared from *T. cruzi* cultures.



Trypanosoma rhodesiense, blood smear
Giemsa stain, magn X 1100

Trypanosoma cruzi, blood smear
Giemsa stain, magn X 1100

FIGURE 4.8

Prophylaxis

Control and elimination of domestic and peridomestic vector bugs would help check the transmission of disease in endemic areas. Triatomine bugs are highly susceptible to chlorinated hydrocarbon insecticides, which form the major weapon for their control. Most human infections are transmitted by bugs living in cracks and crannies in the walls of ill kept tenement dwellings. Provision of better housing would prevent such transmission.

Treatment

No effective specific treatment is available. Nifurtimox and benznidazole have been used with some success in the acute cases. Allopurinol and ketoconazole have also been found useful.

LEISHMANIA

The genus *Leishmania* is named after Sir William Leishman who discovered the flagellate protozoon causing kala-azar, the Indian visceral leishmaniasis. All members of the genus *Leishmania* are obligate intracellular parasites that pass their life cycle in two hosts, the mammalian host and the insect vector, female sandfly. In human and other mammalian hosts, they multiply within macrophages, in which they occur exclusively in the amastigote form, having an ovoid body containing a nucleus and kinetoplast. In the sandfly, they occur in the promastigote form, with a spindle shaped body and a single flagellum arising from the anterior end.

Classification

The taxonomy of leishmaniae is controversial because traditionally they have been classified according to the clinical disease caused and the geographical prevalence. This does not parallel the grouping based on genetic and biochemical features. For medical purposes the old classification is still preferred.

Leishmaniae produce two broad types of clinical disease, visceral and cutaneous (including mucocutaneous) leishmaniases. Leishmaniae parasitic for humans have, therefore been classified into two broad groups.

A. Causing Visceral Leishmaniasis (VL)

1. The *L.donovani* complex infecting internal organs (liver, spleen, bone marrow), causing visceral leishmaniasis.

B. Causing Cutaneous and/or Mucocutaneous Leishmaniasis (CL)

I. *L. tropica, L.major, L. aethiopica*—(Old world CL)
II. *L. mexicana* complex; *L. braziliensis* complex and *L. guyanensis* complex (the 2 latter now regrouped under *Viannia* subgroup)—(New world or American CL).

Each of these complexes contains a number of different varieties and subspecies which differ in several features such as antigenic structure, isoenzymes and other biochemical characteristics, growth properties, ecology and pathogenicity. Based on geographical distribution, they have been classified as "Old World" or " New World" leishminases.

LEISHMANIA DONOVANI

History

Sir William Leishman in 1900 observed the parasite in spleen smears of a soldier who had died of 'Dum Dum fever' or kala-azar contracted at Dum Dum, Calcutta. Leishman reported this finding from London in 1903, in which year Donovan also reported the same parasite in spleen smears of patients from Madras. The name *Leishmania donovani* was therefore given to this parasite. The amastigote forms of the parasite as seen in smears from pateints, are called *Leishman-Donovan* (LD) *bodies*. *L. donovani* causes visceral leishmaniasis or kala-azar. It also causes the condition post-kala-azar dermal leishmaniasis (PKDL).

Leishmaniasis is a major public health problem in many parts of the world. According to the WHO Report of 1990, 1.5 million cases of cutaneous leishmaniasis and 500,000 cases of visceral leishmaniasis occur every year, spread over 82 countries. About 350 million people are at risk of leishmaniasis, with 12 million people currently infected.

Morphology and Life Cycle

The parasite exists in two forms, the amastigote form in humans and other mammals, and the promastigote form in the sandfly and in artificial cultures (Figs 4.9A and B, and 4.10).

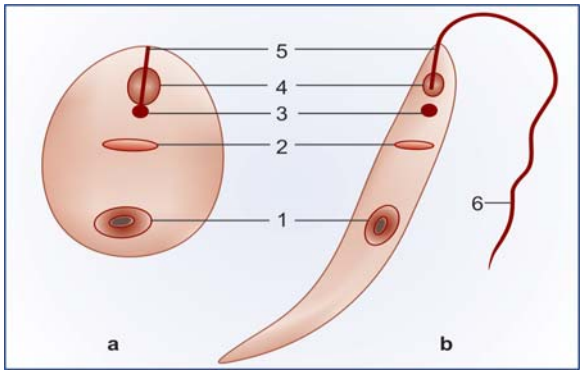


FIGURE 4.9A: Morphology of *Leishmania donovani.* a. Amastigote (LD body). b. Promastigote. 1. Nucleus 2. Parabasal body 3. Blepharoplast 4. Vacuole 5. Axoneme 6. Flagellum

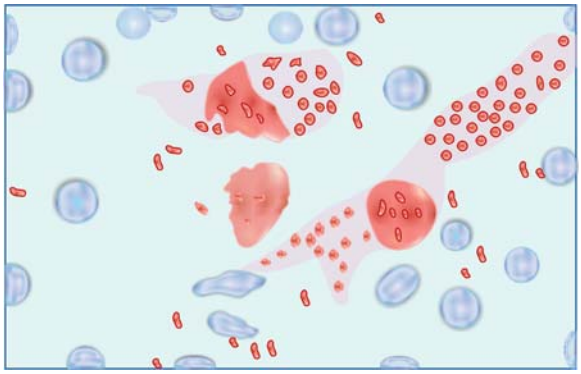
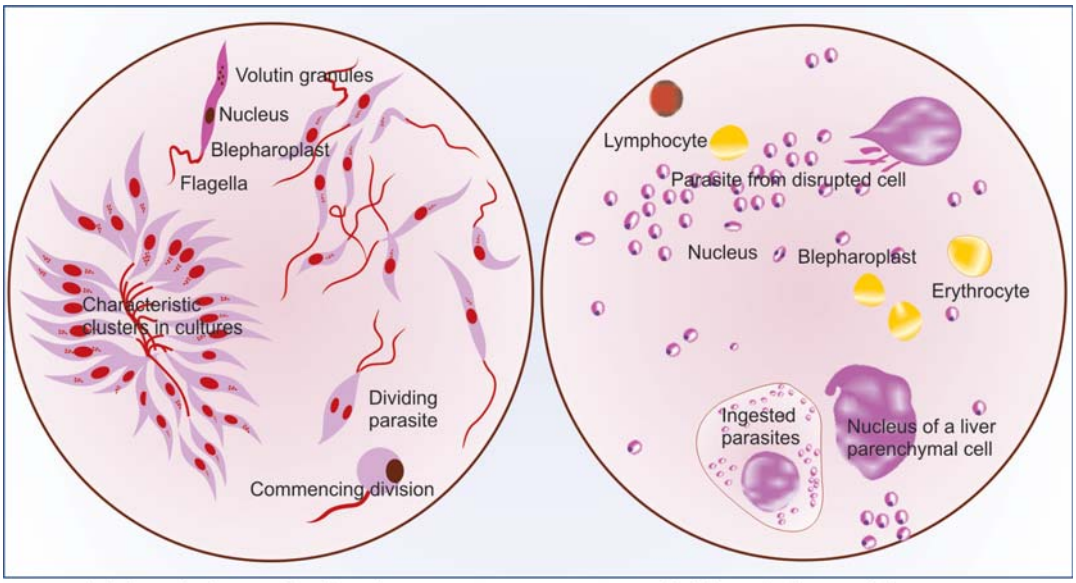


FIGURE 4.9B: LD body in spleen smear of experimentally infected animal (Giemsa stain)

The amastigote form (LD body) is an ovoid or rounded cell, about 2 to 4 µm in size. It is typically intracellular, being found inside macrophages, monocytes, neutrophils or endothelial cells.

Smears stained with Leishman, Giemsa or Wright stains show a pale blue cytoplasm enclosed by a limiting membrane. The large oval or round nucleus is stained red. Lying at right angles to the nucleus is the red or purple stained kinetoplast. In well-stained preparations, the kinetoplast can be seen to consist of the parabasal body and a dot-like blepharoplast with a delicate thread connecting the two. The axoneme arising from the blepharoplast extends to the anterior tip of the cell. Alongside the kinetoplast can be seen a clear unstained vacuole.



***Leishmania donovani*, culture form (Giemsa stain, magn. x 1100)**

***Leishmania donovani*, liver smear (Giemsa stain, magn. x 1100)**

FIGURE 4.10

The habitat of the amastigote LD body is the reticuloendothelial system. They are found mostly within the macrophages in the spleen, liver and bone marrow and less often in other locations such as the skin, intestinal mucosa and mesenteric lymph nodes. They multiply by binary fission, producing numerous daughter cells that distend the macrophage and rupture it. The liberated daughter cells are in turn phagocytosed by other macrophages and histiocytes. Small numbers of LD bodies can be found in peripheral blood inside polymorphonuclear leukocytes or monocytes. Rarely they may be seen in feces, urine and nasal secretions.

When a vector sandfly feeds on an infected person, the amastigotes present in peripheral blood and tissue fluids enter the insect along with its blood meal. In the midgut (stomach) of the sandfly, the amastigote elongates and develops into the promastigote form.

The promastigotes, which are initially short oval or pear-shaped forms, subsequently become long spindle-shaped cells, 15 to 25 µm long, carrying a single flagellum 15 to 30 µm in length. Stained films show pale blue cytoplasm with a red nucleus in the centre. The kinetoplast lies transversely near the anterior end. Near the root of the flagellum is present a vacuole. As the flagellum extends anteriorly without curving back on the body, there is no undulating membrane. Promastigote forms which develop in artificial cultures have the same morphology as those in the sandfly.

The promastigotes multiply by longitudinal binary fission and reach enormous numbers. They may be seen as large rosettes with their flagella entangled. In the

sandfly, they migrate from the midgut to the pharynx and hypostome, where they accumulate and block the passage. Such *blocked sandflies* have difficulty in sucking blood. When they bite a person and attempt to suck blood, plugs of adherent parasites may get dislodged from the pharynx and deposited in the punctured wound. The promastigotes so deposited are phagocytosed by macrophages inside which they change into amastigotes and start multiplying. These, in turn enter the midgut of a sandfly when it bites the infected person. It takes about 6 to 10 days after ingestion of the amastigotes for the promastigotes to reach adequate numbers so as to block the buccal cavity and pharynx of the sandfly. This is, therefore, the duration of the extrinsic incubation period (Fig. 4.11). This is also synchronous with the gonadotropic cycle of the vector so that amastigotes ingested during one blood meal, are ready to be transmitted when the sandfly takes the next blood meal, after its eggs have been laid.

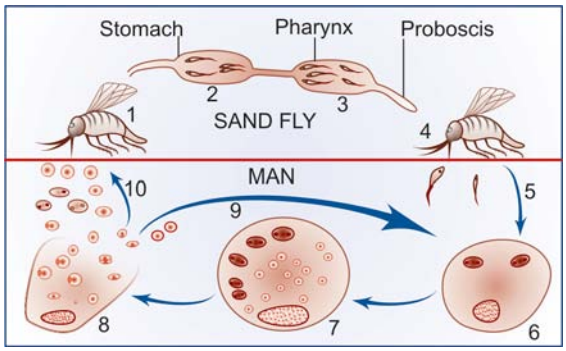


FIGURE 4.11: Life cycle of *Leishmania donovani* 1. Sandfly feeding on infected person ingests amastigotes. 2. In the stomach of the sand-fly, the amastigote becomes promastigote, which multiplies by binary fission. 3. Promastigotes accumulate in pharynx and block the passage. 4. When the sandfly bites a person, 5. The promastigotes get deposited in the puncture wound. 6. They are phagocytosed by macrophage. 7. In which they multiply, distending the cell. 8. The macrophage ruptures, releasing the amastigotes, some of which are 9. Phagocytosed by other macrophages. 10. Amastigotes in peripheral blood and skin are ingested by sandflies while feeding, to repeat the cycle

Ecological Types

The epidemiology and clinical features of visceral leishmaniasis and the ecology of the parasite are very different in different geographical areas. The different clinical syndromes have therefore been considered to be distinct entities and the parasites causing them have been given separate species or subspecies status, as listed below.

i. Indian visceral leishmaniasis caused by *L.donovani* producing the anthroponotic disease kala-azar, and its sequel 'post-kala-azar dermal leishmaniasis' (PKDL). This disease is not zoonotic, humans being the only host and reservoir. Vector is the sandfly *Phlebotomus argentipes*. (In India, classical kala-azar has rarely been seen caused by *L.tropica*).

ii. Mediterranean—Middle Eastern leishmaniasis caused by *L. donovani infantum* (or *L.infantum*) affecting mostly young children. It is a zoonotic disease, the reservoir being dogs or wild canines such as foxes, jackals, and wolves. Vectors are *P. perniciosus* and *P. ariasi*.

iii. East African leishmaniasis caused by *L.d.archibaldi*. The disease is zoonotic, found mainly in rural areas. Reservoirs are dogs, mongoose and wild mammals. Vectors are *P. orientalis* and *P. martini*.

iv. South American leishmaniasis caused by *L.d.chagasi* (*L.chagasi*). The disease is zoonotic. Foxes and wild canines are the reservoirs. Dogs act as the link between the reservoir hosts and humans. The main vector is the sandfly *Lutzomyia longipalpis*.
v. In China, the disease resembles the Mediterranean type (*L. infantum*) in the North West and the Indian type (*L.donovani*) in the East.
vi. American (New World) visceral leishmaniasis is caused by *L.chagasi*. It is present in most parts of LatinAmerica and resembles the disease caused by *L.infantum*.

KALA-AZAR

The disease visceral leishmaniasis was first characterised in India, where it was known under the names, kala-azar (meaning black sickness), Dum Dum fever, Burdwan fever or tropical splenomegaly.

Clinical Features

The infection is transmitted by the bite of the sandfly *P.argentipes*. Instances of transmission of the disease by blood transfusion, sexual contact, inoculation and congenitally have been recorded, but these are extremely rare and of no epidemiological significance. Most infections are inapparent or subclinical and only about 3 per cent develop the typical kala-azar syndrome. The incubation period is usually from 2 to 6 months, though occasionally it may be as short as 10 days or as long as two years. Cutaneous lesion at the site of bite of the sandfly is not seen in Indian patients, but is common in patients in Sudan and the Middle East.

The onset is typically insidious. The clinical illness begins with fever, which may be continuous, remittant or irregular. Splenomegaly starts early and is progressive and massive. Hepatomegaly and lymphadenopathy also occur but are not so prominent. The disease progresses for several months, with periods of apyrexia followed again by fever. Emaciation and anaemia develop. The skin becomes dry, rough arid darkly pigmented (hence the name kala-azar). The hair becomes thin and brittle. Epistaxis and bleeding gums are common. Most untreated patients die in about 2 years due to some intercurrent disease such as dysentery or tuberculosis.

About 10 to 20 per cent of pateints who recover develop post kala-azar dermal leishmaniasis (PKDL). The dermal lesions usually develop about a year or two after recovery from the systemic illness. The lesions are of 3 types—*depigmented macules* which appear commonly on the trunk and extremities, or *erythematous patches* appearing on the face (butterfly patch), both of which develop into painless yellowish pink non-ulcerating *granulomatous nodules*. The parasite can be demonstrated in the lesions. PKDL is seen mainly in India. It is rare in East Africa and China and not seen elsewhere.

Pathology

Kala-azar is a reticuloendotheliosis resulting from the invasion of the reticuloendothelial system by *L.donovani.* Parasitised macrophages disseminate the infection to

all parts of the body. In the spleen, liver and bone marrow particularly, the amastigotes multiply enormously in the fixed macrophages to produce a 'blockade' of the reticuloendothelial system. This leads to a marked proliferation of the reticuloendothelial tissue in these organs.

The spleen is the organ most affected. It is grossly enlarged and the capsule is frequently thickened due to perisplenitis. It is soft and friable and cuts easily without resistance, due to absence of fibrosis. The cut section is red or chocolate in colour due to the dilated and engorged vascular spaces. The trabeculae are thin and atrophic. Microscopically, the reticulum cells are greatly increased in numbers and are loaded with LD bodies. Lymphocytic infiltration is scanty , but plasma cells are numerous.

The liver is enlarged. The Kupffer cells and vascular endothelial cells are heavily parasitised, but hepatocytes are not affected. Liver function is therefore not seriously affected, though prothrombin production is commonly decreased. The sinusoidal capillaries are dilated and engorged. Some degree of fatty degeneration is seen. The cut surface may show a nutmeg appearance.

The bone marrow is heavily infiltrated with parasitised macrophages which may crowd out the haemopoietic tissues. Peripheral lymph nodes and lymphoid tissues of the nasopharynx and intestine are hypertrophic due to infiltration with parasitised cells, though this is not frequently seen in Indian cases.

Anaemia occurs as a result of infiltration of the bone marrow as well as by the increased destruction of erythrocytes due to hypersplenism. Autoantibodies to red cells may contribute to haemolysis. Leucopenia, with marked neutorpenia, and thrombocytopenia are frequently seen. Polyclonal hypergammaglobulinaemia is a common finding.

Immunity

The most important immunological feature in kala-azar is the marked suppression of cell mediated immunity to leishmanial antigens. This makes possible the unrestricted intracellular multiplication of the parasite. Cellular responses to tuberculin and other antigens are also suppressed and may be regained some 6 weeks after recovery from the disease.

In contrast, there is an overproduction of immunoglobulins, both specific anti-leishmania antibodies as well as polyclonal IgG and IgM immunoglobulins. Circulating immune complexes are demonstrable in serum. Complement activation occurs, but the antibodies do not appear to be relevant in defense against the parasites. Patients who have recovered from the infection are considered immune to reinfection. HIV infection heightens susceptibility to visceral leishmaniasis.

Laboratory Diagnosis

Methods employed in laboratory diagnosis are as follows:

1. Demonstration of the parasite in materials obtained from patients, by:
   a. microscopy.
   b. culture.
   c. animal inoculation.

2. Demonstration of antibodies or antigens by using:
   a. specific leishmanial antigens; or
   b. non-specific antigens.
3. Non-specific serum tests.
4. Absence of hypersensitivity to leishmanial antigen.
5. Contributory findings in clinical laboratory tests.

1. *Demonstration of Parasites in Material Obtained from Patients*

a. For microscopic demonstration of the parasite, the materials collected are:
   i. peripheral blood.
   ii. bone marrow, and
   iii. splenic aspirate.

i. *Peripheral blood* contains the amastigotes present inside circulating monocytes and less often in neutrophils, but the numbers are so scanty that a direct blood smear may not show them. Chances of detecting them are somewhat improved by examination of a thick blood film or of the leucocytic edge in a blood smear. It is best to examine buffy coat smears though even these are not often found positive. Buffy coat smears show a diurnal periodicity, more smears being positive when collected during the day than at night.

ii. *Bone marrow aspirate* is the most common diagnostic specimen collected. Generally the sternal marrow is aspirated by puncturing the sternum at the level of the 2nd or 3rd intercostal space, using a sternal puncture needle. This consists of a short stout needle with a stylet. It has a movable guard which is fixed at 1 to 2 cm from the tip, depending on the thickness of the chest wall over the sternum. After disinfecting and anaesthetising the skin, the needle is introduced into the sternal marrow and about 0.5 ml of marrow fluid aspirated using a syringe. The puncture wound is sealed with celloidin or Tr. benzoin. Bone marrow samples can be obtained also by puncturing the iliac crest.

iii. *Spleen aspirates* are richer in parasites and so more valuable for diagnosis. But the procedure can sometimes cause dangerous bleeding and so should be done carefully and only when a marrow examination is inconclusive. To guard against bleeding. prothrombin time and platelet count should be checked before the procedure. The spleen should be palpable at least 3 cm below the costal margin. The spleen is penetrated with a 21-gauge needle attached to a 5 ml syringe and aspiration done by applying gentle suction.

Lymph node aspirates are not useful in the diagnosis of Indian kala-azar, though it is employed in visceral leishmaniasis in some other countries.

The materials collected, as described above can be tested by microscopy, culture and animal inoculation.

a. For *microscopy*, smears are stained by Leishman, Giemsa or Wright stains and examined under the oil immersion objective. Amastigote parasites (LD bodies) can be seen within macrophages, often in large numbers. A few extracellular forms can also be seen usually (see Fig. 4.10).

b. *Cultures* are made on Novy-McNeal-Nicolle (NNN) medium. This is a rabbit blood agar slope having an overlay of Locke's solution with added antibiotics (penicillin, streptomycin, gentamicin) dispensed in screw capped bottles. The material is inoculated into the water of condensation and the culture incubated at 24°C for 7 days. The parasite grows as promastigotes and can be demonstrated by examining a drop of the fluid under high power objective using reduced condenser aperture or preferably, phase contrast illumination. Stained smears can also be examined. If negative, the culture is reincubated and examined weekly for 4 to 6 weeks. Schneider's liquid tissue culture medium with added foetal calf serum is also used for culture.

c. *Animal inoculation* is not used for routine diagnosis. When necessary, hamster is the animal employed. The materials are inoculated intraperitoneally, or intradermally into the skin of the nose and feet. The inoculated animals are kept at 23 to 26°C. In positive cases, the parasite can be demonstrated in smears taken from ulcers or nodules developing at the sites of cutaneous inoculation, or from the spleen. Animal inoculation is a very sensitive method, but takes several weeks to become positive.

2. *Demonstration of Antibodies or Antigens*

a. Specific leishmanial antigens prepared from cultures have been used in a number of tests to demonstrate specific antibodies. These tests include complement fixation. counter immunoelectrophoresis, immunofluorescence and ELISA tests. In kala-azar, the immunofluorescent antibody (IFA) titre usually rises to 64 or above and declines slowly after treatment, eventually becoming negative. The direct agglutination test for anti-leishmanial antibody has been found to be highly specific and sensitive for diagnosis of kala-azar. A specific immunochromatographic dipstick method for antibody has been developed using recombinant leishmanial antigens.

b. Specific antigen detection tests have been developed by immunoblotting and PCR. Nonspecific (nonleishmanial) antigens for serological tests have been used for many decades. The antigen originally used was prepared from human tubercle bacillus by Witebsky, Klingenstein and Kuhn (hence called the WKK antigen). Complement fixation test with WKK antigen becomes positive early in the disease, within weeks of infection. Positive reaction also occurs in some other conditions, including tuberculosis, leprosy and tropical eosinophilia. An antigen prepared from Kedrowsky's acid-fast bacillus is preferred.

3. *Nonspecific Serum Tests*

Some diagnostic tests for kala-azar are based on the greatly increased globulin content of serum in the disease. The two tests widely used are Napier's aldehyde or Formol gel test and Chopra's antimony test.

i. In Napier's aldehyde test 1 ml of clear serum from the patient is taken in a small test tube, a drop of formalin (40% formaldehyde) is added, shaken and

kept in a rack at room temperature. A control tube with normal serum is also set up. A positive reaction is jellification and opacification of the test serum, resembling the coagulated white of egg, appearing within 3 to 30 minutes. About 85 per cent of patients with disease of 4 months or more give positive reaction.

ii. Chopra's antimony test is done by taking 0.2 ml of serum diluted 1 in 10 with distilled water, in a Dreyer's tube and overlaying with 4 per cent solution of urea stibamine in distilled water, run along the side of the tube. In a positive test, a thick flocculent disc forms at the junction of the two liquids in 10 to 15 minutes. This reaction is said to be more sensitive than the aldehyde test. Both tests give false-positive reactions in several other diseases, including tuberculosis and leprosy.

4. Absence of Hypersensitivity to Leishmanial Antigen

A delayed hypersensitivity skin test, first introduced in South America by Montenegro, is known after his name. The Montenegro (leishmanin) skin test is negative in kala-azar. The test is done by injecting intradermally 0.1 ml of killed promastigote antigen. The test is read after 72 hours. It is positive in dermal leishmaniasis and in persons who have recovered from kala-azar , but not in active cases. In endemic areas, a number of healthy persons show a positive reaction, indicating prior exposure to the infection.

5. Contributory Findings in Clinical Laboratory Tests

The following clinical laboratory tests give supportive evidence for the diagnosis of the disease. Blood examination shows a normocytic normochromic anaemia, leucopenia, neutropenia and thrombocytopenia. Serum globulin level is markedly elevated with reversal of albumen-globulin ratio.

Epidemiology

Visceral leishmaniasis occurs in various parts of the world, from South America in the west to China in the east, with local variations with respect to the ecology of the parasite and its vector as well as the clinical manifestations. It is a zoonotic disease in all areas except in India where it is an anthroponotic disease with no known reservoir other than man (Fig. 4.12).

The Indian disease kala-azar usually involves adolescents and young adults, males being affected twice as often as females. The Mediterranean type is seen in infants and children below 5 years of age. The Chinese type is more common among juveniles but also occurs in adults. The Sudanese type is found mostly in adolescents and young adults. This type is generally resistant to pentavalent antimonials. The South American type occurs at all ages.

The vector sandfly species is also different in different geographic areas. In India, the vector is *P.argentipes* which is a house-haunting anthropophilic species highly susceptible to insecticides.

Kala-azar in India was distributed principally along the east, from Assam and Bengal along the Brahmaputra and Ganges, to Bihar , Orissa. Andhra and Tamil Nadu. During the malaria eradication campaign in the 1950s and 1960s, the sandfly .population dwindled and the disease virtually disappeared. However, following cessation of insecticide spraying, the disease staged a come back in the 1970s particularly in Bihar, Orissa, West Bengal and Assam, where epidemics have occurred involving several thousands of cases. Sporadic cases have also been reported from Gujarat, Uttar Pradesh, Punjab, Jammu, Himalayan foothills and Tamil Nadu.

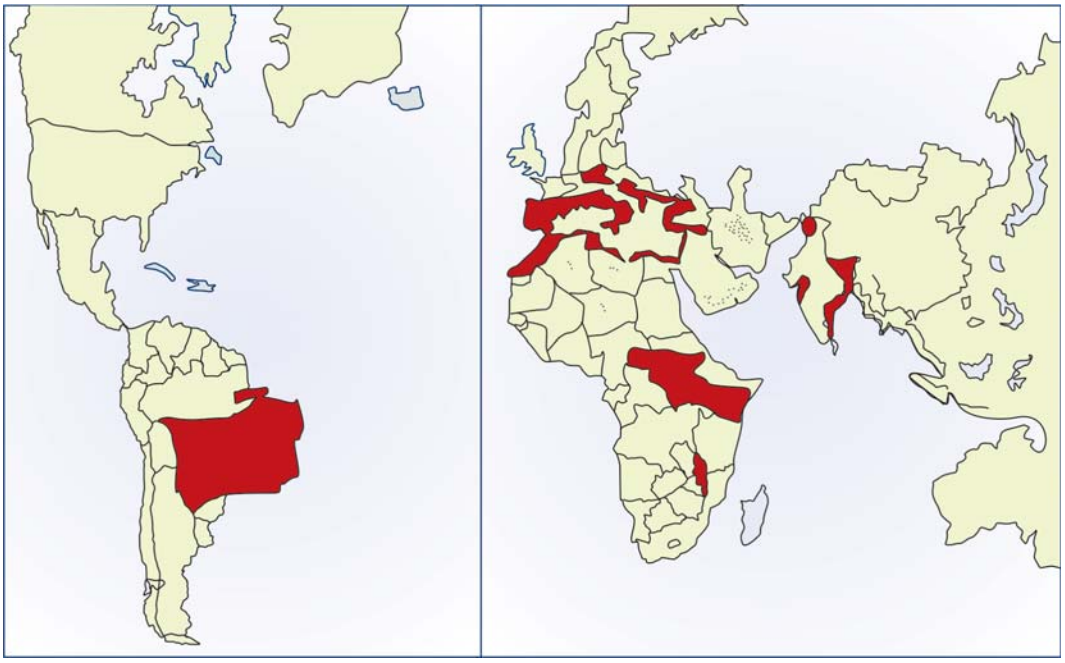


FIGURE 4.12: Geographical distribution of visceral leishmaniasis. Endemic areas shaded; Dots indicate sporadic cases

Kala-azar has become a common opportunistic infection in the HIV infected persons in Mediterranean countries, particularly in injectable drug users. In them the disease shows atypical features, such as involvement of intestines, lungs and CNS. They are generally refractory to treatment.

Treatment

Kala-azar responds to treatment better than other forms of visceral leishmaniasis. The standard treatment is the pentavalent antimonial sodium stibogluconate given intravenously 600 mg daily for 6 days. However, antimony resistance has become a serious problem. The aminoglycoside antibiotic aminosidine (paromomycin) is useful, given alone or with antimonials. The dose is 14 mg/kg body weight daily

given IM or as slow IV infusion once daily for 3 to 4 weeks. An alternative is pentamidine 4 mg/kg/ day given IM for 10 days. If this also does not succeed, amphotericin 0.25 to 1 mg/kg/day may be given as slow infusion for upto 8 weeks. By using liposomal amphotericin, higher doses can be given, improving the cure, without toxicity. Meltefosine is an effective oral treatment.

Prevention

Prophylactic measures consist of treating all cases, eradication of the vector sandfly and personal prophylaxis by using antisandfly measures.

CUTANEOUS LEISHMANIASIS

Causative Agent and Geographical Distribution

Leishmania morphologically similar to *L. donovani* have been responsible for cutaneous lesions in various parts of the world. The disease has been broadly classified as the Old World and New World types of cutaneous leishmaniasis.

In the old world, the disease has been recognised in a wide area, from the countries along the Mediterranean coast, the Middle East and as far east as India. In India, the condition is prevalent in the North West, particularly in Rajasthan. The condition has been called Oriental sore and by various place names such as Delhi boil, Aleppo boil, Bagdad or Biskra button. (Oriental sore is distinct from tropical ulcer, a term applied to ulcers caused by fusospirochaetal infection). The parasites producing Old World cutaneous leishmaniasis belong to the species *L.major, L.tropica* and *L. aethiopica*.

In the New World, the disease occurs in South and Central America and the causative agents have been named *L. braziliensis* and *L. mexicana* (Fig. 4.13).

Morphology and Life Cycle

The morphology and life cycle of these species resemble those of *L.donovani*. The amastigotes are present in the skin, within large mononuclear cells, in neutrophils, inside capillary endothelial cells and also free in the tissues. They are ingested by sandflies feeding near the skin lesions. In the midgut of the sandfly, the amastigotes develop into promastigotes which replicate profusely. These are in turn transmitted to the skin of persons bitten by the sandflies. In the skin, the promastigotes are phagocytosed by the mononuclear cells, in which they become amastigotes and multiply. However, they remain confined to the skin, without being transported to the internal organs as is the case in visceral leishmaniasis.

Though the common mode of infection is through sandflies, infection may also sometimes occur by direct contact. Infection may be transmitted from man-to-man or animal-to-man by direct inoculation of amastigotes. Infection may also occur by autoinoculation. Sometimes, the sandfly may act as a mechanical vector by transmitting the amastigotes from a sore on a patient into the bite wound on another person.

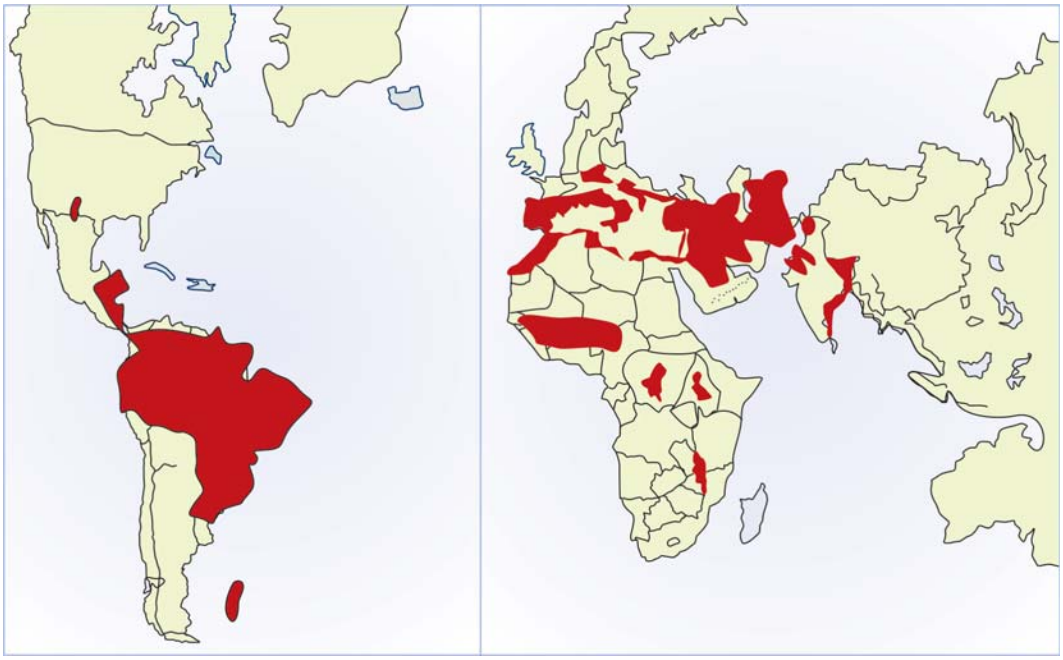


FIGURE 4.13: Geographical distribution of cutaneous and mucocutaneous leishmaniasis. Endemic areas are shaded. Dots indicate sporadic cases (Courtesy WHO)

Clinical Features and Epidemiology

The clinical and epidemiological patterns vary from region-to-region. .

Old World Cutaneous Leishmaniasis

Three distinct patterns have been recognised.

i. The anthroponotic urban type causing painless dry ulcerating lesions, often single, leading to disfiguring scars, caused by the species *L. tropica*. This is seen mainly in children in endemic areas. The incubation period is usually 2 to 8 months. The dry ulcers usually heal spontaneously in about a year. This is prevalent from the Middle East to North Western India. The most important vector is *P. sargenti*.

ii. The zoonotic rural type causing moist ulcers which are inflamed, often multiple, caused by *L.major*. The incubation period is usually less than 4 months. The ulcers heal in several months. This is seen in the lowland zones of Asia, Middle East and Africa. Gerbils, rats and other rodents are the reservoirs. *P.papatasi* is the most important vector.

iii. The non-ulcerative and often diffuse lesions caused by *L.aethiopica* seen in the highlands of Ethiopia and Kenya. *P. longipes* is the usual vector.

The oriental sore begins as a small papule which gradually enlarges to form a raised indurated lesion with surrounding erythema. It often ulcerates. Healing occurs

spontaneously in several months leaving behind a slightly depressed papery scar. The lesions occur mostly on the exposed parts of the body, especially on the face and hands.

Diagnosis is by microscopic demonstration of the amastigote form of the parasite in material obtained by puncturing the edges of the lesion. Cultures may be obtained on NNN medium. The intradermal leishmanin test (Montenegro reaction) is positive, indicating good cell mediated immunity (delayed hypersensitivity) to the parasite.

Most cutaneous lesions heal spontaneously. Pentavalent antimonials are the drugs of choice for treatment. Metronidazole, rifampicin and local application of heat also may give good results. Aminosidine ointment has been found to be a useful local treatment.

Patients lacking adequate cell-mediated immunity may develop diffuse cutaneous leishmaniasis. Numerous nodular non-ulcerating lesions develop, particularly on the face and limbs which resemble the lesions of lepromatous leprosy. American soldiers infected by *L. tropica* during the Gulf war were reported to have developed viscerotropic disease with lymph node and bone marrow involvement.

Leishmaniasis recidivans is a type of lesion seen in persons with a high degree of cell-mediated immunity to the parasite. The lesions are chronic with alternating periods of activity and healing, characterised by a central scar with peripheral activity .The lesions resemble those of lupus or tuberculoid leprosy. Parasites are very scanty in the lesions. The leishmanin test is strongly positive. Chemotherapy is not very useful. Better results follow local application of heat.

New World Cutaneous Leishmaniasis

In the New World two groups of leishmania cause cutaneous lesions, *L. mexicana* causing cutaneous ulcers and occasionally diffuse cutaneous leishmaniasis; and *L.braziliensis* and *L.guyanensis* causing cutaneous lesions which may lead to mucocutaneous leishmaniasis or espundia.

Mucocutaneous leishmaniasis or espundia is seen in South America as a late consequence of cutaneous leishmaniasis. Granulomas develop at mucocutaneous junctions especially around the nose and mouth followed by gross destruction of soft tissue and cartilage leading to marked disfiguration. Secondary anaerobic bacterial infection adds to the severity of the disease.

CHAPTER 5

Malaria Parasites

Malaria is the most important parasitic disease of mankind. It accounts for over 300 million cases and 2 million deaths annually, the large majority of them in Subsaharan Africa. Once prevalent over much of the world, it is now confined to the tropical and subtropical areas of Asia, Africa, South and Central America. Even so, nearly half of the world's population may be exposed to the risk of malaria.

MALARIA

History

Malaria (or *ague*, as it was called earlier) has been known from antiquity . Seasonal intermittent fevers with chills and shivering, recorded in the religious and medical texts of ancient Indian, Chinese and Assyrian civilisations, are believed to have been malaria. Charaka and Susruta have described the disease and noted its association with mosquitoes. Hippocrates in Greece in the 5th century BC gave a detailed account of the clinical picture and observed the prevalence of the disease in certain places and seasons. The relation between the disease and stagnant waters, swamps and marshy lands was recognised and measures to control the disease by effective drainage were practised in Rome and Greece by the 6th century AD. The name malaria (*mal*-bad, *aria*-air) was given in the 18th century in Italy as it was believed to be caused by foul emanations from the marshy soil. Paludism, another name for malaria, also has a similar origin from *palus*, Latin for 'marsh'. The recent demonstration of a specific parasitic antigen in Egyptian mummies indicates that malaria was present thousands of years ago.

The specific causative agent of malaria was discovered in the red blood cells of a patient in 1880 by Alphonse Laveran, a French army surgeon in Algeria. In 1886, Golgi in Italy described the asexual development of the parasite in red blood cells (*erythrocytic schizogony*), which therefore came to be called the *Golgi cycle*. Romanowsky in Russia in 1891, developed a method of staining malaria parasites in blood films. Three different species of malaria parasites infecting man, *Plasmodium vivax, P. malariae* and *P.falciparum* were described in Italy between 1886 and 1890. The fourth species,

P.ovale was identified only in 1922. The mode of transmission of the disease was established in 1897, when Ronald Ross in Secunderabad, India identified the developing stages of malaria parasites in mosquitoes. This led to various measures for the control and possible eradication of malaria by mosquito control. Both Ross (1902) and Laveran(1907) won the Nobel Prize for their discoveries in malaria.

Causative Agents

Four species of plasmodia cause malaria in man, *P. vivax, P. falciparum, P. malariae* and *P. ovale*. Two species, *P.vivax* and *P.falciparum* account for about 95 per cent of all malaria worldwide, the other two being of relatively minor importance.While these four species do not ordinarily infect animals, there is evidence that chimpanzees may act as a reservoir host for *P. malariae* in Africa, providing a possible source of human infection.

Malaria parasites belong to phylum-Apicomplexa, class-Sporozoea, Order- Eucoccidea, Suborder-Haemosporina.

The genus *Plasmodium* is divided into 2 subgenera;—*P. vivax, P. malariae* and *P. ovale* belong to the subgenus *Plasmodium* while *P. falciparum* is allocated to the subgenus Laverania because it differs in a number of respects from the other three species.

Several species of *Plasmodium* cause natural infection in birds and animals. Examples of monkey malaria parasites that have been used widely for experimental studies on malaria are *P.cyanomolgi, P.inui*, and *P.knowlesi.* While it is possible to produce experimental infection in man with some of these simian parasites, there is no evidence that this occurs to any significant extent in nature. *P.knowlesi,* a natural parasite of rhesus monkeys, is found to infect aborigines in the jungles of Malaysia. Examples of plasmodia infecting birds are *P.gallinaceum* and *P.elongatum*.

P. vivax, P. malariae and *P.ovale* are closely related to other primate malaria parasites. *P. falciparum* on the other hand, is more related to bird malaria parasites, and appears to be a recent parasite of humans, in evolutionary terms. Perhaps for this reason, falciparum infection causes the severest form of malaria and is responsible for nearly all fatal cases.

Vectors

Human malaria is transmitted by the female Anopheles mosquito. The male mosquito feeds exclusively on fruit juices, but the female needs at least two blood meals before the first batch of eggs can be laid. Malaria parasites of animals (apes, monkeys, rodents) are transmitted by *Anopheles*, but bird malaria parasites are carried by *Culex, Aedes* and other genera of mosquitoes.

Life Cycle and Morphology

The life cycle of malaria parasites comprises two stages—an *asexual phase* occurring in humans and the *sexual phase* occurring in the mosquito.

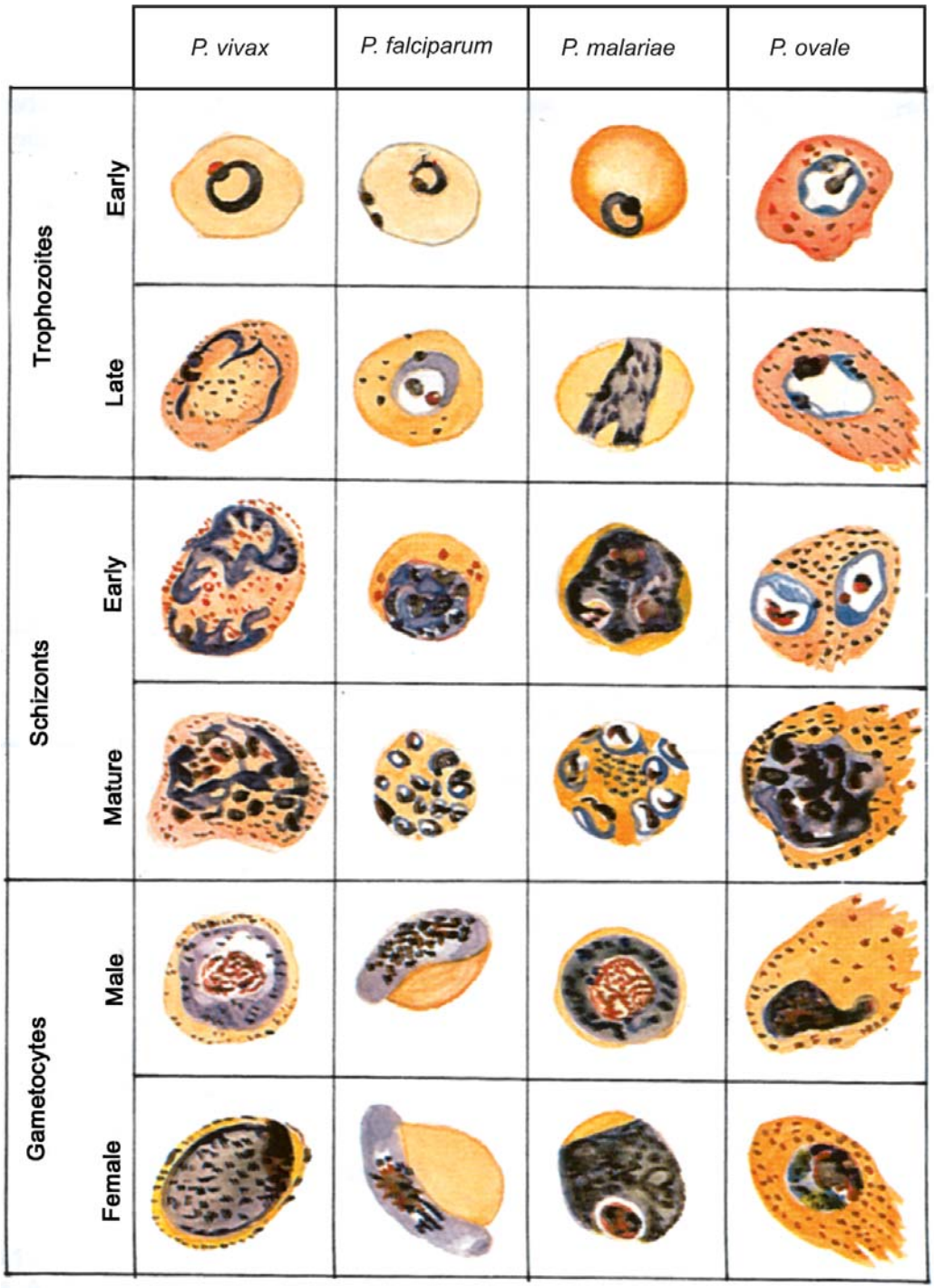


FIGURE 5.1: Malaria parasites—Erythrocytic stages of the four species (Giemsa stain. Magn × 2000)

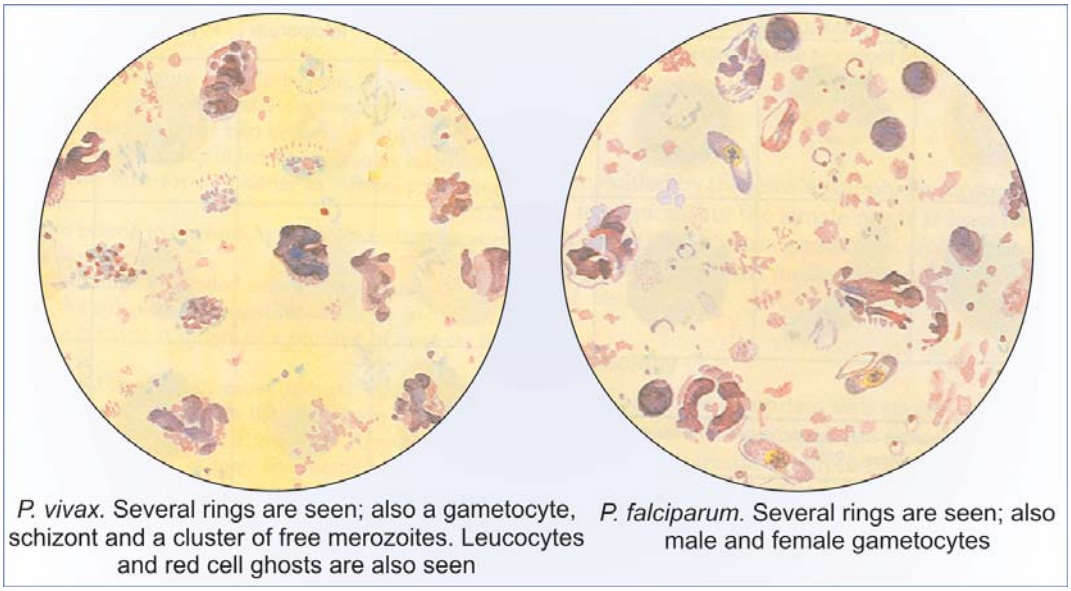


FIGURE 5.2: Malaria parasites in thick blood smear (Giemsa stain, Magn. × 1000)

i. In the asexual phase the parasite multiplies by division or splitting, a process designated *schizogony* (from *schizo*-to split, and *gone*-generation). Because this asexual phase occurs in man it is also called the *vertebrate, intrinsic* or *endogenous phase*. In humans, schizogony occurs in two locations—in the red blood cell (*erythrocytic schizogony*) and in the liver cells (*exoerythrocytic schizogony* or the *tissue phase*). Because schizogony in the liver is an essential step before the parasites can invade erythrocytes, it is called *pre-erythrocytic schizogony*. The products of schizogony, whether erythrocytic or exoerythrocytic, are called *merozoites* (*meros*-a part, *zoon*-animal).
ii. The sexual phase takes place in the female Anopheles mosquito, even though the sexual forms of the parasite (*gametocytes*) originate in human red blood cells. Maturation and fertilisation take place in the mosquito, giving rise to a large number of sporozoites (from *sporos*-seed). Hence this phase of sexual multiplication is called *sporogony*. It is also called the *invertebrate, extrinsic*, or *exogenous phase*.

There is thus an *alternation of generations* in the life cycle of malaria parasites—asexual and sexual generations alternatively. There also occurs an *alternation of hosts*, as the asexual phase takes place in humans followed by the sexual phase in the mosquito. Therefore, the complete life cycle of the malaria parasite comprises an alternation of generations with an alternation of hosts. As the sexual phase occurs in the mosquito, it is considered the *definitive host* of malaria parasites. Humans are the *intermediate host* as the human phase consists of asexual multiplication.

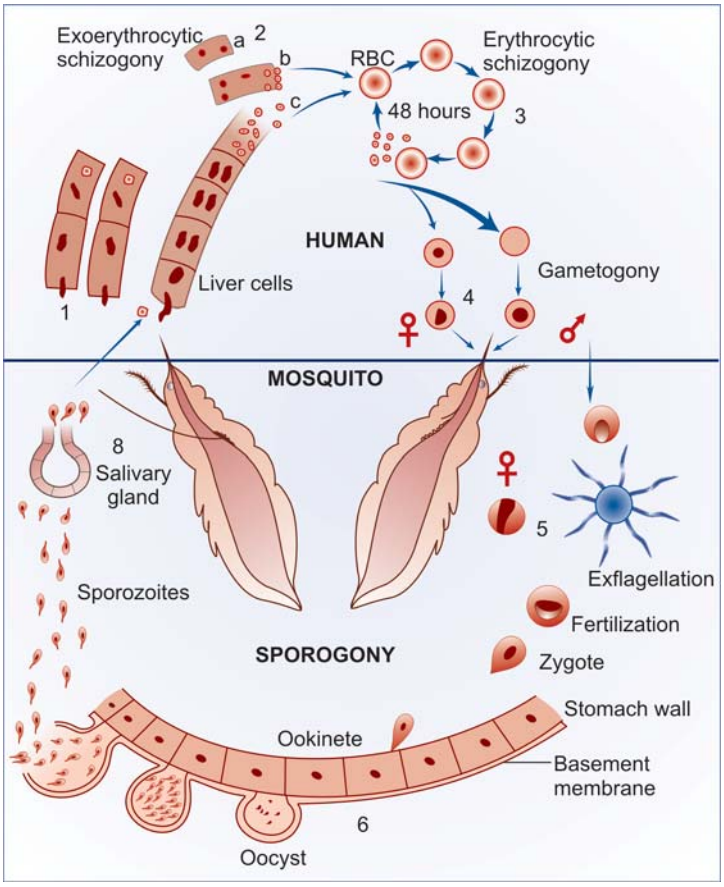


FIGURE 5.3: Life cycle of *Plasmodium vivax*. (1) Sporozoites present in the salivary gland of female Anopheles mosquito are injected into skin capillaries when the mosquito bites humans. (2) They enter liver cells to initiate exoerythrocytic schizogony. Some sporozoites become dormant hypnozoites (2a,b) which are reactivated after varying intervals to produce relapses. Most sporozoites complete the pre-erythrocytic schizogony (2c). to form merozoites which infect red blood cells, to initiate the cycle of erythrocytic schizogony (3) which is repeated every 48 hours. Some merozoites initiate gametogony, forming male and female gametocytes (4) which are ingested by mosquito in its blood meal. Male gametocyte undergoes exflagellation.(5) one male gamete fertilises female gamete to form zygote. It develops into the motile ookinete, which penetrates the stomach wall and becomes the oocyst inside which sporozoites develop. (6) Sporozoites released by rupture of mature oocyst (7) enter the haemocoele (8) and reach the salivary glands of the mosquito

The Human Phase

Human infection comes through the bite of the infective female Anopheles mosquito. The sporozoites which are infective forms of the parasite are present in the salivary gland of the mosquito. They are injected into blood capillaries when the mosquito

feeds on blood after piercing the skin. Usually 10 to 15 sporozoites are injected at a time, but occasionally many hundreds may be introduced. The sporozoites pass into the blood stream, where many are destroyed by the phagocytes, but some reach the liver and enter the parenchymal cells (hepatocytes).

Exo-erythrocytic (Tissue) Stage

Within an hour of being injected into the body by the mosquito, the sporozoites reach the liver and enter the hepatocytes to initiate the stage of pre-erythrocytic schizogony or *merogony*. The sporozoites which are elongated spindle-shaped bodies become rounded inside the liver cells. They enlarge in size and undergo repeated nuclear division to form several daughter nuclei, each of which is surrounded by cytoplasm. This stage of the parasite is called the *pre-erythrocytic* or *exoerythrocytic schizont* or *meront*. The hepatocyte is distended by the enlarging schizont and the liver cell nucleus is pushed to the periphery. Unlike in erythrocytic schizogony, there is no pigment in liver schizonts. In 5.5 to 15 days the schizont becomes mature and bursts, releasing thousands of merozoites. They enter the blood stream and infect the erythrocytes by a process of invagination. The interval between the entry of the sporozoites into the body and the first appearance of the parasites in blood is called the prepatent period. The duration of the pre-erythrocytic phase in the liver, the size of the mature schizont and the number of merozoites produced vary with the species of the parasite.

Pre-erythrocytic schizogony involves only a very small proportion of liver cells and causes no significant damage or clinical illness. Liver schizonts cannot be demonstrated in natural human infections, but have been observed in splenectomised chimpanzees or human volunteers experimentally infected with very large numbers of sporozoites (Table 5.1).

Table 5.1: Features of pre-erythrocytic schizogony in human malaria parasites

	P.vivax	*P.falciparum*	*P.malariae*	*P.ovale*
Pre-erythrocytic stage (days).	8	5.5	15	9
Diameter of pre-erythrocytic schizont (µm)	45	60	55	60
No. of merozoites in pre-erythrocytic schizont	10,000	30,000	15,000	15,000

Formerly, it was postulated that some merozoites released after the primary exoerythrocytic schizogony invaded other hepatocytes to initiate the secondary exoerythrocytic schizogony. Such exoerythrocytic schizogony was believed to be repeated for a few generations and was considered to explain the occurrence of relapses in *P. vivax* and *P. ovale* infections. This view is no longer held.

In *P.vivax* and *P. ovale*, two kinds of sporozoites are seen, some which multiply inside hepatocytes promptly to form schizonts and others which remain dormant. These latter forms are called *hypnozoites* (from *hypnos*-sleep). Hypnozoites remain inside the hepatocytes as uninucleated forms, 4 to 5 µm in diameter, for long periods. From time-to-time, some are activated to become schizonts and release merozoites, which go on to infect erythrocytes, producing clinical relapses. This is the present concept of relapses in vivax and ovale malaria. Secondary exoerythrocytic schizogony is not believed to occur (Fig. 5.3).

In *P.falciparum* and *P.malariae* no hypnozoites are formed and the parasites do not persist in the exoerythrocytic phase. However, a small number of erythrocytic parasites persist in the blood stream, and in course of time, multiply to reach significant numbers, resulting in clinical disease (*short-term relapse* or *recrudescence*). In falciparum malaria recrudescences are seen for one or two years, while in *P.malariae* infections, they may last for long periods, even up to 50 years.

Erythrocytic Stage

The merozoites released by pre-erythrocytic schizonts invade the red blood cells. The receptor for merozoites is glycophorin, which is a major glycoprotein on the red cell. The differences in the glycophorins of red cells of different species may account for the species specificity of malaria parasites. Merozoites are pear-shaped bodies about 1.5 µm in length, possessing an *apical complex* (*rhoptry*). They attach to erythrocytes by their apex, which has certain organelles that secrete a substance producing a pit on the erythrocyte membrane. The merozoite then enters the erythrocyte by endocytosis and the red cell membrane seals itself to form a vacuole (*parasitophorous vacuole*) enclosing the merozoite. The process of entry into the red cell takes about 30 seconds. Once inside the red cell the merozoite rounds up and loses its internal organelles.

In the erythrocyte, the merozoite appears as a rounded body having a vacuole in the centre with the cytoplasm pushed to the periphery and the nucleus situated at one pole. When stained with Giemsa or other Romanowsky stains, the cytoplasm is stained blue and the nucleus red the central vacuole remaining unstained. This gives the parasite an annular or signet ring appearance. These young parasites are therefore called the *ring forms.*

The parasite feeds on the haemoglobin of the erythrocyte. It does not metabolise haemoglobin completely and so leaves behind as residue a haematin-globin pigment. called the malaria pigment (formerly known as haemozoin pigment). These iron-containing pigments accumulate in the body of the parasite as dark granules which become more prominent as the parasite grows. The appearance of malaria pigments varies in the different species as follows:

P. vivax	Numerous fine golden brown dust-like particles.
P. falciparum	Few (one to three) solid blocks of black pigment.
P. malariae	Numerous coarse dark brown particles.
P. ovale	Numerous blackish brown particles.

The malaria pigment released when the parasitised cells rupture is taken up by reticuloendothelial cells. Such pigment laden cells in the internal organs provide histological evidence of previous malaria infection.

As the ring form develops it enlarges in size becoming irregular in shape and shows amoeboid motility .This is called the *amoeboid form*. Bits of membrane from developing parasites accumulate on the inner surface of the erythrocyte and these appear as stippling or clefts on the erythrocyte surface. When the amoeboid form reaches a certain stage of development its nucleus starts dividing. The parasite within the erythrocyte till the time its nucleus starts dividing is called the *trophozoite* (from *trophos*—growth). The ring form is called the *early trophozoite* and the amoeboid from the *late trophozoite*.

From the time the nucleus starts dividing, the parasite within the erythrocyte is called the *schizont* or *meront* (formerly also known as segmenter or rosette forms). At first only the nucleus divides into a variable number of small nuclei, the cytoplasm remaining entire and undivided. This stage is called the *early schizont*. This continues into the *late schizont* stage when each daughter nucleus becomes surrounded by cytoplasm. The *mature schizont* is the fully grown form, in which a number of small merozoites are seen, each having a nucleus with surrounding cytoplasm. The mature schizont bursts releasing the merozoites into the circulation. The residual mass of unutilised cytoplasm containing all the accumulated malarial pigment is also released at the same time into the circulation. This is phagocytosed and can be seen as pigment granules within polymorphs and macrophages. The merozoites invade fresh erythrocytes in which they go through the same process of development. This cycle of erythrocytic schizogony or merogony is repeated sequentially, leading to progressive increase in the intensity of parasitaemia till it is arrested by the development of immune response in the host.

The rupture of the mature schizont releases large quantities of pyrogens. This is responsible for the febrile paroxysms characterising malaria. The interval between the entry of the sporozoite into the host and the earliest manifestation of clinical illness is the incubation period. This is different from the prepatent period, which is the time taken from the entry of the sporozoite to the first appearance of malaria parasites in peripheral blood.

The duration of the erythrocytic schizogony varies according to the species of the parasite. An important feature determining the clinical manifestations of malaria is the tendency for the erythrocytic schizogonic cycles to become synchronised, so that all the mature schizonts in the body burst at the same time releasing merozoites and other pyrogens into circulation, causing the febrile paroxysms. It has been suggested that this schizogonic periodicity is related to the human circadian rhythm of approximately 24 hours. Thus the schizogonic periodicity is about 48 hours in *P. vivax*, *P. falciparum* and *P. ovale* while in *P.malariae* it is 72 hours. This in turn, is reflected in the periodicity of the bouts of fever in these different infections. Malarial periodicity has been recognised from early times and the colloquial terms tertian, quartan and quotidian had been applied to the different types of malaria as detailed below.

P.vivax: *Benign tertian* or *BT* malaria. (Tertian, because the fever recurs after intervals of 48 hours or every third day, according to the Greek or Roman system of counting, which counts the first and last days also. Benign, because it is relatively less dangerous than falciparum malaria which is called malignant tertian).

P.falciparum: *Malignant tertian* or *MT* malaria. (Also called subtertian. because the cycles are often poorly synchronised and febrile paroxysms recur at intervals of less than the expected 48 hours. It was also called pernicious malaria because of its lethal nature, and aestivo-autumnal referring to its seasonal prevalence).

P. malariae: *Quartan* malaria. (Occurring every fourth day, as it has a cycle of 72 hours).

P. ovale: *Ovale tertian*. (Because of its tertian periodicity and the irregular oval shape of infected RBCs).

Sometimes, especially in early *P.vivax* infections, there may be two independent broods of parasites with overlapping cycles so that there may be daily paroxysms. This is called *quotidian* periodicity.

Gametogony

After a few cycles of erythrocytic schizogony, some merozoites that infect red cells do not proceed to become schizonts, but instead develop into sexually differentiated forms, the gametocytes. They grow in size till they almost fill the red cell, but the nucleus remains undivided. Development of gametocytes generally takes place within the internal organs such as spleen and bone marrow, and only the mature forms appear in circulation. The mature gametocytes are round in shape, except in *P.falciparum*, in which they are crescent-shaped. In all species, the female gametocyte is larger (*macrogametocyte*) and has cytoplasm staining dark blue with a small compact nucleus staining deep red. In the smaller male gametocyte (*microgametocyte*), the cytoplasm stains pale blue or pink and the nucleus is larger, pale stained and diffuse. Pigment granules are prominent. Female gametocytes are generally more numerous than the male.

Gametocytes appear in circulation 4 to 5 days after the first appearance of asexual form in the case of *P.vivax* and 10 to 12 days in *P.falciparum*. A person with gametocytes in circulation is a carrier or reservoir. Children are more effective carriers than adults. Gametocytes are more numerous in the early phase of infection.

The gametocytes do not cause any clinical illness in the host, but are essential for transmission of the infection. The gametocytes do not develop further or divide in the vertebrate host and unless taken up by the vector mosquito, they die in a few days. A gametocyte concentration of 12 or more per c.mm of blood in the human host is necessary for mosquitoes to become infected.

The Mosquito Phase

When a female Anopheles mosquito ingests parasitised erythrocytes along with its blood meal, the asexual forms of malaria parasites are digested, but the gametocytes are set free in the stomach and undergo further development. Within 15 minutes

of entry into the stomach of the mosquito, the male gametocyte divides into 8 nuclei, from each of which protrudes a long, actively motile whip-like filament. These flagella which are the male gametes (*microgametes*) lash about for sometime and then break free. This process of formation of male gametes from the gametocyte is called *exflagellation*. This can take place outside the body of the mosquito also and can be observed under the microscope.

Exflagellation can be demonstrated by making a thick film of freshly drawn blood containing mature gametocytes on a slide and placing it in a warm moist chamber, such as a Petri dish containing filter paper soaked in warm water. When examined under the microscope after about 10 minutes, the male gametocyte can be seen to shed its erythrocytic envelope and put forth up to eight slender active flagella containing nuclear material from the original nucleus. Detaching from the cell body, the flagella lash about vigorously in the plasma. At 25°C, the exflagellation is complete in 15 minutes for *P. vivax* and *P. ovale*, and 15 to 30 minutes for *P.falciparum*.

The female gametocyte does not divide but undergoes a process of maturation to become the female gamete or *macrogamete*. It is fertilised by one of the microgametes to produce the zygote. Fertilisation occurs in half to two hours after the blood meal.

The zygote, which is initially a motionless round body elongates and within 18 to 24 hours, becomes a vermicular motile form with an apical complex anteriorly. This is called the *ookinete* ('travelling vermicule'). It penetrates the epithelial lining of the mosquito stomach wall and comes to lie just beneath its basement membrane. It becomes rounded into a sphere with an elastic membrane. This stage is called the *oocyst*. There may be up to several hundred pigmented oocysts in the stomach of a mosquito. It was the discovery by Ronald Ross, of pigmented oocysts in the stomach walls of dissected mosquitoes that established the mosquito transmission of malaria.

The oocyst matures, increasing in size, with the nucleus undergoing multiple divisions. This *sporogony* leads to the development within the oocyst of about a thousand sporozoites, 10 to 15 µm in length, each with a central nucleus and an anterior apical complex. The mature oocyst which may be about 500 µm in size bulges into the body cavity of the mosquito, and when it ruptures the sporozoites enter the haemocoele. The sporozoites reach the salivary glands situated in the thorax of the mosquito, penetrate the acinar cells and enter the salivary ducts. The mosquito is now infective and when it feeds on humans, the sporozoites are injected into the skin capillaries to initiate human infection (Fig. 5.4). The time taken for completion of sporogony in the mosquito is about 1 to 4 weeks; depending on the environmental temperature and the species.

The characteristics of the four species of plasmodia infecting man are listed in Table 5.2.

Plasmodium Vivax

P. vivax has the widest geographical distribution, extending through the tropics, subtropics and temperate regions. It is believed to account for 80 per cent of all malaria infections. It is the most common species of malaria parasite in Asia and

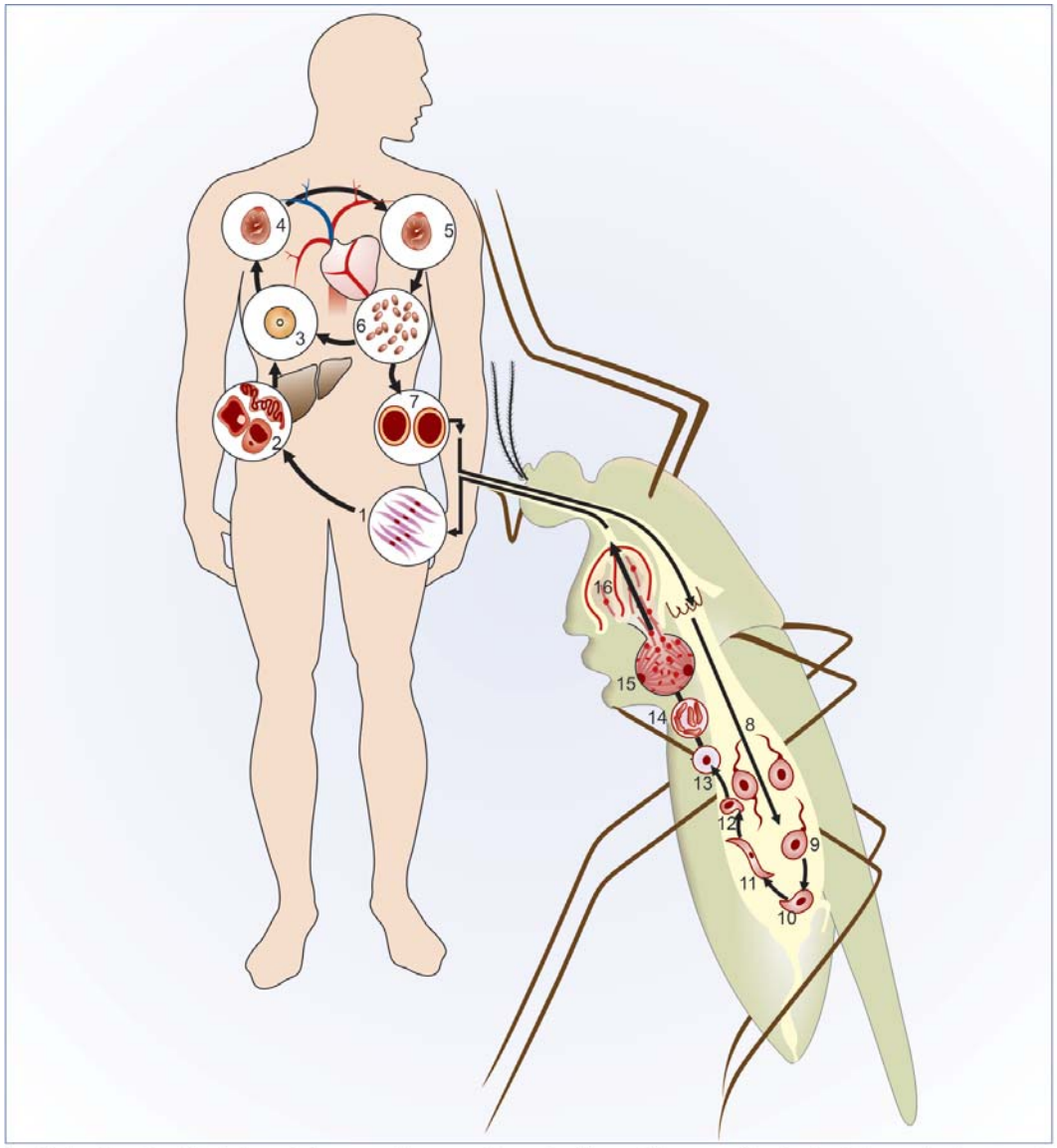
Table 5.2: Comparison of the characteristics of plasmodia causing human malaria

	P.vivax	*P. falciparum*	*P. malariae*	*P. ovale*
Hypnozoites	Yes	No	No	Yes
Erythrocyte preference	Reticulocytes	Young erythrocytes, but can infect all stages	Old erythrocytes	Reticulocytes
Stages found in peripheral blood	Rings, trophozoites, schizonts, gametocytes	Only rings and gametocytes	As in vivax	As in vivax
Ring stage	Large, 2.5 µm usually single, prominent chromatin	Delicate small, 1.5 µm double chromatin and multiple rings common, Accole forms found	Similar to vivax, but thicker	Similar to vivax, more compact
Late trophozoite	Large irregular, actively amoeboid, prominent vacuole	Compact, seldom seen in blood smear	Band from characteristic	Compact coarse pigment
Schizont	Large filling red cell	Small, compact, seldom seen in blood smear	Medium size	Medium size
Number of merozoites	12-24 in irregular grape-like cluster	8-24 in grape-like cluster	6-12 in daisy-head or rosette pattern	6-12 irregularly arranged

Contd...

Table 5.2: Contd...

	P. vivax	*P. falciparum*	*P. malariae*	*P. ovale*
Microgametocyte	Spherical, compact, pale blue cytoplasm, diffuse nucleus	Sausage or banana-shaped pale blue or pink cytoplasm, large diffuse nucleus	As in vivax	As in vivax
Macrogametocyte	Large, spherical, deep blue cytoplasm, compact nucleus	Crescentic, deep blue cytoplasm, compact nucleus	As in vivax	As in vivax
Infected erythrocyte	Enlarged, pale, with Schuffner's dots	Normal size, Maurer's clefts, sometimes basophilic stippling	Normal, occasionally Ziemann's stippling	Enlarged, oval fimbriated, prominent Schuffner's dots
Duration of schizogony (days)	2	2	3	2
Prepatent period (days)	8	5	13	9
Average incubation period (days)	14	12	30	14
Appearance of gametocyte after parasite patency (days)	4-5	10-12	11-14	5-6
Duration of sporogony in mosquito (25°C) (days)	9-10	10-12	25-28	14-16
Average duration of untreated infection (years)	4	2	40	4



1. By the bite of an infected anopheles mosquito sporozoites are inoculated into the capillary vessels of the skin, and thence enter the bloodstream.
2. The sporozoties are taken up by tissue cells, mainly of the liver, and the development of the exoerythrocytic forms (pre-erythrocytic) begins. After the incubation period the merozoties are released from these cells, enter into the general circulation and commence the erythrocytic (schizont) cycle.
3. Ring form in erythrocyte.
4. Late trophozoite in erythrocyte.
5. Mature schizont in erythrocyte.
6. Free merozoites develop from the rupture of the schizont, and these penetrate new erythrocytes.
7. Some of the merozoites develop into gametocytes—a male and female gametocyte are shown in an erythrocyte.
8. Flagellate male and spherical female gametocyte in the mosquito.
9. Fertilization of female gamete by a male gamete.
10. Commencing ookinete development.
11. Ookinete.
12. Penetration of stomach wall of mosquito by ookinete.
13. and 14. Development of oocytes.
15. Mature oocysts liberating sporozoites.
16. Sporozites in the mosquito's salivary glands.

FIGURE 5.4: Cycle of the malaria parasite (text shows development of *Plasmodium vivax* in man and mosquito)

America, but is much less common in Africa. It causes benign tertian malaria with frequent relapses.

The sporozoites of *P.vivax* are narrow and slightly curved. On entering the liver cells, the sporozoites initiate two types of infection. Some develop promptly into exoerythrocytic schizonts, while others persist in the dormant state for varying periods as hypnozoites. There may be two distinct types of sporozoites, the tachysporozoite (*tachy*-fast) which develops into the primary exoerythrocytic schizont and the bradysporozoite (*brady*—slow) which becomes the hypnozoite.

P. vivax shows strain differences with respect to the proportion of sporozoites that develop into hypnozoites. Strains prevalent in the temperate zones (*P.vivax hibernans*) produce a high proportion of hypnozoites, thereby causing relapses after long periods of time. This feature may provide survival advantage to the parasite by avoiding possible cessation of its transmission, due to the absence of vector mosquitoes during overwintering or drought seasons. Tropical strains produce fewer hypnozoites. This apparently does not affect survival prospects of the parasite as vector mosquitoes are constantly present in the tropics.

The pre-erythrocytic schizogony lasts for 8 days and the average number of merozoites per tissue schizont is 10,000. Merozoites of *P.vivax* preferentially infect reticulocytes and young erythrocytes. All stages of erythrocytic schizogony can be seen in peripheral smears. The degree of parasitisation is not generally heavy , each infected red cell usually having only one trophozoite and not more than 2 to 5 per cent of the red cells being affected. Reticulocytes are preferentially infected.

The trophozoite is actively motile, as indicated by its name *vivax*. The ring form is well-defined, with a prominent central vacuole. One side of the ring is thicker and the other side thin. Nucleus is situated on the thin side of the ring. The ring is about 2.5 to 3 µm in diameter, about a third of the size of an erythrocyte. The cytoplasm is blue and the nucleus red in stained films. The ring develops rapidly to the amoeboid form and accumulates malarial pigment. The infected erythrocytes are enlarged and show red granules known as *Schuffner's dots* on the surface. They become irregular in shape, lose their red colour and present a washed out appearance. A few of the parasitised erythrocytes retreat into the blood spaces of the internal organs.

The schizont appears in about 36 to 40 hours. It occupies virtually the whole of the enlarged red cell. The schizont matures in the next 6 to 8 hours, with the development of merozoites, each with its central nucleus and surrounding cytoplasm. The pigment granules agglomerate into a few dark brown collections at the centre, and with the merozoites around it, this stage presents a rosette appearance. There are about 12 to 24 (usually 16) merozoites per schizont. Erythrocytic schizogony takes approximately 48 hours. The red cell, which now measures about 10 µm in diameter is heavily stippled and often distorted. It bursts to liberate the merozoites and pigment. The pigment is phagocytosed by reticuloendothelial cells. The merozoites measure about 1.5 µm and have no pigment.

Gametocytes appear early, usually within 4 days after the trophozoites first appear. Both male and female gametocytes are large, nearly filling the enlarged red cell.

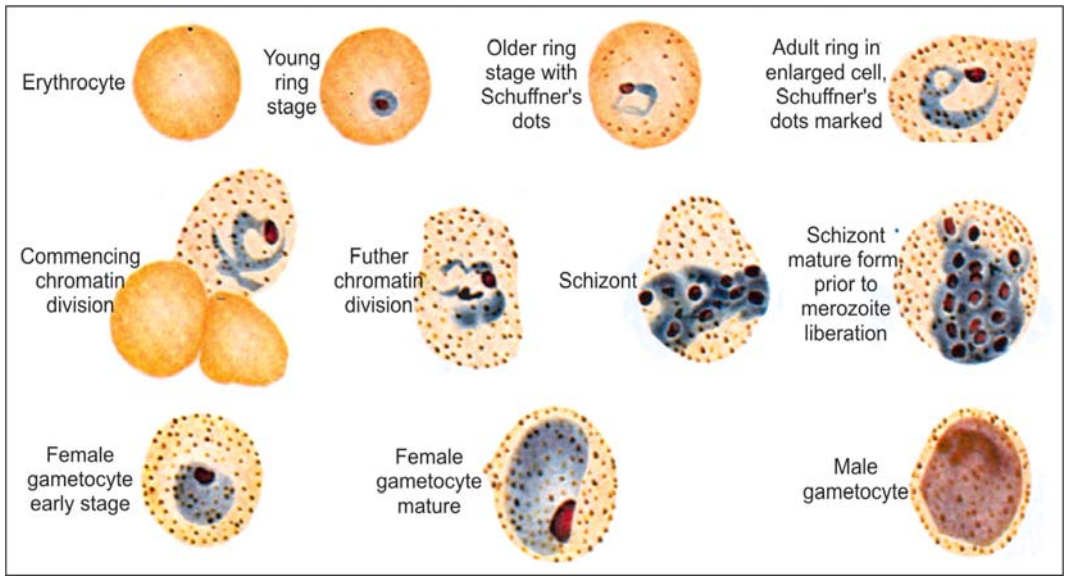


FIGURE 5.5: *Plasmodium vivax* (Giemsa stain, magn x 2000)

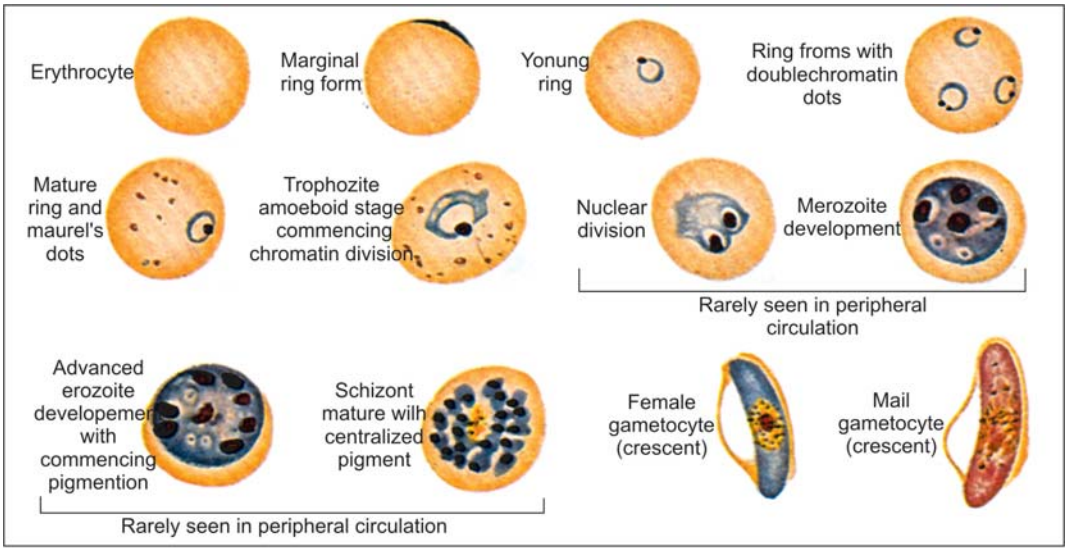


FIGURE 5.6: *Plasmodium falciparum* (Giemsa stain, magn x 2000)

The macrogametocyte has dense cytoplasm staining deep blue and a small compact nucleus. The microgametocyte has pale staining cytoplasm and a large diffuse nucleus. Pigment granules are prominent in the gametocytes (Fig. 5.5).

Plasmodium falciparum

The name falciparum comes from the characteristic sickle shape of the gametocytes of this species (*falx*-sickle, *parere*-to bring forth). This is the most highly pathogenic of all the plasmodia and hence the name malignant tertian or pernicious malaria for its infection. The disease has a high rate of complications and unless treated is often fatal. The species is responsible for almost all deaths caused by malaria. It is deeply entrenched in tropical Atrica and some parts of Asia. It is limited to the tropical and subtropical regions because at temperatures below 20°C, its development in the mosquito is greatly retarded. This is the species of the greatest public health importance due to its increasing resistance to antimalarial drugs and its spread to new areas. In India, it has been spreading widely, causing large epidemics in some places.

The sporozoites are sickle-shaped. The tissue phase consists of only a single cycle of pre-erythrocytic schizogony. No hypnozoites occur. The mature liver schizont releases about 30,000 merozoites. They attack both young and mature erythrocytes and so the population of cells affected is very large. Infected erythrocytes present a brassy colouration.

The early ring form in the erythrocyte is very delicate and tiny, measuring only a sixth of the red cell diameter. Rings are often seen attached along the margin of the red cell, the so-called *form applique* or *accole*. Binucleate rings are common resembling stereo headphones in appearance. Several rings may be seen within a single erythrocyte. In course of time, the rings become larger, about a third of the size of the red cell and may have one or two grains of pigment in its cytoplasm.

The subsequent stages of the asexual cycle—late trophozoite, early and mature schizonts—are not ordinarily seen in peripheral blood, except in very severe or pernicious malaria. The presence of *P.falciparum* schizonts in peripheral smears indicates a grave prognosis. The trophozoites usually disappear from peripheral circulation after about 24 hours. By then, a strain-specific high molecular weight antigen appears on the surface of the infected red cells, associated with knob-like projections on the erythrocyte membrane. Such red cells disappear from peripheral circulation and adhere to the walls of venules and capillaries in internal organs—brain, heart, kidney, lungs, spleen, intestine, bone marrow, placenta. This cytoadherence causes sequestration of infected red cells in, these sites and is responsible for many of the serious complications of falciparum malaria, such as cerebral malaria.

The mature schizont is smaller than in any other species and has 8 to 24 (usually 16) merozoites. The erythrocytic schizogony takes about 48 hours or less, so that the periodicity of febrile paroxysms is 36 to 48 hours. Very high intensity of parasitisation is seen in falciparum malaria. In very severe infections the rate of parasitised cells may even be up to 50 per cent. The infected erythrocytes are of

normal size. They show a few (6-12) coarse brick-red dots which are called *Maurer's clefts*. Some red cells show basophilic stippling (Fig. 5.6).

Gametogony begins after several generations of schizogony. Gametocytes are seen in circulation about 10 days after the ring stage first appears. The early gametocytes seldom appear in peripheral circulation. The mature gametocytes which are seen in peripheral smears are curved oblong structures variously described as crescentic, sickle, sausage or banana-shaped. They are usually referred to as crescents. The male gametocytes are broad and sausage-shaped or kidney-shaped; with blunt rounded ends as compared to the female gametocytes which are thinner and more typically crescentic, with sharply rounded or pointed ends. The mature gametocyte is longer than the diameter of the red cell and so produces gross distortion and sometimes even apparent disappearance of the infected red cell. The red cell is often seen as a rim on the concave side of the gametocyte. The cytoplasm in the female gametocyte is deep blue, while in the male it is pale blue or pink. The nucleus is deep red and compact in the female, with the pigment granules closely aggregated around it, while in the male it is pink, large and diffuse, with the pigment granules scattered in the cytoplasm. Falciparum crescents can survive in circulation for up to 60 days, much longer than in other species. Gametocytes are most numerous in the blood of young children, 9 months to 2 years old. They therefore serve as the most effective source of infection to mosquitoes.

Plasmodium malariae

This was the species of malaria parasite first discovered by Laveran in 1880 and the name malariae is the one given by him. It causes quartan malaria, in which febrile paroxysms occur every fourth day, with 72 hours' interval between the bouts. The disease is generally mild, but is notorious for its long persistence in circulation in undetectable levels, for 50 years or more. Recrudescence may be provoked by splenectomy or immunosuppression. The development of the parasite, in man and mosquito is much slower than with other species. Chimpanzees may be naturally infected with *P.malariae* and may constitute a natural reservoir for quartan malaria. *P.brasilianum,* a parasite of South American monkeys is virtually identical with *P.malariae*. *P.malariae* occurs in tropical Africa, Sri Lanka, Burma and parts of India, but its distribution is patchy.

The sporozoites are relatively thick. Pre-erythrocytic schizogony takes about 15 days, much longer than in other species. Each schizont releases about 15,000 merozoites. Hypnozoites do not occur. The long latency of the infection is believed to be due to persistence of small numbers of erythrocytic forms in some internal organs. *P.malariae* preferentially infects older erythrocytes and the degree of parasitisation is low.

The ring forms resemble those of *P.vivax*, though thicker and more intensely stained. The older trophozoites are sometimes seen stretched across the erythrocyte as a broad band. These *band forms* are a unique feature of *P. malariae*. Numerous large pigment granules are seen.

The schizonts appear in about 50 hours and mature during the next 18 hours. The mature schizont has an average of 8 merozoites, which usually present a rosette appearance (Fig. 5.7).

The infected erythrocytes may be of the normal size or slightly smaller. Fine stippling called Ziemann's stippling may be seen with special stains. The degree of parasitisation is lowest in *P. malariae*. Erythrocytic schizogony takes 72 hours.

The gametocytes develop in the internal organs and appear in the peripheral circulation when fully grown. Gametocytes occupy nearly the entire red cell. The male has pale blue cytoplasm with a large diffuse nucleus, while the female has deep blue cytoplasm and a small compact nucleus.

Plasmodium ovale

This parasite produces a tertian fever resembling vivax malaria, but with milder symptoms, prolonged latency and fewer relapses. It is the rarest of all plasmodia infecting humans and is seen mostly in tropical Africa, particularly along the West Coast.

The pre-erythrocytic stage extends for 9 days. Hepatocytes containing schizonts usually have enlarged nuclei. The mature liver schizont releases about 15,000 merozoites. Hypnozoites are present.

The trophozoites resemble those in vivax malaria, but are usually more compact, with less amoeboid appearance. Schuffner's dots appear earlier and are more abundant and prominent than in vivax infection. The infected erythrocytes are slightly enlarged. In thin films, many of them present an oval shape with fimbriated margins. This oval appearance of the infected erythrocyte is the reason for the name ovale given to this species (Fig. 5.8).

The schizonts resemble those of *P. malariae*, except that the pigment is darker and the erythrocyte usually oval, with prominent Schuffner's dots.

Mixed Infections

In endemic areas it is not uncommon to find mixed infections with two or more species of malaria parasites in the same individual. Mixed infection with *P.vivax* and *P. falciparum* is the most common combination with a tendency for one or the other to predominate. The clinical picture may be atypical with bouts of fever occurring daily. Diagnosis may be made by demonstrating the characteristic parasitic forms in thin blood smears.

Culture of Malaria Parasites

Attempts to culture malaria parasites *in vitro* were started in 1912 by Bass and Johns who obtained limited multiplication of human plasmodia. The breakthrough came in 1976 with the discovery by Trager and Jensen of a simple method for the continuous culture of *P. falciparum*. The technique has been extended to culture other species also.

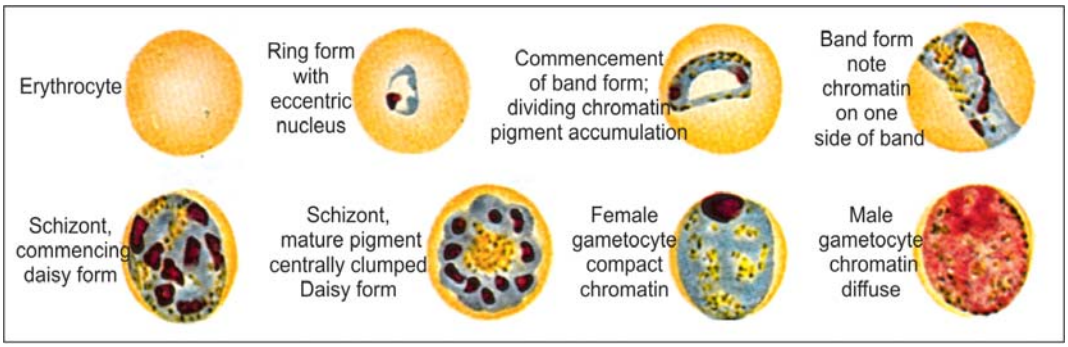


FIGURE 5.7: *Plasmodium malariae* (Giemsa stain, magn x 2000)

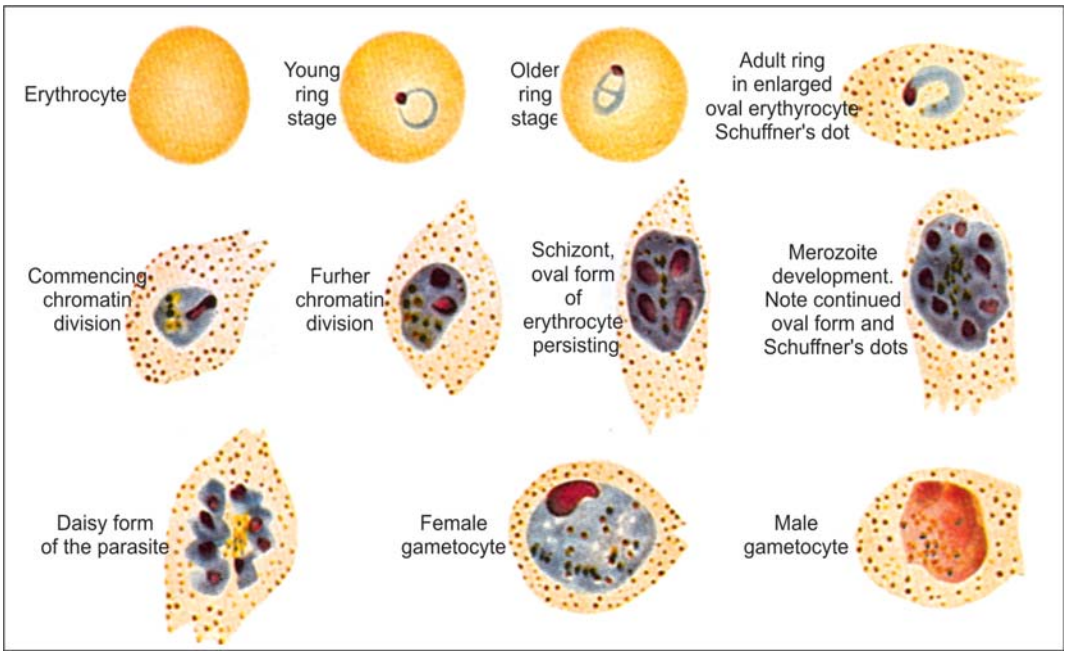


FIGURE 5.8: *Plasmodium ovale* (Giemsa stain, magn x 2000)

The original method of Petri dish culture employed a candle-jar to provide an atmosphere of 3 per cent CO_2 and 10 per cent O_2 and a relatively simple culture medium supplemented with human, rabbit or calf serum to maintain infected erythrocytes. Fresh red cells were added periodically for continuation of the growth and multiplication of plasmodia. The continuous flow method devised by Trager enables the prolonged maintenance of stock cultures. Computer-controlled culture systems introduced subsequently provide a steady abundant supply of parasites. Several culture lines have been established from blood of infected *Aotus monkey* or directly from human patients.

Schizogony proceeds normally in culture. Gametocytes are formed infrequently. Pre-erythrocytic stages of some species have been obtained in tissue cultures. Plasmodia retain their infectivity in culture.

Culture of plasmodia provides a source of the parasites for study of their antigenic structure, for use in seroepidemiologic surveys, for drug sensitivity tests and for studies in immunoprophylaxis.

Pathogenesis and Clinical Picture

The incubation period varies usually from 8 to 40 days, being shortest in *P.falciparum* and longest in *P. malariae* infec.tions. The average incubation periods are 8-11 days for falciparum, 10 to 12 days for vivax and ovale and 18 to 40 days for quartan malaria. However, very much longer incubation periods, up to 9 months have been recorded with some strains of *P. vivax* (*P. vivax hibernans*).

The incubation period is to be distinguished from the prepatent period, which is the interval between the entry of the parasites into the host and the time when they first become detectable in blood. The minimum level of parasitaemia for their microscopic detection is called the *microscopic threshold*. This is about 20 to 25 parasites per cu. mm. Clinical disease develops only later, when after a number of further cycles of multiplication, the level of parasitaemia rises high enough to cause fever, the so-called *fever threshold* or *pyrogenic density*. The first clinical illness marking the end of the incubation period is called the primary attack.

The typical picture of malaria consists of periodic bouts of fever with rigor, followed by anaemia and splenomegaly. True rigor is typically present in vivax malaria and is less common in falciparum infection. The febrile paroxysm comprises three successive stages. In the cold stage, lasting for 15 to 60 minutes, the patient experiences intense cold and uncontrollable shivering. This is followed by the hot stage, lasting for 2 to 6 hours, when the patient feels intensely hot. The fever mounts to 41°C or higher. Severe headache, nausea and vomiting are common. Afterwards comes the sweating stage, when the patient is drenched in profuse sweat. The temperature drops rapidly and the patient usually falls into deep sleep, to wake up refreshed. The paroxysm usually begins in the early afternoon and lasts for 8 to 12 hours.

The periodicity of the attack varies with the species of the infecting parasite. The periodicity is approximately 48 hours in tertian and 72 hours in quartan malaria. Quotidian periodicity , with the fever occurring at 24 hour intervals may be due to two broods of tertian parasites maturing on successive days, or due to mixed infection. Regular periodicity is seldom seen in the primary attack, but is established usually only after a few days of continuous, remittent or intermittent fever.

All clinical manifestations in malaria are due to the products of erythrocytic schizogony and the host's reactions to them. The exoerythrocytic liver cycle and gametogony do not appear to contribute to clinical illness. The febrile paroxysms follow the completion of erythrocytic schizogony, when the mature schizont ruptures, releasing red cell fragments, merozoites, malarial pigment and other parasitic debris. Macrophages and polymorphs phagocytose these and release large quantities of

endogenous pyrogens, leading to elevation of temperature. Cytokines such as tumour necrosis factor (TNF) and interleukin-l may play a pivotal role in the pathogenesis of malarial fever.

Recrudescence and Relapse

After a number of paroxysms, the primary attack subsides with the development of partial immunity in the host. This is followed by a period of latency during which there is no clinical illness or sometimes even parasitaemia. The parasites are not, however, eliminated at this stage, but persist in some erythrocytes, though the level of parasitaemia is below the fever threshold, or sometimes even below the microscopic threshold. Erythrocytic schizogony continues in the body at low levels and gradually the numbers of parasites build up to cross the fever threshold. Fresh malarial attacks then develop. These new malarial attacks that appear after a period of latency usually within eight weeks after the culmination of the primary attack and resulting from persistence of the erythrocytic cycle of the parasites are called *recrudescences*. Recrudescence may be due to waning immunity of the host or possibly to antigenic variations in the parasite. There may be several such recrudescences, which are generally milder than the primary attack. After a varying number of such attacks, the infection is eliminated in *P. falciparum* and *P. malariae* infections.

In *P. vivax* and *P. ovale* infections the parasites may survive for long periods in a dormant exoerythrocytic stage as hypnozoites in liver cells. Reactivation of hypnozoites leads to initiation of fresh erythrocytic cycles and new attacks of malarial fever. Such new attacks of malaria caused by the dormant exoerythrocytic forms being reactivated after long periods, usually from 24 weeks to 5 years after the primary attack are called *relapses*.

The term recurrence has been used to refer to both recrudescence and relapse and so carries no specific meaning. Several factors including stress, intercurrent infection, pregnancy and alcoholism have been proposed as precipitating causes for recurrences.

Malignant Tertian Malaria

The most serious and fatal type of malaria is malignant tertian (MT) malaria caused by *P. falciparum*. When not treated promptly and adequately, dangerous complications develop. The term pernicious malaria has been applied to a complex of life-threatening complications that sometimes supervenes in acute falciparum malaria. These may present in various forms, the most important of which are the cerebral, algid and septicaemic varieties. These occur following heavy parasitisation of red cells. The parasitised red cells become deformed, sticky and adhere on the capillary endothelium in internal organs causing anoxic damage, oedema and inflammatory reaction. Cerebral malaria is characterised by hyperpyrexia, coma and paralysis. Algid malaria resembles surgical shock, with cold clammy skin, peripheral circulatory failure and profound hypotension. Gastrointestinal symptoms such as vomiting, dysenteric or choleraic diarrhoea may occur. Some cases develop severe hiccup, with profuse bilious vomiting,

a condition formerly called bilious remittent fever. In septicaemic malaria, characterised by a high degree of prostration, there is high continuous fever with involvement of various organs. Acute renal failure and acute pulmonary oedema are other serious complications.

Blackwater Fever

A syndrome called *blackwater fever* (malarial haemoglobinuria) is sometimes seen in falciparum malaria, particularly in patients who have experienced repeated infections and inadequate treatment with quinine. Patients with G6PD deficiency may develop this condition after taking oxidant drugs, even in the absence of malaria. Clinical manifestations include bilious vomiting and prostration, with passage of dark red or blackish urine (blackwater). The pathogenesis is believed to be massive intravascular haemolysis caused by antierythrocyte autoantibodies, leading to haemoglobinaemia and haemoglobinuria.

Anaemia

Anaemia occurs in all types of malaria, but is most pronounced in falciparum infections. The type of anaemia is haemolytic, normocytic, normochromic. The degree of anaemia is greater than what could be explained by the destruction of parasitised red cells. In addition, there occurs increased destruction of red cells possibly by autoimmune mechanisms, and decreased erythropoiesis.

Splenomegaly

The spleen is invariably affected, being always enlarged in malaria. The initial change is congestion, leading to a soft enlargement. Later, it becomes dark due to accumulated malarial pigment. Diffuse cellular hyperplasia, dilated sinusoids and accumulation of macrophages accentuate the enlargement of spleen, which becomes hard due to fibrosis.

Tropical Splenomegaly Syndrome

Tropical splenomegaly syndrome (TSS) also known as hyper-reactive malarial splenomegaly (HMS) is a chronic benign condition seen in some adults in endemic areas, mainly tropical Africa, New Guinea and Vietnam. This results from an abnormal immunological response to malaria and is characterised by enormous splenomegaly, high titres of circulating antimalaria antibody and absence of malaria parasites in peripheral blood smears. Hyperimmunoglobulinaemia (IgM, but not IgG), cryoglobulinaemia, reduced C3 and presence of rheumatoid factor without arthritis are other features. A normocytic normochromic anaemia is present, not responding to haematinics or anthelmentics. TSS differs from various other types of splenomegalies seen in the tropics in its response to antimalarial treatment, and histological changes in spleen (dilated sinusoids lined with reticulum cells showing erythrophagocytosis, lymphocytic infiltration of pulp) and liver (marked sinusoidal infiltration with lymphocytes).

The liver is also congested, enlarged and pigmented. Numerous pigment-laden Kupffer cells dot the liver. Changes are also seen in bone marrow, kidney and adrenals.

Cerebral Malaria

In cerebral malaria, lesions occur in the central nervous system. These consist of congestion of the meninges and brain, occlusion of capillaries in brain, numerous petechial perivascular haemorrhages, and necrotic lesions in mid zonal brain tissue, with peripheral glial reaction (*malarial granuloma*) around occluded blood vessels.

Merozoite-induced Malaria

Natural malaria is sporozoite-induced, the infection being transmitted by sporozoites introduced through the bite of vector mosquitoes. Injection of merozoites can lead to direct infection of red cells and erythrocytic schizogony with clinical illness. Such merozoite-induced malaria may occur in the following situations.

Blood transfusion can accidentally transmit malaria if the donor is infected with malaria. The parasites may remain viable in bank blood for 1 to 2 weeks. The incubation period in *transfusion malaria* depends on the number of parasites introduced and the species. It varies from 10 days in *P. falciparum* to 40 days or longer in *P. malariae*.

Malaria can also be transmitted by procedures other than transfusion when small quantities of blood are conveyed from one person to another. *Shared syringes* among drug addicts may be responsible. Renal *transplantation* may lead to malaria if the donor had parasitaemia.

Therapeutic malaria is a special type of merozoite-induced malaria which was used formerly as a treatment for late syphilis.

Congenital malaria a natural form of merozoite-induced malaria where the parasite is transmitted transplacentally from the mother to the foetus.

Merozoite-induced malaria causes febrile paroxysms as in the natural disease. But it is self-limited and undergoes spontaneous cure due to the absence of any exoerythrocytic stage.

Immunity

Immunity in malaria may be classified into innate and acquired types.

Innate Immunity

Only little is known about innate immunity in malaria, but a few naturally occurring examples illustrate its importance.

The invasion of red cells by merozoites requires the presence of specific glycoprotein receptors on the erythrocyte surface. It has been found that persons who lack the Duffy blood group antigen (Fya Fyb) are refractory to infection by *P. vivax*. This blood group antigen appears to be the receptor for the malarial parasite. Duffy blood group is absent in the native population of West Africa. This may be one reason why vivax malaria is not prevalent there.

P. falciparum does not multiply properly in sickle red cells containing the abnormal haemoglobin S. Sickle cell trait is very common in Africa where falciparum infection is hyperendemic. It has been proposed that the sickle cell trait, which is otherwise undesirable has been conserved there because of the survival advantage it offers in falciparum malaria. Haemoglobin F present in neonates protects them from malaria.

Innate immunity to malaria has also been related to the G6PDH deficiency found in the Mediterranean coast, Africa, the Middle East and India. HLA-B53 is associated with protection from malaria.

There is some evidence that severe malnutrition and iron deficiency may confer some protection against malaria. It was observed that during severe famine in North Africa malaria was rare, but on providing food and iron supplements, the patients began to develop clinical malaria. Falciparum malaria is more severe in pregnancy, particularly in primigravida, and may be enhanced by iron supplementation.

The spleen appears to play an important role in immunity against malaria. Splenectomy enhances susceptibility to malaria.

Acquired Immunity

Infection with malaria parasites induces specific immunity which can bring about clinical cure, but cannot lead to complete elimination of parasites from the body. It can prevent superinfection, but is not powerful enough defence against re-infection. This state of resistance in an infected host, which is associated with continued asymptomatic parasitic infection is called *premunition*. The host is resistant to fresh infection (superinfection) as long as the pre-existing infection continues even though in subclinical form. But once the infection is eradicated, the immunity does not persist for long and is not capable of preventing subsequent infection (re-infection).

Specific immunity is evident in endemic areas where infants below the age of 3 months are protected by passive maternal antibodies. Young children are highly susceptible to malaria. As they grow up they acquire immunity by subclinical or clinical infections so that the incidence of malaria is low in older children and adults.

The antigenic fractions of malaria parasites have been investigated in detail. The four species of human parasites have both common as well as species-specific antigens. Within each species, the different stages in the life-cycle have stage-specific antigens. The practical importance of these studies is in the development of vaccines and for serological diagnosis of malaria. Immunity appears to be strain-specific and one infection may not be protective against infection by a different strain of the same species of thc parasite. In endemic areas, repeated infections by multiple strains broadens the scope of immunity.

Experimental studies on immunisation against malaria date back to early in the 20th century. Injection of erythrocytic parasites with Freund's complete adjuvant was shown to induce immunity in monkey malaria, but this was not practicable in humans. It is only recently, after successful continuous culture of malaria parasites, the availability of monoclonal antibodies and the development of cloning techniques

that significant progress was achieved in this field. Several possibilities are being tested for the immunoprophylaxis of malaria.

Immunisation against the sporozoite antigens could check the first step in human infection by blocking the invasion of liver cells. An antigenic surface component has been identified on the sporozoites. This 'circum-sporozoite protein' has been cloned and its immunodominant epitope identified. The epitope sequence consisting of a small number of amino acids has been chemically synthesised. Its gene has been introduced into the vaccinia virus and the recombinant virus has been shown to produce the sporozoite antigen.

Several other antigens have been considered as potential vaccines, including the merozoite surface protein-l, apical membrane antigen, erythrocyte binding antigen, a soluble antigen released during rupture of parasitised erythrocytes and a zygote antigen Pfs 25.

A vaccine that has undergone several field trials is the *spf 66 vaccine* developed by Manuel Patarroyo in Columbia. This is a synthetic peptide containing the amino acid sequences of three *P.falciparum* merozoite proteins linked together by a tetrapeptide from the circumsporozoite protein. Field trials in South America and Tanzania showed moderate protection, but in Gambia and Thailand it was much less effective.

A method of blocking mosquito transmission has been proposed, by immunising malaria patients or carriers with vaccine containing gamete or zygote antigens. When mosquitoes feed on them, the antibodies sucked in along with gametocytes prevent sporogony taking place. This method has been termed *transmission blocking immunity*.

An ideal malaria vaccine should be one inducing multistage, multivalent, multi-immune response. Nothing approaching this is available at present. Much work is being done in developing DNA vaccines to meet these requirements.

Cell-mediated immunity is operative in malaria, but little is known about its scope and importance. Malaria does not appear to be aggravated by AIDS.

Immunopathology

Malaria is known to produce some depression of the immune system. It has been suggested that immune depression caused by endemic malaria is responsible for the Burkitt's lymphoma seen in African children. While the Epstein-Barr virus causes asymptomatic infection or infectious mononucleosis in immunocompetent persons, in African children whose immune system is severely compromised by recurrent malaria infection, the virus leads to lymphoma.

Parasitised erythrocytes may undergo antigenic changes, which may lead to autoimmune phenomena. Immune complexes occur in malaria. These may lead to nephropathies.

Laboratory Diagnosis

The most important method for the diagnosis of malaria is the demonstration of the parasite in blood. Clinical diagnosis of malaria can be made with considerable

confidence in residents of endemic areas and recent visitors, but confirmation requires the finding of parasites in blood smear.

All asexual erythrocytic stages as well as gametocytes can be seen in peripheral blood in infection with *P.vivax, P. ovale* and *P. malariae* but in *P. falciparum* infection, only the ring form and gametocytes can be seen. Late trophozoites and schizont stages of *P.falciparum* are usually confined to the internal organs and appear in peripheral blood only in severe or pernicious types of MT malaria.

The parasites are most abundant in peripheral blood late in the febrile paroxysm, a few hours after the peak of the fever. Therefore. blood smears ideally should be collected at this period. In practice, it is advisable to obtain a blood smear when the patient is first seen, and then a few hours after the height of the fever. In smears taken between paroxysms the parasites may be scanty or absent. This is particularly so in falciparum malaria. Repeated blood smears have to be examined before a negative result is given. If finger prick smears cannot be obtained, blood sent in EDTA tubes may be used instead for making smears.

Two types of blood films are prepared for examination—the *thick and the thin films*. They can be made on separate slides, or more conveniently on the same slide. After cleaning with ether or spirit and drying, the finger tip is pricked and gently squeezed till a good drop of blood exudes. The drop of blood is touched with a clean dry slide, near one end. The blood on the slide is spread with the corner of another slide to produce a square or circular patch of moderate thickness. This is the thick film. When correctly prepared the thick film will just allow printed letters to be read through it. For preparing a thin film. collect a small drop of blood on the slide, away from the thick film and separated from it by a line drawn with a glass marking pencil. The blood is spread evenly and thinly with the edge of a spreader slide. A properly made thin film will consist of an unbroken smear of a single layer of red cells, ending in a tongue which stops a little short of the edge of the slide. The slide is kept flat protected from dust, to dry.

Chinese workers recommend intradermal smears taken from multiple punctures on the upper forearm using a 25-gauge needle. The punctures should not bleed, but a serosanguinous fluid can be expressed on to a slide by squeezing. This is claimed to be more sensitive than peripheral blood smears.

The thin film is fixed in methanol for 30 seconds. The thick film is not to be fixed as it is to be dehaemoglobinised. Diluted Giemsa stain is applied over both thick and thin films and allowed to stand for half to two hours. The slide is then washed and dried. A rapid method of staining, particularly useful in field work is the Field's stain. The rapid method commonly employed in India is the JSB stain named after Jaswant Singh and Bhattacharji.

The stained film is examined under the oil immersion microscope. The thick film is more sensitive, when examined by an experienced person, because it concentrates 20 to 30 layers of blood cells in a small area. The dehaemoglobinised and stained thick film does not show any red cells, but only leucocytes and, when present, the parasites. But the parasites are often distorted in form, and as the diagnostic changes in blood cells such as enlargement and stippling cannot be made out, species

identification is difficult. In falciparum malaria, the presence of gametocyte crescents makes species identification simple. It is recommended that 200 oil immersion fields should be examined before a thick film is declared negative (see Fig. 5.2).

When parasites are found, an approximate quantitative estimate may be given as follows.

\+ 1-10 parasites per l00 thick films fields
++ 11-100 parasites per 100 thick film fields
+++ 1-10 parasites per each thick fIlm field
++++ More than 10 parasites per each thick film field.

The morphology of the parasites is preserved in thin films and so species identification is easy in them.

Some other tests have been introduced to simplify malaria diagnosis. In the *QBC test* (Beckton-Dickinson, USA), a small quantity of blood (50 to 110 µl) of blood is spun in the QBC centrifuge. The parasites get concentrated near the tip of the RBC column. Pre-coating of the tube with acridine orange induces a fluorescence on the parasites which can then be readily visualised under the oil immersion microscope. The QBC method is sensitive and specific and has been widely accepted as a rapid test for malaria. Many other similar tests have been developed, but none can replace the thick and thin smear which alone can reveal the parasite morphology clearly enough for accurate identification of the species. A careful and patient smear examination still remains as the 'gold standard' in malaria diagnosis.

Another useful approach is immunodiagnosis of malaria by detection of parasite-specific antigens using monoclonal antibodies The *Para Sight–F test* (BD) is a dip-stick antigen capture test targeting the "histidine-rich protein–2" (HRP-2), specific for *P.falciparum.* The test is sensitive, specific and rapid, results being ready in ten minutes. It is of interest that the Para Sight-F test has enabled the diagnosis of malaria in ancient Egyptian and Nubian mummies, demonstrating the remarkable stability of the HRP-2 antigen over thousands of years.

A dip-stick test targeting species specific lactic dehydrogenase is also available. It is useful not only in diagnosing malaria, but also in confirming cure after treatment, because the test will detect only live parasites, and so will be negative if the parasites have been killed by treatment. Dipstick tests are also available for vivax malaria. Molecular methods such as dot-blot assay, DNA probes and PCR amplification are not useful in routine diagnosis, but may be used in special situations.

Serological tests are not employed for routine diagnosis. Several serological tests have been described for detection of specific malaria antibodies. These include indirect immunofluorescence test (IFAT), indirect haemagglutination (IHA), immuno-precipitation (gel diffusion), ELISA, RIA. IFAT uses erythrocytic schizonts on a slide as antigen. It detects IgM, IgG and IgA antibodies. Antibodies appear within a few days of clinical illness and persist for months or years. It is of some use for diagnosis of cases, particularly in between recrudescences in nonendemic areas. IFAT can also be used to detect parasitaemia. lHA uses antigen-coated erythrocytes. The technique is simple and suitable for testing large numbers of sera. The test becomes positive even before parasites appear in blood. However, false-positives are common. Gel

diffusion percipitation is simple and convenient for screening purposes, but its sensitivity is low. ELISA is highly specific, but less sensitive. It is best suited for screening large batches of sera. RIA is costly, complex and suited only for research. Serological tests are more often employed for seroepidemiological surveys than for diagnosis of individual cases as presence of antibody need not indicate active infection. It is better to use homologous antigens for the test, but in their absence, related monkey malaria antigens can also be employed.

Epidemiology

Malaria had been recorded in places as far north as Archangel, Russia and as far south as Cordoba. Argentina, in places as low as the Dead Sea (400 metres below sea level) and as high as Cochabamba, Bolivia (2800 metres above sea level). However, malaria is essentially a focal disease and its distribution is patchy in most parts (Fig. 5.9).

Till the 19th century, it was prevalent in Northern Europe, Russia and North America, but it has disappeared from those areas and is now confined to the tropics and subtropics. However, imported cases of malaria are not infrequent in nonendemic areas, due to the entry of infected persons. Thousands of such cases are recorded in the USA and Europe every year .In addition, a few instances of introduced malaria occur in nonendemic areas through infected mosquitoes from endemic areas entering as stowways in jet planes. These infect residents near airports and, if vector mosquitoes are present, can initiate small local outbreaks.

The relative prevalence of the four species of malaria parasites varies in different geographical regions. *P. vivax* is the most widely distributed, being common in Asia, North Africa and Central and South America. *P.falciparum*, the predominant species in Africa, Papua New Guinea and Haiti, is rapidly spreading in South East Asia and India. *P.malariae* is present in most places but is rare, except in Africa. *P.ovale* is virtually confined to West Africa where it ranks second after *P.falciparum*.

Malaria may occur in endemic as well as epidemic patterns. It is described as endemic when it occurs constantly in an area over a period of several successive years and as epidemic when periodic or occasional sharp rises occur in its incidence. The terms stable and unstable malaria have been frequently employed to refer, respectively to endemicity without fluctuation, and to highly variable degrees of malaria transmission.

Endemic malaria has been classified into the following types:
Hypoendemic: When transmission is low and malaria is not an important problem in the area.

Mesoendemic: Intensity of transmission is moderate and varies depending on local circumstances.

Hyperendemic: When transmission is intense, but seasonal.

Holoendemic: When transmission of high intensity is constantly present.

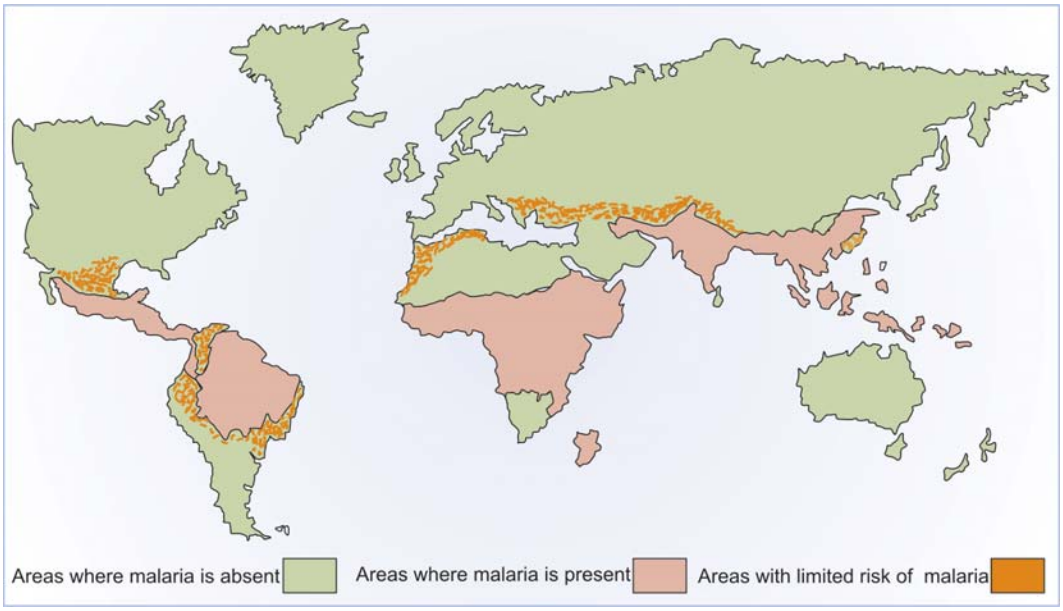


FIGURE 5.9: Global distribution of malaria

The above classification is based on the results of malaria surveys. The basic investigations in malaria surveys concern data regarding the human host, the vector mosquito as well as environmental conditions. Two measurements made in the population are the spleen rate, which is the proportion of children aged 2 to 10 years in a population, with enlarged spleens, and the parasite rate, which is the proportion of persons in the population who show malaria parasites in blood.

The WHO has recommended the classification of endemicity depending on the spleen or parasite rate in a statistically significant sample in the populations of children (2 to 9 yr) and adults. According to this:

Hypoendemic: Spleen or parasite rate in children 10 per cent or less.

Mesoendemic: Spleen or parasite rate in children 11 to 50 per cent.

Hyperendemic: Spleen or parasite rate 50 to 75%, in adults over 25 per cent.

Holoendemic: Spleen or parasite rate in children over 75 per cent but low in adults.

The prevalence of malaria is intimately connected with the distribution and habits of its vector mosquito, as also the seasonal and climatic variations. Several species of Anopheles act as the vectors in different geographical areas. In India, the major vectors are *A. culicifacies* in the mainland and *A. fluviatilis* and *A. stephensi* in the coastal areas.

Treatment

Antimalaria therapy should ideally destroy all asexual forms of the parasite in order to cure the clinical illness, eliminate sporozoites and exoerythrocytic forms to prevent

relapse and kill gametocytes to block transmission to the vector mosquito. No single drug satisfies all these objectives and combinations of drugs are therefore necessary. The strategy of treatment is to cure the clinical disease with blood schizonticidal drugs such as chloroquine (600 mg statum, 300 mg after 6 hours, 300 mg daily for the next two days). Single dose regimens have also been advocated but may not always be adequate. Chloroquine does not destroy exoerythrocytic parasites and so in *P. vivax* and *P. ovale* infections, a tissue schizonticidal drug such as primaquine (15 mg daily for 5 days) should be administered. Primaquine is also active against gametocytes.

A serious problem is posed by the development and spread of drug resistance in *P.falciparum*, which is now widespread in South America and South East Asia, including India. A combination of sulphadoxine and pyrimethamine (fansidar) or mefloquine is useful in such cases. In severe drug resistant falciparum malaria, intravenous quinine may be lifesaving.

The traditional Chinese medicine Qinghaosu (from the shrub *Artemesia anhua*) and its derivatives artemether, artesunate and others have been found to be highly effective and safe antimalarials.

Prophylaxis

Personal prophylaxis consists of avoiding mosquito bites by suitable clothing, use of bed nets (particularly nets impregnated with permethrin or other insecticides which have been shown to be very effective) and insect repellant applications (such as diethyltoluamide) on exposed skin.

For travellers visiting endemic areas, chemoprophylaxis provides effective protection. Prophylaxis should begin on the day of arrival and be continued for 4 to 6 weeks after departure. The drug recommended for chemoprophylaxis are chloroquine, amodiaquin and fansidar in weekly doses or doxycycline daily. No vaccine is now available.

Control

Malaria control has a long history, beginning with early attempts by drainage of marshy lands in Roman times. The introduction of DDT and other insecticides after the Second World War gave a new dimension to antivector activities.

As malaria has no extra-human reservoir. Its eradication is theoretically possible by elimination of the vector mosquito and treatment of patients and carriers. In India, the National Malaria Control Programme operated very succesfully for 5 years, bringing down the annual incidence of malaria from 75 million in 1953 to 2 million in 1958. The National Malaria Eradication Programme was introduced in 1958 with the objective of the ultimate eradication of the disease. By 1961, the incidence dropped to an all-time low of 50,000 cases and no deaths. However, there have been set backs from 1970 and by 1976, the incidence rose to 6.4 million cases. There have also been regular and extensive epidemics in different parts of the country . Vector control by insecticides became impracticable due to their high cost and increasing

resistance to them in mosquitoes. Integrated control, including additional methods of vector reduction, bioenvironmental modification and personal protection measures have been proposed.

In the meantime, malaria has continued to spread and by 1995, has covered virtually all parts of India. Particularly distressing has been the spread of *P.falciparum* which has become increasingly drug resistant.

CHAPTER 6

Miscellaneous Sporozoa and Microspora

Protozoan parasites characterised by the production of spore-like oocysts containing sporozoites were known as sporozoa. They live intracellularly, at least during part of their life cycle. At some stage in their life cycle they possess a structure called the apical complex, by means of which they attach to and penetrate host cells. These protozoa are therefore grouped under the Phylum Apicomplexa. The medically important parasites in this group are the malaria parasites (considered in Chapter 5), Coccidia and Babesia. Some unclassified organisms of uncertain origin (e.g. *Pneumocystis carinii*) were also grouped with sporozoa.

Their life cycles show an alternation of generations—a sexual sporogonic phase and an asexual schizogonic phase. Many of them also show an alternation of hosts—a vertebrate host and an insect vector, or a definitive and an intermediate host. The adult forms have no organs of locomotion. Motility when present is by flexion or gliding. Flagella are present in the microgametes of some species.

Many minute intracellular protozoa formerly grouped as sporozoa have been reclassified because of some structural differences. These are now called microspora. They infect a large spectrum of hosts including vertebrates and invertebrates. Infection is mostly asymptomatic, but clinical illness is often seen in the immunodeficient.

Many parasites considered in this chapter have acquired great prominence due to their frequent association with HIV infection.

TOXOPLASMA GONDII

History

Toxoplasma gondii is an obligate intracellular coccidian parasite, first described in 1908 by Nicolle and Manceaux in spleen and liver smears of a small North African rodent called gondi, *Ctenodactylus gundi*. Its importance as a human pathogen was recognised only much later, when Janku in 1923 observed the cyst in the retina of a child with hydrocephalus and microphthalmia, Wolf and Cowen in 1937 identified the first congenital brain infection , and Pinkerton and Weinman recorded postnatal infection in 1940. With the discovery in 1948, of the Sabin-Feldman dye test, the first serological

assay for toxoplasma antibody, the scope and extent of the infection became open for study.

The name toxoplasma is derived from the Greek word *Toxon* meaning arc or bow, referring to the curved shape of the trophozoite. Toxoplasmosis is now recognised as the most common protozoan parasite globally, with the widest range of hosts spread over 200 species of birds, reptiles and mammals, including humans.

The life cycle of the parasite became clear only in 1970 when the domestic cat was identified as its definitive host, only in which it undergoes the sexual sporogony. All other species are merely intermediate hosts, in which only asexual schizogony takes place. Though human infection is very common, perhaps involving a third of the human race, clinical disease is relatively rare, being mostly opportunistic in nature.

Morphology

T. gondii occurs in three forms—trophozoite, tissue cyst and oocyst (Fig. 6.1).

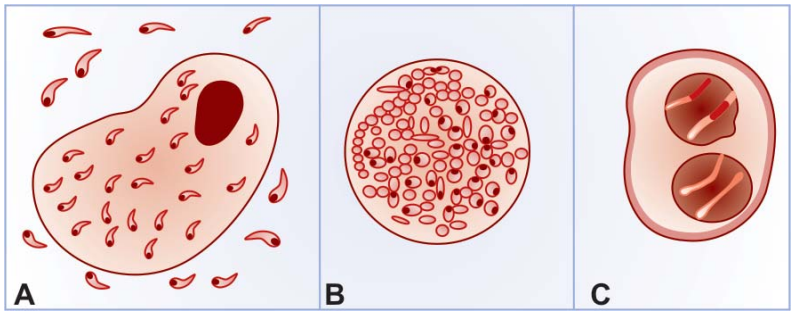


FIGURE 6.1: *Toxoplasma gondii.* (A) Smear from peritoneal fluid of infected mouse, showing crescentic tachyzoites—extracellular trophozoites and intracellular form within macrophage. (B) Thick-walled tissue cyst containing rounded forms-bradyzoites. (C) Oocyst containing two sporocysts with sporozoites inside

The trophozoite and tissue cyst represent stages in asexual multiplication (schizogony), while the oocyst is formed by sexual reproduction (gametogony or sporogony). All three forms occur in the domestic cat and other felines which are the definitive hosts and which support both schizogony and gametogony. Only the asexual forms, trophozoites and tissue cysts are present in other animals, including humans, and birds, which are the intermediate hosts. Both oocysts and tissue cysts are infective by ingestion.

Trophozoite

The trophozoite is crescent-shaped, with one end pointed and the other end rounded. It measures approximately 3 µm by 7 µm. The nucleus is ovoid and situated near the blunt end of the parasite. Electron microscopy reveals an apical complex at the pointed end (Figs 6.2A and B). The trophozoite stains well with Giemsa stain, the cytoplasm appearing azure blue and the nucleus red. It is seen intracellularly in various tissues and organs during the early acute phase of infection. Extracellular trophozoites

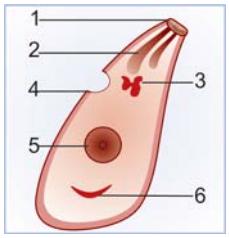


FIGURE 6.2A: *Toxoplasma gondii*. Trophozoite (tachyzoite), fine structure seen by electron microscopy. 1. Conoid, 2. Rhoptery 3. Golgi body 4. Cytostome 5. Nucleus 6. Mitochondrion

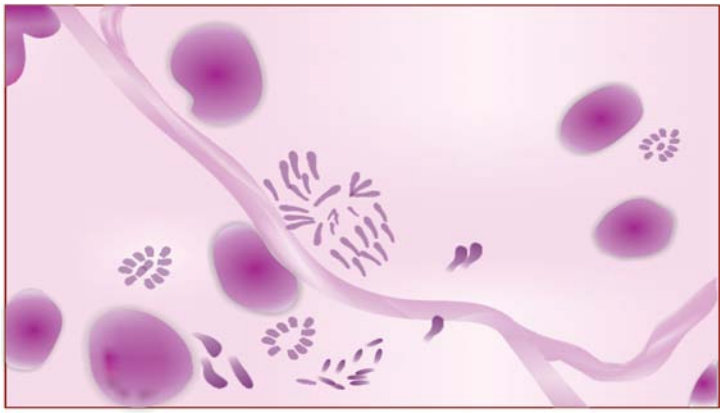


FIGURE 6.2B: *T gondii.* Trophozoite grows in tissue culture. Smear shows trophozoites arranged in different patterns—singly, in cluster or as rosette (Giemsa stain)

can also be seen in impression smears. It can invade any nucleated cell and replicate within cytoplasmic vacuoles by a process called *endodyogeny* or *internal budding*—daughter trophozoites being formed, each surrounded by its own memrane, while still within the parent cell. When the host cell becomes distended with the parasites, it disintegrates releasing the trophozoites which infect other cells.

During acute infection, the proliferating trophozoites within a host cell may appear rounded and enclosed by the host cell membrane. This appearance, called the '*pseudocyst*' or '*colony*' can be differentiated from true tissue cysts by its staining reactions. The rapidly proliferating trophozoites in acute infection are called *tachyzoites*. Trophozoites can be propagated in the laboratory in eggs, tissue culture and in the peritoneum of mice, for maintenance of strains and preparation of antigens for serological tests. The trophozoites are susceptible to drying, freeze-thawing and to gastric digestion.

Tissue Cyst

The tissue cyst is formed during the chronic phase of the infection and can be found in the muscles and various other tissues and organs, including the brain. The parasite multiplying slowly within the host cell, produces a cyst wall within the host cell membrane. The cyst wall is eosinophilic and stains with silver, in contrast to the 'pseudocyst'. With periodic acid Schiff stain, the cyst wall stains weakly and the parasites inside deeply. The slowly multiplying parasites within the cyst are called

bradyzoites. The cyst is round or oval, 10 to 200 µm in size and contains numerous bradyzoites. Cysts remain viable in tissue for several years. In immunologically normal hosts, the cysts remain silent, but in the immunodeficient subjects they may get reactivated, leading to clinical disease. It is relatively resistant and when meat containing the cysts is eaten raw or undercooked, infection occurs. The cyst wall is disrupted by peptic or tryptic digestion and the released parasites initiate infection by invading intestinal epithelial cells. They reach various tissues and organs through blood and lymphatic dissemination. Cysts are susceptible to desiccation, freeze-thawing and heat above 60°C.

Oocyst

Oocysts develop only in definitive hosts—in the intestines of cats and other felines. When cats get infected by ingestion of either tissue cysts or oocysts, the parasites develop in the intestinal epithelial cells, where both schizogony and gametogony take place. Male and female gametocytes develop and after fertilisation, the zygote gets surrounded by a thin, but extremely resistant wall. This is the oocyst, which is spherical or ovoid. about 10 to 12 µm in size and contains a sporoblast. Cats shed millions of oocysts per day in faeces for about two weeks during the primary infection. The freshly passed oocyst is not infectious. It becomes infectious only after development in soil or water for a few days. During this state of sporulation, the sporoblast divides into two sporocysts and four sporozoites develop inside each sporocyst. The mature oocyst containing eight sporozoites is the infective form. It is very resistant to environmental conditions and can remain infective in soil for about a year. When the infective oocyst is ingested, it releases sporozoites in the intestine, which initiate infection.

Life Cycle

The life cycle of the parasite consists of 3 stages as follows—(a) Tachyzoites, the rapidly multiplying trophozoites which invade and multiply within cells, (b) bradyzoites, the slowly multiplying forms inside tisssue cysts, seen during latent and chronic infection, and (c) sporozoites inside oocysts, which are shed in cat feces and remain in the environment.

In cats, which are the definitive hosts. both schizogony and gametogony take place in the epithelial cells of the small intestine. This is known as the *enteric cycle*. The oocysts produced by gametogony are shed in faeces. They develop into infective forms in soil or water. They may be ingested by felines to repeat the cycle.

When ingested by other animals or birds. which are intermediate hosts. The oocysts release sporozoites which infect the intestinal epithelial cells. Here, they multiply by endodyogeny to form tachyzoites. The host cell ruptures releasing numerous trophozoites which spread through blood and lymph infecting any type of nucleated cell in various tissues and organs. This is known as the *exoenteric cycle*.

Primary infection with the parasite may be asymptomatic, acute or chronic. In chronic infections tissue cysts are produced within muscles and other tissues. When

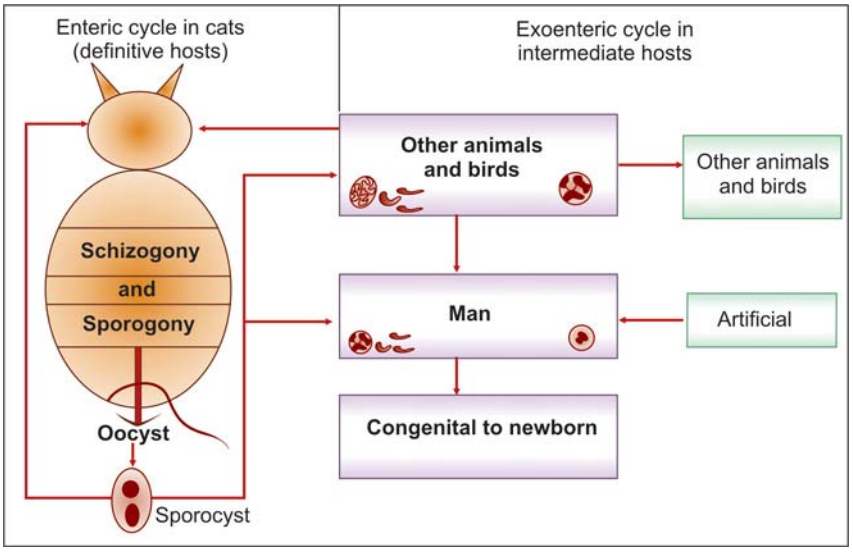


FIGURE 6.3: Life cycle of *Toxoplasma gondii*. Cats and other felines are the definitive hosts in which the enteric cycle takes place involving both schizogony (asexual) and sporogony (gametogony). Cat is infected either by ingestion of oocyst shed in its faeces or by eating flesh of other animals or birds containing tissue cysts. Oocysts shed in cat faeces sporulate, developing sporocysts containing sporozoites. When ingested these infect animals, humans and birds. Human infection also occurs from flesh of animals and birds containing tissue cysts. Artificial methods of human infection are laboratory contamination, blood transfusion and organ transplantation. Congenital infection also occurs

other intermediate hosts ingest these tissue cysts, the asexual cycle is repeated. When cats ingest the tissue cysts they become infected and in them both asexual and sexual cycles are repeated (Fig. 6.3).

Human infection is a dead end for the parasite. Human infection is obtained in the following ways—

a. Eating uncooked or undercooked infected meat containing tissue cysts.
b. Ingestion of mature oocysts through food, water or fingers contaminated with cat feces directly or indirectly.
c. Intrauterine infection from infected mothers to babies.
d. Rarely by blood transfusion or transplantation from infected donors.

Clinical Features

Most human infections are asymptomatic. Clinical toxoplasmosis may be congenital or acquired.

Congenital Toxoplasmosis

Congenital toxoplasmosis results when infection is transmitted transplacentally from mother to foetus. This occurs when the mother gets primary toxoplasma infection, whether clinical or asymptomatic during the pregnancy. The risk of foetal infection

rises with the progress of gestation, from 25 per cent when the mother acquires primary infection in the first trimester, to 65 per cent in the third trimester. Conversely the severity of foetal damage is highest when infection is transmitted in early pregnancy. Mothers with chronic or latent Toxoplasma infection acquired earlier do not ordinarily infect their babies, but in some women with latent or chronic infection, the tissue cyst may be reactivated during pregnancy and liberate trophozoites which may reach the fetus *in utero*. Most infected newborns are asymptomatic at birth and may remain so throughout. Some develop clinical manifestations of toxoplasmosis weeks, months or even years after birth. The manifestations may be chorioretinitis, strabismus, blindness, deafness, epilepsy or mental retardation. A few are born with manifestations of acute toxoplasmosis, which may include fever, jaundice, diarrhoea, petechial rashes, hydrocephalus, microcephaly, cerebral calcifications, microphthalmia, cataract, glaucoma, chorioretinitis, optic atrophy, lymphadenitis, pneumonitis, myocarditis and hepatosplenomegaly.

Acquired Toxoplasmosis

Infection acquired postnatally is mostly asymptomatic. Clinical toxoplasmosis may present in different forms. The most common manifestation of acute acquired toxoplasmosis is lymphadenopathy, the cervical lymph nodes being most frequently affected. Fever, headache, myalgia and splenomegaly are often present. The illness may resemble mild 'flu' or infectious mononucleosis and is self-limited, though the lymphadenopathy may persist. In some there may be a typhus-like exanthem,with pneumonitis, myocarditis and meningoencephalitis, which may be fatal. Another type of toxoplasmosis is ocular. Approximately 35 per cent of cases of chorioretinitis in the USA and Europe have been reported to be due to toxoplasmosis. While most of these follow congenital infection, it may sometimes be due to postnatal infection. Some cases may be so severe as to require enucleation. Toxoplasmosis primarily involving the central nervous system is usually fatal and often found in AIDS.

Toxoplasmosis is particularly severe in the immunodeficient, particularly in AIDS patients, whether it be due to reactivation of latent infection or to new acquisition of infection. In them brain involvement is common.

Host defence against toxoplasma infection involves both humoral and cellular responses. Specific IgG antibody can lyse extracellular trophozoites. But activated T cells and natural killer cells appear to be more important in containing the infection and preventing clinical disease.

Diagnosis

Laboratory diagnosis may be made by microscopic demonstration of the parasite, by its isolation or by serological tests. Giemsa stained impression smears of lymph nodes, bone marrow, spleen or brain may occasionally show the trophozoites, which can be readily identified by their morphology. Tissue sections may show the cyst forms.

Isolation may be intraperitoneally is made by injecting body fluids or ground tissues into cell cultures or immunosuppressed mice. Peritoneal fluid and spleen smears may show the trophozoites after 7 to 10 days. Serial blind passages may often be necessary for isolation. Sera of inoculated animals may also be tested for antibodies.

The most common method of laboratory diagnosis is by serology. Several serological tests are available. These include the Sabin-Feldman dye test, indirect immunofluorescence, indirect haemagglutination, complement fixation and ELISA. The Sabin-Feldman dye test is based on the specific inhibition by antibody of the staining of the trophozoite by alkaline methylene blue. Toxoplasma trophozoites propagated in mice peritoneal cavity are used. An accessory factor present in fresh normal serum is essential for the reaction. The highest dilution of the test serum which inhibits the staining is the titre. The test becomes positive within 1 to 2 weeks after infection, reaching titres of 1000 or more in 4 to 8 weeks and remaining positive at lower titres for years. Fluorescent antibody test results are similar. The CF test becomes positive only 3 to 8 weeks after infection rises in titre over the next 2 to 8 months and declines to low or undetectable levels within a year. The dye test was the first serological test for toxoplasmosis and remained the gold standard for many decades. But as the test required live toxoplasma, it could be done only in select laboratories. It is seldom done now because of its complexity and as better tests like ELISA are avialable. The standard test used now is ELISA, separately for IgM and IgG antibodies.

The presence of IgM antibody in the absence of IgG denotes current infection; IgM antibody with high titre IgG suggests infection in the recent past; Negative IgM with positive IgG indicates past infection.This is subject to individual variation. In some cases IgM antibody may persist up to 18 months. Serial ELISA provides better information than a sinlge test.

Epidemiology

The infection is worldwide, being found wherever there are cats. Numerous species of mammals, reptiles and birds are naturally infected. The full natural cycle is maintained predominantly by cats and mice. The mice eat materials contaminated with oocysts shed by cats. Mice get infected and develop cysts in their tissues. When such mice are eaten by cats they get infected. Infected cats shed oocysts in faeces. Besides this cycle, several others have been documented.

Human toxoplasmosis is a zoonosis. It is acquired through food or water contaminated with mature oocysts or by ingestion of raw or undercooked meat containing tissue cysts. Pork and beef frequently have tissue cysts. Flies and cockroaches may act as mechanical vectors by contaminating food with oocysts from soil. Infection may be water borne when the source of water is contaminated with cat faeces. Rarely infection may be transmitted through blood or leucocyte transfusion or organ transplantation. Toxoplasmosis may also be acquired by laboratory infection. The incubation period is usually 1 to 3 weeks.

The outcome of infection depends on the immune status of the infected person. Active progression of infection is more likely in immunocompromised individuals. Toxoplasmosis has acquired great importance as one of the major fatal complications in AIDS.

The incidence of congenital toxoplasmosis is estimated as approximately 1 in 1000 live births.Because of the public health importance of congenital toxoplasmosis, serological surveys for toxoplasma antibodies are conducted in many advanced countries in women of childbearing age, antenatal women and newborns.

Prevention

Eradication of toxoplasmosis appears unlikely because it is so widely disseminated in nature. But some simple measures may reduce the risk of infection. These include proper cooking of meat and washing of hands before eating to safeguard against soil contamination of fingers.

Treatment

Combined treatment with pyrimethamine and sulphonamides or cotrimoxazole may lead to clinical cure, though the parasites may not be eliminated. Spiromycin and clindamycin have also been used. Treatment is effective only against trophozoites and not against cysts.

SARCOCYSTIS

Sporozoa of the genus *Sarcocystis* show alternation of generations and alternation of hosts. Three species can infect humans—*S. hominis* (Fig. 6.4). *S. suihominis, S. lindemanni.* Humans are the definitive host for the first two, and the intermediate host for the third. Sarcocystis species produce cysts in the muscles of intermediate hosts. These cysts, called *sarcocysts* contain numerous merozoites (bradyzoites). When eaten by the definitive host. the merozoites are released in the intestine, where they develop into male and female gamonts. After fertilisation the zygote develops into an oocyst containing two sporocysts each having four sporozoites. The oocysts are shed in faeces and are ingested by the intermediate host. In them the sporozoites invade the bowel wall and reach the vascular endothelial cells, where they undergo schizogony producing merozoites (tachyzoites). These spread to muscle fibres and develop into sarcocysts.

FIGURE 6.4: *Sarcocystis hominis* oocyst

Cow is the intermediate host for *S. hominis*. Human infection is acquired by eating raw or undercooked beef. Oocysts are shed in human faeces which contaminate grass and fodder eaten by cows. In the case of *S. suihominis* the pig is the intermediate host and human infection is obtained through pork. Human infection with *S. hominis* and *S. suihominis* is related to food habits. Clinical symptoms are minimal.

Humans are the intermediate host for *S. lindemanni*, the definitive host of which is not yet known. It is believed that *S. lindemanni* may not be a single species but

a group of as yet unidentified species. Humans apparently get infected by ingestion of oocysts. Sarcocysts develop in the human skeletal muscle and myocardium. Clinical symptoms are insignificant and the diagnosis is made incidentally at biopsy or autopsy.

ISOSPORA BELLI

Isospora belli is a coccidian parasite which can cause diarrhoea in humans. It was originally described by Virchow in 1860, but was named only in 1923. The name *belli* (from *bellum*—meaning war) was given for its association with war, because several cases of infection with this parasite were seen among troops stationed in the Middle East during the First World War. The parasite resides in the epithelial cells of the small intestine where schizogony and sporogony take place. Human infection is acquired by ingestion of mature oocysts in food or drink. There is no evidence for any animal reservoir for this parasite. There are several other species of isospora parasitic in animals but they do not appear to infect humans.

Numerous slender sickle-shaped merozoites are produced by schizogony in the intestinal epithelial cells. After release from the ruptured schizonts, the merozoites infect other epithelial cells. Male and female gametocytes develop in some infected cells. After fertilisation, the zygote becomes an oocyst which is shed in faeces. The oocyst is oval or flask-shaped, thin-walled, transparent, about 25 µm by 15 µm and contains a single sporoblast. The oocyst matures outside the body and develops two sporocysts containing four sporozoites each. This is the infective stage. On being swallowed, the sporozoites escape and infect the intestinal epithelial cells and initiate schizogony (Fig. 6.5).

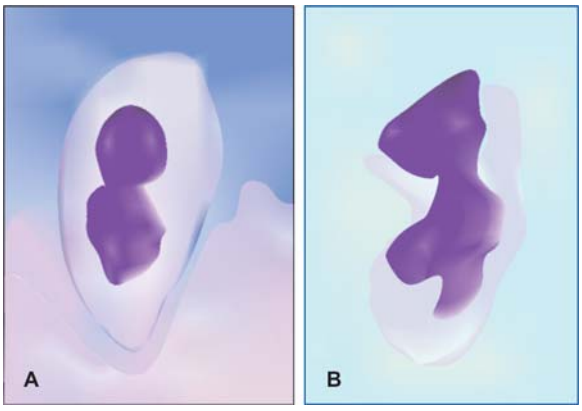


FIGURE 6.5: Oocysts of *Isospora belli*—(A) Oocyst showing two sporoblasts (B) Mature oocyst with two sporocysts containing sporozoites

Infection is usually asymptomatic. Clinical illness including abdominal discomfort, mild fever and diarrhoea develops a week after exposure. The illness is usually self-limited but protracted diarrhoea, lasting for several years, can be produced in immunocompromised persons, particularly in the HIV infected.

Diagnosis may be made by demonstration of the oocysts in fecal smears. They may be scanty and because of their transparent nature may be overlooked in unstained films. They stain red by the cold acid-fast technique. Zinc sulphate or formol-ether techniques can be employed for concentration.

Treatment with cotrimoxazole is effective.

CRYPTOSPORIDIUM PARVUM

Cryptosporidia were first observed in the gastric mucosal crypts of laboratory mice by Tyzzer in 1907. Its importance as a pathogen causing diarrhoea in animals was recognised in 1971 and the first case of human infection reported in 1976. *Cryptosporidium* has assumed great importance as a frequent cause of intractable diarrhoea in AIDS patients. It can lead to acute self-limited diarrhoea in previously healthy persons and chronic life-threatening diarrhoea in immunocompromised subjects.

Cryptosporidium is a minute coccidian parasite of worldwide distribution. Natural infection with *C. parvum* is present in many species of birds such as chicken, turkey, and of animals including cattle, sheep, goats and cats, besides humans. The parasite does not appear to be host-specific and infection can spread from one host species to another. The parasite completes its life cycle, sexual and asexual phases in a single host (*monoxenous*) (Fig. 6.6).

Infection is acquired by ingesting the oocyst in contaminated food or drink. The, oocyst contains four sporozoites which are released in the intestine. They infect the intestinal epithelial cells, remaining just within the brush border. There they develop

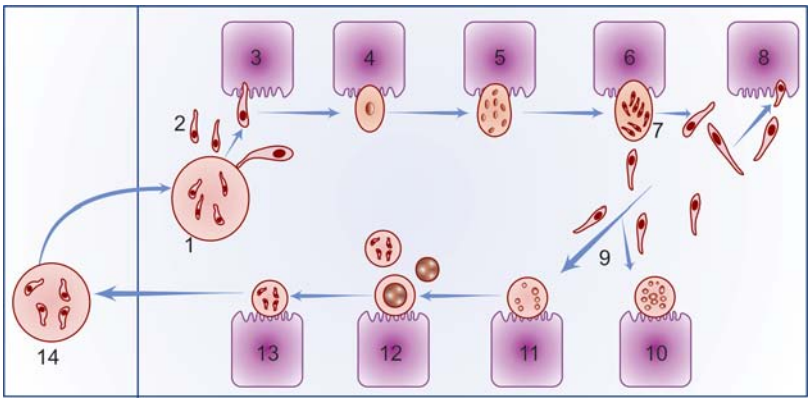


FIGURE 6.6: Life cycle of *Cryptosporidium parvum.* 1. Infection occurs by ingestion of oocyst containing four sporozoites. 2. In the small intestine, excystation releases the sporozoites. 3. The motile sporozoites penetrate enterocytes, remaining intracellular but extracytoplasmic. 4 to 6. Trophozoite, early schizont and mature schizont respectively, comprising the asexual cycle (merogony). 7.Release of merozoites from schizont. 8. Merozoites infect neighbouring enterocytes to continue merogony. 9. Initiation of sexual cycle (sporogony). 10. Macrogametocyte. 11. Microgametocyte. 12. Zygote. 13. Oocyst. 14. Thick-walled oocyst the infective form, shed to exterior

into the trophozoites, which undergo asexual multiplication (schizogony) and release merozoites. These, in turn infect the neighouring epithelial cells and repeat schizogony. Some develop into micro- and macrogametocytes. After fertilisation, the zygote develops into the oocyst, which is shed in feces. It is fully mature on release and is infective immediately without further development. The oocyst is about 5 µm in diameter. It can remain viable in the environment for long periods, as it is very hardy and resistant to most disinfectants and temperature up to 60°C. *C. parvum* contamination of water supplies can cause outbreaks of food poisoning. It can survive in chlorinated water, but sequential application of ozone and chlorine has been found effective.

Infection in previously healthy persons may be asymptomatic or cause a sef-limited febrile illness with watery diarrhoea. It can also cause childhood and traveller's diarrhoea, as well as water-borne outbreaks. But in the HIV infected and other immunodeficient persons, infection leads to severe protracted diarrhoea, fever and emaciation. In AIDS, the parasite may invade the bronchial and biliary tracts and can be demonstrated in sputum.

Diagnosis is made by demonstration of the oocyst in feces. With Jenner-Giemsa stain, the oocysts in faeces smears appear as blue spherical bodies containing a few eosinophilic granules. By cold Ziehl-Neelsen technique, the internal structures appear acid fast (Fig. 6.7). Fluorescent staining with auramine phenol has been reported to be a useful technique. Definitive identification can be made by indirect immunofluorescence using specific antibody. In acute diarrhoea, the oocysts are abundant, but when they are scanty, concentration by the formol-either technique may be employed.

Seroconversion can be demonstrated within 2 months of acute infection. Antibodies persist for at least one year and can be demonstrated by ELlSA or immuno-fluorescence.

An ELISA for detection of cryptosporidium in stools using monoclonal antibody has been said to be highly sensitive and specific.

No specific treatment is available. In persons with normal immune response, the disease undergoes spontaneous cure.

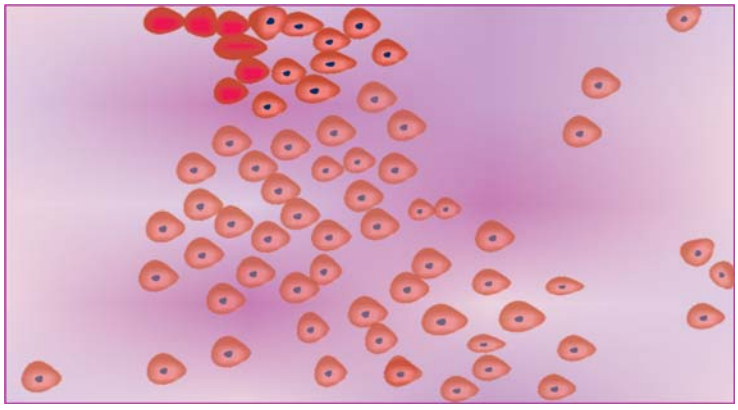


FIGURE 6.7: *Cryptosporidium* sp oocyst stained by Ziehl-Neelsen technique

CYCLOSPORA CAYETANENSIS

This is one among the many new intestinal pathogens that have come to light in association with HIV-related diarrhoea. It was first reported from Nepal, where it caused seasonal outbreaks of prolonged diarrhoea, with peak prevalence in the warm rainy months.

Oocysts shed in faeces sporulate *in vitro.* Excystation of the sporocyst releases two crescentic sporozoites measuring about 9 × 1.2 µm. The sporozoites infect enterocytes in the small intestine and cause diarrhoea.

Diagnosis is by demonstration of oocysts in feces. They can be induced to sporulate in presence of 5 per cent potassium dichromate solution. Concentration methods facilitate demonstration of cysts. The parasite can also be seen in small bowel biopsy material by electron microscopy.

Infection is through faecal-oral route with an incubation period of 1 to 7 days. Drinking water is the most important vehicle of transmission. No specific therapy is known.

MICROSPORIDIA

Microsporidia are minute intracellular prasites. They reproduce by spores, 2 to 4 µm in size, which have polar filaments or tubules. The spore can survive outside the host cell and is the infective form. Infection is acquired by ingestion of the spores, which transmit the sporoplasm through the tubules into enterocytes. Merogony and sporogony take place and the spores are shed in feces. Infection may also take place outside the intestine, in any viscus, muscle or in the central nervous system.

Microsporidia are of historical interest as the first protozoon parasite to have been successfully studied and controlled. In the 19th century Europe, the silkworm disease pebrine caused great damage to the silk industry. Louis Pasteur was assigned the problem in 1863 and by 1870 he published his findings and recommendations which led to the control of pebrine and the rescue of the silk industry in France. It was this experience which led Pasteur to his epochal work on human and animal diseases that formed the foundation of Microbiology. The causative agent of pebrine is *Nosema bombycis,* a microsporidian parasite.

Microsporidia are classified under the Phylum Microspora within the Protozoa. Microsporidia had been known as animal parasites for long, but their role as human pathogens was recognised only in the mid-1980s with the spread of AIDS. Some 9 genera and 13 species of microspora are associated with human disease, particularly in the HIV infected and other immunocompromised subjects. They can cause a wide range of illness, from diarrhoea to involvment of the CNS, eyes, viscera, muscles and diseminated disease.The most common microsporidium involved is *Enterocytozoon biuneusi.* Others include *Encephalitozoon, Brachiola, Nosema, Pleistophora* and *Vittaforma* species.

Diagnosis is by demonstration of the spores in stools or tissues after apropriate staining or by elctron microscopy.Serology and culture are not useful. Antigen detection by immunofluorescence is promising. Metronidazole, primaquine and albendazole have been used in treatment.

BABESIA

Babesia is so named after Babes who in 1888 described the intraerythrocytic parasite in the blood of cattle and sheep in Romania. In 1893, the parasite was shown to cause the tick-borne disease Texas fever, an acute haemolytic disease of cattle in southern USA. This was the first arthropod-borne disease to have been identified.

Infection in vertebrates is acquired through the bite of ixodid ticks in which the parasite undergoes its sexual cycle. The sporozoites present in the tick salivary glands are inoculated into the vertebrate host, enter the blood stream and invade erythrocytes in which they undergo asexual multiplication by budding. Release of daughter parasites leads to invasion of fresh red cells and further asexual multiplication. The intraerythrocytic parasite is typicalty pear-shaped, about 2 to 5 µm in size and usually occur in pairs. The appearance in Giemsa-stained films may be mistaken for *Plasmodium falciparum* rings. The parasite digests haemoglobin, but no pigment is formed, in contrast to plasmodia. Ticks feeding on vertebrates get infected. The parasite can be maintained through successive generations in ticks by transovarial transmission. Ticks can therefore act as reservoir hosts as well as vectors.

The name *Piroplasma* was given to Babesia because of its pear shape (from *pyrum*—pear) and the disease caused by the parasite was known as piroplasmosis.

Human infections caused by *B.bovis*, *B.divergans* and *B.microti* have been recorded in Europe and USA. After an incubation period of about 2 to 3 weeks, the illness starts with fever, chills, headache and myalgia. Anaemia and jaundice may follow. The disease may be fatal in splenectomised individuals. Recovery may be followed by prolonged carrier stage.

Diagnosis can be made by demonstration of the intraerythrocytic parasites in Giemsa-stained blood films. Blood from suspected cases may be inoculated into hamsters and after a month blood films from the inoculated animals may show the parasites in large numbers. Pentamidine with cotrimoxazole, clindamycin and oral quinine have been used for treatment.

PNEUMOCYSTIS CARINII

Pneumocystis carinii was first described by Chagas (1909) and Carini (1910) in the lungs of guinea pigs in Brazil. Human infection was recognised only in 1942 and subsequently several cases of interstitial pneumonia caused by the parasite have been observed, particularly in malnourished and premature infants. *P.carinii* has received much attention from the 1980s as it is one of the characteristic opportunistic infections seen in AIDS patients. It is far more commonly seen in AIDS patients in America and Europe than in Asia and Africa.

The taxonomic status of *P.carinii* has been uncertain. While it was generally considered as a sporozoan parasite, analysis of its chromosomal and mitochondria genes indicates its closer relationship to fungi than to protozoa. Natural infection with *P.carinii* occurs in many species of animals. It has been reported that human infection may come from dogs or other domestic animals, but this is considered much less important than inter-human spread.

P. carinii lives within the alveoli of the lungs. It occurs in two forms, trophozoite and cyst. The trophozoite is 1 to 5 µm in size, amoeboid in shape and has a central nucleus. Its cytoplasm contains mitochondria, ribosomes, endoplasmic reticulum and various granules.It divides by binary fission. Some trophozoites become encysted and produce within the cyst eight daughter trophozoites, also known as intracystic bodies or sporozoites. The mature cyst is thick-walled and measures up to 10 µm in diameter. The cyst collapses, releasing trophozoites which initiate another cycle of multiplication, either in the same host, or in another if they have been spread by coughing. The collapsed cysts can be seen as irregular crescentic bodies (Figs 6.8 and 6.9).

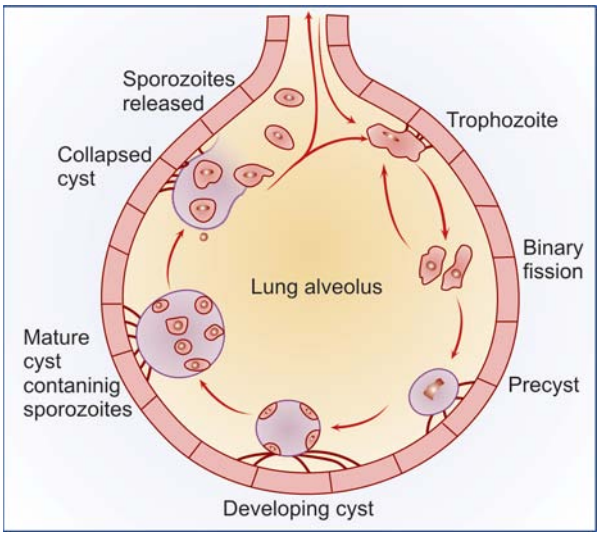


FIGURE 6.8: Life cycle of *Pneumocystis carinii.* The parasite enters the lung in respiratory droplets and gets attached to alveolar epithelium. It divides by binary fission. Some form a thick-walled cyst within which sporozoites develop. When mature cysts rupture sporozoites are released which initiate fresh cycles of infection

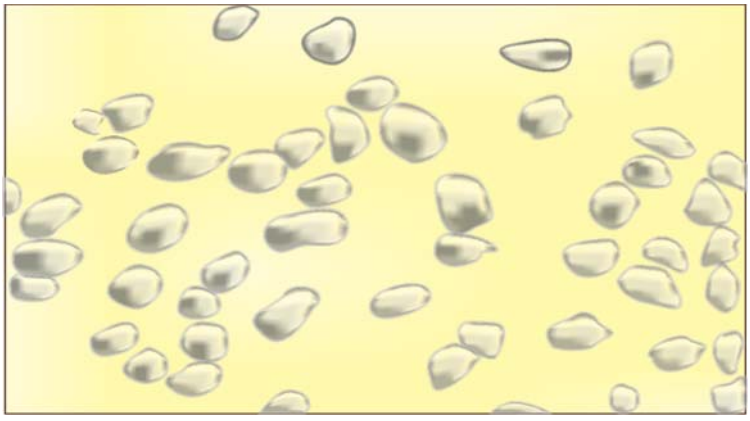


FIGURE 6.9: *P.carinii* cyst (methanamine silver stain)

P.carinii is normally a commensal in the lung, spread by respiratory droplets. Many healthy persons have been reported to carry the organisms in the lungs. Pneumocystosis is an opportunistic infection, clinical disease being found only when the resistance is very low, as in premature and malnourished infants and in AIDS and other immunodeficiencies. The multiplication of the parasite in the lungs induces a hyaline or foamy alveolar exudate containing numerous lymphocytes, macrophages and plasma cells, but no polymorphs. In stained sections, the exudate filling the alveoli shows a characteristic honycomb pattern. The disease presents with nonproductive cough, breathlessness and cyanosis. *P.carinii* pneumonia has been reported to be a very common life-threatening opportunistic infection in AIDS patients in the West.

Diagnosis may be made by demonstrating the parasite in sputum, tracheobronchial lavage or transbronchial biopsy specimens. Sputum examination is less satisfactory. Open lung biopsy may sometimes be necessary .Diagnosis is often made from autopsy speciments. The cysts can be stained by Giemsa or methanamine-silver techniques. Immunofluorescence has been used for demonstrating cysts. *P.carinii* antigen can be demonstrated by ELISA.

Cotrimoxazole and pentamidine have been used in treatment. But the prognosis is poor in pneumocystis pneumonia in the immunodeficient subjects.

PROTOTHECA

Prototheca are algae and not protozoan parasites, but they are considered here because they may cause opportunist infections in compromised hosts, like many parasites dealt with in this Chapter.

Prototheca infections usually involve the skin and underlying tissues, producing papules, plaques or erythematous lesions. The infection may become disseminated in the immunodeficient, involving the liver, gall bladder and peritoneum. Diagnosis is easy if the condition is suspected. Biopsy of the lesion shows sporangia with multiple septa, containing spores, 5-15 µm in diameter. Cultures can be obtained in routine media incubated at 30°C. On Sabouraud's medium, creamy yeast-like colonies are formed. Cycloheximide should be avoided in the medium as it is inhibitory.

Treatment is not very effective. Tetracycline and amphotericin B have been tried. In some cases surgical measures may be necessary.

CHAPTER 7

Ciliate Protozoa

BALANTIDIUM COLI

History and Distribution

The only ciliate protozoan parasite of humans is *Balantidium coli*. This was first described by Malmsten in 1857 in the faeces of dysenteric patients. It is the largest protozoan parasite of humans. It is present worldwide, but the prevalence of the infection is very low. Balantidiasis is a zoonosis, the principal reservoir being the pig, monkeys and rats are also infected.

Morphology and Life Cycle

B. coli occurs in two stages—the trophozoite and cyst (Fig. 7.1).

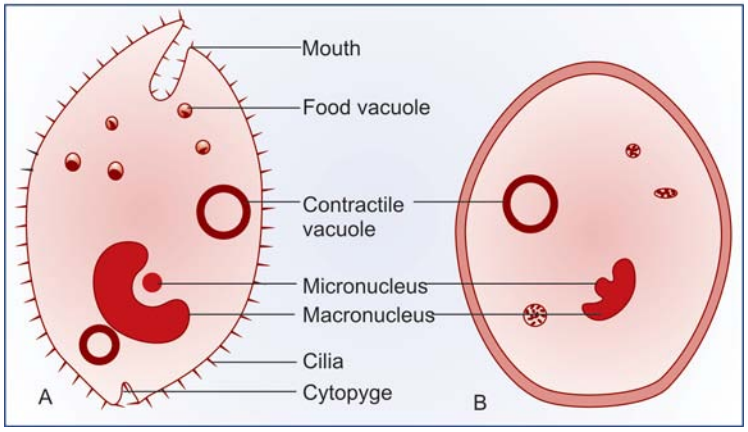


FIGURE 7.1: Morphology of *Balantidium coli*. A. Trophozoite B. Cyst

Trophozoite

The trophozoite lives in the large intestine, feeding on cell debris, bacteria, starch grains and other particles. The trophozoite is a large ovoid cell, about 60 to 70 µm

in length and 40 to 50 µm in breadth. Very large cells,measuring up to 200 µm are sometimes seen. The cell is enclosed within a delicate pellicle showing longitudinal striations. The anterior end is narrow and the posterior broad. At the anterior end is a groove (*peristome*), leading to the mouth (*cytostome*) and a short blind gullet (*cytopharynx*). Posteriorly there is a small anal pore (*cytopyge*). The cell is covered all over with short delicate cilia. The cilia around the mouth are larger (*adoral cilia*). The cell has two nuclei—a large kidney-shaped macronucleus and lying in its concavity a small micronucleus. The cytoplasm has one or two contractile vacuoles and several food vacuoles. The trophozoite is motile, being propelled forwards by a vigorous synchronous motion of the cilia.

Cyst

Encystation occurs as the trophozoite passes down the colon or in the evacuated stool. The cell rounds up and secretes a tough cyst wall around it.The cyst measures 50 to 60 µm in diameter. The macronucleus, micronucleus and vacuoles are present in the cyst also. The cyst remains viable in feces for a day or two.

Multiplication during the trophozoite stage is by transverse binary fission. Conjugation occurs infrequently, during which reciprocal exchange of nuclear material takes place between two trophozoites enclosed within a single cyst wall.

B.coli can be grown in culture in the media used for growing *E.histolytica.*

Pathogenicity

Infection is acquired from pigs and other animal reservoirs or from human carriers. The infective form is the cyst, which is ingested in contaminated food or drink. Excystment takes place in the small intestine and the liberated trophozoites reach the large intestine where they feed and multiply as lumen commensals. Infection is very often confined to the lumen and is asymptomatic. Clinical disease results only when the trophozoites burrow into the intestinal mucosa, set up colonies and initiate inflammatory reaction. This leads to mucosal ulcers and submucosal abscesses. resembling the lesions in amoebiasis. Clinically also, balantidiasis resembles amoebiasis, causing diarrhoea or frank dysentery with abdominal colic, tenesmus, nausea and vomiting. Occasionally there may occur intestinal perforation with peritonitis and rarely involvement of genital and urinary tracts.

Diagnosis and Treatment

Diagnosis is established by demonstration of the parasite in faeces. While motile trophozoites occur in diarrhoeic faeces, cysts are found in formed stools. Treatment with tetracycline 500 mg every six hours, for 10 days is successful. Metronidazole and nitroimidazole have also been reported to be useful. Prophylaxis consists of avoidance of contamination of food and drink with human or animal faeces.

CHAPTER 8

Helminths: General Features

Helminths are bilaterally symmetrical metazoa belonging to the Phylum-Scolecida. The term "helminth" (Greek *helmins*-'worm') originally referred to intestinal worms, but now comprises many other worms, including tissue parasites as well as many free-living species.

Helminths have an outer protective covering, the cuticle or integument which may be tough and armed with spines or hooks. The mouth may be provided with teeth or cutting plates. Many helminths possess suckers or hooks for attachment to host tissues. The cuticle of live helminths is resistant to intestinal digestion. They do not possess organs of locomotion, but in some species the suckers assist in movement. Locomotion is generally by muscular contraction and relaxation.

Helminths do not possess a true coelomic or body cavity. In some parasitic helminths the digestive system is absent or rudimentary as they depend on predigested nutrients available from the host. Many helminths have a primitive nervous system. The excretory system is better developed. The greatest development is seen in the reproductive system. Helminths may be *monoecious* (with functioning male and female sex organs in the same individual) or *diecious* (the two sexes, male and female, separate). In the hermaphroditic helminths both male and female reproductive systems are present in the same worm and self-fertilisation as well as cross-fertilisation take place. In the diecious species males and females are separate, the male being smaller than the female. Rarely the female is parthenogenic, being able to produce fertile eggs or larvae without mating with males.

The eggs or larvae are produced in enormous numbers—as many as 200,000 or more per female per day. This seemingly wasteful excess is necessary as only few of them survive and manage to infect a suitable host. It has been estimated that their chance of survival and subsequent infection may be less than one in a million. Survival and development are further complicated by the fact that many helminths require more than one intermediate host for completion of their life cycle. The process of development in some helminths is extremely complex and is influenced by various factors such as environmental conditions and human customs and practices.

Helminths differ from protozoa in their inability to multiply in the body of the host. Protozoa multiply in the infected person so that disease could result from a

single infection. But helminths apart from very rare exceptions do not multiply in the human body so that a single infection does not generally lead to disease. Heavy worm load follows multiple infections.

CLASSIFICATION

Though the term helminth suggests a long cylindrical worm-like shape, not all of them possess this feature. Some are flat and ribbon-like, while some others are leaf-shaped. Based on their shape and other characteristics. Helminths are classified into two broad groups—the cylindrical worms belonging to the Phylum Nemathelminthes (Class Nematoda) commonly called Nematodes (from Nema—thread) and the flat worms belonging to the Phylum Platyhelminthes (from Platys—flat). The flat worms. in turn are classified into two categories—the leaf-like Trematodes (Class Trematoda) or flukes and the tape-like Cestodes (Class Cestoda) or tapeworms.

Nematodes are elongated cylindrical worms with an unsegmented body. They possess a relatively well-developed alimentary canal, complete with the anus. The head does not have either suckers or hooks, but may have a buccal capsule with teeth or cutting plates. The sexes are separate.

Trematodes have flat or fleshy leaf-like unsegmented bodies. The alimentary canal is present but incomplete, without an anus. They possess suckers, but no hooks. The sexes are separate in the schistosomes, while the other flukes are hermphroditic.

Cestodes have tape-like segmented bodies. They do not possess an alimentary system. The head carries suckers and some also have hooks. They are monoecious.

A simplified zoological classification of helminths infecting humans is given below.

PHYLUM PLATYHELMINTHES

Class Trematoda

Possess oral and ventral suckers; bifurcated gut, ending blind

A. Blood Flukes (Sexes separate, infection by cercarial penetration)
   Family Schistosomatidae (Schistosomes).

B. Hermaphroditic Flukes (Bisexual, infection by ingestion of cercariae)
   Family Fasciolidae (Large flukes, cercariae encyst on aquatic vegetation).
      Genus *Fasciola, Fasciolopsis*
   Family Paramphistomatidae (Large ventral sucker posteriorly)
      Genus *Gastrodiscoides*
   Family Echinostomatidae (Collar of spines behind oral sucker, cercariae encyst in mollusc or fish)
      Genus *Echinostoma*
   Family Triglotrematidae (Testes side by side behind ovary, cercariae encyst in crustacea)
      Genus *Paragonimus*
   Family Opisthorchidae (Testes in tandem behind ovary, cercariae encyst in fish)
      Genus *Clonorchis, Opisthorchis*

Family Dicrocoelida (Testes in front of ovary cercariae encyst in insects)
Genus *Dicrocoelium*
Family Heterophyidae (Minute flukes, Cercartae in fish)
Genus *Heterophyes, Metagonimus*

Class Cestoda (Scolex, with ribbon of proglottids, no gut)

Order Pseudophyllidea (Scolex has grooves)
Genus *Diphyllobothrium*
Order Cyclophyllidea (Scolex has suckers)
Family Taeniidae, (Proglottid longer than broad numerous testes, one genital pore, larva in vertebrates)
Genus *Taenia, Multiceps, Echinococcus*
Family Hymenolepidiidae (Transverse proglottids, one genital pore, larva in insects)
Genus *Hymenolepis*
Family Dilepidiidae (Two genital pores)
Genus *Dipylidium.*

PHYLUM NEMATHELMINTHES

Class Nematoda

Subclass Adenophorea or Aphasmidia . No phasmids, no caudal papillae in male)
Order Enoplida
Superfamily Trichuroidea (Anterior part narrow, male has one spicule, female has one ovary
Genus *Trichuris, Trichinella, Capillaria*
Subclass Secernentea or Phasmidia (Phasmids present, numerous caudal papillae)
Order Rhabditida (Free-living and parasic generations, parasitic female parthenogenic)
Genus *Strongyloides*
Order Strongylida (Males have copulatory bursa, mouth has no lips)
Superfamily Ancylostomatoidea (Prominent buccal capsule with teeth or cutting plates)
Genus *Ancylostoma, Necator*
Order Ascaridida (Large worms mouth has three lips)
Genus *Ascaris, Toxocara Anisakis*
Order Oxyurida (Live in large gut oesophagus has posterior bulb)
Genus *Enterobius*
Order Spirurida (Tissue parasites arthropod or crustacean intermediate hosts)
Genus *Gnathostoma*
Superfamily Filarioidea (Tissue parasites viviparous, insect vector)
Genus *Wuchereria. Brugia. Loa, Onchocerca. Mansonella*
Superlamily Dracunculoidea (Very long female viviparous larvae escaping from ruptured uterus)
Genus *Dracunculus.*

CHAPTER 9

Trematodes: Flukes

Termatodes are unsegmented helminths which are flat and broad, resembling the leaf of a tree or a flatfish (hence the name *Fluke*, from the Anglo-Saxon word *floc* meaning flatfish). The name Trematode comes from their having large prominent suckers with a hole in the middle (Greek *trema*—hole, *eidos*—appearance).

They vary in size from the species just visible to the naked eye, like *Heterophyes* to the large fleshy flukes, like *Fasciola* and *Fasciolopsis*. Medically important members of the class Trematoda belong to the subclass Digenea, as they are digenetic, i.e. require two hosts. The definitive hosts in which they pass the sexual or adult stage are mammals, humans or animals, and the intermediate hosts in which they pass their asexual or larval stages are freshwater molluscs or snails.

FLUKES: GENERAL CHARACTERS

Flukes are hermaphroditic (monoecious) except for schistosomes in which the sexes are separate (Fig. 9.1).

A conspicuous feature is the presence of two muscular cup-shaped suckers (hence called Distomata)—the *oral sucker* surrounding the mouth at the anterior end and the *ventral sucker* or *acetabulum* in the middle, ventrally. The body is covered by an

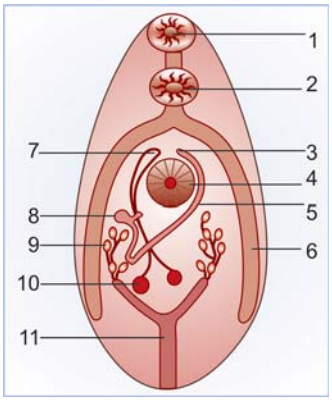


FIGURE 9.1: Morphology of a hermaphroditic trematode: 1. Oral sucker 2. Pharynx 3. Genital pore 4. Ventral sucker 5. Uterus 6. Caecum 7. Cirrus 8. Ovary 9. Flame cell 10. Testis 11. Excretory bladder

integument which often bears spines, papillae or tubercles. They have no body cavity, circulatory or respiratory organs. The alimentary system consists of the mouth surrounded by the oral sucker, a muscular pharynx and the oesophagus which bifurcates anterior to the acetabulum to form two blind caeca, which reunite in some species. The alimentary canal therefore appears like an inverted Y. The anus is absent, the excretory system consists of flame cells and collecting tubules which lead to a median bladder opening posteriorly. There is a rudimentary nervous system consisting of paired ganglion cells. The reproductive system is well-developed. The hermaphroditic flukes have both male and female structures so that self-fertilisation takes place, though in many species cross-fertilisation also occurs. In the schistosomes the sexes are separate, but the male and female live in close apposition (*in copula*), the female fitting snugly into the folded ventral surface of the male, which forms the *gynaecophoric canal*.

Trematodes are oviparous and lay eggs which are operculated, except in the case of schistosomes. The eggs hatch in water to form the first stage larva, the motile ciliated *miracidium* (Greek *miracidium*—a 'little boy'). The miracidium infects the intermediate host snail in which further development takes place. The miracidium sheds its cilia and becomes the sac-like *sporocyst* (meaning a 'bladder containing seeds'). Within the sporocyst, certain cells proliferate to form the *germ balls*, which are responsible for asexual replication. In schistosomes, the sporocyst develops into the second generation sporocyst in which the infective larvae *cercariae* are formed by sexual multiplication. But in the hermaphroditic trematodes, the sporocyst matures into a more complex larval stage name *redia* (after the 17th century Italian naturalist Francesco Redi), which produce cercariae. Cercariae are tailed larvae and hence their name (Greek *kerkos*—tail). In schistosomes, cercariae have a forked tail and infect the definitive host by direct skin penetration. In the hermaphroditic flukes, the cercariae have an unsplit tail, and they encyst on vegetables or within a second intermediate host, fish, or crab, to form the *metacercariae*, which are the infective forms, infection is acquired by ingesting metacercariae encysted on vegetables (*F. hepatica, F. buski, W. watsoni*), in fish (*C. sinensis, H. heterophyes*) or crabs (*P. westermani*). The asexual multiplication during larval development is of great magnitude, and in some species, a single miracidium may give rise to over half a million cercariae.

Trematodes infecting humans can be classified as follows:

A. Diecious blood flukes or Schistosomes which live inside veins in various locations:
   1. In the vesical and pelvic venous plexuses—*Schistosoma haematobium*.
   2. In the inferior mesenteric vein—*S. mansoni*
   3. In the superior mesenteric vein—*S. japonicum*

B. Hermaphroditic flukes which live in the lumen of various tracts:
   1. Biliary tract (liver flukes); *Clonorchis sinensis. Fasciola hepatica. Opisthorchis* sp.
   2. Gastrointestinal tract (Intestinal flukes): .
      a. Small intestine—*Fasciolopsis buski, Heterophyes heterophyes, Metagonimus yokogawai, Watsonius watsoni*
      b. Large intestine—*Gastrodiscoides hominis*
   3. Respiratory tract (Lung fluke)—*Paragonimus westermani*.

SCHISTOSOMES: BLOOD FLUKES

Schistosomes are diecious trematodes in which the sexes are separate. The male is broader than the female, and its lateral borders are rolled ventrally into a cylindrical shape, producing a long groove or trough called the gynaecophoric canal, in which the female is held. It appears as though the body of the male is split longitudinally to produce this canal—hence the name Schistosome (Greek *schisto*—split and *soma*—body). Schistosomes were formerly called *Bilharzia* after Theodor Bilharz who in 1851, first observed the worm in the mesenteric veins of an Egyptian in Cairo. All schistosomes live in venous plexuses in the body of the definitive host, the location varying with the species (Fig. 9.2).

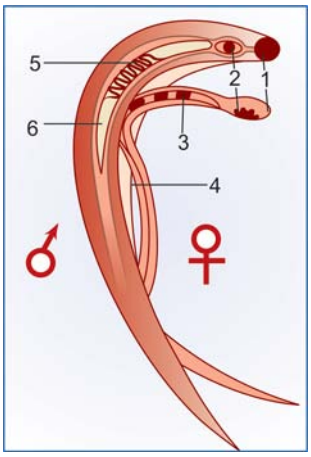


FIGURE 9.2: Morphology of Schistosomes: Male and female in copula. 1. Oral sucker 2. Ventral sucker 3. Uterus 4. Gynaecophoric canal 5. Testis 6. Caecum

Schistosomes differ from the hermaphroditic trematodes in many respects. They lack a muscular pharynx. Their intestinal caeca reunite after bifurcation to form a single canal. They produce non-operculate eggs. They have no redia stage in larval development. The cercariae have forked tails and infect by penetrating the unbroken skin of definitive hosts. Schistosomiasis (bilharziasis) is a water-borne disease constituting an important public health problem affecting millions of persons in Africa, Asia and Latin America. It is estimated that over 100 milion persons are infected with *S. haematobium, S. mansoni* and *S. japonicum* each.

SCHISTOSOMA HAEMATOBIUM

History

This vesical blood fluke, formerly known as *Bilharzia haematobium* has been endemic in the Nile valley in Egypt for millenia. Its eggs have been found in the renal pelvis of an Egyptian mummy dating from 1250-1000 B.C. Schistosome antigens have been identified by ELISA in Egyptian mummies of the Predynastic period, 3100 B.C. The adult worm was described in 1851 by Bilharz in Cairo. Its life cycle, including the larval stage in the snail was worked out by Leiper in 1915 in Egypt.

Geographical Distribution

Although maximally entrenched in the Nile valley, *S. haematobium* is also endemic in most parts of Africa and in West Asia. An isolated focus of endemicity in India was identified in Ratnagiri, south of Mumbai by Gadgil and Shah in 1952.

Morphology and Life Cycle

The adult worms live in the vesical and pelvic plexuses of veins. The male is 10 to 15 mm long by 1 mm thick and covered by a finely tuberculated cuticle. It has two muscular suckers, the oral sucker being small and the ventral sucker large and prominent. Beginning immediately behind the ventral sucker and extending to the caudal end is the gynaecophoric canal in which the female worm is held. The adult female is long and slender, 20 mm by 0.25 mm with the cuticular tubercles confined to the two ends.

The gravid worm contains 20 to 30 eggs in its uterus at anyone time and may pass up to 300 eggs a day. The eggs are ovoid, about 150 µm by 50 µm, with a brownish yellow transparent shell carrying a terminal spine at one pole (the *terminal spine* is characteristic of the species). The eggs are laid usually in the small venules of the vesical and pelvic plexuses, though sometimes they are laid in the mesenteric portal system, pulmonary arterioles and other ectopic sites. The eggs are laid one behind the other with the spine pointing posteriorly. From the venules, the eggs make their way through the vesical wall by the piercing action of the spine, assisted by the mounting pressure within the venules and a lytic substance released by the eggs. The eggs pass into the lumen of the urinary bladder together with some extravasated blood. The eggs are discharged in the urine, particularly towards the end of micturition. For some unknown reasons, the eggs are passed in urine more during midday than at any other time of the day or night. The eggs laid in ectopic sites generally die and evoke local tissue reactions. They may be found, for instance in rectal biopsies, but are seldom passed live in feces.

The eggs that are passed in water hatch, releasing the ciliated miracidia. They swim about in water and on encountering a suitable intermediate host, penetrate into its tissues and reach its liver. The intermediate hosts are snails of *Bulinus* species in Africa. In India, the intermediate host is the limpet *Ferrisia tenuis*.

Inside the snail, the miracidia lose their cilia and in about 4 to 8 weeks, successively pass through the stages of the first and second generation sporocysts. Large number of cercariae are produced by asexual reproduction within the second generation sporocyst. The cercaria has an elongated ovoid body and forked tail (*furcocercous cercaria*). Swarms of cercariae swim about in water for 1 to 3 days. If during that period they come into contact with persons bathing or wading in the water, they penetrate through their unbroken skin. Skin penetration is facilitated by lytic substances secreted by penetration glands present in the cercaria.

On entering the skin, the cercariae shed their tails and become *schistosomulae* which enter the peripheral venules. They then start a long migration, through the vena cava into the right heart, the pulmonary circulation, the left heart and the systemic circulation, ultimately reaching the liver. In the intrahepatic portal veins, the

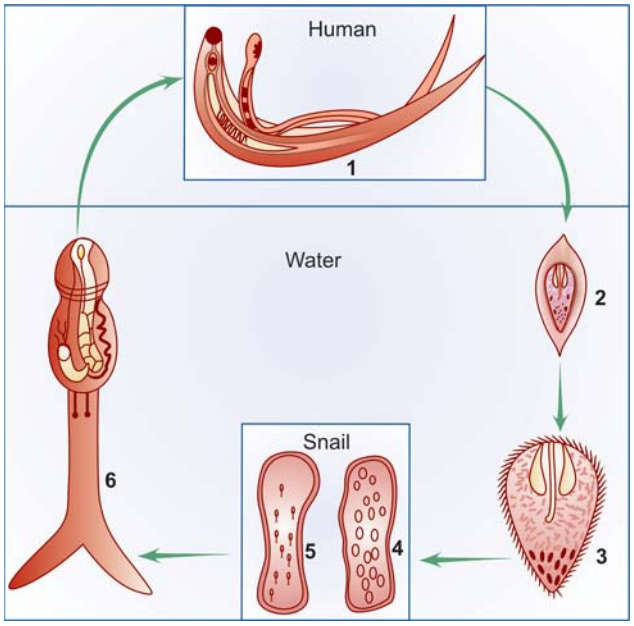


FIGURE 9.3: Life cycle of *Schistosoma haematobium*. 1. Adult male and female in copula in the vesical venous plexus. 2. Egg containing ciliated embryo passed in urine reaches water. 3. Miracidium hatches out of egg and enters the snail liver. 4. Development in snail—Sporocyst first generation. 5. Sporocyst second generation. 6. Cercaria with forked tail released into water. Human infection by skin penetration

schistosomulae grow and become sexually differentiated adolescents about 20 days after skin penetration. They then start migrating against the blood stream into the inferior mesenteric veins, ultimately reaching the vesical and pelvic venous plexuses where they mature, mate and begin laying eggs. Eggs start appearing in urine usually 10 to 12 weeks after cercarial penetration. The adult worms may live for 20 to 30 years (Figs 9.3 to 9.6).

Humans are the only natural definitive hosts. No animal reservoir is known.

Pathogenicity and Clinical Features

Clinical illness caused by schistosomes can be classified depending on the stages in the evolution of the infection, as follows:

i. Skin penetration and incubation period;
ii. Egg deposition and extrusion; and
iii. Tissue proliferation and repair.

The clinical features during the incubation period may be local cercarial dermatitis or general anaphylactic or toxic symptoms. Cercarial dermatitis consists of transient itching petechial lesions at the site of entry of the cercariae. This is seen more often in visitors to endemic areas than in locals who may be immune due to repeated contacts. It is particularly severe when infection occurs with cercariae of nonhuman

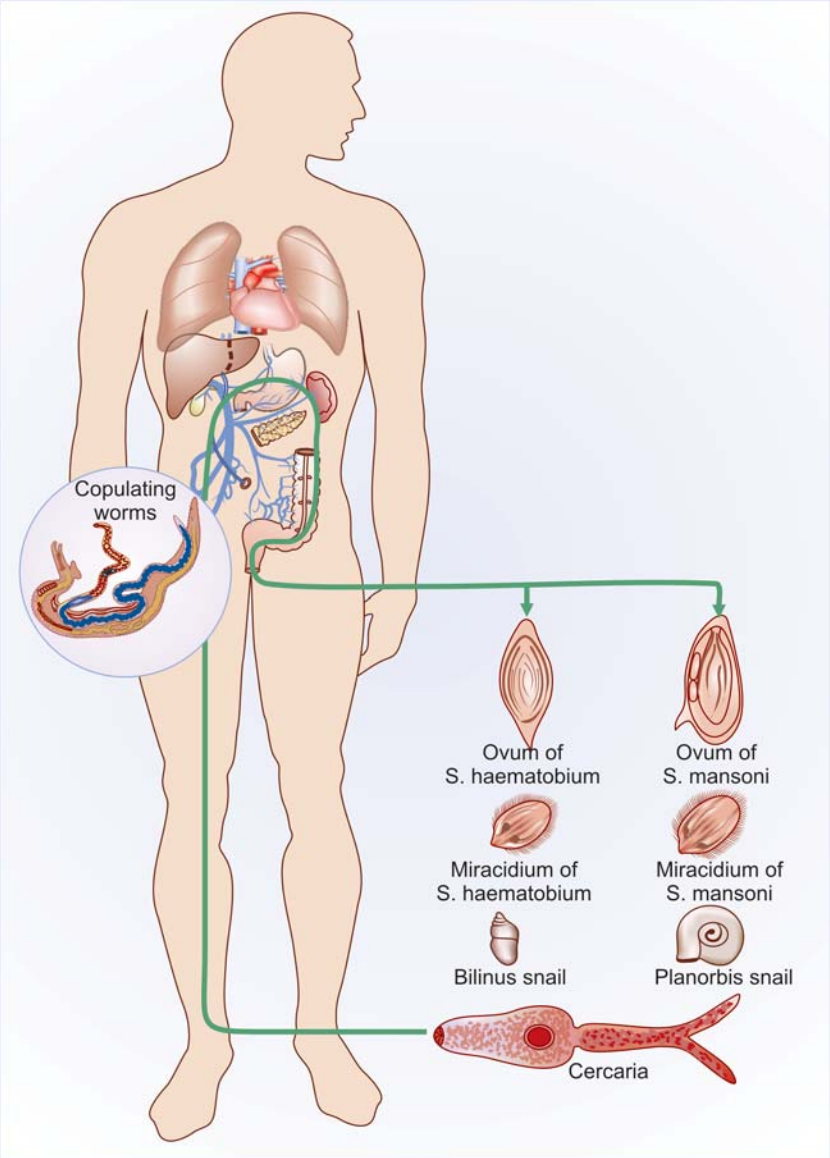


FIGURE 9.4: *S. haematobium:* developmental stages

schistosomes. Anaphylactic or toxic symptoms include fever, headache, malaise and urticaria. This is accompanied by leucocytosis, eosinophilia, enlarged tender liver and a palpable spleen. This condition is more common in infection with *S. japonicum* (*Katayama fever*).

The typical manifestation caused by egg laying and extrusion is painless terminal haematuria (*endemic haematuria*). Haematuria is initially microscopic, but becomes gross if infection is heavy. Most patients develop frequency of micturition and burning.

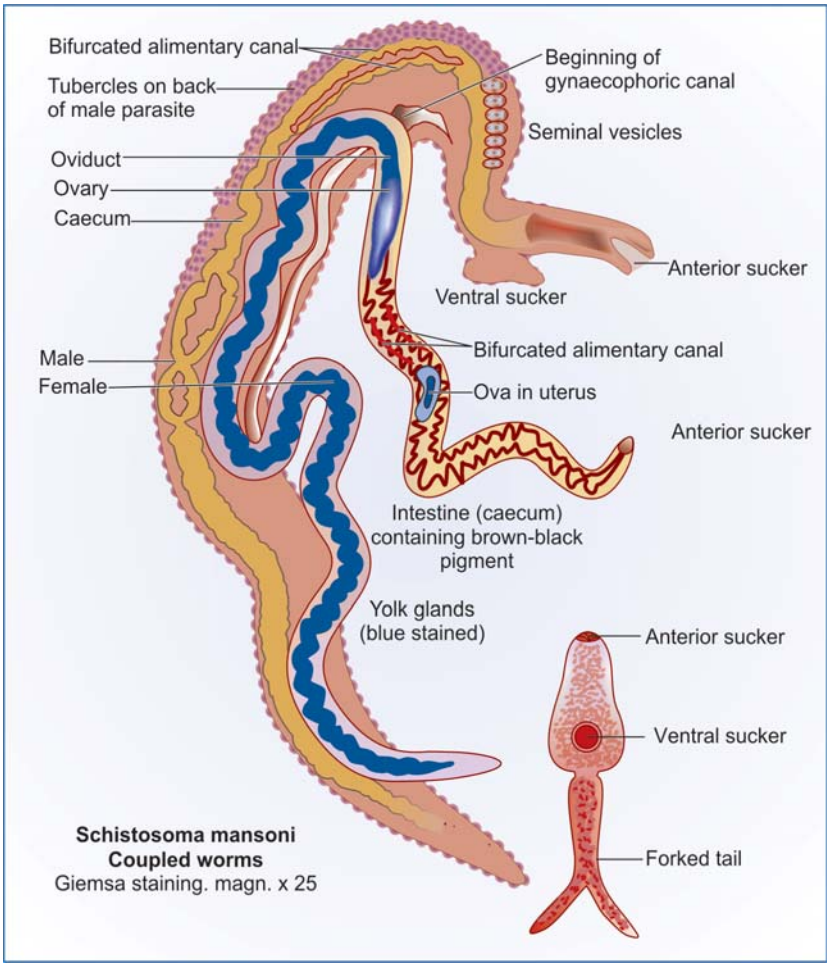
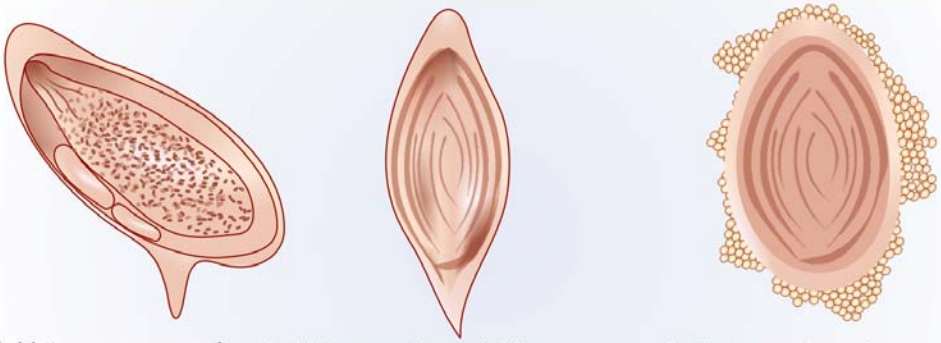


FIGURE 9.5: Schistosoma in coupled

Cystoscopy shows hyperplasia and inflammation of bladder mucosa with minute papular or vesicular lesions.

In the chronic stage there is generalised hyperplasia and fibrosis of the vesical mucosa with a granular appearance (*Sandy patch*). At the sites of deposition of the eggs, dense infiltration with lymphocytes, plasma cells and eosinophils leads to pseudoabscesses. Initially the trigone is involved, but ultimately the entire mucosa becomes inflamed, thickened and ulcerated. Secondary bacterial infection leads to chronic cystitis. Calculi form in the bladder due to deposition of oxalate and uric acid crystals around the eggs and blood clots. There may be obstructive hyperplasia of the ureters and urethra. Schistosomiasis favours urinary carriage of typhoid bacilli. Chronic schistosomiasis has been associated with bladder cancer, though a causative relationship is not proved.



Schistosoma mansoni
ova from stool, containing viable miracidium spine at side

Schistosoma haematobium
ova from urine, containing miracidium spine at end

Schistosoma japonicum
ova from stool, containing miracidium. Note characteristic surround of tissue particles. Spin only rudimentary and not always visible

FIGURE 9.6

Diagnosis

The eggs with characteristic terminal spines can be demonstrated by microscopic examination of centrifuged deposits of urine. Eggs are more abundant in the blood and pus passed by patients at the end of micturition. They can also be seen in seminal fluid. They may occasionally be found in feces, or more often in vesical or rectal biopsies.

A refinement of diagnosis by demonstration of eggs is to hatch shed eggs into motile miracidia.

Another diagnostic method is by detection of specific schistosome antigens in serum or urine. Two glycoprotein antigens associated with the gut of adult schistosomes (circulating anodic and cathodic antigens, CAA and CCA) can be demonstrated by ELISA using monoclonal antibodies. The test is very sensitive and specific, but is available only in specialised laboratories. Skin tests are group specific and give positive results in all schistosomiases. The intradermal allergic test (Fairley's test) uses antigen from infected snails, from cercariae, eggs and adult schistosomes from experimentally infected laboratory animals.

Several serological tests have been described but are not very useful. These include complement fixation, bentonite flocculation, indirect haemagglutination, immunofluorescence, gel diffusion and ELISA. Two special tests are circumoval precipitation (globular or segmented precipitation around schistosome eggs incubated in positive sera) and "cercarien-hullen" reaction (development of pericercarial membranes around cercariae incubated in positive sera). Animal schistosomes can be used as antigens in these tests. Ultrasonography is useful in diagnosing *S. haematobium* infection.

Treatment

Metriphonate is the drug of choice in schistosomiasis due to haematobium. Praziquantel is effective against all schistosomes and also against many other trematode and cestode infections.

Prevention and Control

Prophylactic measures include eradication of the intermediate molluscan hosts. prevention of environmental pollution with urine and feces and effective treatment of infected persons.

SCHISTOSOMA MANSONI

History and Distribution

The discovery by Manson in 1902 of eggs with lateral spines in the feces of a West Indian patient led to the recognition of this second species of human schistosomes. It was therefore named *S. mansoni*. It is widely distributed in Africa, South America and the Caribbean islands.

Morphology and Life Cycle

S. mansoni resembles *S. haematobium* in morphology and life cycle. The adult worms are smaller and their integuments studded with prominent coarse tubercles. In the gravid female the uterus contains very few eggs usually 1 to 3 only. The prepatent period (the interval between cercarial penetration and beginning of egg laying) is 4 to 5 weeks. The egg has a characteristic lateral spine (Fig. 9.7).

The intermediate hosts are planorbid fresh-water snails of the Genus *Biomphalaria*. Humans are the only natural definitive hosts, though in endemic areas monkeys and baboons have been found infected.

In humans the schistosomulae mature in the liver and the adult worms move against the blood stream into the venules of the inferior mesenteric group in the sigmoidorectal area. Eggs penetrate the gut wall, reach the colonic lumen and are shed in feces.

Pathogenicity and Clinical Features

Following skin penetration by cercariae a pruritic rash may develop locally. During the stage of egg deposition the symptomatology is mainly intestinal. This condition is therefore known as intestinal bilharziasis or schistosomal dysentery. Patients develop colicky abdominal pain and bloody diarrhoea which may go on intermittently for many years.The eggs deposited in the gut wall cause inflammatory reactions leading to micro-abscesses, granulomas, hyperplasia and eventual fibrosis. Ectopic lesions include hepatosplenomegaly and portal hypertension.

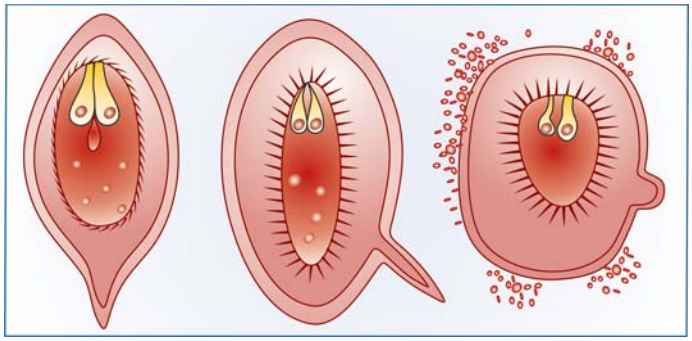


FIGURE 9.7: Schistosome eggs. 1. *S. haematobium*—Oval, with terminal spine, 2. *S. mansoni*—Oval, with lateral spine. 3. *S. japonicum*—Roundish, with lateral knob. Small granules of tissue debris adherent to shell.

Diagnosis

Eggs with lateral spines may be demonstrated microscopically in stools. Concentration methods may be required when infection is light. Proctoscopic biopsy snips of rectal mucosa reveal eggs when examined as fresh squash preparation between two slides.

Treatment

Oxamniquine is the drug of choice.

Prevention and Control

These are based on control of the snail hosts, prevention of fecal pollution and treatment of infected persons.

SCHISTOSOMA JAPONICUM

Distribution

Known as the Oriental blood fluke, *S. japonicum* is found in the Far East, Japan, China, Taiwan, Philippines and Sulawesi.

Morphology and Life Cycle

These are generally similar to the schistosomes described above. The adult worms are seen typically in the venules of the superior mesenteric vein draining the ileocaecal region. They are also seen in the intrahepatic portal venules and in the haemorrhoidal plexus of veins.

The adult male is comparatively slender (0.5 mm thick) and does not have cuticular tuberculations. In the gravid female, the uterus contains as many as 100 eggs at one time and up to 3500 eggs may be passed daily by one worm. The prepatent period

is 4 to 5 weeks. The eggs are smaller and more spherical than those of *S. haematobium* and *S. mansoni*. The egg has no spine, but shows a lateral knob.

Eggs deposited in the mesenteric venules penetrate the gut wall and are passed in feces. They hatch in water and the miracidia infect the intermediate hosts, amphibian snails of the genus Oncomelania. Man is the definitive host but in endemic areas, natural infection occurs widely in several domestic animals and rodents, which act as reservoirs of infection.

Pathogenesis and Clinical Features

Disease caused by *S. japonicum* is also known as Oriental schistosomiasis or Katayama disease. Its pathogenesis is similar to that in other schistosomiases, but probably because of the higher egg output, the clinical manifestations are more severe.

The acute illness consisting of fever, abdominal pain, diarrhoea and allergic manifestations is called Katayama fever. It is an immune complex disease caused by antibodies to the schistosomulae, adult worms and eggs.

In the chronic illness, the liver is the site maximally affected. There is initial hepatomegaly followed by fibrosis. Portal hypertension leads to oesophageal varices and gastrointestinal bleeding. The spleen is secondarily enlarged. Cerebral and pulmonary involvement may occur in some cases.

Diagnosis is by demonstration of the eggs in feces.

Treatment

S. japonicum infection is more resistant to treatment than other schistosomiases. A prolonged course of intravenous tartar emetic gives good results. Praziquantel has also been reported useful.

Prevention and Control

Prevention of fecal population of soil and water, treatment of infected persons and snail control help to contain the infection. But the presence of animal reservoirs in endemic areas makes eradication difficult.

SCHISTOSOMA INTERCALATUM

This species, first recognised in 1934 is found in West Central Africa. The eggs have terminal spines, but are passed exclusively in stools.

SCHISTOSOMA MEKONGI

This species first recognised in 1978 is found in Thailand and Cambodia, along the Mekong river. It is closely related to *S. japonicum*.

HERMAPHRODITIC FLUKES: LIVER FLUKES

The adult forms of all hermaphroditic flukes infecting humans live in the lumen of the biliary, intestinal or respiratory tracts. This location affords the parasites considerable protection from host defense mechanisms and also facilitates dispersal of eggs to the environment.

Flukes inhabiting the human biliary tract are *Clonorchis sinensis*, *Fasciola hepatica*, less often *Opisthorchis* species, and rarely *Dicrocoelium dendriticum*.

CLONORCHIS SINENSIS

History and Distribution

Commonly known as the Chinese liver fluke, *C. sinensis* was first described in 1875 by McConnell in the biliary tract of a Chinese in Calcutta. Human clonorchiasis occurs in Japan, Korea, Taiwan, China and Vietnam affecting about ten million persons.

Morphology and Life Cycle

Humans are the principal definitive host, but dogs and other fish-eating canines act as reservoir hosts. Two intermediate hosts are required to complete its life cycle, the first being snail and the second fish. The adult worm lives in the human biliary tract for 15 years or more. It has a flat, transparent, spatulate body; pointed anteriorly and rounded posteriorly, 10 to 25 mm long and 3 to 5 mm broad. It discharges eggs into the bile duct. The eggs are broadly ovoid, 30 µm by 15 µm with a yellowish brown shell. It has an operculum at one pole and a small hook-like spine at the other.

The eggs passed in feces contain the ciliated miracida. They do not hatch in water, but only when ingested by suitable species of operculate snails, such as *Parafossarulus*, *Bulimus* or *Alocinma* species. The miracidium develops through the sporocyst and redia stages to become the lophocercus cercaria with a large fluted tail in about 3 weeks. The cercariae escape from the snail and swim about in water, waiting to get attached to the second intermediate host, suitable fresh-water fish of the carp family. The cercariae shed their tails and encyst under the scales or in the flesh of the fish to become, in about 3 weeks the metacercariae which are the infective stage for humans. Infection occurs when such fish are eaten raw or inadequately processed by human or other definitive hosts. Frozen, dried or pickled fish may act as source of infection. Infection may also occur through fingers or cooking utensils contaminated with the metacercariae during preparation of the fish for cooking.

The metacercariae excyst in the duodenum of the definitive host. The adolescaria that come out enter the common bile duct through the ampulla of Vater and proceed to the distal bile capillaries where they mature in about a month and assume the adult form (Fig. 9.8).

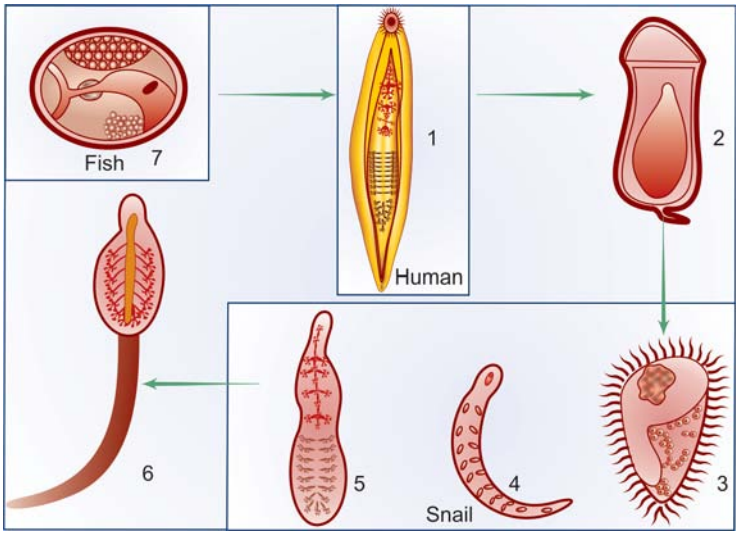


FIGURE 9.8: Life cycle of Clonorchis sinensis. 1. Adult fluke in biliary tract of humans or animals. 2. Eggs passed in stools reach water and are ingested by the first intermediate host snail. 3. Miracidium emerges from egg and penetrates into tissues of snail. 4. Sporocyst containing rediae. 5. Redia showing cercariae developing inside. 6. Cercariae leave the snail and swim about in water to infect the second intermediate host fish. 7. Encysted metacercaria develop in the muscles of fish. This is the infective form for human or other definitive hosts.

Pathogenicity

The migration of the larva up the bile duct induces desquamation, followed by hyperplasia and sometimes adenomatous changes. The smaller bile ducts undergo cystic dilatation. The adult worm may cause obstruction and blockage of the common bile duct leading to cholangitis. Chronic infection may result in calculus formation. A few cases go on to biliary cirrhosis and portal hypertension. Some patients with chronic clonorchiasis tend to become biliary carriers of typhoid bacilli. Chronic infection has also been linked with cholangiocarcinoma.

Patients in the early stage have fever, epigastric pain, diarrhoea and tender hepatomegaly. This is followed by biliary colic, jaundice and progressive liver enlargement. Many infections are asymptomatic.

Diagnosis

The eggs may be demonstrated in feces or aspirated bile. They do not float in concentrated saline. Several serological tests have been described including complement fixation and gel precipitation but extensive cross-reactions limit their utility. Indirect haemagglutination with a saline extract of etherised worms has been reported to be sensitive and specific. Intradermal allergic tests have also been described.

Treatment

Chemotherapy has not been very successful. Chloroquine and praziquantel have been reported to be useful. Surgical intervention may become necessary in cases with obstructive jaundice.

Prophylaxis

Proper cooking of fish can prevent the infection. Health education, proper disposal of feces and snail control measures help to limit the infection in endemic areas.

OPISTHORCHIS SPECIES

Some species of Opisthorchis which resemble *C. sinesis* can cause human infection. *O. felineus*, the cat liver fluke which is common in Europe and the erstwhile Soviet Union may infect humans. Infection is usually asymptomatic but may sometimes cause liver disease resembling clonorchiasis. *O. viverrini* is common in Thailand where the civet cat is the reservoir host. Human infection is usually asymptomatic.

FASCIOLA HEPATICA

Fasciola hepatica or the sheep liver fluke was the first trematode to have been discovered as early as 1379 by de Brie. It is the largest and most common liver fluke found in humans, but its primary host is the sheep, and to a less extent cattle. It is worldwide in distribution, being found mainly in sheep-rearing areas. It causes the economically important disease 'liver rot' in sheep.

Morphology and Life Cycle

The adult worm lives in the biliary tract of the definitive host for many years—about 5 years in sheep and 10 years in humans. It is a large leaf-shaped fleshy fluke, 30 mm long and 15 mm broad, grey or brown in colour. It has a conical projection anteriorly and is rounded posteriorly. The eggs are large, ovoid, operculated, bile stained and about 140 µm by 80 µm in size. They are laid in the biliary passages and shed in feces. The embryo matures in water in about 10 days and the miracidium escapes. It penetrates the tissues of intermediate host, snails of the genus *Lymnaea*. In snail, the miracidium progresses through the sporocyst, the first and second generation redia stages to become the cercariae in about 1 to 2 months. The cercariae escape into the water and encyst on aquatic vegetation or blades of grass to become metacercariae which can survive for long periods. Sheep, cattle or humans eating watercress or other water vegetation containing the metacercaria become infected. The metacercariae excyst in the duodenum and pierce the gut wall to enter the peritoneal cavity. They penetrate the Glisson's capsule, traverse the liver parenchyma and reach the biliary passages, where they mature into the adult worms in about 3-4 months (Fig. 9.9).

FIGURE 9.9A

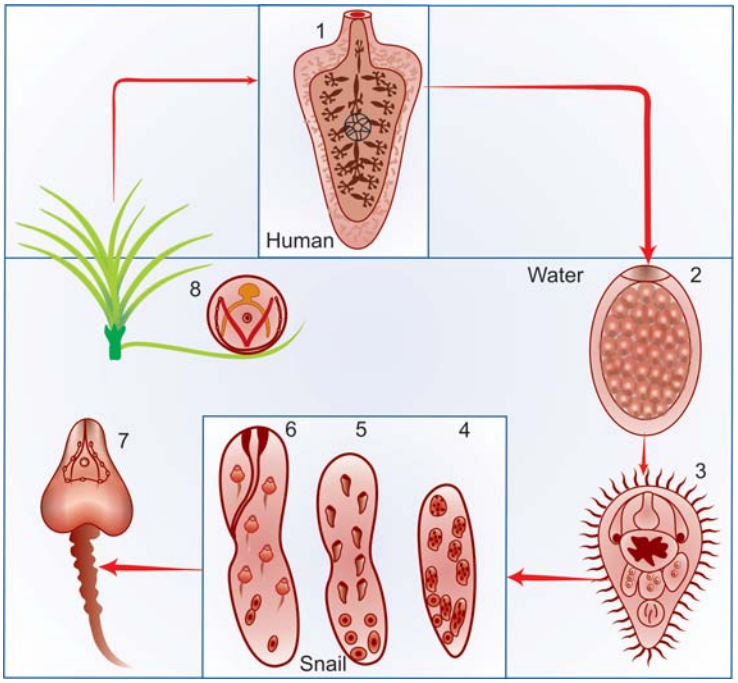


FIGURE 9.9B: Life cycle of *Fasciola hepatica.* 1. Adult in biliary tract of sheep and humans. 2. Egg passed in stools reaches water. 3. Miracidium escapes and penetrates tissues of snail in which it develops successively into 4. Sporocyst and 5. Redia first generation and 6. Second generation. 7. Cercaria released into water encysts on water plants to become 8. Metacercaria which is infective to definite hosts by ingestion

Pathogenicity

Fascioliasis differs from clonorchiasis in that *F. hepatica* is larger and so causes more mechanical damage. In traversing the liver tissue it causes parenchymal injury. As humans are not its primary host, it causes more severe inflammatory response. Some larvae penetrate right through the liver and diaphragm ending up in the lung. Patients present initially with fever, eosinophilia and tender hepatomegaly. Later they develop acute epigastric pain, obstructive jaundice and anaemia. Cholelithiasis is a common late complication.

Occasionally, ingestion of raw liver of infected sheep results in a condition called *halzoun* (meaning suffocation). The adult worms in the liver attach to the pharyngeal mucosa causing oedematous congestion of the pharynx and surrounding areas, leading to dyspnoea, dysphagia, deafness and rarely asphyxiation. However, this condition is more often due to pentastome larvae. Halzoun is particularly common in Lebanon and other parts of the Middle East and North Africa.

Diagnosis

Demonstration of eggs in feces or aspirated bile is the best method of diagnosis. Eosinophilia is constantly present. Serological tests such as immunofluorescence, immunoelectrophoresis and complement fixation may be helpful.

Treatment

Oral bithionol is the treatment of choice. Intramuscular emetine has been used successfully.

Prophylaxis

Health education, preventing pollution of water courses with sheep, cattle and human feces, and proper disinfection of watercresses and other water vegetations before consumption can limit the infection.

F. gigantica, a related species is a common parasite of herbivores in Africa and has caused occasional human infection. It is also prevalent in Indian herbivores.

DICROCOELIUM DENDRITICUM

Known also as the 'lancet fluke' because of its shape, *D. dendriticum* is a very common biliary parasite of sheep and other herbivores in Europe, North Africa, Northern Asia and parts of the Far East. Eggs passed in feces are ingested by land snails. Cercariae appear in slime balls secreted by the snails and are eaten by ants of the genus *Formica*, in which matacercariae develop. Herbivores get infected when they accidentally eat the ants while grazing. Reports of human infection have come from Europe, Middle East and China. However, spurious infection is more common. In the latter, the eggs can be passed in feces for several days by persons eating infected sheep liver.

Eurytrema pancreaticum, a related fluke is commonly present in the pancreatic duct of cattle, sheep and monkeys. Occasional human infection has been noticed in China and Japan.

INTESTINAL FLUKES

A number of flukes parasitise the human small intestine. These include *Fasciolopsis buski, Heterophyes, Metagonimus yokogawai, Watsonius watsoni and Echinostoma*. Only one fluke *Gastrodiscoides hominis* parasitises the human large intestine (Fig. 9.10).

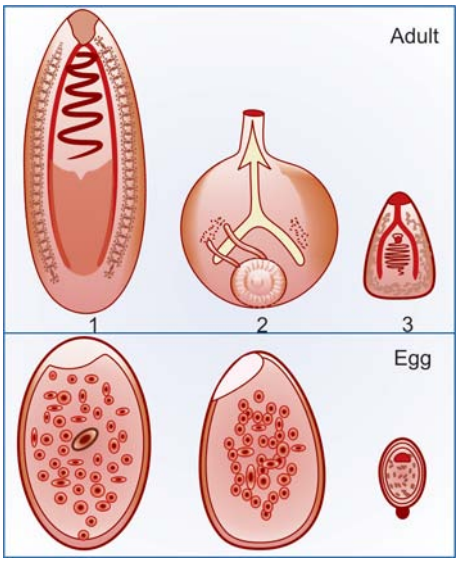


Fig. 9.10: Some intestinal flukes and their eggs.
1. Fasciolopsis buski 2. Gastrodiscoides hominis
3. Heterophyes heterophyes

FASCIOLOPSIS BUSKI

History and Distribution

Also called the giant intestinal fluke, *Fasciolopsis buski* is the largest trematode infecting humans. It was first described by Busk in 1843 in the duodenum of an East Indian sailor who died in London. It is a common parasite of man and pigs in China and in South East Asian countries. In India it occurs in Assam and Bengal.

Morphology and Life Cycle

The adult is a large fleshy worm, 20 to 75 mm long and 8 to 20 mm broad. It is elongated ovoid in shape, with a small oral sucker and a large acetabulum. It has no cephalic cone as in *F. hepatica*. The adult lives in the duodenum or jejunum and has a lifespan of about 6 months. The operculated eggs are similar to those of *F. hepatica*. Eggs are laid in the lumen of the intestine in large numbers, about 25,000 per day. The eggs passed in feces hatch in water in about 6 weeks, releasing the miracidia which swim about. On contact with a suitable milluscan intermediate host, snails of the genus *Segmentina*, they penetrate its tissues to undergo development in the next few weeks as sporocyst, first and second generation rediae and cercariae. The cercariae which escape from the snail encyst on the roots of the lotus, bulb of the water chestnut and on other aquatic vegetation. When they are eaten, the metacercariae excyst in the duodenum, become attached to the mucosa and develop into adults in about 3 months.

Pathogenicity

The pathogenesis of fasciolopsiasis is due to traumatic, mechanical and toxic effects. Larvae that attach to the duodenal and jejunal mucosa cause inflammation and local ulceration. In heavy infections, the adult worms cause partial obstruction of the bowel. Intoxication and sensitisation also account for clinical illness.

The initial symptoms are diarrhoea and abdominal pain. Toxic and allergic symptoms appear, usually as oedema, ascites, anaemia, prostration and persistent diarrhoea.

Diagnosis

History of residence in endemic areas suggests the diagnosis which is confirmed by demonstration of the egg in feces, or of the worms after administration of a purgative.

Treatment

Hexylresorcinol and tetrachlorethylene have been found useful. Dichlorophen and praziquantel are effective.

Prophylaxis

Adequate washing of water vegetables, preferably in hot water affords protection against infection. Preventing contamination of ponds and other waters with pig or human excreta, sterilisation of night soil before use as fertiliser, and anti-snail measures help in limiting the infection.

HETEROPHYES

This is the smallest trematode parasite of man, measuring about 1.5 mm in length and 0.3 mm in breadth. The definitive hosts, besides humans, are cats, dogs, foxes and other fish eating mammals. The infection is prevalent in the Nile Delta, Turkey and in the Far East. The worm has been reported in a dog in India.

The adult worm lives in the small intestine and has a lifespan of about 2 months. The minute operculated egg 30 µm by 15 µm are passed in faeces and hatch after ingestion by intermediate molluscan host, snails of the genera *Pironella* and *Cerithidea*. After passing through the sporocyst and one or two redia stages, the cercariae escape and encyst on suitable fishes, such as the mullet and telapia. When the infected fish are eaten raw or inadequately cooked, the definitive hosts become infected.

In the small intestine, it can induce mucous diarrhoea and colicky pains. Occasionally, the worms burrow into the gut mucosa, and their eggs are carried in the lymphatic and portal circulation to ectopic sites such as the brain, spinal cord and myocardium, where they induce granulomas. Rarely the worms themselves may be carried to these sites as emboli.

METAGONIMUS YOKOGAWAI

This minute worm, generally resembling *H. heterophyes* occurs in the Far East, Northern Siberia, Balkan states and Spain. The definitive hosts are humans, pigs, dogs, cats and pelicans. The first intermediate host is a fresh water snail and the second a fish. Definitive hosts are infected by eating raw fish containing the metacercariae.

Pathogenic effects consist of mucous diarrhoea and ectopic lesions in myocardium and central nervous system as in heterophyasis. A number of other heterophyid worms can cause occasional human infections.

WATSONIUS WATSONI

This trematode infects various primates in Asia and Africa. Only one instance of human infection has been reported.

ECHINOSTOMA

Echinostomes are medium sized flukes causing small intestinal infection in Japan, Philippines and all along the Far East. The worm is less than 20 mm long and 2 mm wide. The characteristic feature is a crown of spines on a disc surrounding the oral sucker, justifying its name 'echinostoma' which means 'spiny mouth '. Its eggs resemble those of fasciolopsis. Mild infections are asymptomatic, but diarrhoea and abdominal pain follow heavy infection. *E. ilocanum* is the species usually seen in human infections.

GASTRODISCOIDES HOMINIS

G. hominis is the only fluke inhabiting the human large intestine. It was discovered by Lewis and McConnell in 1876 in the caecum of an Indian patient. It is a common human parasite in Assam. Cases have also been reported from Bengal, Bihar and Orissa. It also occurs in Vietnam, Philippines and some parts of erstwhile USSR. Pigs are the reservoir hosts. Monkeys have been found naturally infected.

The adult worm is pyriform, with a conical anterior end and a discoidal posterior part. It is about 5-14 mm long and 4-6 mm broad. The eggs are operculated and measure 150 µm by 70 µm. The miracidia invade the tissues of the intermediate molluscan host. The cercariae encyst on water plants. Infected persons develop mucoid diarrhoea. Tetrachlorethylene is useful in treatment.

LUNG FLUKES

PARAGONIMUS WESTERMANI

History and Distribution

Also known as the Oriental lung fluke, *Paragonimus westermani* was discovered in 1878 by Kerbert in the lungs of Bengal tigers that died in the zoological gardens

at Hamberg and Amsterdam. The parasite is endemic in the Far East—Japan, Korea, Taiwan, China, and South East Asia—Sri Lanka and India. Cases have been reported from Assam, Bengal, Tamil Nadu and Kerala.

P. mexicanus is an important human pathogen in Central and South America.

Morphology and Life Cycle

The adult worm is egg-shaped about 10 mm long, 5 mm broad and 4 mm thick. Adults worms live in the lungs, usually in pairs in cystic spaces that communicate with bronchi. They have a lifespan of up to 20 years in humans. Besides humans other definitive hosts include cats, tigers, leopards, foxes, dogs, pigs, beavers, civet-cats, mongoose and many other crab-eating mammals.

The eggs are operculated, golden brown, about 100 µm by 50 µm. Eggs escape into the bronchi and are coughed up and voided in sputum or swallowed and passed in faeces. The eggs mature in about 2 weeks and hatch to release free-swimming miracidia. These infect the first intermediate molluscan host, snails belonging to the genera *Semisulcospira* and *Brotia*. Cercariae that are released from the snails after several weeks are microcercus, having a short stumpy tail. The cercariae that swim about in streams are drawn into the gill chambers of the second intermediate crustacean host, crabs or crayfish. They encyst in the gills or muscles as metacercariae. Definitive hosts are infected when they eat such crabs or crayfish raw or inadequately cooked. The metacercariae excyst in the duodenum and the adolescariae penetrate the gut wall reaching the abdominal cavity in a few hours. They then migrate up through the diaphragm into the pleural cavity and lungs finally reaching near the bronchi, where they settle and develop into adult worms in 2 to 3 months (Figs 9.11 and 9.12). The worm is hermaphroditic but usually it takes two for fertilisation.

Sometimes the migrating larvae lose their way and reach ectopic sites such as the mesentery, groin or brain.

Pathogenicity

In the lungs the worms lie in cystic spaces surrounded by a fibrous capsule formed by the host tissues. The cysts, about a centimetre in diameter are usually in

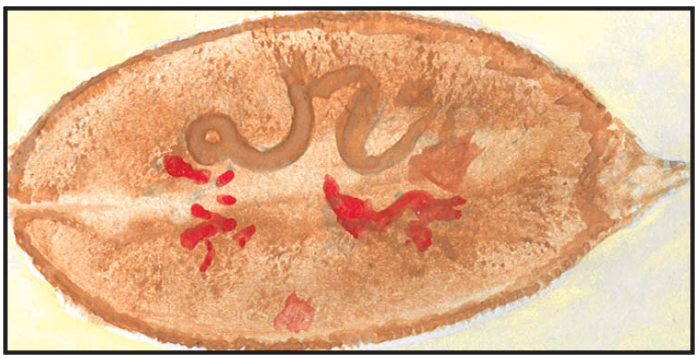


FIGURE 9.11: *P. westermani* morphology

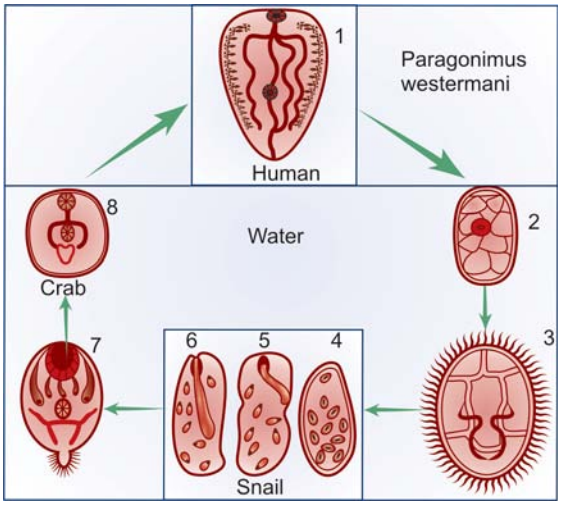


FIGURE 9.12: Life cycle of *Paragonimus westermani*. 1. Adult in human or animal lung. 2. Egg shed in sputum or stools reaches water, infects the first intermediate host. 3. Snail in which it develops into 4. Sporocyst. 5. Redia. first generation. 6. Redia. second generation, which releases 7. Cercaria with short slumpy tail. It enters the second intermediate host, crab or other crustaceans, in which it encysts to become 8. Metacercaria, which is infective for definitive hosts by ingestion

communication with a bronchus. Inflammatory reaction to the worms and their eggs lead to peribronchial granulomatous lesions, cystic dilatation of the bronchi, abscesses and pneumonitis. Patients present with cough, chest pain and haemoptysis. The viscous sputum is speckled with the golden brown eggs. Occasionally, the haemoptysis may be profuse. Chronic cases may resemble pulmonary tuberculosis.

Paragonimiasis may also be extrapulmonary, the clinical features varying with the site affected. In the abdominal type there may be abdominal pain and diarrhoea. The cerebral type resembles cysticercosis and may cause Jacksonian epilepsy. Glandular involvement causes fever and multiple abscesses.

Diagnosis

Demonstration of the eggs in sputum or faeces provides definitive evidence. Complement fixation test is positive only during and shortly after active infection, while the intradermal test remains positive for much longer periods.

Treatment

Bithionol, praziquantel and niclofolan are effective in treatment.

Prophylaxis

Adequate cooking of crabs and crayfish and washing the hands after preparing them for food can prevent human infections.

Many other species of Paragonimus which normally live in animals can, on occasion, infect man.

CHAPTER 10

Cestodes: Tapeworms

Cestodes (Greek *Kestos*—girdle or ribbon) are segmented tape-like worms whose sizes vary from a few millimetres to several metres. The adult worm consists of three parts—the head, neck and trunk. The head (*scolex*) carries grooved or cup-like *suckers*, which are the organs of attachment to the intestinal mucosa of the definitive host, human or animal. The neck, immediately behind the head is the region of growth, where the segments of the body are being continuously generated. The trunk (called *strobila*) is composed of a chain of *proglottides* or *segments*. The proglottides near the neck are the young immature segments, behind them are the mature segments and at the hind end are the gravid segments (Fig. 10.1).

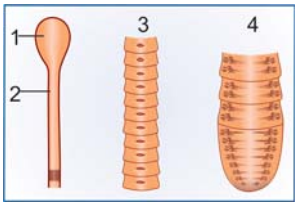


FIGURE 10.1: Tapeworm: 1. Scolex or head. 2. Neck, leading to the region of growth below, showing immature segments. 3. Mature segments 4. Gravid segments filled with eggs

TAPEWORMS: GENERAL CHARACTERS

Tapeworms do not have a body cavity or alimentary canal. Rudimentary excretory and nervous systems are present. The reproductive system is well-developed and the proglottides are practically filled with reproductive organs. Tapeworms are hermaphrodites (monoecious) and every mature segment contains both male and female sex organs. In the immature segments the reproductive organs are not well-developed. They are well-differentiated in the mature segments. The gravid segments are completely occupied by the uterus filled with eggs.

The embryo inside the egg is called the *oncosphere* (meaning 'hooked ball') because it is spherical and has *hooklets*. Oncospheres of human tapeworms typically have 3 pairs of hooklets and so are called *hexacanth* (meaning six-hooked) embryos.

Humans are the definitive host for most tapeworms which cause human infection. An important exception is the dog tapeworm *Echinococcus granulosus* for which dog is the definitive host and man the intermediate host. For the pork tapeworm *Taenia solium* man is ordinarily the definitive host, but its larval stages also can develop in the human body.

Clinical disease can be caused by the adult worm or the larval form. In general, adult worm causes only minimal disturbance, while the larvae can produce serious illness, particularly when they lodge in critical areas like the brain or the eyes.

Tapeworms that infect man belong to two orders—*Pseudophyllidea* and *Cyclophyllidea*, the former bearing slit-like grooves (*bothria*) and the latter cup-like suckers (*acetabula*) on their scolices. Pseudophyllidean tapeworms have an unbranched convoluted uterus which opens through a pore, possess ventrally situated genital pores, and produce operculated eggs that give rise to ciliated larvae. In Cyclophyllidean tapeworms the uterus is branched and does not have an opening. They have lateral genital pores, and produce non-operculated eggs that yield larvae which are not ciliated. Their larvae are called '*bladder worms*' and occur in four varieties, cysticercus, cysticercoid, coenurus and echinococcus.

Medically important tapeworms are classified into the following:

A. Pseudophyllidean tapeworms

1. *Diphyllobothrium latum*, the fish tapeworm
   Adult worm in human intestine
2. *Sparganum mansoni, S. proliferum*
   Larval stages in tissues, causing Sparganosis.

B. Cyclophyllidean tapeworms

1. Genus *Taenia*
   a. *T. saginata*, the beef tapeworm.
      Adult worm in human intestine
   b. *T. solium*, the pork tapeworm.
      Adult worm in human intestine.
      Larval form also can cause disease in man (cysticercus cellulosae)
2. Genus *Echinococcus*
   a. *E. granulosus* the dog tapeworm.
      Larval form causes hydatid disease in man.
   b. *E. multilocularis* Larval stage causes alveolar or multilocular hydatid disease.
3. Genus *Hymenolepis*
   a. *H. nana*, the dwarf tapeworm.
      Adult and larval stages in human intestine.
   b. *H. diminuta*, the rat tapeworm.
      Adult worm rarely in human intestine.
4. Genus *Dipylidium*
   D. caninum, the double-pored dog tapeworm. Adult rarely in human intestine.
5. Genus *Multiceps*
   M. multiceps and other species. Larval stage may cause coenurosis in man.

PSEUDOPHYLLIDEAN TAPEWORMS

DIPHYLLOBOTHRIUM LATUM

History and Distribution

This pseudophyllidean tapeworm, formerly called *Dibothriocephalus latus* is commonly known as the *fish tapeworm* or the *broad tapeworm* (Greek diphyllobothrium-having

two leaf-like grooves; dibothriocephalus—having two grooves in the head; latus—broad). Infection with this tapeworm is called *diphyllobothriasis*. The head of the worm was found by Bonnet as early as 1777 but it was only in 1917 that its life cycle was worked out by Janicki and Rosen. Diphyllobothriasis occurs in central and northern Europe, particularly in the Scandinavian countries. It is also found in Siberia, Japan, North America and Central Africa. It has not been reported from India.

Morphology and Life Cycle

Humans are the optimal definitive host, though dogs, cats and their wild relatives may also act as definitive hosts. The adult worm is found in the small intestine, usually in the ileum, where it lies folded in several loops, in contact with the mucosa. It is ivory-coloured and very long, measuring upto 10 metres or more. The scolex (head) is *spatulate* or spoon-shaped, about 2 to 3 mm long and 1 mm broad. It carries two slit-like longitudinal sucking grooves (bothria), one dorsal and the other ventral. Immediately behind the scolex is the thin unsegmented neck region, several times longer than the head. The proglottides (commonly, though inaccurately called segments) extend from the neck posteriorly, the youngest being next to the neck and the oldest hindmost. The strobila may have 3000 or more proglottides, consisting of immature, mature and gravid segments in that order from the front backwards.

The mature proglottid is broader than long, about 2 to 4 mm long and 10 to 20 mm broad and is practically filled with male and female reproductive organs. The testes are represented by numerous minute follicles situated laterally in the dorsal plane. The female reproductive organs are arranged along the midline, lying ventrally. The ovary is bilobed. The large uterus lies convoluted in the centre. Three genital openings are present ventrally along the midline—the openings of the vas deferens, vagina and uterus in that order, from front backwards. The fertilized ova develop in the uterus and are discharged periodically through the uterine pore. *D. latum* is a prolific egg layer and a single worm may pass about a million eggs a day. The terminal segments become dried up after delivering many eggs and are discharged in strands of varying length (Fig. 10.2).

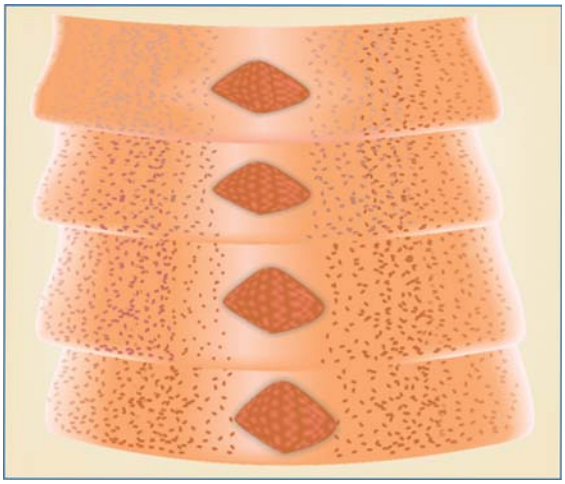


FIGURE 10.2: *D. latum* proglottide

The eggs are passed in faeces in large numbers. They are broadly ovoid, about 65 µm by 45 µm, with a thick, light brown shell. It has an operculum at one end and often a small knob at the other. The eggs do not float in saturated salt solution. They are not infective to humans (Fig. 10.3).

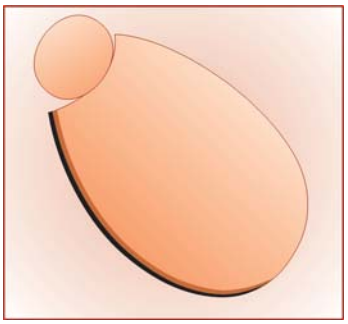


FIGURE 10.3: *D. latum* egg

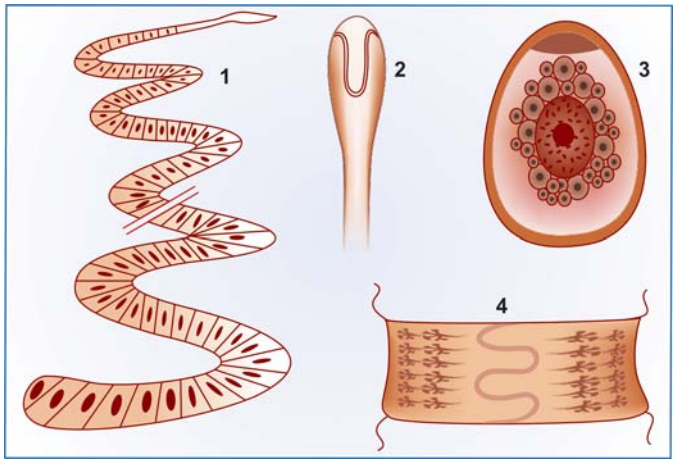


FIGURE 10.4: *Diphyllobothrium latum*. Morphology of body parts. 1. Adult worm showing spatulate scolex, neck and strobila. 2. Scolex showing slit-like sucking grooves. 3. Operculated egg. 4. Mature proglottid showing male and female reproductive structures

The freshly passed egg contains an immature embryo surrounded by yolk granules. The eggs are resistant to chemicals but are killed by drying. The embryo with six hooklets (hexacanth embryo) inside the egg is called the oncosphere. In water it matures in about 10 to 15 days and emerges through the operculum as the ciliated first stage larva, called *coracidium*, which swims about. It can survive in water for about 12 hours, by which time it should be ingested by the fresh water copepod *cyclops*, which is the first intermediate host. In the midgut of the cyclops, the coracidium casts off its ciliated coat and by means of its six hooklets, penetrates into the haemocoele (body cavity). In about 3 weeks, it becomes transformed into the elongated second stage larva about 550 µm long, which is called the *procercoid* larva. It has a rounded caudal appendage (*cercomer*) which bears the now useless hooklets. If the infected cyclops is now devoured by a freshwater fish (which is the second intermediate host), the procercoid larva penetrates the intestine of the fish and grows. It looses its caudal appendage and develops into the third stage larva called the

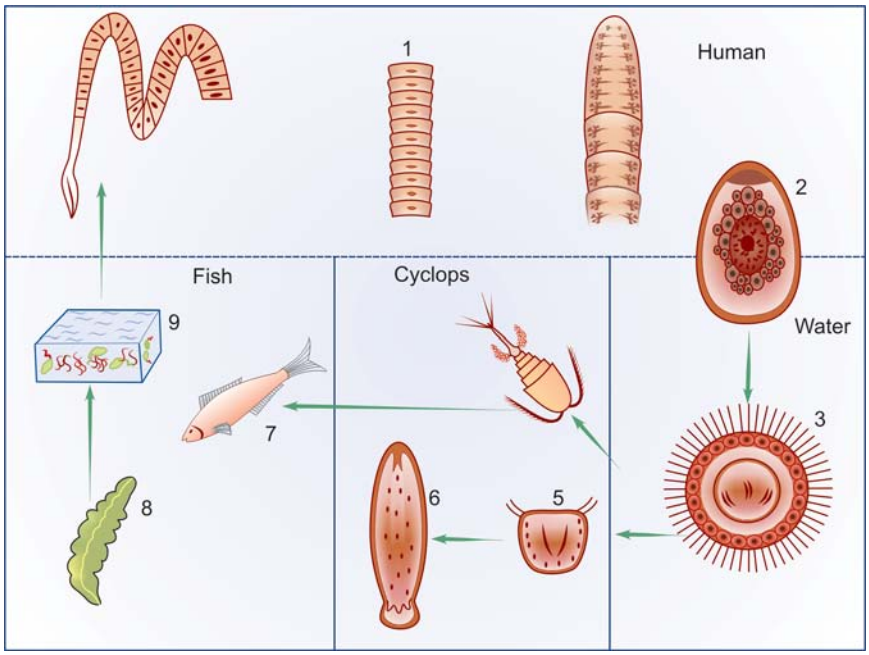


FIGURE 10.5: Life cycle of *Diphyllobothrium latum*. 1. Adult worm in human small intestine. 2. Operculated egg passed in stools reaches water. 3. Ciliated embryo coracidum develops in egg and escapes out into water to be ingested by. 4. Cyclops, the first intermediate host. 5. The hexacanth embryo sheds its cilia and the oncosphere penetrates the gut wall of cyclops to develop into the elongated, 6 Procercoid larva. 7. The cyclops containing procercoid larva is ingested by the second intermediate host, fish in which, 8. The plerocercoid larva develops. 9. When fish flesh containing the plerocercoid larva is eaten, humans become infected

plerocercoid larva or *sparganum*. This is a glistening white flattened unsegmented vermicule, with a wrinkled surface, about 1 to 2 cm long and with a rudimentary scolex. This is the infective stage for humans. When fish containing plerocercoid larva is eaten uncooked or undercooked, the larva develops into the adult worm in the small intestine. The worm attains maturity in about 5 to 6 weeks and starts laying eggs. The worm may live for about 10 years or more (Fig. 10.5).

Pathogenicity

The pathogenic effects of diphyllobothriasis depend on the mass of the worm, absorption of its byproducts by the host and deprivation of the host's essential metabolic intermediates. In some persons, infection may be entirely asymptomatic, while in others there may be evidence of mechanical obstruction. Patients may be frightened by noticing the strands of proglottides passed in their faeces. Abdominal discomfort, diarrhoea, nausea and anaemia are the usual manifestations. A kind of pernicious anaemia sometimes caused by the infection is called bothriocephalus anaemia. This is believed to be racially determined, being common in Finland and rare elsewhere.

Epidemiology

The prevalence of the disease depends on the presence of infected human or animal definitive hosts, suitable intermediate hosts and the extent of faecal pollution of natural fresh waters by the definitive hosts. Though dogs, cats, foxes, jackals, mongoose, pigs and many wild animals may be naturally infected, human cases are primarily responsible for the propagation of the infection. Human cases depend on traditional food habits. Where uncooked, undercooked or inadequately processed fish or fish products are eaten, infection is likely to be present. In countries like India, where fish is eaten only after cooking, the infection does not occur.

Diagnosis

Eggs are passed in very large number in faeces, and therefore their demonstration offers an easy method of diagnosis. The proglottides passed in faeces can be identified by their morphology.

Treatment

Praziquantel in a single dose of 10 mg/kg is effective. Niclosamide has also been used.

Prophylaxis

Infection can be prevented by proper cooking of fish, prevention of fecal pollution of natural waters and periodical deworming of pet dogs and cats.

SPARGANOSIS

The term sparganosis is used for ectopic infection by sparganum (plerocercoid larva) of miscellaneous pseudophyllidean tapeworms, found in abnormal hosts. Human sparganosis may result from ingestion of cyclops containing procercoid larva, ingestion of plerocercoid larva present in uncooked meat of animals or birds, or local application of raw flesh of infected animals on skin or mucosa. The last method follows the practice prevalent among the Chinese, of applying split frogs on skin or eye sores.

In most cases, the species of tapeworm cannot be identified. The two species often recognised have been *Spirometra mansoni* and *S. proliferum*. The sparganum is usually found in the subcutaneous tissues in various parts of the body, but may also present in the peritoneum, abdominal viscera or brain. Diagnosis is usually possible only after surgical removal of the worm.

Sparganosis has been reported mostly from Japan and South East Asia, less often from America and Australia. A few cases have been reported from India also.

CYCLOPHYLLIDEAN TAPEWORMS

TAENIA SAGINATA

History and Distribution

Commonly called the *beef tapeworm, Taenia saginata* has been known as an intestinal parasite of man from very ancient times. But it was only in 1782 the Goeze differentiated it from the pork tapeworm *T. solium*. Its life cycle was elucidated when Leuckart in 1861 first experimentally demonstrated that cattle serve as the intermediate host for the worm.

The name *taenia* is derived from the Greek word meaning tape or band. It was originally used to refer to most tapeworms, but is now restricted to the members of the Genus *Taenia*.

T. saginata is worldwide in distribution, but the infection is not found in vegetarians and those who do not eat beef.

Morphology and Life Cycle

The adult worm lives in the human small intestine, commonly in the jejunum with its head embedded in the mucosa. The worm is an opalescent white in colour. It is usually about 5 metres in length, but may on occasion be much longer, about 25 metres or more, thus being the largest helminth causing human infection.

The scolex (head) is about 1-2 mm in diameter, quadrate in cross section, bearing 4 hemispherical suckers situated at its four angles. They may be pigmented. The scolex has no rostellum or hooklets (which are present in *T. solium*). *T. saginata* is therefore called the unarmed tapeworm. The suckers serve as the sole organs for attachment.

The neck is long and narrow. The strobila (trunk) consists of 1000 to 2000 proglottides or segments—immature, mature and gravid in that order from front backwards.

The gravid segments are nearly four times as long as they are broad, about 20 mm long and 5 mm broad. The segment contains male and female reproductive structures. The testes are numerous, 300 to 400 (twice as many as in *T. solium*. The gravid segment has 15 to 30 lateral branches (as against 7 to 13 in *T. solium*). It differs from *T. solium* also in having a prominent vaginal sphincter and in lacking the accessory ovarian lobe. The common genital pore opens on the lateral wall of the segments (Fig. 10.6A).

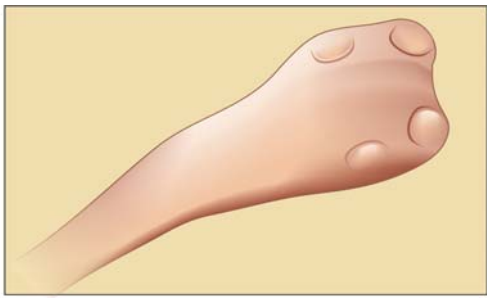


FIGURE 10.6A: Scolex of *T. saginata* with 4 suckers and no hook

The gravid segments break away and are expelled singly, actively forcing their way out through the anal sphincter. As there is no uterine opening, the eggs escape from the uterus through its ruptured wall. The eggs cannot be differentiated from those

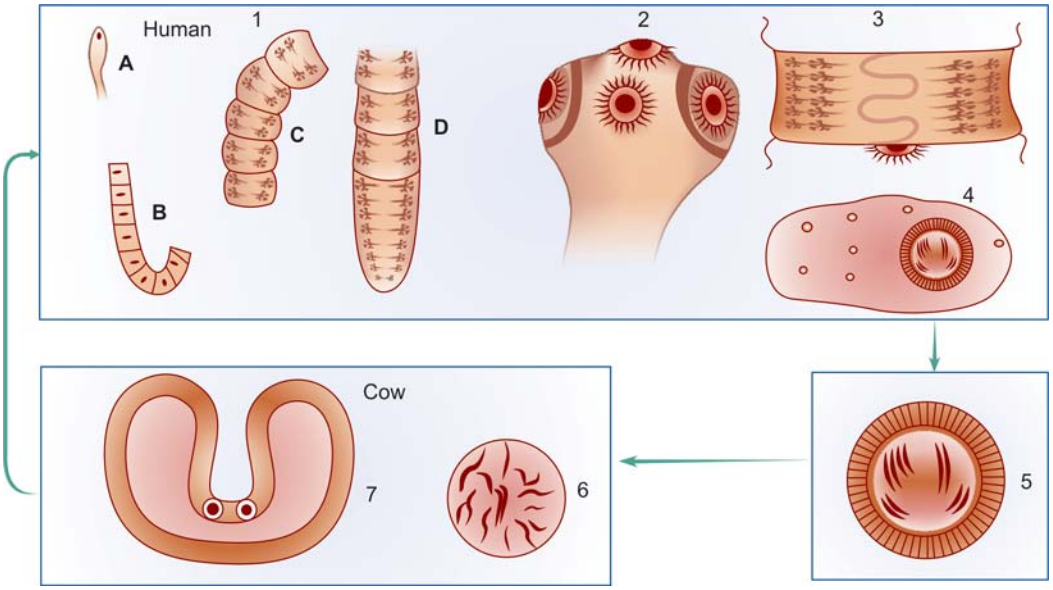


FIGURE 10.6B: Life cycle of *Taenia saginata*. 1. Adult worm in human small intestine. A. Scolex and neck. B. Immature segments. C. Mature segments, showing genital pore opening laterally irregularly alternating between right and left. D. Gravid segments. 2. Scolex bearing four suckers. No rostellum or hooks. 3. Mature segment much longer than broad. Uterus has several branches (15-30). 4. Immature egg with hyaline embryonic membrane around it. 5. Mature egg deposited in soil, ingested by cattle. 6. Oncosphere penetrates intestinal wall. 7. Cysticercus bovis develops in muscle—measly beef—the infective stage for man

of other species of Taenia, Multiceps or Echinococcus. The spherical eggs measure 30 to 40 µm in diameter. When freshly released from the proglottid, the egg has a thin hyaline embryonic membrane around it, which soon disappears. The thick outer wall is radially striated and is brown due to bile staining. In the centre is a fully developed embryo with three pairs of hooklets (hexacanth embryo). The eggs do not float in saturated salt solution. *T. saginata* is a prolific egg producer, with a daily output of about 50,000 eggs for 10 years or more.

The eggs deposited in soil remain viable for several weeks. They are infective to cattle which ingest the eggs while grazing. When ingested by cattle (cows or buffaloes), the egg-shell ruptures and the oncosphere hatches out in the duodenum. The oncospheres, with their hooklets penetrate the intestinal wall, reach the mesenteric venules or lymphatics and enter the systemic circulation. They get filtered out in the striated muscles, particularly in the muscles of the tongue, neck, shoulder, ham and in the myocardium. In these sites, the oncospheres lose their hooks and in about 60 to 70 days develop into the mature larva, the *bladder worm* or *cysticercus bovis*. (The name cysticercus is derived from the Greek *kystis*—bladder and *kerkos*—tail). The cysticercus is an ovoid, milky white opalescent fluid-filled vesicle measuring about 5 mm by 10 mm and contains the invaginated unarmed scolex. The cysticerci can be seen on visual inspection as shiny-white dots in the infected beef (measly beef) (Fig. 10.7).

When such infected beef is eaten raw or inadequately cooked, the cysticerci are digested out of the meat in the stomach. In the upper part of the small intestine, the head evaginates out of the cysticercus, becomes attached to the mucosa and by gradual strobilisation develops into the adult worm in about 2 to 3 months. The adult worm has a lifespan of 10 years or more. Infection is usually with a single worm, but sometimes multiple infection is seen and 25 or more worms have been reported in some patients.

Pathogenesis

The adult worm, in spite of its large size causes surprisingly little inconvenience to the patient. It may lead to vague abdominal discomfort, indigestion and diarrhoea. Occasional cases of acute intestinal obstruction and acute appendicitis have been reported. The proglottides crawling out the anus, particularly during the day time may cause alarm or embarrassment.

The larva of *T. saginata* (*cysticercus bovis*) is not found in humans.

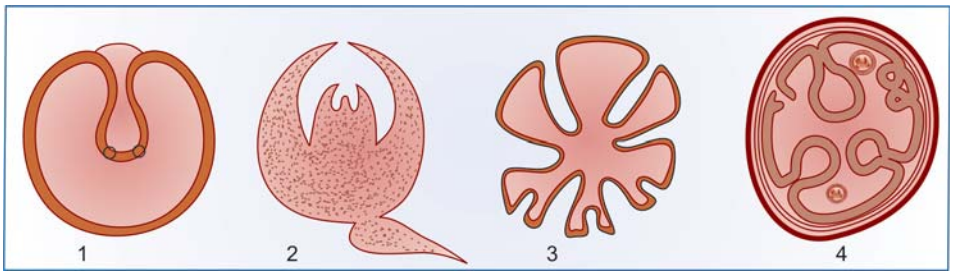


Fig. 10.7: Larvae of cyclophyllidean tapeworms. 1 Cysticercus, a typical bladder with the invaginated protoscolex. e.g. *T. solium*. 2. Cysticercoid, a fleshy larva with the head withdrawn and surrounded by a double fold of integument. e.g. *H. nana*. 3. Coenurus larva with multiple invaginated protoscolices, e.g. *M. multiceps*. 4. Echinococcus. Hydatid cyst with internal budding producing brood capsules with multiple scolices. e.g. *E. granulosus*

Epidemiology

Human infection follows consumption of raw and undercooked beef and so is related to local eating habits. The formerly popular practice of prescribing raw or rare beef or beef juice for debilitated persons had been responsible for many infections in the West.

Diagnosis

The diagnosis is often made by the patient who feels the proglottides crawling down the anus unexpectedly or notices them in stools. Microscopic examination of faeces shows the eggs. Salt floatation is not suitable for concentrating eggs in faeces; formol-ether sedimentation method is useful. Species identification cannot be made from the eggs. This can be done by examining with a hand lens, the gravid proglottid pressed between two slides, when uterine branching can be made out (15 to 20 lateral branches in *T. saginata*; under 13 in *T. solium*).

Treatment

Niclosamide and praziquantel are effective. Purgation is not considered necessary.

Prophylaxis

Beef should be subjected to effective inspection for cysticerci and should be eaten only after proper cooking. The critical thermal point for cysticerci is 56°C.

Other preventive measures consist of prevention of faecal pollution of soil and proper disposal of sewage.

TAENIA SOLIUM

History and Distribution

Commonly called the *pork tapeworm*, this has been known from the time of Hippocrates. However, it was differentiated from the beef tapeworm only by Kuchenmeister (1855) and Leuckart (1956) who worked out its life cycle and demonstrated the larval stage in the pig. Kuchenmeister fed a condemned prisoner with 20 cysticercus cellulosae from a pig and when the criminal was executed four months later, 19 adult *T. solium* were recovered from his intestines.

Various derivations have been proposed for the name 'solium'—from the Latin solus meaning solitary because usually only a single worm is found in infected persons, or sol meaning sun from a fancied resemblance of the rostellum with hooks to the sun and its rays, and from a Syrian word meaning a 'chain'.

T. solium is worldwide in distribution, except in the countries and communities which proscribe pork as taboo.

Morphology and Life Cycle

The adult worm lives in the human intestine, usually in the jejunum, where it lies in several folds in the lumen. Commonly only a single worm is present, but rarely several worms may be seen, upto 25 or more in a patient.

The adult worm is usually 2 to 3 metres long. The scolex is roughly quadrate about 1 mm in diameter, with 4 large cup-like suckers (0.5 mm in diameter) and a conspicuous rounded rostellum, armed with a double row of alternating round and small dagger-shaped hooks, 20 to 50 in number. The neck is short and half as thick as the head.

The proglottides number less than a thousand. They resemble those of *T. saginata* in general. The gravid segments are twice as long as broad, 12 mm by 6 mm. The testes are composed of 150 to 200 follicles. There is an accessory lobe for the ovary. The vaginal sphincter is absent. The uterus has only 5 to 10 (under 13) thick lateral branches. A lateral thick-lipped genital pore is present, alternating between the right and left sides of adjacent segments.

The gravid segments are not expelled singly, but pass passively out as short chains. The eggs escape from the ruptured wall of the uterus. The eggs are indistinguishable

from those of *T. saginata*. They remain infective for several weeks in soil. They can infect pigs as well as humans (Fig. 10.8).

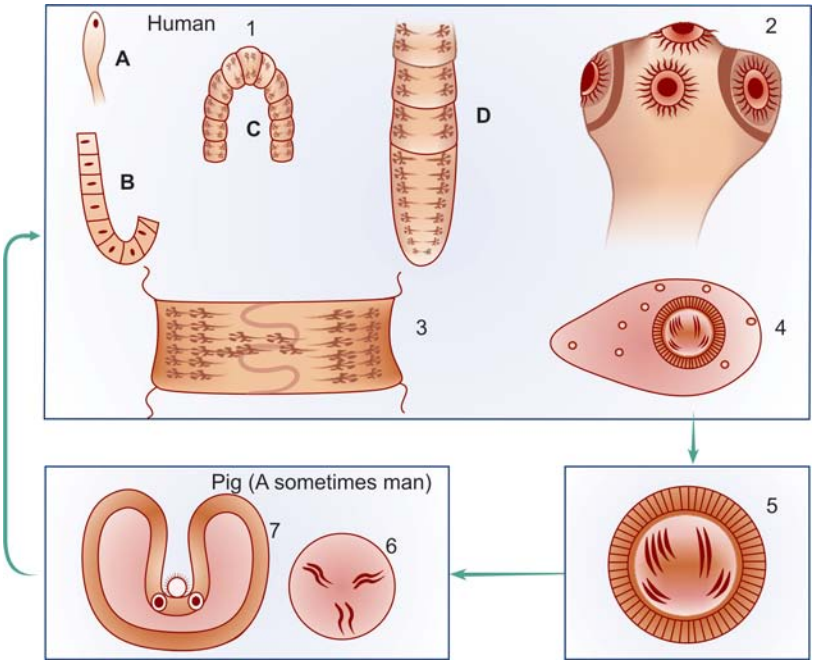


FIGURE 10.8: Life cycle of *Taenia solium*. 1. Adult worm in human small intestine. A. Scolex and neck. B. Immature segments. C. Mature segments, showing genital pore opening laterally alternating between right and left . D. Gravid segments. 2. Scolex bearing four suckers and a rostellum with a double row of hooks (Fig. 10.9) 3. Mature segment longer than broad. Uterus has few branches. (5-10). 4. Immature egg with hyaline embryonic membrane around it. 5. Mature egg deposited in soil, ingested by pig, or occasionally by man. 6. Oncosphere penetrates intestinal wall. 7. Cysticercus cellulosa develops in muscle (measly pork), the infective stage for humans

When the eggs are ingested by pig or humans, the embryos are released in the duodenum or jejunum. The oncospheres penetrate the intestinal wall, enter the mesenteric venules or lymphatics and are carried in systemic circulation to the different parts of the body. They are filtered out principally in the muscles where they develop into the larval stage, *cysticercus cellulosae* in about 60 to 70 days.

The cysticercus cellulosae or 'bladder worm' is an ovoid opalescent milky-white bladder or vesicle surrounded by a fibrous capsule. It contains a thick fluid, rich in protein and salt. The scolex of the larva, with its suckers, lies invaginated within the bladder and can be seen as a thick white spot. It remains viable for several months. The cysticercus measures usually about 5 mm by 10 mm, but can be much larger when it occurs in the brain or subarachnoid space.

Cysticercus cellulosae can develop in humans or pigs. In humans it is a dead end and the larvae die without further development. When pork containing cysticercus cellulosae (measly pork) is consumed inadequately cooked, the larvae are digested

FIGURE 10.9: *T. solium* hooks

out of the meat in the stomach and duodenum. The head evaginates out of the bladder and becomes attached to the jejunal mucosa. In 5-12 weeks it develops into a mature worm. *T. solium* has a long lifespan of about 25 years or more.

Pathogenesis

The adult worms do not cause any disturbance apart from vague abdominal discomfort, indigestion or alternating diarrhoea and constipation. It is the larval stage that can cause serious trouble.

Cysticercus cellulosae develop in humans following ingestion of *T. solium* eggs in water or vegetables. In persons harbouring the adult worm in the intestine, autoinfection and infection of close contacts can take place by finger contamination with eggs from the perineal skin or faeces. Autoinfection can also occur by the gravid segments reaching the stomach by retrograde peristalsis from the jejunum, whereupon they are digested and thousands of eggs released.

Cysticercus cellulosae may be solitary or more often multiple, commonly numerous. Any organ or tissue may be involved, the most common being subcutaneous tissues and muscles. It may also affect the eyes, brain, and less often the heart, liver, lungs, abdominal cavity and spinal cord. The symptomatology depends on the site affected.

The cysticercus is surrounded by a fibrous capsule except in the eye and ventricles on the brain. The larvae evoke a cellular reaction starting with infiltration of neutrophils, eosinophils, lymphocytes, plasma cells and at times giant cells. This is followed by fibrosis and death of the larva with eventual calcification.

In cysticercosis of the brain, symptoms are more often due to the dead and calcified larvae than the living larvae. Epilepsy is the most common manifestation, but it can also cause behavioural disorders, pareses or hydrocephalus. Ocular cysticercosis may cause blurring of vision, uveitis, iritis and ultimately blindness.

Epidemiology

Intestinal infection with *T. solium* occurs only in persons eating undercooked pork and so is related to food habits. It is therefore absent in those with religious or

other reservations against eating pork. But cysticercosis may occur in any person residing in endemic areas, even in vegetarians because the mode of infection is contamination of food or drink with eggs deposited in soil.

Diagnosis

Infection with the adult worm is diagnosed by demonstration of eggs, or more specifically of proglottides in faeces. It can be differentiated from *T. saginata* on the characteristics of the proglottides.

Definitive diagnosis of cysticercosis is by biopsy of the lesion and its microscopic examination to show the invaginated scolex with suckers and hooks. Cysticercosis in the subcutaneous tissue and muscles, particularly in the buttocks and thighs can be made out by radiological demonstration of the calcified larvae. Radiography is helpful for diagnosis of cerebral cysticercosis also, but CT scan is much more useful. Ocular cysticercosis can be made out by ophthalmoscopy.

Eosinophilia usually occurs during the early stage of cysticercosis, but is not constant. An indirect haemagglutination test has been repored using an antigen obtained from cysticercus from pigs.

Treatment

Praziquantel and niclosamide are useful in treatment of infection with the adult worm. For cysticercosis excision is the best method wherever possible. Praziquantel and metrifonate have been reported to be effective in cysticercosis.

Prevention

Proper meat inspection in slaughter houses to eliminate measly pork, adequate cooking of pork, clean personal habits and general sanitary measures can prevent the infection. For control of cysticercosis, prevention of faecal contamination of soil, proper disposal of sewage and avoiding raw vegetables grown in polluted soil are useful measures. It is important to detect and treat persons harbouring adult worms as they can develop cysticercosis due to autoinfection.

ECHINOCOCCUS GRANULOSUS

History and Distribution

Tapeworms belonging to the Genus *Echinococcus* have, as their definitive host a carnivorous predator that preys on the intermediate host which is usually a herbivorous mammal. The domesticated example of this is *Echinococcus granulosus*, the dog tapeworm or the hydatid worm (formerly called *Taenia echinococcus*), which has the dog as the definitive host and sheep and humans as the principal intermediate hosts. In humans it causes unilocular echinococcosis or *hydatid disease*.

Hydatid cysts had been described by Hippocrates and other ancient physicians. It was only in 1782 that Goeze recognised their relationship to tapeworms by studying their scolices.

The disease is prevalent in most parts of the world, though it is most extensive in the sheep and cattle-raising areas in Australasia, parts of Africa and South America. It is also common in Europe, China and the Middle East. It occurs in many parts of India. It is seen more often in temperate than in tropical regions.

Morphology and Life Cycle

The dog is the principal definitive host. The adult worm lives in the jejunum and duodenum of dogs and other canine carnivora, with its scolex buried in the mucosa, between the villi. Enormous numbers of them may be seen in infected dogs.

It is a small tapeworm, measuring only 3-6 mm in length. It consists of a scolex, a short neck and the trunk composed of only 3 proglottides, the anterior immature, the middle mature and the posterior gravid.

The scolex is pyriform, with 4 suckers and a prominent rostellum bearing two circular rows of hooklets. The terminal proglottid is longer and wider than the rest of the worm and contains the branched uterus filled with eggs.

The eggs are indistinguishable from those of *Taenia* species. They are passed in dog faeces. Sheep and cattle ingest them while grazing. The egg-shell disintegrates in the duodenum setting free the hexacanth embryos which penetrate the intestinal wall and enter the portal venules, to be carried to the liver along the portal circulation. The liver acts as the first filter for the embryos which get arrested in the sinusoidal capillaries. Of the embryos that escape, many get filtered out in the pulmonary capillaries, so that the lung acts as the second filter. A few enter the systemic circulation and get lodged in various organs and tissues such as the spleen, kidneys, eye, brain or bones (Fig. 10.10).

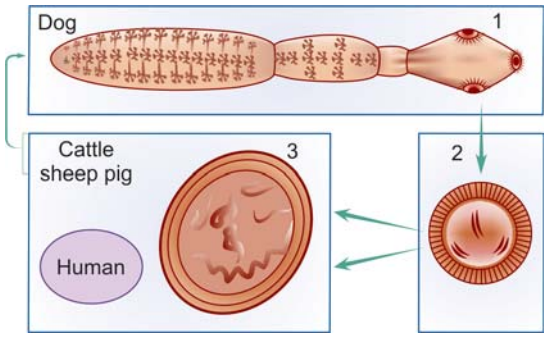


FIGURE 10.10: Life cycle of *Echinococcus granulosus*. 1. Adult worm in intestine of dogs. It consists of a pyriform scolex with four suckers and rostellum bearing hooklets and three proglottides—immature, mature and gravid, 2. Egg deposited in soil. 3. When ingested by animals (or humans) hexacanth embro penetrating intestine, settles in liver, lung, or other sites to form hydatid cystid containing protoscolices which are infective to dogs, hydatid cyst in humans is a blind end

At the site of deposition the embryo develops into a bladder or cyst filled with fluid. This becomes the hydatid cyst (Greek hydatis—a drop of water). It enlarges slowly and reaches a diameter of 0.5 to 1 cm in about 6 months. The growing cyst evokes host tissue reaction leading to the deposition of a fibrous capsule around it. The cyst has a thick opaque white outer *cuticle* or *laminated layer*, and a thin inner *germinal layer* containing nucleaed cells. The germinal layer is the site of asexual reproduction. It also secretes the hydatid fluid which fills the cyst. The fluid is clear, colourless or pale yellow, with a pH of about 6.7, containing salts and protein. It

is a good antigen which sensitises the host. The fluid was used as the antigen for Casoni's intradermal test and other diagnostic serological tests (Fig. 10.11).

From the germinal layer, small knob-like excrescences or *gemmules* protrude into the lumen of the cyst. These enlarge, become vacuolated and filled with fluid. These are called *brood capsules*. They are initially attached to the germinal layer by a stalk, but later escape free into the fluid filled cyst cavity. From the inner wall of the brood capsule, *protoscolices* develop, which represent the head of the potential adult worm, complete with invaginated scolex, bearing suckers and hooklets. Each of these is a potential tapeworm. Several thousands of protoscolices develop in a mature hydatid cyst, so that this represents an asexual reproduction of great magnitude. Many of the scolices float free in the cyst fluid. These, together with the free brood capsules are called the *hydatid sand*.

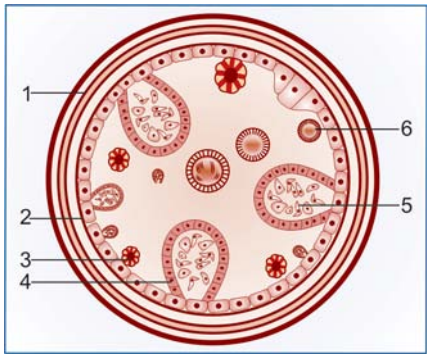


FIGURE 10.11: Hydatid cyst. 1. Outer laminated layer 2. Germinal layer 3. Gemmule 4. Brood capsule 5. Protoscolex 6. Sterile daughter cyst

Inside mature hydatid cysts, further generations of cysts may develop—daughter cysts and granddaughter cysts. The cyst grows slowly, often taking 20 years or more to become big enough to cause clinical illness. Unilocular cysts are usually less than 5 cm in diameter, but occasionally may grow to 20 cm or more in size, with about 2 litres of fluid inside. *E. granulosus* typically forms unilocular hydatid cysts, but may rarely produce multilocular cysts. Sometimes the scolices may escape from the cyst and get transported to other parts of the body, where they may initiate secondary hydatid cysts. Some cysts are sterile and may never produce brood capsules, while some brood capsules may not produce scolices. These are called *acephalocysts*.

When hydatid cysts form inside bones, because of the confinement by dense osseous tissue, the laminated layer is not well-developed. The parasite migrates along the bony canals as naked excrescences that erode the bone tissue. This is called the *osseous hydatid*. When sheep or cattle harbouring hydatid cysts die or are slaughtered, dogs may feed on the carcass or offal. Inside the intestine of dogs, the scolices develop into the adult worms that mature in about 6 to 7 weeks and produce eggs to repeat the life cycle. The adult worm lives from 6 to 30 months.

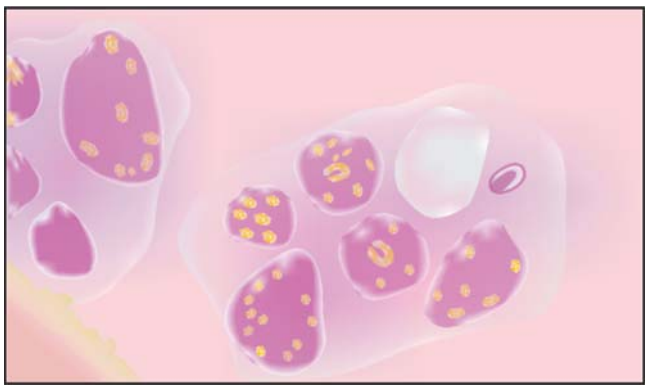


FIGURE 10.12: E. granulosus brood capsules in cross section

The above is the natural cycle of the parasite. When infection occurs in humans, the cycle comes to a dead end, because the human hydatid cysts are unlikely to be eaten by dogs.

Pathogenesis

Human infection follows ingestion of the eggs passed by infected dogs. This may occur by eating raw vegetables or other food items contaminated with dog faeces. Fingers contaminated with the eggs while fondling pet dogs may carry them to the mouth. Kissing pet dogs may cause the eggs to be transferred directly to the mouth.

Infection is often acquired during childhood when intimate contact with pet dogs is more likely. But the clinical disease develops only several years later, when the hydatid cyst has grown big enough to cause obstructive symptoms. Disease results mainly from pressure effects caused by the enlarging cysts.

In about half the cases the primary hydatid occurs in the liver, mostly in the right lobe. Hepatomegaly, pain and obstructive jaundice are the usual manifestations. The next common site is the lung, usually in the lower lobe of the right lung. Cough, haemoptysis, chest pain and dyspnoea constitute the clinical picture. In the kidney, hydatid cyst causes pain and haematuria. Other sites affected include the spleen, brain, orbit and bones. Erosion of bone may lead to pathological fractures.

A second pathogenic mechanism in hydatid disease is hypersensitivity to the echinococcal antigen. The host is sensitised to the antigen by minute amounts of hydatid fluid seeping out through the capsule. Hypersensitivity may cause urticaria. But if a hydatid cyst ruptures spontaneously or during surgical interference, massive release of hydatid fluid may cause severe, even fatal anaphylaxis.

Epidemiology

Human hydatid disease is only a tangenital accident in the natural cycle of the hydatid worm. The natural intermediate reservoir hosts are sheep, cattle, pigs and a large variety of herbivores, from elks to elephants. The dog is the usual definitive host,

although several wild canines have been found infected in nature. The definitive hosts are predators and the intermediate hosts the preys, except for humans who constitute a blind alley in the cycle of transmission.

Diagnosis

Radiological examinations and other imaging techniques such as ultrasonography and CT scan reveal the diagnosis in most cases. Blood eosinophilia is often present, but is not constant or diagnostic. Exploratory puncture of the cyst yields hydatid fluid and demonstration of scolices in the hydatid sand provides conclusive diagnosis. But this procedure is risky and not recommended as it may cause escape of hydatid fluid and consequent anaphylaxis.

Immunological methods employed include the Casoni's intradermal test and serological tests. The Casoni's test is an immediate hypersensitivity test originally introduced by Casoni in 1911. The antigen is hydatid fluid collected from animal or human cysts and sterilised by Seitz or membrane filtration and is injected (0.2 ml) intradermally on one arm and an equal volume of saline as control on the other arm. In positive cases a large wheal, about 5 cm in diameter, with multiple pseudopodial projections appears within 20 to 30 minutes at the test site and fades in an hour. A secondary reaction consisting of oedema and induration appears after 8 hours. The test is very sensitive, but not specific and false-positive reactions may appear in a number of other conditions. Casoni's test is little used now and has been supplanted by serological tests.

An active cyst is associated with the presence of circulating antibodies, which increase in titre when there is a leak of hydatid fluid. High levels of antibodies are seen with cysts in the liver, though lung cysts may not cause a similar antibody response. Following surgical removal, suppuration or calcification of the cysts, antibody levels decline.

The serological tests used are CFT, IHA, latex agglutination, immunofluorescence, immunoelectrophoresis and ELISA. CFT is not very sensitive and false-positive reaction is seen in those receiving neural antirabic vaccine. CFT is useful after surgical removal of cysts, when a negative test has a better prognostic value. The slide latex agglutination test and IEP using hydatid fluid fraction 5 antigen are widely used. ELISA for demonstration of circulating hydatid antigen is also helpful in diagnosis. Specific molecular diagnostic methods have been developed involving DNA probes and polymerase chain reaction, but their application is limited by their technical complexity.

Treatment

Surgical removal offers the best mode of treatment where the cysts are accessible. But recurrence after surgery is common. Drug treatment has only limited application. Mebendazole, albendazole and praziquantel have been used.

Prophylaxis

Infection of dogs can be prevented by ensuring that they do not eat animal carcass or offal. Destruction of stray dogs has been found to be helpful. Periodical deworming of pet dogs is useful. It is essential to wash the hands after touching dogs. Kissing of pet dogs should be discouraged.

ECHINOCOCCUS MULTILOCULARS

This causes the rare but serious condition of *alveolar* or *multilocular hydatid* disease in humans. It is found in the northern parts of the world, from Siberia in the East to Canada in the West. The adult worm is smaller than *E. granulosus* and lives in the intestines of foxes, dogs and cats. Human infection develops from eating fruits or vegetables contaminated with their faeces. Rodents are the main intermediate hosts.

The liver is the organ most often affected. The multilocular infiltrating lesion appears like a grossly invasive growth that can be mistaken for a malignant tumour. It may also metastasize to the lungs and brain.

The prognosis is very grave. Surgical removal, when possible is the best method of treatment. Mebendazole has been reported to be of some value.

HYMENOLEPIS NANA

Commonly known as the *dwarf tapeworm*, *Hymenolepis nana* is the smallest and the most common tapeworm found in the human intestine. The name Hymenolepis refers to the thin membrane covering the egg (Greek *hymen*—membrane, *lepis*—rind or covering) and nana to its small size (nanus—dwarf). It is cosmopolitan in distribution but is more common in the warm than in cold climates. Infection is most common in school children and institutional populations. It is unique that it completes its life cycle in one host, the parasite being maintained by transmission between humans, and even in a single individual, who can act as both the definitive and intermediate host.

The adult worm lives in the human intestine, often in large numbers. It is 5 to 45 mm long and less than 1 mm thick. The scolex has 4 suckers and a retractile rostellum with a single row of hooklets. The long slender neck is followed by the strobila consisting of 200 or more proglottids, which are much broader than long. Eggs are released in the intestine by disintegration of the distal gravid segments. The egg is roughly spherical or ovoid, 30 to 45 µm in size, with a thin colourless outer membrane and an inner embryophore enclosing the hexacanth oncosphere. The space between the two membranes contains yolk granules and 4 to 8 polar filaments arising from two knobs on the embryophore. The eggs float in saturated salt solution (Fig. 10.13).

Infection occurs by ingestion of the eggs, by faecal oral transmission from person to person or in the same individual. Internal autoinfection may also occur when the eggs released in the intestine hatch there itself. No intermediate host is required.

H.nana is unusual in that it undergoes multiplication in the body of the definitive host.

When the eggs are swallowed, or in internal autoinfection, they hatch in the duodenum or jejunum. The hexacanth embryo penetrates a jejunal villus and develops into the cysticercoid larva. This is a solid pyriform structure, with the vesicular, anterior end containing the invaginated scolex and a short conical posterior end. After about 4 days, the mature larva emerging out of the villus evaginates its scolex and attaches to the mucosa. It starts strobilisation, to become the mature worm which begins producing eggs in about 25 days.

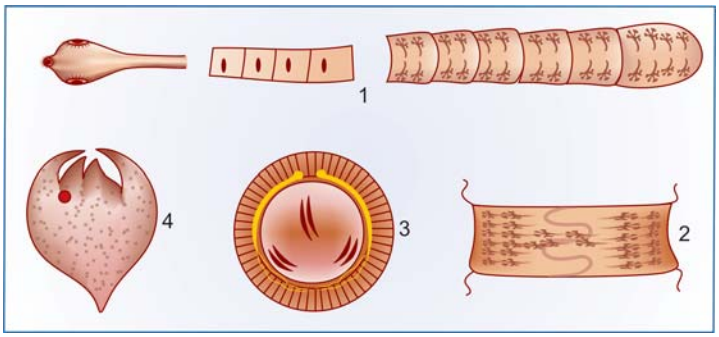


FIGURE 10.13: Life cycle of *Hymenolepis nana*. 1. Adult worm in human intestine, showing scolex with four suckers and retractile rostellum bearing hooklets, a slender neck and strobila. 2. Mature proglottid much broader than long. 3. Egg passed in stools, showing hexacanth embryo, polar filaments and outer membrane. 4. When egg is ingested by humans, cysticercoid larva develops in intestine and grows into adult worm. Entire life cycle can be passed in one host

A different strain of *H. nana* infects rats and mice. The eggs passed in rodent faeces are ingested by rat fleas (*Xenopsylla cheopis* and others) which act as the intermediate host. The eggs develop into cysticercoid larvae in the haemocele of these insects. Rodents get infected when they eat these insects. The murine strain does not appear to infect man. However, the human strain may infect rodents, which may, therefore, constitute a subsidiary reservoir of infection for the human parasite.

Infection with *H. nana* does not generally produce any illness. Symptoms may sometimes occur due to an allergic response. These include abdominal discomfort diarrhoea and pruritus. The diagnosis is made by demonstration of the eggs in faeces. Praziquantel and niclosamide are effective in treatment. Prevention is by proper personal hygiene.

HYMENOLEPIS DIMINUTA

This is called the rat tapeworm and is a common parasite of rats and mice. The name "diminuta" is a misnomer as it is larger than *H. nana* being 10 to 60 cm in length. Its life cycle is similar to that of the murine strain of *H. nana*. Rarely, human infection follows accidental ingestion of infected rat fleas. Human infection is asymptomatic.

DIPYLIDIUM CANINUM

This common parasite of dogs and cats may rarely cause human infection, mainly in children. The adult worm in the intestine is about 10 to 70 cm long. The scolex has 4 prominent suckers and a retractile rostellum with up to 7 rows of spines. The mature proglottid has two genital pores, one on either side, hence the name Dipylidium (dipylos—two entrances). The eggs passed in faeces are eaten by larval stages of dog and cat fleas, *Ctenocephalus canis* and *C. felis*. The embryo develops into a tailed cysticercoid larva. When the adult fleas containing the larvae are eaten by dogs, cats, or rarely humans, infection is transmitted (Fig. 10.14).

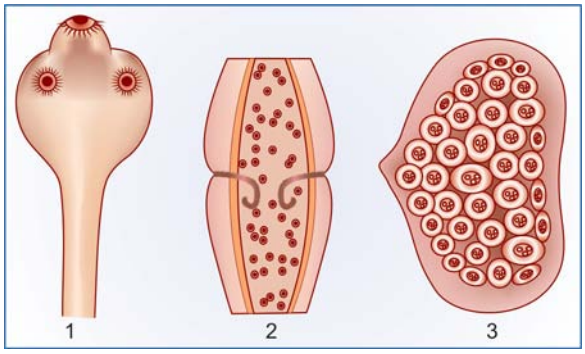


FIGURE 10.14: *Dipylidium caninum*. 1. Scolex showing suckers and rostellum with multiple rows of hooklets. 2. Mature proglottid showing two genital pores, one on either side. 3, Eggs found in clusters enclosed in a membrane

Human infection is generally asymptomatic, but the actively motile proglottids passed in stools may cause alarm. Treatment with niclosamide or quinacrine is effective.

COENUROSIS

Tapeworms of the Genus *Multiceps* (*M. multiceps, M. serialis, M. glomeratus*, etc.) are widespread natural parasites of dogs and other canines. Sheep and other ruminants are the natural intermediate hosts, in which the larval stage known as *coenurus* develops. Occasionally man can become an abnormal intermediate host, a condition known as coenurosis.

Coenurus is a roughly spherical or ovoid bladder, up to 3 cm in size and bearing multiple invaginated postoscolices. In sheep, coenurus is typically seen in the brain and spinal cord. Affected sheep develop cerebellar ataxia, giving the disease its name 'staggers'. Human coenurosis has been reported from Africa, Europe and the USA. The sites affected mainly are the orbit, brain and subcutaneous tissue. Clinical disease is due to pressure effects and also to allergic reactions. Surgical removal, where feasible is the only mode of treatment.

CHAPTER 11

Nematodes: General Features

Nematodes are said to be the most worm-like of all helminths. This is because they generally resemble in appearance the commom earth worm, which is considered to be the prototype of 'worms'. However, taxonomically earthworms are not nematodes as they are segmented worms of the Phylum Annelida.

Nematodes are elongated, cylindrical, unsegmented worms with tapering ends. The name 'nematode' means'thread-like', from *nema*, thread. They are bilaterally symmetrical, with a secondary triradiate symmetry at the anterior end. The adults vary greatly in size, from about a millimetre to a metre in length.

The body is covered with a tough cuticle, which may be smooth, striated, bossed or spiny. They move by sinuous flexion of the body. The body cavity is a pseudocoele in which all the viscera are suspended.

The digestive system consists of the anteriorly placed mouth, leading to the oesophagus which characteristically varies in shape and structure in different groups. The intestine is lined with a single layer of columnar cells and leads to the rectum, opening through the anus. In the male, the rectum and the ejaculatory duct open into the cloaca. Nematodes have simple excretory and nervous systems.

The sexes are separate. The male reproductive system consists of a single delicate tubule differentiated into testis, vas deferens, seminal vesicle and ejaculatory duct which opens into the cloaca. The female reproductive system consists of the ovary, oviduct, seminal receptacle, uterus and vagina.

Nematodes may produce eggs (*oviparous*) or larvae (*viviparous*). Some lay eggs containing larvae which immediately hatch out (*ovoviviparous*). The life cycle consists typically of four larval stages and the adult form. The cuticle is shed in passing from one stage to another.

Unlike trematodes and cestodes, all of which are parasitic, most nematodes are free-living forms found in soil and water. Several species are parasites of plants, of great economic importance. Many nematodes parasitise invertebrate and vertebrate animals. The largest number of helminthic parasites of humans belong to the class of nematodes. There are an estimated 500,000 species of nematodes.

CLASSIFICATION

Nematode parasites may be classified in various ways.

A. Location of Adult in the Body

1. Intestinal Nematodes
   a. Small intestine: Ascaris, Ancylostoma, Necator, Strongyloides,Trichinella.
   b. Large intestine: Enterobius, Trichuris.

2. Tissue Nematodes
   a. Lymphatic: Wuchereria, Brugia.
   b. Subcutaneous: Loa loa. Onchocerca, Dracunculus
   c. Mesentery: Mansonella
   d. Conjunctiva: Loa loa.

B. Mode of Infection

1. *By Ingestion:*
   a. Eggs: Ascaris, Enterobius. Trichuris.
   b. Larvae within intermediate host: Dracunculus.
   c. Encysted larvae in muscle: Trichinella.
2. *By Penetration of Skin:* Ancylostoma, Necator, Strongyloides.
3. *By Blood Sucking Insects:* Filariae.
4. *By Inhalation of Dust Containing Eggs:* Ascaris, Enterobius.

C. Based on Whether they Lay Eggs or Larvae

1. *Oviparous* -Laying eggs:
   a. Unsegmented eggs: Ascaris. Trichuris.
   b. Segmented eggs: Ancylostoma, Necator.
   c. Eggs containing larvae: Enterobius.
2. *Viviparous*—Producing larvae:
   Trichinella, Wuchereria, Brugia, Dracunculus.
3. *Ovoviviparous*-Laying eggs containing fully formed larvae which hatch out immediately: Strongyloides.

D. Zoological Classification

A simplified zoological classification of nematodes parasitic for man is given below:

PHYLUM NEMATHELMINTHES

Class Nematoda

Nematodes are divided into 2 subclasses based on the absence or presence of 'Phasmids' which are caudal chemoreceptors. The 2 subclasses were called Aphasmidia and Phasmidia, now renamed Adenophorea and Secernentea respectively.

Subclass Adenophorea (Aphasmidia)

(No phasmids; no caudal papillae in male; eggs usually unsegmented with polar plugs, or hatching in uterus).

Order Enoplida

Superfamily Trichuroidea (Anterior part of body narrower than posterior) Trichuris, Trichinelia, Capillaria.

Subclass Secernentea (Phasmidia)

(Phasmids present; numerous caudal papillae).

Order Rhabditida

Superfamily Rhabdisoidea (alternation of free-living and parasitic generations; parasitic females parthenogenetic).

Strongyloides

Order Strongyloida

Superfamily Ancylostomatoidea (Prominent buccal capsule with teeth or cutting plates)
Ancylostoma, Necator

Superfamily Metastrongyloidea (Tissue parasites; inconspicuous buccal capsule; have intermediate hosts).
Angiostrongylus

Order Ascaridida

Stiperfamily Ascaridoidea (Large worms of gut lumen. Mouth has 3 lips)—
Ascaris, Toxocara, Anisakis.

Order Oxyurida

Stiperfamily Oxyuroidea (Male has no caudal bursa; short stout body; oesophagus has prominent bulb; eggs planoconvex embryonate in uterus).
Enterobius

Order Spirudida

Stiperfamily Filarioidea (tissue parasites; viviparous; insect vector) *Wuchereria, Brugia, Onchocerca, Loa, Mansonelia, Dirofilaria.*

Superfamily Dracunculoidea (Very long female and small male; viviparous; larvae escape from ruptured uterus).
Dracunculus

Superfamily Gnathostomatoidea (Spiny body with bulbous head).
Gnathostoma

CHAPTER 12

Trichinella Spiralis

HISTORY AND DISTRIBUTION

Trichinella spiralis or the trichina worm, the causative agent of trichinosis was first observed in 1821 in the muscles of a patient at autopsy by James Paget who was then a first year medical student at St. Bartholomew's Hospital, London. Owen in 1835 described the encysted larval form in muscles and named it *Trichina spiralis*. Leuckart, Virchow and Zenker (1853-60) independently proved the infectivity of the encysted larvae when fed to experimental animals. They traced the life cycle of the worms from the encysted larvae in muscles, to adults in the duodenum and then again to encystment in muscles. The major source of human infection was shown to be the consumption of inadequately cooked pork. The name *trichinella* is derived from the minute size of the adult, (Gr. *trichos*—hair; *ella*. suffix for diminutive): *spiralis* refers to the spirally coiled appearance of larvae in muscles.

Trichinosis is recognised as an important public health problem in Europe and America, but is much less common in the tropics. In Asia, the disease had been reported from Malaysia, Vietnam, Thailand, China and Syria. Human trichinosis had not been recorded in India till 1996, when the first case was reported from Punjab.

Morphology and Life Cycle

The infective form is the encysted larvae found in the muscles of pigs and other animals. When such meat is eaten without adequate cooking, the cysts are digested by the gastric juice and viable larvae are released (excystation) in the stomach, duodenum and jejunum. The larvae immediately penetrate the mucosal epithelium, moult four times and rapidly develop into adults, either male or female, by the second day of infection.

The adult *T. spiralis*, a white worm just visible to the naked eye, which inhabits the small intestine is one of the smallest nematodes infecting humans. The male measures about 1.5 mm by 0.04 mm and the female about 3 mm by 0.06 mm. The anterior half of the body is thin and pointed, well-adapted for burrowing into the mucosal epithelium. The posterior end of the male has a pair of pear-shaped clasping papillae, one on each side of the cloacal orifice.

Insemination occurs by the second day of infection. The male dies soon afterwards. The female worm is viviparous. The fertilised females start releasing motile larvae by the sixth day of infection. Larvae continue to be discharged during the remaining part of the lifespan of the worm, which ranges from 4 weeks to 4 months. Each female gives birth to some 1000 larvae. These larvae enter the intestinal lymphatics or mesenteric venules and are transported in circulation to different parts of the body. They get deposited in the muscles, central nervous system and other sites. While they die in most other situations, they grow and develop in the skeletal muscles. Deposition in the muscles occurs mostly during the second week of infection. Larval development in muscles takes place during the next three or four weeks. After this, they become encysted and remain as infective larvae inside the cysts for many years (Fig. 12.1).

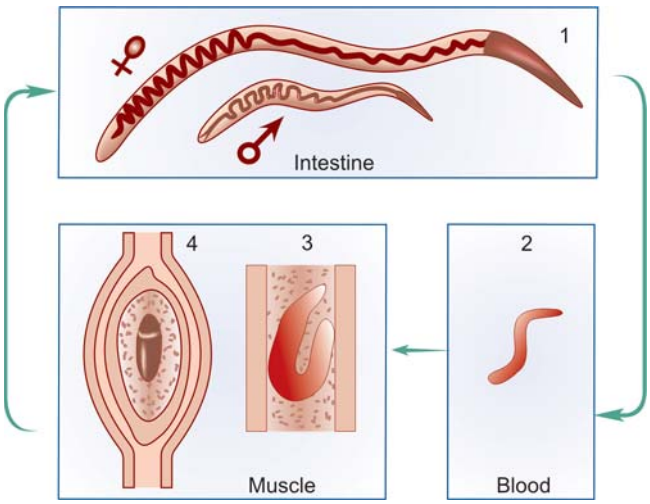


Fig. 12.1: Life cycle of *Trichinella spiralis*. 1. Adult male and female in intestine. Female viviparous. 2. Larva discharged into blood stream. 3. Young larva in striated muscle undergoing development. 4. Encysted larva in muscles; infective stage

At the time of deposition in the muscle fibres, the larvae are about 100 µm by 6 µm in size. They grow in size, becoming about 1 mm long, but remain tightly coiled and enclosed within a fibrous capsule. The cyst is formed by the tissue reaction around the encapsulated larvae. Cysts are usually ovoid. measuring about 400 µm by 250 µm, lying longitudinally along the muscle fibres. They may get calcified in about two years, but the larvae often remain viable even inside calcified cysts. Cysts develop preferentially in muscles relatively poor in glycogen. Therefore, the diaphragm, intercostal, pectoral girdle, cervical, tongue, jaw and extraocular muscles, which are constantly active are the ones most heavily affected. Cysts are more abundant near the sites of attachment of muscles to tendons and bones than in other parts. They are also more frequent in the superficial muscles or superficial parts of muscles. The deltoid being easily accessible is chosen for taking diagnostic muscle biopsies. In heavy infection, there may be about 1000 cysts per gram of muscle.

Pathogenesis and Clinical Features

The disease caused by *T.spiralis* is called *trichinosis* or less commonly trichinelliasis or trichiniasis. The manifestations vary from asymptomatic infection, which is very common, to an acute fatal illness, which is extremely rare. The clinical features may be classified according to the stage in the life cycle of worm.

1. *Stage of Intestinal Invasion (Enteric Phase)*

This occurs during the early stage of infection when the larvae excyst, invade the intestinal epithelium in the duodenum and jejunum and develop into adults. Symptoms are gastrointestinal—nausea, diarrhoea, abdominal cramps and sometimes vomiting. This is diagnosed as acute food poisoning particularly when it occurs in groups of persons who have partaken the same food. In some, constipation is seen instead of diarrhoea. The onset of illness may be from 2 to 30 hours of ingestion of the infective food.

2. *Stage of Muscle Invasion (Migratory Phase)*

This occurs during the release of larvae, their migration, deposition and encapsulation in muscles. The typical presentation is with fever, oedema of face, swelling and weakness of affected muscles. Eosinophilia is a constant feature. Myocarditis and encephalitis are serious and potentially fatal complications. Respiratory symptoms may occur. This stage appears usually one to four weeks after infection.

3. *Stage of Encapsulation (Encystment Phase)*

During this period, lasting for one to eight months after infection, the fever and other symptoms subside. After this stage, the cysts begin to calcify.

The clinical disease is self-limited and usually lasts 2 to 3 weeks in light and 2 to 3 months in heavy infections.

Diagnosis

Clinical diagnosis is helped by the history of consumption of inadequately cooked pork or other meat, particularly when a number of persons sharing the same food are affected.

Demonstration of adult worm in faeces or of larvae in blood is seldom possible. Muscle biopsy is useful for demonstration of encysted larvae, from three to four weeks after infection. Biopsy bits from the deltoid or gastrocnemius can be examined microscopically after crushing between glass slides or digestion in artificial gastric juice.

For xenodiagnosis, biopsy bits are fed to laboratory rats, which are killed a month or so later. The larvae can be demonstrated more easily in the muscles of such infected rats. The Brachman intradermal test uses a 1 : 5000 or 1: 10,000 dilution of the larval antigen. An erythematous wheal appears in positive cases within 15

to 20 minutes. The test remains positive for years after infection. Bentonite flocculation test and latex fixation test for demonstration of antibodies have been widely used. A positive test indicates recent infection. IFA and ELISA have also been described. Calcified cysts may be demonstrated in skiagrams. Blood examination shows eosinophilia.

Epidemiology

The entire life cycle can be passed in one host, starting with the ingestion of infective encysted larvae, to the development of adults in the upper intestine, larviposition, deposition of larvae in muscle, and their development into infective cysts. But only a single cycle occurs in one host and for continuation of the cycle and maintenance of the species, it is necessary for the infection to be transmitted to another host, of the same species or of different species.

T. spiralis is maintained in nature principally in three cycles:

1. Pig to pig: This is facilitated by the custom of feeding pigs with untreated household garbage. Such garbage may contain bits of pork with infective cysts.
2. Rat to rat: Rats may get infected by eating household garbage or by cannibalistically eating one another.
3. Feral cycle: This involves wild animals such as wild boar, wild rodents and various carnivores. In the Arctic, where the infection is important, the cycle involves walruses, polar bears, foxes, wolves, and dogs. In Africa, the infection is maintained by warthogs, bushpigs and the carnivores such as leopards, lions, hyenas and jackals.

Human infection comes mainly by eating undercooked pork or inadequately processed sausage or other meat products. Human infection is a dead end as the cysts in human muscles are unlikely to be eaten by another host.

Treatment

Thiabendazole is effective if treatment is started soon after infection. Mebendazole also may be useful.

Prevention

The best safeguard against human infection is proper cooking of pork and other meat likely to be infected. When pork and pork products are to be eaten raw they should be adequately processed. Smoking, salting and drying of meat may not ensure killing of infective trichina larvae. Strains of *T. spiralis* appear to show differences in susceptibility to refrigeration and freezing.

The most effective method of control is to stop the practice of feeding pigs with raw garbage. Extermination of rats from pig farms limits the spread of infection.

CHAPTER 13

Whipworm

TRICHURIS TRICHIURA

History and Distribution

Trichuris trichiura, the human whipworm was first described by Linnaeus in 1771. It is worldwide in distribution, but is much more common in the tropics. Some 750 million people are estimated to be infected with this worm. While whipworm infection is extremely frequent, whipworm disease is relatively rare.

The name *Trichuris* means a hair-like tail (Greek *trichos*—hair, *oura*—tail). This name is not quite correct because it is the anterior end that is hair-like, and not the tail. The name *whipworm* is more apt as the thick posterior part resembles the stock and the thin anterior the lash of a whip.

The antiquity of the whipworm as a human parasite is indicated by the demonstration of its eggs in colonic contents of a young man who died on the Alps some 5300 years ago and whose well-preserved body was discovered in 1990.

The adult worms are found attached to the wall of the caecum and appendix. The male is 30 mm to 45 mm long, while the female is slightly larger, about 40 mm to 50 mm. The worm is flesh coloured. In shape it resembles a whip, with the anterior three-fifth thin and thread-like, and the posterior two-fifth thick and fleshy, appearing like the handle of a whip. The attenuated anterior portion which contains the capillary oesophagus, is embedded in the mucosa. The posterior part contains the intestines and reproductive organs. The posterior end of the male is coiled ventrally, while the hind end of the female is straight, blunt and rounded. The worm has a lifespan of 5 to 10 years.

The fertilised female lays about 5000 eggs per day. The egg has a characteristic appearance. It is brown, being bile stained. It has a triple shell, the outermost layer of which is stained brown. It is barrel-shaped, about 50 µm long and 25 µm wide in the middle, with a projecting mucous plug at each pole. The egg floats in saturated salt solution.

The egg passed in feces contains an unsegmented ovum. At this stage it is not infective for humans. The egg undergoes development in soil, optimally under warm,

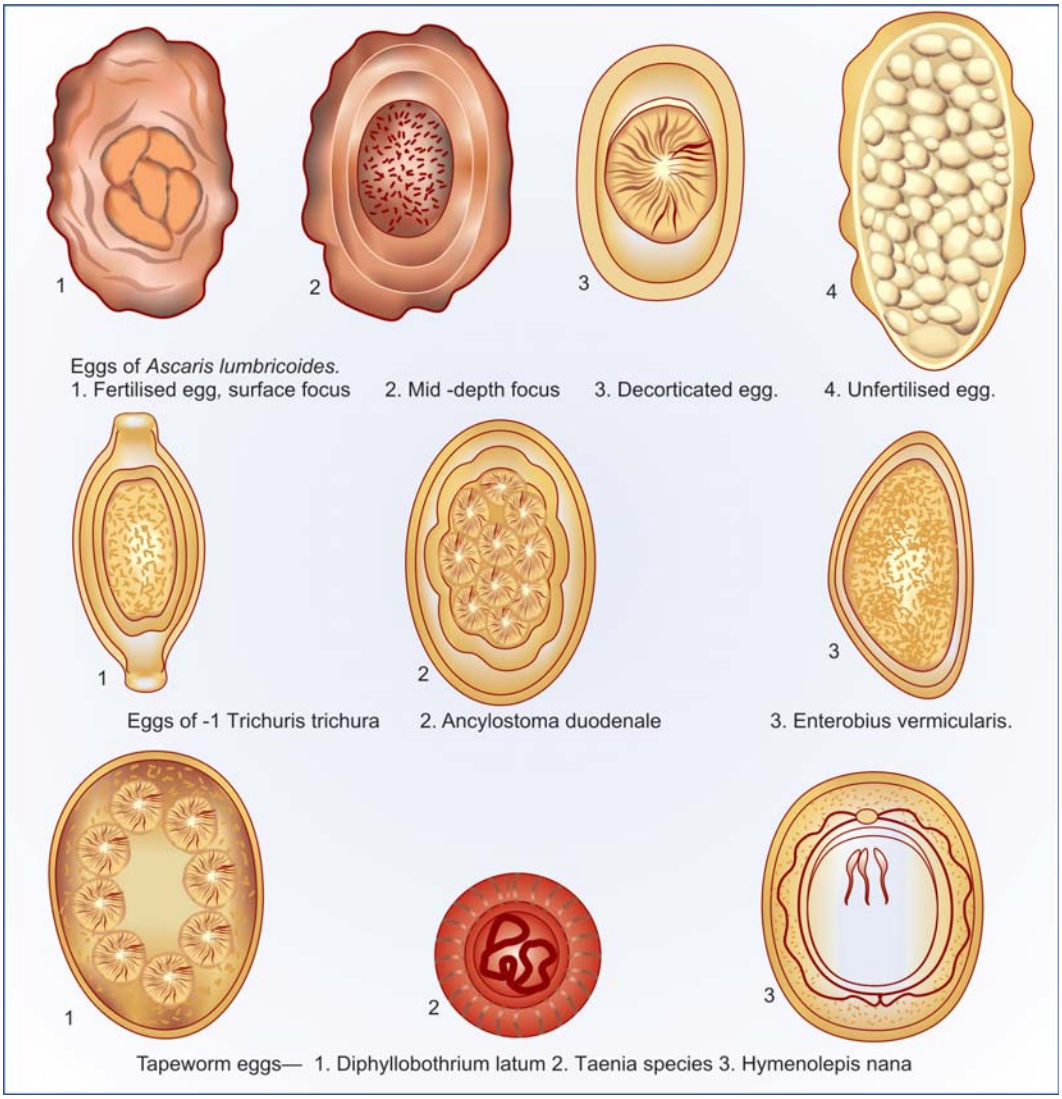


FIGURE 13.1: Helminth eggs in human stools

moist, shady conditions, when the infective rhabditiform larva develops within the egg in 3 to 4 weeks. At lower temperatures this may be delayed for 3 months or more (Fig. 13.2).

Infection occurs when the mature embryonated eggs containing the infective larvae are swallowed in food or water. The eggs hatch in the small intestine and the larva which emerges through the pole of the egg passes down into the caecum. In about 2 to 3 months they become mature adults and lie embedded on the caecal wall, with the thread-like anterior portion piercing the mucosa and the thick posterior end projecting out. Eggs start appearing in faeces usually about 3 months after infection.

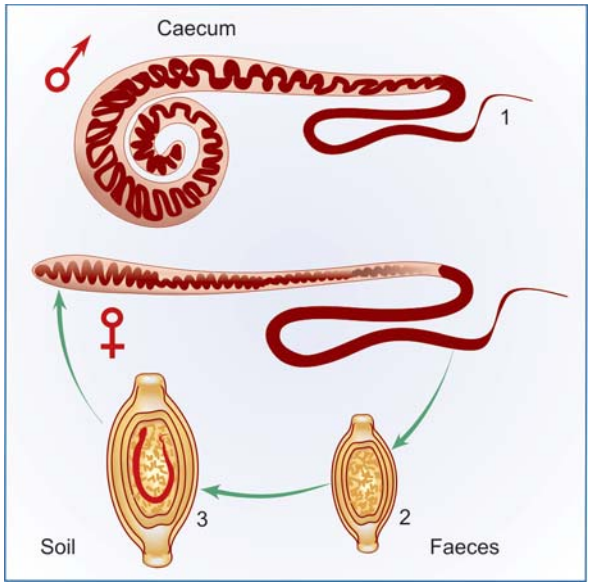


FIGURE 13.2: Life cycle of *Trichuris trichiura*. 1. Adult male and female in human caecum. 2 Freshly passed egg in faeces, containing unsegmented ovum. Not infective. 3. Mature egg in soil, containing coiled rhabditiform larva. Infective stage

The entire life cycle can be passed in one host, from the ingested infective egg to the development of the adults and the release of their eggs in faeces. But for transmission of infection to other hosts and perpetuation of the species, the egg has to undergo development in the soil and then infect another person. Humans are the only natural host for *T.trichiura*, but morphologically similar worms are found to infect pigs and some monkeys.

Pathogenesis and Clinical Features

Infection with *T.trichiura* (*trichuriasis. whipworm infection or trichocephaliasis*) is asymptomatic except when the worm load is heavy. Disease may result either due to mechanical effects or allergic reaction.

The worms lie threaded into the caecal mucosa and even though it is not a blood feeder, oozing of blood may occur at the sites of attachment. The blood loss is about 0.005 ml per worm per day. Over a period of time this may lead to anaemia and malnutrition.

It has been suggested that mechanical blockage of the appendiceal lumen by masses of whipworms may cause acute appendicitis. In heavy infection, the worm may be abundant on the colonic mucosa, even upto the rectum. Mucus diarrhoea, chronic dysentery and abdominal pain are frequently seen in such cases. Some patients, particularly young children may develop rectal prolapse.

Diagnosis

The characteristic eggs are found in stools. The degree of infection can be assessed by egg counts. Less than 10 eggs per smear in direct stool preparation is considered light infection and more than 50 as heavy .Light infection is not considered to cause clinical disease. Proctoscopy is useful as worms are found on the rectal mucosa in whipworm diarrhoea and dysentery. Charcot-Leyden crystals are usually abundant in stools of patients with whipworm dysentery.

Treatment

Mebendazole and albendazole are effective in treatment.

Prophylaxis

Prevention of promiscuous defecation and proper disposal of feces would eliminate transmission of infection. Checking the consumption of unwashed fruits and vegetables grown on polluted fields can minimise the risk of infection.

CHAPTER 14

Strongyloides

STRONGYLOIDES STERCORALIS

History

Normand (1876) observed minute cylindrical worms in the diarrhoeic feces and intestinal walls of some French soldiers in Cochin-China. These were named *Strongyloides stercoralis* (*strongylus*—round; *eidos*—resembling; *stercoralis*-fecal). It is found mainly in the warm moist tropics, but may also occur in the temperate regions. It is common in Brazil, Columbia and in the Far East—Myanmar, Thailand, Vietnam. Malaysia, Philippines.

Morphology and Life Cycle

The life cycle of *Str. stercoralis* is complex because of the multiplicity of pathways through which it can develop. It is unique among human nematodes in that it has, in addition to the parasitic cycle, a free-living soil cycle, in which it can persist for long periods in soil, feeding on soil bacteria, passing through several generations (Fig. 14.1, and Table 14.1).

Parasitic Phase

The adult worm is found in the human intestine embedded in the mucosa of the duodenum and upper jejunum. The individual worm has a lifespan of 3 or 4 months, but because it can cause autoinfection, the infection may persist for years. Only the female worms are seen in the intestine. It was believed that they are parthenogenetic and can produce offspring without being fertilised by the male. But it has since been established that parasitic males do exist. They are shorter and broader than the female. They can be demonstrated in experimentally infected dogs. They are not seen in human infections because they do not invade the intestinal wall and so are eliminated from the bowel soon after the females begin to oviposit. However, the majority of females are probably parthenogenetic.

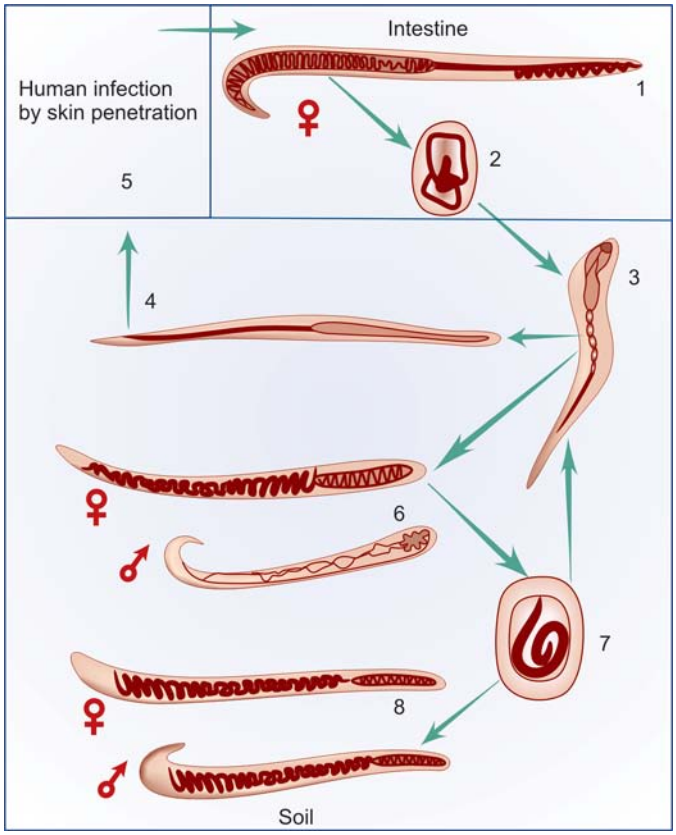
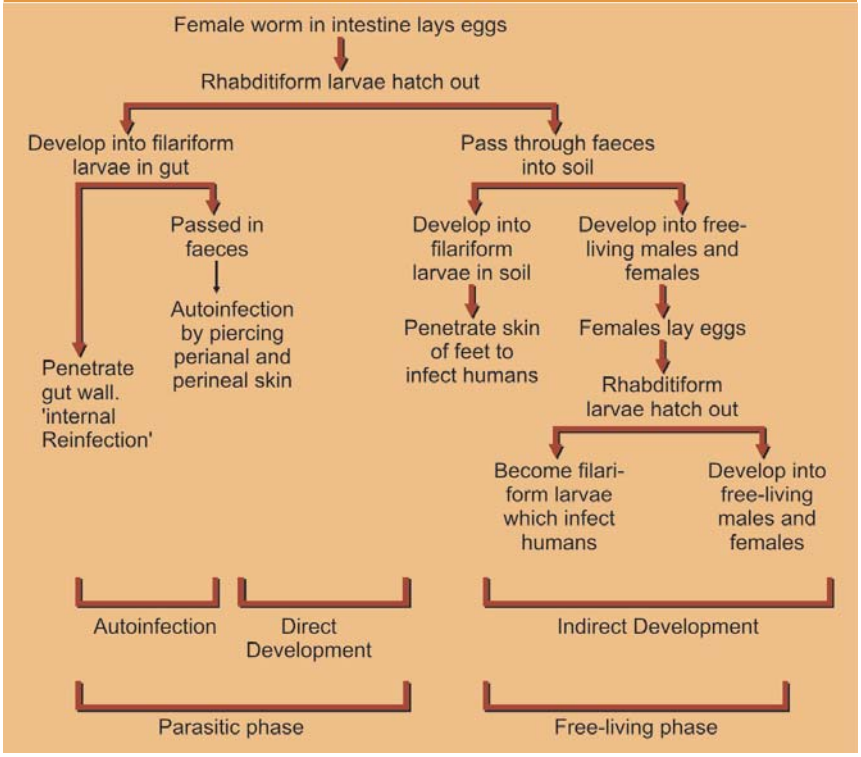


Fig. 14.1: Life cycle of *Strongyloides stercoralis*. 1. Parasitic adult female worm in human intestine. 2. Eggs laid in mucosa hatch immediately. 3. Rhabditiform larva passed in faeces to soil. 4. Filariform larva. 5. Infects humans by penetrating skin of feet, circulates in blood, migrates through lungs and reaches jejunum where it develops into adult. (Steps 1 to 5 constitute 'Direct Development'). 6. Some rhabditiform larvae develop into free-living male and female worms which mate in soil, 7. The egg laid hatches into rhabditiform larva, which may develop into filariform larva and infect humans, or 8. become free-living worms in soil. (Steps 6 to 8 constitute 'Indirect Development'. Autoinfection cycles are not shown in the diagram)

The female worm is thin, transparent, about 2.5 mm long and 0.05 mm in breadth. It has a cylindrical oesophagus occupying the anterior third of the body, and the intestines in the posterior two-thirds opening through the anus situated ventrally, a little in front of the pointed tail tip. Paired uteri lead to the vulva situated at the junction of the middle and posterior thirds of the body. In the gravid female, the uteri contain thin-walled transparent ovoid eggs, 50 µm by 30 µm in size. The worm is ovoviviparous. The eggs laid in the mucosa hatch immediately, releasing rhabditiform (first stage) larvae, about 0.25 mm long, with a relatively short muscular oesophagus ending in an enlarged bulb. The rhabditiform larvae migrate into the

lumen and pass down the gut to be released in feces. On reaching the soil, they moult twice to become the infective filariform (third stage) larvae. The filariform larvae are slender, about 0.55 mm in size, with a long oesophagus of uniform width. Its tail tip is notched and finely triradiate. The filariform larvae are nonfeeding and can live in soil only for about 12 days.

Table 14:1: Life cycle of *Strongyloides stercoralis*



When a person walks barefoot on soil containing the infective filariform larvae, they penetrate the skin, usually on the sides of the feet or between toes, enter the cutaneous lymphatics or blood vessels and are carried along the venous circulation to the right side of the heart and to the lungs. Here they escape from the pulmonary capillaries into the alveoli, migrate up the respiratory tract to the pharynx and are swallowed, reaching their final destination, the duodenum and jejunum, where they burrow into the mucosa. There they mature in 15 to 20 days and start laying eggs. This mode of life cycle is called the *direct development* and is the usual mode of human infection. It resembles the life cycle of the hookworm.

The worm also has a cycle of *autoinfection*. Here the rhabditiform larvae mature into the infective third stage larvae during their passage down the gut. These filariform larvae cause reinfection by piercing the perianal and perineal skin during defecation. The larvae wander in the dermis of the perianal region for sometime, causing a radiating perianal creeping eruption, a form of *cutaneous larva migrans*. They ultimately

enter the lymphatics or venules and are carried to the right heart and the lungs to complete the life cycle as above. This ability to cause autoinfection explains the persistence of the infection in patients for long periods, even 30 to 40 years, after leaving the endemic areas.

In another type of autoinfection, seen typically in immunodeficient hosts, the rhabditiform larvae released into the bowel walls mature into the infective filariform larvae there itself. They penetrate the deeper layers of the intestine, to reach the mesenteric venules and are carried in circulation to complete the life cycle. This mode of autoinfection is called *internal reinfection*. It may lead to very heavy infection causing serious and sometimes even fatal illness.

Free-living Phase

The rhabditiform larvae passed in stools develop in moist soil into free-living males and females. The female is 1 mm and the male 0.7 mm long. They mate in soil. The fertilised female lays eggs which hatch to release the next generation of rhabditiform larvae. These may repeat the free-living cycle, or may develop into the filariform larvae which infect humans and initiate the parasitic phase.

Humans are the natural host of *Str. stercoralis*, though dogs and cats are found infected with morphologically indistinguishable strains. *Str.fullerborni* is widely prevalent in African monkeys. It infects pygmies in the forests of Zaire and Zambia. It also causes human infection in Papua New Guinea. *Trichostrongylus*, a parasite of sheep and goats, seen in Africa and Asia (including India) may cause human infection which is usually asymptomatic.

Pathogenesis and Clinical Features

Strongyloidosis is generally benign and asymptomatic, blood eosinophilia and larvae in stools being the only indications of infection. But it may sometimes cause clinical manifestations, which may be severe and even fatal, particularly in those with defective immune response. The clinical disease may be classified as cutaneous, pulmonary and intestinal. The overwhelming severe disease seen in the immunocompromised is known as *hyperinfection*. *Generalised strongyloidosis* may be seen in AIDS.

Cutaneous

There may be a dermatitis, with erythema and itching at the site of penetration of the filariform larvae, particularly when large numbers of larvae enter the skin. In those sensitised by prior infection, there may be an allergic response. This may prevent circulation in the blood of the larvae, which may instead migrate in skin, leading to a form of creeping eruption or larva migrans. The term *larva currens* (meaning 'racing larva') has been applied to this rapidly progressing linear urticarial tracks caused by migrating strongyloides larvae. These often follow autoinfection and start perianally.

Pulmonary

During escape of the larvae from the pulmonary capillaries into the alveoli, small haemorrhages occur, along with cellular infiltration into alveoli and bronchioles. Bronchopneumonia may be present, which may, in some go on to chronic bronchitis and asthmatic symptoms. Larvae may be found in the sputum.

Intestinal

The symptoms may resemble those of peptic ulcer or of malabsorption syndrome. Mucus diarrhoea is often present. In heavy infection, the mucosa may be honeycombed with the worm and there may be extensive sloughing, causing dysenteric stools.

Hyperinfection

In debilitated individuals, and particularly in those with cellular immune defects, extensive internal reinfection takes place, leading to enormous numbers of adult worms in the intestines and lungs, and larvae in various tissues and organs. This is known as hyperinfection. Severe malnutrition, lepromatous leprosy, lymphoreticular malignancies, AIDS, immunosuppressive drugs and other situations in which cell-mediated immunity is defective, predispose to this condition. Hyperinfection is an important hazard of steroid therapy and other instances of prolonged immunosuppression as in tansplant patients. Manifestations depend on the sites affected. Brain abscess, meningitis and peritonitis are major fatal complications.

It has been reported that circulating strongyloides larvae may carry intestinal bacteria causing septicaemia.

Diagnosis

Demonstration of the rhabditiform larvae in freshly passed stools is the most important method of specific diagnosis. Larvae found in stale stools have to be differentiated from larvae hatched from hookworm eggs. Larvae may sometimes be present in sputum and gastric aspirates.

When larvae are scanty in stools, diagnosis may be facilitated by stool culture. The larvae develop into free-living forms and multiply in charcoal cultures set up with stools. Large numbers of free-living larvae and adults can be seen after 7 to 10 days.

Serological tests have been described, using strongyloides or filarial antigens. Complement fixation, indirect haemagglutination and ELISA have been reported. But the antigens are not freely available, and extensive cross reactions limit the utility of these tests. Radiological appearances in intestinal infection are said to be characteristic and helpful in diagnosis. Peripheral eosinophilia is a constant finding. However, in severe hyperinfection eosinophilia may sometimes be absent.

Treatment

All cases of strongyloidosis, whether symptomatic or not should be treated to prevent severe invasive disease. Thiabendazole, mebendazole and ivermectin are effective.

Prevention

Prevention of soil contamination with feces and avoiding contact with infective soil and contaminated surface waters constitute the general methods of prevention.

CHAPTER 15

Hookworm

HISTORY AND DISTRIBUTION

Hookworms have been known from very ancient times. They have been referred to in the Ebers papyrus (Circa 1600 B.C.). Hookworm disease is prevalent throughout the tropics and subtropics. Even though it has been controlled in the advanced countries, it is estimated that it still affects some 900 million people, causing the loss of about 9 million litres of blood overall each day. Two species of hookworms are human parasites, *Ancylostoma duodenale* and *Necator americanus*.

Ancylostoma duodenale (Greek *ankylos*—hooked; *stoma*—mouth) was originally described by Dubini in 1843 in Italy. The life cycle of the worm was worked out by Looss in 1898 in Egypt. The second species *Necator americanus* was identified by Stiles in 1902 in specimens obtained from Texas, USA. The name literally means the 'American murderer' (Latin *necator*—murderer). It is called the American or the 'New World' hookworm and *A.duodenale* the 'Old World' hookworm. But it is believed that *N.americanus* actually originated in Africa and was transported to America with the slave trade.

A. duodenale was distributed predominantly to the north and *N. americanus* to the south in the endemic zones. *A. duodenale* was prevalent along the Mediterranean coast of Europe and Africa, in Northern India, China and Japan while *N. americanus* was prevalent in Central and South America, Central and Southern Africa, Southern India, the Far East and the Southern Pacific region. However, in more recent times, movement of infected persons has blurred the geographic differences in distribution of the two species. For example, *A. duodenale* is now commonly seen along with *N. americanus* in South India and S.E. Asia.

ANCYLOSTOMA DUODENALE

Morphology

The adult worms live in the small intestines of infected persons, mostly in the jejunum, less often in the—duodenum and infrequently in the ileum. They are relatively stout

cylindroidal worms. They are pale pink or greyish white, but may appear reddish brown due to ingested blood. The body is curved with the dorsal aspect concave and the ventral aspect convex. The anterior end is somewhat constricted and bent dorsally. This cervical curvature gave it the name hookworm. The mouth is not at the tip but directed dorsally. The prominent buccal capsule, reinforced with a hard chitin-like substance carries two pairs of hook-like teeth ventrally and a dental plate with a median cleft dorsally.

The male worm is about 8 to11 mm in length and about 0.4 mm thick. The posterior end of the male is expanded into a copulatory bursa supported by fleshy rays. The pattern of the rays helps in distinguishing between different species. The cloaca into which the rectum and genital canal open is situated within the bursa. There are two long retractile bristle-like copulatory spicules, the tips of which project from the bursa.

The female is larger, 10 to 13 mm long and 0.6 mm thick. Its hind end is conoid, with a subterminal anus situated ventrally. The vulva opens ventrally at the junction of the middle and posterior thirds of the body. The vagina leads to two intricately coiled ovarian tubes which occupy the hind and middle parts of the worm. During copulation the male attaches its copulatory bursa to the vulva. The copulating pair therefore presents a Y-shaped appearance (Fig. 15.1).

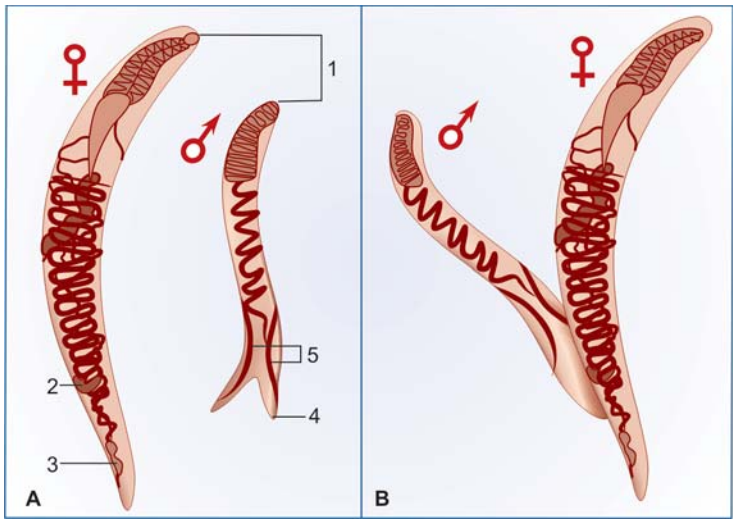


FIGURE 15.1: (A) Morphology of *A.duodenale*. The body is curved, with dorsal surface concave and ventral surface convex. 1. Buccal capsule. 2. Vulva. 3. Anal pore. 4. Copulatory bursa. 5. Copulatory spicutes (B) Male and female in copulation, forming a Y-shaped figure

The eggs are oval or elliptical, measuring 60 µm by 40 µm, colourless, not bile stained, with a thin transparent hyaline shell membrane. When released by the worm in the intestine, the egg contains an unsegmented ovum. During its passage down the intestine, the ovum develops. When passed in feces, the egg contains a segmented ovum, usually with 4 or 8 blastomeres. There is a clear space between the segmented

ovum and the egg shell. The eggs float in saturated salt solution. A single female worm lays about 25,000 to 30,000 eggs a day and some 18 to 54 million during its life time.

Life Cycle

Humans are the only natural host. Eggs freshly passed in feces are not infective for humans. When deposited in the soil, the embryo develops inside the eggs. Its development takes place optimally in sandy loamy soil with decaying vegetation under a moist warm, shady environment. In about 2 days, a rhabditiform larva, about 250 µm long, hatches out of the egg. It feeds on bacteria and other organic matter in the soil, grows in size and moults twice, on the 3rd and 5th days after hatching. to become the third-stage infective filariform larva. It is about 500 to 600 µm long, with a sharp pointed tail. The filariform larvae are non-feeding. They can live in the soil for about 5 weeks, with their heads waving in the air, waiting for their hosts. They can also ascend on blades of grass or other vegetation, being carried in capillary water films on their surface. Direct sunlight, drying or salt water can kill the larvae (Fig. 15.2).

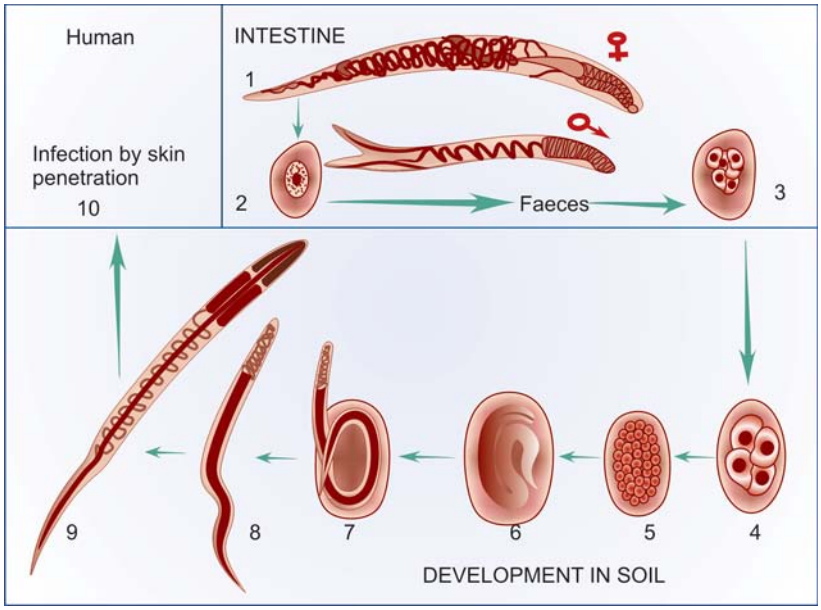


FIGURE 15.2: Life cycle of *Ancylostoma duodenale*. 1. Adult male and female in human intestine. 2. Egg released by worm has an unsegmented ovum. 3. Egg passed in faeces has 4 to 8 blastomeres 4-7. Embryo undergoes development in soil. 8. Rhabditiform larva hatches out. 9. Filariform larva develops, which, 10. Infects humans by penetrating skin of feet. It circulates in blood, migrates through lungs on to pharynx and the intestine

When a person walks barefooted on soil containing the filariform larvae they penetrate the skin and enter the subcutaneous tissue. The common sites of entry

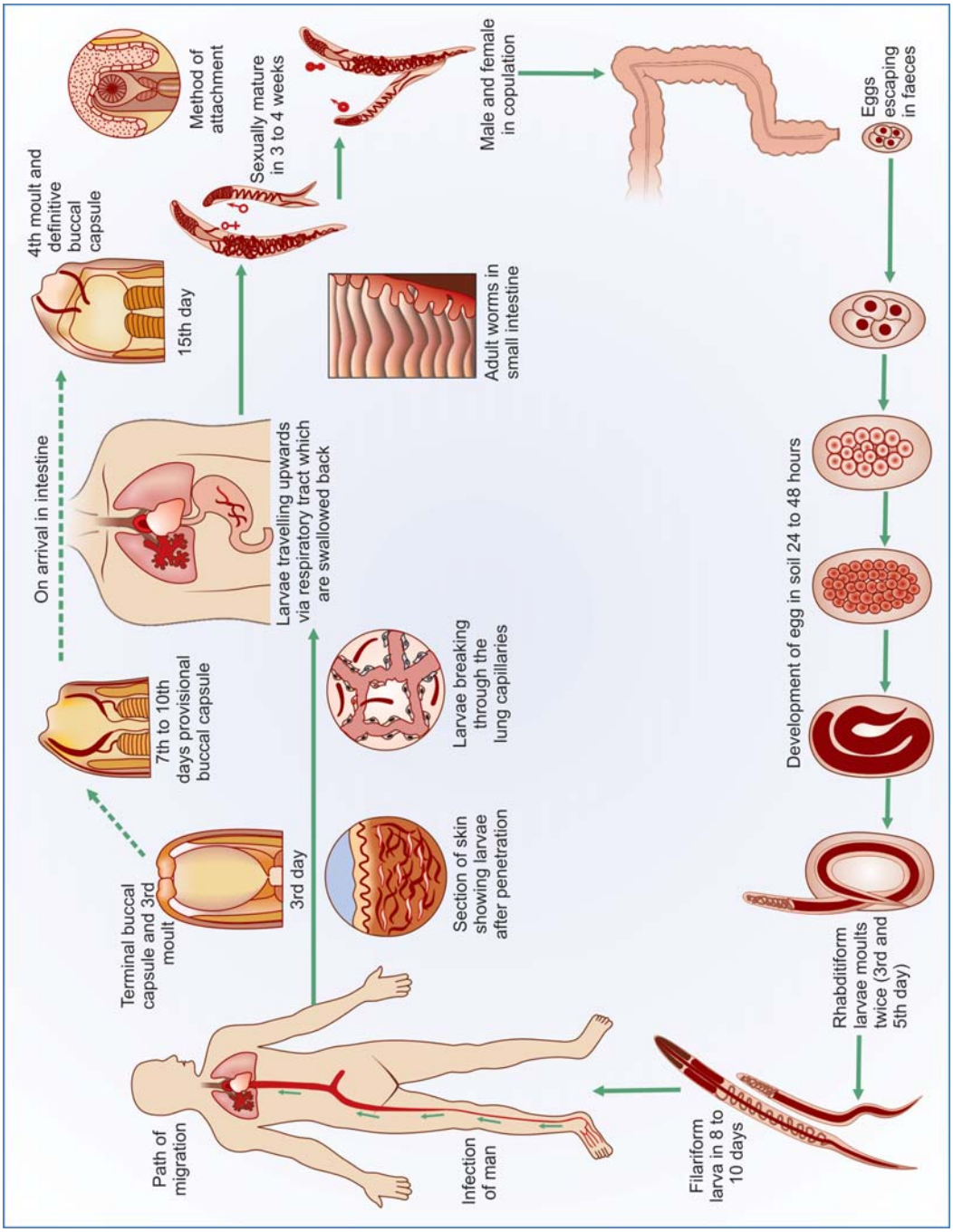


FIGURE 15.3: Life cycle of hook worm (*Courtesy* CIPLA)

are the skin between the toes, the dorsum of the foot and the medial aspect of the sole. In farm workers and miners the larvae may penetrate the skin of the hands. Rarely entry may be through skin on the other parts of the body. In the subcutaneous tissue the larvae enter the venules and are carried in circulation to the right heart and to the lungs. In the lungs. they break out of the capillaries to reach the alveoli, from where they migrate up the respiratory tract to the epiglottis. They crawl over the epiglottis to the pharynx and are swallowed. During migration or on reaching the jejunum, they moult and develop a temporary buccal capsule by which they get attached to the gut mucosa. They feed and grow in size, undergo a fourth and final moulting, develop the buccal capsule and grow into adults. There is no multiplication in the host and one infective larva develops into a single adult, male or female. It takes usually about 6 weeks from the time of infection for the adult worms to become sexually mature and start laying eggs. But sometimes, there may be an arrest in development and the process may take much longer, 6 months or more.

Rarely infection may take place by the oral route, the filariform larvae being carried on contaminated vegetables or fruits. The larvae may penetrate the buccal mucosa to reach the venous circulation and complete their migration via the lungs. Alternatively the larvae may be swallowed and may develop directly into adults in the small intestine without a tissue phase. Transmammary and transplacental transmission has been reported for Ancylostome, but not for Necator.

NECATOR AMERICANUS

Morphology

The adult worms are slightly smaller than *A.duodenale*, the male being 7 to 9 mm by 0.3 mm and the female 9 to 11 mm by 0.4 mm. The anterior end is bent in a direction opposite to the general curvature of the body while in *A.duodenale* the bend is in the same direction. They have a smaller buccal capsule with 2 pairs of semilunar cutting plates instead of teeth as in *A.duodenale.* The copulatory bursa of the male is long and wide. The copulatory spicules are fused at the ends to form a barbed tip. In the female the vulva is placed in the middle of the body or anterior to it (Fig. 15.4).

The eggs are identical with those of *A. duodenale.* The life cycle is similar to that of *A. duodenale.* The lifespan is much longer being about 4 to 20 years in Necator and 2 to 7 years in Ancylostoma.

Pathogenesis and Clinical Features

Clinical disease in hookworm infection may be due to larvae or adult worms. When the filariform larvae enter the skin, they cause severe local itching. An erythematous papular rash may develop, becoming vesicular. Scratching and secondary bacterial infection may follow. This condition, known as *ground itch* occurs when large numbers of larvae penetrate the skin and is more common in infection with Necator than with Ancylostome. The condition is self-limited, lasting for 2-4 weeks.

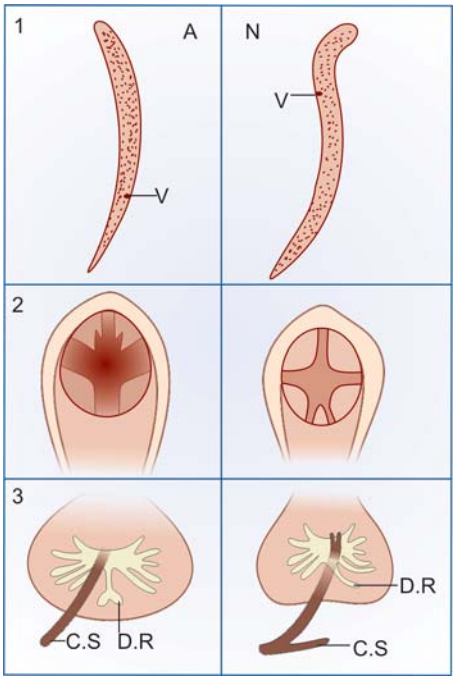


FIGURE 15.4: Major distinguishing features between *A.duodenale*, (A) and *N.americanus* (N): 1. *Adult female* in Ancylostome—anterior curvature uniform with body curve; in Necator- anterior curvature in opposite direction to body curve. Vulva opens at junction of middle and posterior thirds in (A); in (N) it opens a little in front of the middle. 2. *Buccal capsule*. (A). has 2 pairs of hook-like teeth ventrally and a dental plate with median cleft dorsally: (N) has two pairs of semilunar cutting plates instead of teeth. 3 *Copulatory bursa*. In (A) the dorsal ray (D.R.) is single with a split end, making a total of 13 rays: (N) has a paired dorsal ray, making a total of 14 rays. Copulatory spicules (C.S.) separate in (A): they are fused at the tip in (N)

The larvae may sometimes cause *creeping eruption (cutaneous larva migrans).* This is more common in infections with animal hookworms than with human hookworms. The larvae migrate in tortuous tunnels between stratum germinativum and stratum corneum of the skin, causing serpigenous vesicular lesions. With advancing movement of the larvae, the rear portions of the lesions become dry and crusty. The lesion can be intensely pruritic.

When larvae break out of the pulmonary capillaries and enter the alveoli, they may cause minute local hemorrhages. But clinical pneumonitis develops only in massive infections. The pulmonary syndrome, Loeffler'syndrome, commonlly seen in ascariasis is rare in hookworm infection.

The more important manifestations of ancylostomiasis (hookworm disease) are caused by the adult worms in the intestine. The worms attach themselves to the gut mucosa by their buccal capsules. They suck into their mouth a portion of intestinal villi. They utilise gut epithelial cells and plasma for their food. Because of the pumping action of the oesophagus, the worm sucks in blood, which passes out undigested and unutilised through its intestines. An adult Ancylostome can suck about 0.2 ml blood a day, while the smaller Necator sucks in about 0.03 ml per day. The worms frequently leave one site and attach themselves to another site. As the secretions of the worm contain anticoagulant activity, bleeding from the site may continue for sometime. This adds to the blood loss. This chronic blood loss over a period of time leads to a microcytic hypochromic type of iron deficiency anaemia. The speed of onset of anaemia and its severity depend on the intensity of infection, body iron

store and availability of dietary iron. The degree of anaemia is directly proportional to the worm burden. Worm loads of up to 100 worms are light and may cause no symptoms. Loads of 500 to 1000 or more cause significant blood loss and anaemia. The worm load is indicated by the egg count of feces. A count of less than 5 eggs per mg of faeces seldom causes clinical disease, while counts of 20 eggs or more are associated with significant anaemia. Egg counts of 50 or more represent massive infection. In hookworm disease, intestinal absorption of iron is apparently normal so that oral administration of iron can correct the anaemia. However, cure depends on elimination of the worms.

Hookworm infection may cause an intestinal syndrome resembling peptic ulcer, with epigastric pain, dyspepsia and vomiting. There may be diarrhoea, the stool being reddish or black. This is more often seen in the acute stage, when the infection is heavy.

Hookworm anaemia leads to severe lassitude and dullness, affecting the working and learning capacities of patients. The haemoglobin level may drop drastically causing a characteristic sallow appearance of the skin—conjunctiva and tongue. Hypoproteinaemia develops, which is in excess of the red cell loss, and leads to protein—losing enteropathy, oedema and effusion in serous cavities. Severe hookworm anaemia commonly leads to cardiac failure. Patients present with exertional dyspnoea, palpitation, dizziness and generalised puffy oedema.

Diagnosis

Demonstration of the eggs in faeces by direct microscopy or by concentration methods is the diagnostic test. In stool samples examined 24 hours or more after collection, the eggs may have hatched and rhabditiform larvae may be present. These have to be differentiated from strongyloides larvae. Egg counts give a measure of the intensity of infection.

Adult hookworms may sometimes be seen in feces.

Treatment

For specific anthelmintic treatment mebendazole and pyrantel pamoate are the drugs of choice. Thiabendazole is less effective. The old drug tetrachlorethylene is active, but toxic. Bephenium hydroxynaphthoate is active against Ancylostoma but not against Necator.

Treatment of hookworm disease includes relief of anaemia. Oral iron is effective, but in severe cases a preliminary packed cell transfusion may be needed. When the haemoglobin level is very low anthelmintic drugs should not be used before correcting the anaemia.

Epidemiology and Prevention

The conditions required for maintenance of endemic hookworm infection are the presence of infected persons, dispersal of eggs in soil due to indiscriminate defecation

and inadequate processing of excreta, appropriate environmental factors facilitating development of eggs in soil, and opportunity for the larvae to infect people through their exposed skin surfaces. These conditions prevail throughout the year in most parts of the tropics, but in subtropical areas, these conditions exist only seasonally, being limited to the warmer months.

Control depends on prevention of soil pollution with feces and proper disposal of night soil. The use of footwear prevents entry of larvae through the skin of the foot. Gloves give similar protection to the hands of farm workers. Treatment of patients and carriers, preferably all at the same time, limits the source of infection.

OTHER HOOKWORMS

A.ceylanicum naturally parasitises cats and wild felines in S.E. Asia, but can occasionally infect man. *A.braziliense* a parasite of cats and dogs, and some other species of animal ancylostomes have been reported to infect man, but they tend to cause creeping eruption (larva migrans) rather than intestinal infection.

TRICHOSTRONGYLIASIS

Trichostrongylus species normally parasitic in sheep and goats can also cause human infections. The life cycle is similar to that of hookworms. Human infection is usually acquired by ingestion of leafy vegetables carrying the third-stage larvae. Adults attach themselves to small intestinal mucosa, suck blood and live for long periods. Infection is mostly asymptomatic, but epigastric discomfort and anaemia with marked eosinophilia occur in massive infections. The eggs passed in faeces resemble hookworm eggs, but are larger, with more pointed ends and show greater segmentation with 16 to 32 blastomers.

The infection is present in some parts of India. Though trichostrongyliasis is primarily zoonotic, human-to-human transmission can also occur. This is particularly likely where the use of night soil as manure is prevalent. Metronidazole is effective in treatment.

CHAPTER 16

Pinworm

ENTEROBIUS VERMICULARIS

History and Distribution

Enterobius vermicularis, the human pinworm, threadworm or seatworm, formerly called *Oxyuris vermicularis* has been known from ancient times. The name *Enterobius vermicularis* means a tiny worm living in the intestine (Greek *enteron*—intestine, *bios*—life and *vermiculus*—small worm). The term Oxyuris means 'sharp tail', a feature of the female worm, from which the name 'pinworm' is also derived.

It is worldwide in distribution. Unlike the usual situation where helminthic infections are more prevalent in the poor people of the tropics, *E.vermicularis* is one worm infestation which is far more common in the affluent nations in the cold and temperate regions.

Morphology

The adults are short, white, fusiform worms with pointed ends, looking like bits of white thread. The mouth is surrounded by three wing-like cuticular expansions (cervical alae) which are transversely striated. The oesophagus has a double-bulb structure, a feature unique to this worm.

The male is 2 to 5 mm long and 0.1 to 0.2 mm thick. Its posterior end is tightly curved and carries a prominent copulatory spicule. The female is 8 to 13 mm long and 0.3 to 0.5 mm thick. Its posterior third is drawn into a thin pointed pin-like tail. The vulva is located just in front of the middle third of the body and opens into the single vagina which leads to the paired uteri, oviducts and ovaries. In the gravid female, virtually the whole body is filled by the distended uteri carrying thousands of eggs.

Biology and Life Cycle (Fig. 16.1)

E. vermicularis is monoxenous, passing its entire life cycle in the human host. It has no intermediate host.

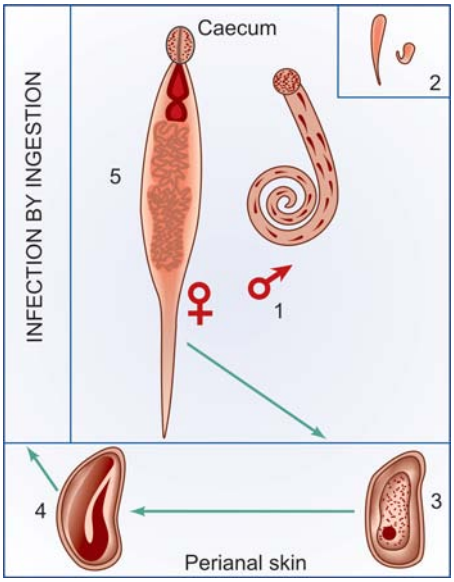


Fig. 16.1: Life cycle of *Enterobius vermicularis.* 1. Adult worms in caecum. Note cervical alae and oesophagus with double-bulb. Body of gravid female filled with paired uteri loaded with eggs. Posterior third pin-like. Male has tightly coiled posterior. 2. Inset showing actual sizes of adult female and male. 3. Plano-convex egg containing tadpole-shaped embryo, deposited by gravid female worm on perianal skin. 4. Mature egg containing infective larva. 5. Infection by ingestion of mature egg

The adult worms live in the caecum, appendix and adjacent parts of the ascending colon. The male is seldom seen as it does not migrate. It usually dies after mating and is passed in the feces. The gravid female migrates down the colon to the rectum. At night when the host is in bed, the worm comes out through the anus and crawls about on the perianal and perineal skin to lay its sticky eggs. The worm may retreat into the anal canal and come out again to lay more eggs. The worm may wander into the vulva, vagina and even into the uterus and fallopian tubes, sometimes reaching the peritoneum. A single worm lays from 5000 to 17,000 eggs. When the eggs are all laid, the worm dies or gets crushed by the host during scratching. The worm may often be seen on the feces, having been passively carried from the rectum. The eggs, however, are only infrequently found in feces.

The egg is colourless and not bile stained. It has a characteristic shape, being elongated ovoid. flattened on one side and convex on the other (planoconvex), measuring 50 to 60 µm by 20 to 30 µm.The egg shell is double layered and relatively thick, though transparent. The outer albuminous layer makes the eggs stick to each other and to clothing and other objects. The egg contains a tadpole shaped coiled embryo which is fully formed, but becomes infectious only some 6 hours after being deposited on the skin. Under cool moist conditions, the egg remains viable for about 2 weeks.

When eggs containing infective larvae are swallowed, they hatch out in the intestine. They moult in the ileum and enter the caecum, where they mature into adults. It takes from 2 weeks to 2 months from the time the eggs are ingested, to the development of the gravid female, ready to lay eggs.

Clinical Features

The infection occurs mostly in children. It is more common in females than in males. The worm produces intense irritation and pruritus of the perianal and perineal area

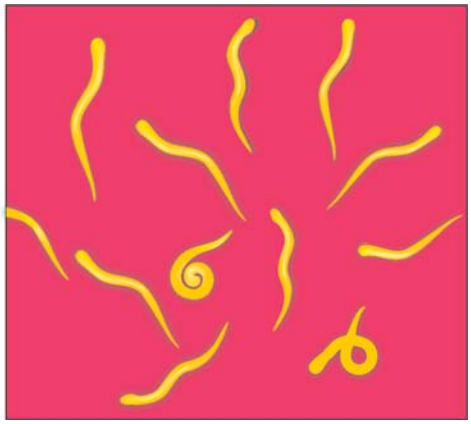


FIGURE 16.2: Pinworm adult

when it crawls out of the anus to lay eggs. This leads to scratching and excoriation of the skin around the anus. As the worm migrates out at night, it disturbs sleep. Nocturnal enuresis is sometimes seen.

The worm crawling into the vulva and vagina causes irritation and a mucoid discharge. It may migrate upto the uterus, fallopian tubes and into the peritoneum. This may cause symptoms of chronic salpingitis.

The worm is sometimes found in surgically removed appendix and has been claimed to be responsible for appendicitis.

Epidemiology

Enterobiasis is generally a group infection, found in a group of children in a class or boarding school or in a family.

Enterobiasis is less common in the tropics probably because children there often wear less underclothes and wash more frequently. The eggs are destroyed by the desiccation in the hot weather. In the cold countries people wear close fitting undergarments and use many layers of bed clothes. This facilitates transmission of the infection.

The source of infection is an infected person. Thousands of eggs are laid on the perianal skin. Scratching transfers them to the fingers, in the dirt beneath the nails. These are carried to the patient's own mouth (auto-infection) during eating or nailbiting, and to contacts either directly or through food and fomites. The eggs survive in the dust for some days and get airborne during sweeping or bedmaking. When inhaled, the eggs may stick to mucus and be swallowed. A process of retrograde infection (retrofection) has been described, in which the eggs laid on the perianal skin hatch there itself and the larvae migrate back to the anus and up the colon to the caecum, to develop into adults.

As the worm does not multiply in the host and has a lifespan of only about 2 weeks to 2 months, the infection should get automatically eliminated after that period.

However, in some children, the infection persists for long periods. This is due to autoinfection. The importance of retrofection in prepetuation of infection is not known.

Diagnosis

Pinworm infestation can be suspected from the history of perianal pruritus. Diagnosis depends on the demonstration of the eggs or adult worms. Eggs are present in the feces only in a small proportion of patients and so feces examination is not useful in diagnosis. They are deposited in large numbers on the perianal and perineal skin at night and can be demonstrated in swabs collected from the sites early morning, before going to the toilet or bathing. Swabs from perianal folds are most often positive. The NIH swab (named after National Institutes of Health, USA) has been widely used for collection of specimens. This consists of a glass rod at one end of which a piece of transparent cellophane is attached with a rubber band. The glass rod is fixed on a rubber stopper and kept in a wide test tube. The cellophane part is used for swabbing by rolling over the perianal area (Fig. 16.3). It is returned to the test tube and sent to the laboratory, where the cellophane piece is detached, spread over a glass side and examined microscopically. Another method for collection of specimens is with Scotch tape (adhesive transparent cellophane tape) held sticky side out, on a wooden tongue depressor. The mounted tape is firmly pressed against the anal margin, covering all sides. The tape is transferred to a glass slide, sticky side down, with a drop of toluene for clearing and examined under the microscope.

The eggs may sometimes be demonstrated in the dirt collected from beneath the finger nails in infected children. The adult worms may sometimes be noticed on the surface of stools. They may occasionally be found crawling out of the anus while the children are asleep. They may be demonstrated in stools collected after an enema.

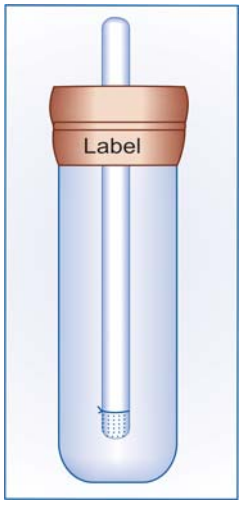


FIGURE 16.3: NIH swab. A piece of transparent cellophane is attached with rubber band to one end of a glass rod which is fixed on a rubber stopper and kept in a wide test tube

Treatment

Several effective drugs are available for the treatment of enterobiasis. Pyrantel, pyrvinium and mebendazole can be used for single dose therapy, while piperazine has to be given daily for one week. It is necessary to repeat the treatment after two weeks to take care of autochthonous infections and ensure elimination of all worms. As pinworm infection usually affects a group, it is advisable to treat the whole family, or group of children, as the case may be.

Control

Health education on personal and community hygiene and group chemotherapy constitute the control measures.

CHAPTER 17

Roundworm

ASCARIS LUMBRICOIDES

History and Distribution

The roundworm, *Ascaris lumbricoides* is the largest nematode parasite in the human intestine. It had been observed and described from very ancient times, when it was sometimes confused with the earthworm. Its specific name *lumbricoides* is derived from this resemblance (*Lumbricus*, meaning earthworm in Latin). It is the most common of human helminths and is distributed worldwide. A billion people are estimated to be infected with roundworms. The individual worm burden could be very high, even up to over a thousand. An editorial in the *Lancet* in 1989 observed that if all the roundworms in all the people worldwide were placed end-to-end they would encircle the world 50 times.

Morphology and Life Cycle

The adult worms live in the small intestines of infected persons. They are large cylindrical worms, with tapering ends, the anterior end being more pointed than the posterior. They are pale pink or flesh coloured when freshly passed in stools, but become white outside the body. The mouth at the anterior end has three finely denticulated lips, one dorsal and two ventro-lateral.

The male measures 15 to 30 cm in length and 2 to 4 mm in thickness. Its posterior end is curved ventrally to form a hook and carries two copulatory spicules. The female is larger, 20 to 40 cm long and 3 to 6 mm thick. Its posterior extremity is straight and conical. The vulva is situated mid-ventrally, near the junction of the anterior and middle thirds of the body. A distinct groove is often seen surrounding the worm at the level of the vulvar opening. This is called the vulvar waist or genital girdle and is believed to facilitate mating (Fig. 17.1). The vulva leads to a single vagina, which branches into a pair of genital tubules that lie convoluted through much of the posterior two thirds of the body. The genital tubules of the gravid worm contain an enormous number of eggs as many as 27 million at a time. A single worm lays up to 200,000 eggs per day. The eggs are passed in faeces.

Two types of eggs are passed by the worms. The fertilised eggs, laid by females inseminated by mating with a male, are embryonated and develop into the infective eggs. The uninseminated female also lays eggs, but these are non-embryonated and cannot become infective. These are called *unfertilised eggs*.

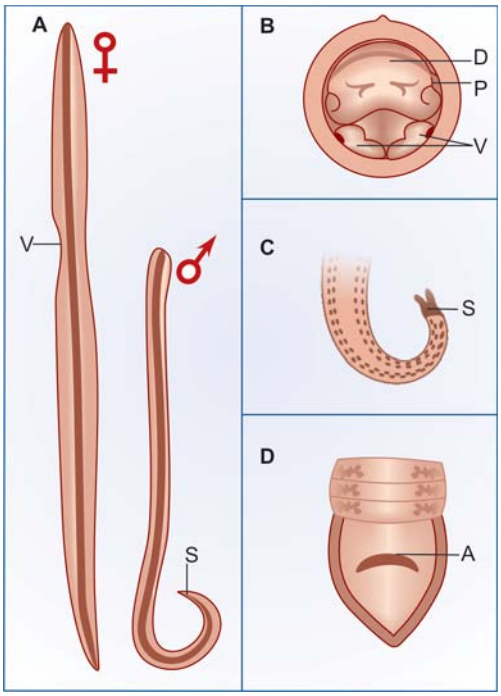


FIGURE 17.1: *Ascaris lumbricoides*. A. Adult male and female worms. Note the vulvar waist (v) in the female and the ventrally curved posterior end in the male with copulatory spicules(s). B. Anterior end of worm. head-on view, showing one dorsal (D) and two ventral. (V) lips, with papillae (P). C. Posterior end of male, showing two protruding copulatory spicules(s). D. Posterior end of female, showing anal opening (A) a little above the conical tip

The fertilised ascaris egg is spherical or ovoid, bile stained to a golden brown colour and measures 60 to 75 µm in length and 40 to 50 µm in breadth. It is enclosed in a stout translucent shell consisting of three layers, the outer coarsely mamillated albuminoid coat a thick transparent middle layer and the inner lipoidal vitelline membrane. Some eggs are found in feces without the outer mamillated coat. They are called the *decorticated eggs*. In the middle of the egg is a large unsegmented ovum, containing a mass of coarse lecithin granules. It nearly fills the egg, except for a clear crescentic area at either pole.

The unfertilised egg is longer, up to 90 µm, and more elliptical. The shell is thinner with the outer mamillary coat scanty and irregular. The ovum is atrophic and contains numerous disorganised, highly refractile granules of various sizes. The unfertilised egg is relatively heavy and does not float in saturated salt solution used for concentration by salt floatation while the fertilised eggs float. Stool samples may show both fertilised and unfertilised eggs, or either type alone (Fig. 17.2).

The fertilised egg passed in feces, is not immediately infective. It has to undergo a period of incubation in soil before acquiring infectivity .The eggs are resistant to adverse conditions and can survive for several years. The development of the

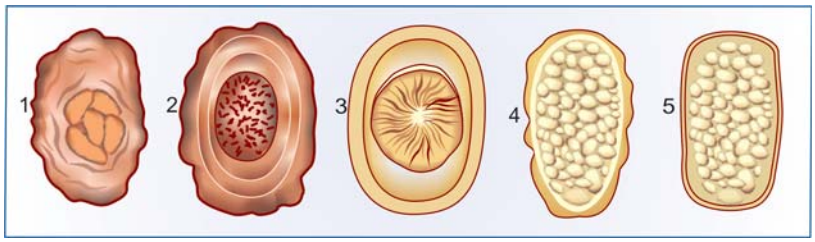


FIGURE 17.2: Types of ascaris eggs found in stools. 1. Fertilised egg surface focus. showing outer mamillary coat. 2. Fertilised egg. median focus. showing unsegmented ovum surrounded by three layers of coats. 3. Decorticated fertilised egg. The mamillary coat is absent. 4. Unfertilised egg. Elongated, with atrophic ovum. 5. Decorticated unfertilised egg

egg in soil depends on the nature of the soil and various environmental factors. A heavy clayey soil and moist shady location, with temperature between 20° and 30°C are optimal for rapid development of the embryo. The development usually takes from 10 to 40 days, during which time the embryo moults twice and becomes the infective rhabditiform larva, coiled up within the egg.

Infection occurs when the egg containing the infective rhabditiform larva is swallowed. A frequent mode of transmission is through fresh vegetables grown in fields manured with human feces ('night soil'). Infection may be transmitted through contaminated drinking water. Children playing about in mud can transmit eggs to their mouth through dirty fingers. Where soil contamination is heavy due to indiscriminate defecation, the eggs sometimes get airborne along with windswept dust and inhaled. The inhaled eggs get swallowed.

When the swallowed eggs reach the duodenum, the larvae hatch out. The rhabditiform larvae, about 250 µm in length and 14 µm in diameter, are actively motile. They penetrate the intestinal mucosa, enter the portal vessels and are carried to the liver. They then pass via the hepatic vein, inferior vena cava and the right heart, and in about four days reach the lungs, where they grow and moult twice. After development in the lungs, in about 10 to 15 days, the larvae pierce the lung capillaries and reach the alveoli. Then they crawl up or are carried up the respiratory passage to the throat and are swallowed. The larvae moult and develop into adults in the upper part of the small intestine. They become sexually mature in about 6 to 12 weeks and the gravid females start laying eggs. to repeat the cycle. The adult worm has a lifespan of 12 to 20 months (Fig. 17.3).

Pathogenesis and Clinical Features

Clinical manifestations in ascariasis can be caused by either the migrating larvae or the adult worms.

The pathogenic effects of larval migration are due to allergic reaction and not the presence of larvae as such. Therefore, the initial exposure to larvae is usually asymptomatic, except when the larval load is very heavy .But when reinfection occurs subsequently there may be intense cellular reaction to the migrating larvae in the

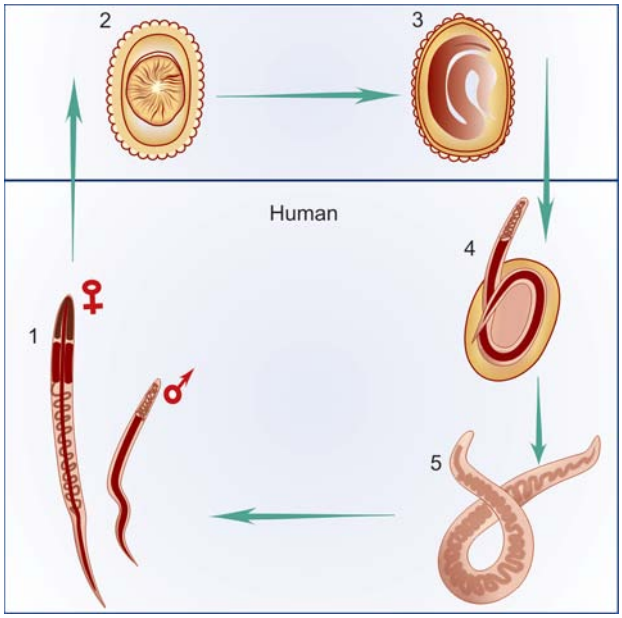


FIGURE 17.3: Life cycle of *Ascaris lumbricoides*. 1. Adult worms in small intestine of man. 2. Egg passed in feces reaches soil. 3. Mature egg containing larva— infective for humans. 4. When swallowed, larva hatches out in duodenum. 5. Rhabditiform larva penetrates gut wall, circulates in blood stream, moults in lung, reaches pharynx and is swallowed to develop into the adult in intestine

lungs, with infiltration of eosinophils, macrophages and epithelioid cells. This ascaris pneumonia is characterised by low grade fever, dry cough, asthmatic wheezing, urticaria, eosinophilia and mottled lung infiltration in the chest radiograph. The sputum may contain Charcot-Leyden crystals. The larvae may occasionally be found in the sputum, but are seen more often in gastric washings. This condition is called *Loeffler's syndrome*. The clinical features generally clear in one or two weeks, though it may sometimes be severe and rarely even fatal. Loeffler's syndrome can also be caused by hypersensitivity to other agents, both living and non-living. Allergic inflammatory reaction to migrating larvae may involve other organs such as the kidney or liver. Very rarely the larvae may occlude a small vessel in the heart or brain.

Clinical manifestations due to adult worm vary from asymptomatic infection to severe and even fatal consequences. It is not unusual to find children apparently unaffected in spite of heavy infestation with the worms. The pathological effects, when present, are caused by (i) Spoliative action, (ii) Toxic action, and (iii) Mechanical effects.

i. The *spoliative* or *nutritional* effects are usually seen when the worm burden is heavy.The worms may be present in enormous numbers, sometimes exceeding 500, in small children, occupying a large part of the intestinal tract. This interferes with proper digestion and absorption of food. Ascariasis may contribute to

protein-energy malnutrition and vitamin A deficiency. Patients have loss of appetite and are often listless. Abnormalities of the jejunal mucosa are often present, including broadening and shortening of villi, elongation of crypts and round cell infiltration of lamina propria. These changes are reversed when the worms are eliminated.

ii. The so called *toxic* effects are due to hypersensitivity to the worm antigens and may be manifested as fever, urticaria, angioneurotic oedema, wheezing and conjunctivitis. These are more often seen in persons who come into contact with the worm occupationally, as in laboratory technicians and abattoir workers (who become sensitive to the pig ascarid *A. suum*), than in children having intestinal infestation.

iii. The mechanical effects are the most important manifestations of ascariasis. Mechanical effects can be due to masses of worms causing luminal occlusion or even a single worm infiltrating into a vital area. The adult worms live in the upper part of the small intestine, where they maintain their position due to their body muscle tone, spanning the lumen.

They may stimulate reflex peristalsis, causing recurrent and often severe colicky pain in the abdomen. The worms may be clumped together into a mass, filling the lumen, leading to volvulus, intussusception or intestinal obstruction.

The worms are restless wanderers apparently showing great inquisitiveness, in that they tend to probe and insinuate themselves into any aperture they find on the way. The wandering is enhanced when the host is ill, particularly when febrile, with temperature above 39°C. The male worm is more responsive to illness of the host, than the female. The worm may wander up or down along the gut. Going up, it may enter the opening of the biliary or pancreatic duct causing acute biliary obstruction or pancreatitis. It may enter the liver parenchyma, where it may lead to abscesses. The worm may go up the oesophagus and come out through the mouth or nose. It may crawl into the trachea and the lung causing respiratory obstruction or lung abscesses. Migrating downwards, the worm may cause obstructive appendicitis. It may lead to peritonitis when it perforates the intestine, generally at weak spots such as typhoid or tuberculous ulcers or through suture lines. This tendency makes preoperative deworming necessary before gastrointestinal surgery in endemic areas. The wandering worm may reach the kidneys, lungs or other organs and cause ectopic lesions.

Diagnosis

In the early stages of infection, when migrating larvae cause Loeffler's syndrome, the diagnosis may be made by demonstrating the larvae in sputum, or more often in gastric washings. Presence of Charcot-Leyden crystals in sputum and an attendant eosinophilia support the diagnosis. At this stage no eggs are seen in feces.

The most important method for the diagnosis of ascariasis is the demonstration of eggs in feces. Ascarides are prolific egg layers. A single female may account

for about 3 eggs per mg of feces. At this concentration, the eggs can be readily seen by microscopic examination of a saline emulsion of feces. Both fertilised and unfertilised eggs are usually present. Occasionally only one type is seen. The fertilised eggs may sometimes appear decorticated. Rarely, when the infestation is light, eggs are demonstrable only by concentration methods. The unfertilised eggs are not detectable by salt floatation. Eggs may not be seen if only male worms are present, as may occasionally be the case. Fecal films often contain many artefacts resembling ascaris eggs and care must be taken to differentiate them.

Sometimes the diagnosis becomes evident when the worm is passed either through the anus, or through the mouth or nose.

A skin test with ascaris antigen gives a positive result, but is unreliable and not used for diagnosis. Serological tests are not useful in diagnosis.

Diagnosis may often be made by barium contrast radiography of the abdomen.

Treatment

Several safe and effective drugs are now available. These include pyrantel pamoate, albendazole, mebendazole and piperazine citrate.

Prevention

Ascariasis can be eliminated only if fecal contamination of soil can be prevented. The ascaris egg is highly resistant. Therefore the use of night soil as manure will lead to spread of the infection unless destruction of the eggs is ensured by proper composting. Treatment of vegetables and other garden crops with water containing iodine 200 ppm for 15 minutes kills the eggs and larvae of ascaris and other helminths.

OTHER ROUNDWORMS

TOXOCARIASIS

Toxocara canis and *T. cati*, natural parasites of dogs and cats respectively can cause aberrant infection in humans leading to *visceral larva migrans* (VLM). Infection is acquired in pups by transmission of larvae transplacentally or lactogenically (through breast milk), but in kittens, only lactogenic transmission is recorded. Older animals are infected by ingestion of mature eggs in soil or of larva by eating infected rodents, birds or other paratenic hosts. Eggs are shed in feces and become infective in 2-3 weeks.

Human infection is by ingestion of eggs. Larvae hatch out in the small intestine, penetrate the mucosa and reach the liver, lungs or other viscera. They do not develop any further. Most infections are asymptomatic, but in some, particularly in young children VLM develops, characterised by fever, hepatomegaly, cough, pulmonary infiltrates, high eosinophilia and hyperglobulinaemia. In some, the eye is affected (*ophthalmic larva migrans*—OLM).

Baylisascaris procyonis, an ascarid parasite of raccoons in North America is known to cause serious zoonotic infections leading to VLM, OLM and *neural larva migrans* (NLM). Complications include blindness and central nervous system lesions ranging from minor neuropsychiatric conditions to seizure, coma and death.

GEOHELMINTHS

Soil-transmitted intestinal nematodes are called Geohelminths. In all of them eggs passed in feces undergo maturation in soil. They are classified into three categories based on their life cycle.

1. *Direct*: Ingested infective eggs directly develop into adults in the intestine, e.g. whipworms.
2. *Modified direct*: Larvae from ingested eggs penetrate intestinal mucosa enter blood stream and through the liver, heart, lungs, bronchus and oesophagus, reach the gut to develop into adults, e.g. roundworms.
3. *Skin penetrating*: Infective larvae in soil penetrate host skin, reach the lung and proceed to the gut as in the modified direct method, e.g. hookworms.

Geohelminths pose a serious health problem in poor countries, particularly among children. Their control requires general measures such as personal hygeine, sanitation and health education, besides provision of diagnostic and treatment facilities.

CHAPTER 18

Filarial Worms

Nematodes belonging to the superfamily Filarioidea are slender thread-like worms (Latin, *filum*—thread) which are transmitted by the bite of blood-sucking insects. In the bodies of infected vertebrate hosts, they occur both as adults and the embryos, which are known as microfilariae. In some species, the microfilariae retain their egg membranes which envelope them as a sheath. These are known as 'sheathed' microfilariae, in contrast to others which rupture their egg membranes and come out as 'unsheathed' or naked microfilariae.

Eight species of filarial worms infect humans, who are the definite hosts. Of them 6 are pathogens—*Wuchereria bancrofti, Brugia malayi*, and *B.timori* cause lymphatic filariasis; *Loa loa* causes calabar swellings and allergic lesions; *Onchocerca volvulus* causes eye lesions and dermatitis; *Mansonella streptocerca* leads to skin diseases; and 2 of them, *Mansonella ozzardi* and *M. perstans* are virtually nonpathogenic. They can be classified according to their sites of election in the body and the characteristics of their microfilariae (Table 18.1, Fig. 18.1).

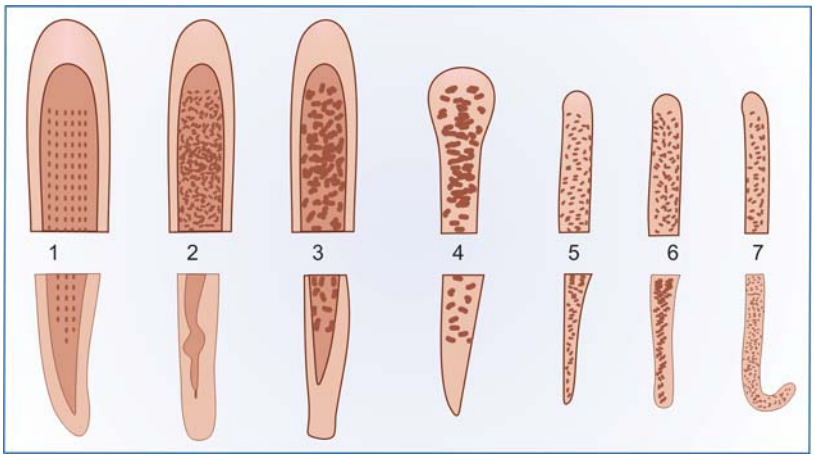


FIGURE 18.1: Head and tail ends of microfilariae found in humans. 1. *Mf. bancrofti.* 2. *Mf. malayi.* 3. *Mf. loa.* 4. *Mf. volvulus* 5. *Mf. ozzardi* 6. *Mf. perstans* 7. *Mf. streptocerca.*

Table 18.1: Filariae infecting humans

Parasite	*Location in body*		*Characteristics of Microfilaria*	*Principal vector*
	Adult	*Microfilaria*		
I. Lymphatic filariasis				
Wuchereria bancrofti	Lymphatics	Blood	Sheathed, pointed tall tip free of nuclei	*Culex quinquefasciatus*
Brugia malayi	Lymphatics	Blood	Sheathed, blunt tail tip with two terminal nuclei	*Mansonia spp*
Brugia timori	Lymphatics	Blood	Sheathed, longer than *Mf. malayi*	*Anopheles barbirostris*
II. Subcutaneous filariasis				
Loa loa	Connective tissue, conjunctiva	Blood	Sheathed, nuclei extending up to pointed tail tip	*Chyrsops spp*
Onchocerca volvulus	Subcutaneous nodules	Skin, eyes	Unsheathed, blunt tail tip free of nuclei	*Simulium spp*
Mansonella streptocerca	Subcutaneous	Skin	Unsheathed: blunt tail tip with nuclei	*Culicoides*
III. Serous cavity filariasis				
Mansonella ozzardi	Peritoneum and pleura	Blood	Unsheathed, pointed tail tip without nuclei	*Culicoides*
Mansonella perstans	Peritoneum and pleura	Blood	Unsheathed, pointed tail tip with nuclei	*Culicoides*

According to the normal habitat of the adult worm, human filarial infections can be classified as follows.

I. Lymphatic filariasis
W.bancrofti
B.malayi
B.timori

II. Subcutaneous filariasis
Loa loa
Onchocerca volvulus
Mansonella streptocerca

III. Serous cavity filariasis
Mansonella ozzardi
Mansonella perstans

Infection with any of the filarial worms may be called filariasis, but traditionally, the term filariasis refers to lymphatic filariasis caused by *Wuchereria* or *Brugia* species.

LYMPHATIC FILARIASIS

WUCHERERIA BANCROFTI

History

Filariasis has been known from antiquity. Elephantiasis had been described in India by Sushrutha (circa 600 BC) and in Persia by Rhazes and Avicenna. The term 'Malabar leg' was applied to the condition by Clarke in 1709 in Cochin.

Microfilaria was first observed by Demarquay (1863) in the hydrocoele fluid of a patient from Havana, Cuba. The genus is named after Wucherer, a Brazilian physician who reported microfilariae in chylous urine in 1868. Microfilaria was first demonstrated in human blood in Calcutta by Lewis (1872), who called it Filaria sanguinis hominis. The female adult worm was described by Bancroft (1876) in Brisbane, Australia and the male worm by Bourne (1888). Manson (1878) in China identified the Culex mosquito as the vector. This was the first discovery of insect transmission of a human disease. Manson (1879) also demonstrated the nocturnal periodicity of microfilariae in peripheral blood.

Distribution

W. bancrofti is distributed widely in the tropics and subtropics of Asia, Africa and South America (Fig. 18.2). Over 900 million persons live in areas endemic for lymphatic filariasis and are therefore at risk of infection. In 1999, over 90 million persons were estimated to be infected, with or without clinical manifestations—over 81 million with *Wuchereria* and over 8 million with *Brugia*.

The largest number of cases of filariasis occur in India, where over 300 million people live in endemic zones. It is estimated that at least 6 million attacks of acute filarial disease occur every year in India and that over 15 million persons have chronic filarial disease. The endemic areas are mainly along the sea coast and along the

banks of the large rivers, though infection occurs virtually in all states, except in the North West.

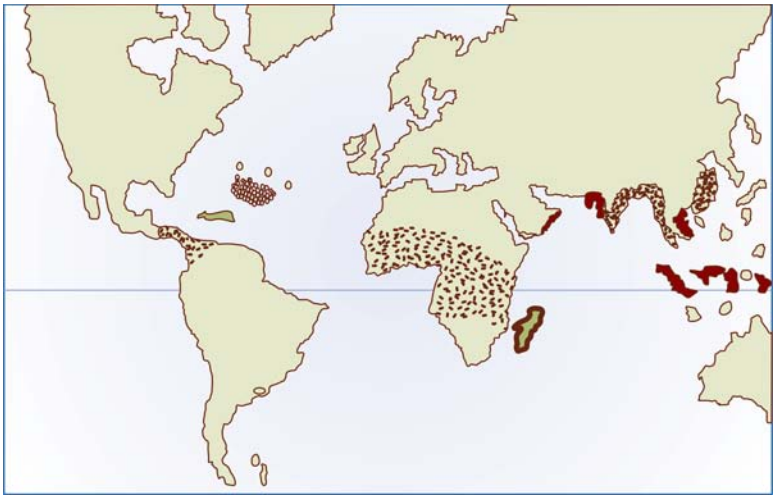


FIGURE 18.2: Geographical distribution of *Wuchereria bancrofti*

Morphology and Life Cycle

The adults are whitish, translucent, thread-like worms with smooth cuticle and tapering ends. The female is larger (70-100 × 0.25 mm) than the male (25-40 × 0.1 mm). Males and females remain coiled together usually in the abdominal and inguinal lymphatics and in the testicular tissues. The adult worms live for many years, probably 10 to 15 years or more.

The worm is ovoviviparous. The embryo (microfilaria) is released encased in its elongated egg-shell, which persists as a *sheath*. The microfilaria has a colourless. translucent body with a *blunt head* and *pointed tail*. It measures 250 to 300 µm in length and 6 to 10 µm in thickness. It is actively motile and can move forwards and backwards within the sheath, which is much longer than the embryo (Fig. 18.3).

When stained with Leishman or other Romanowsky stains, structural details can be made out. Along the central axis of the microfilaria can be seen a column of granules, which are called *somatic cells* or *nuclei*. The granules are absent at certain specific locations—a feature which helps in the identification of the species. The specific locations are the following.

a. At the head end is a clear space devoid of granules, called the *cephalic space*. In *Microfilaria bancrofti*, the cephalic space is as long as it is broad while in *M. malayi*, it is longer than its breadth. With vital stains a *stylet* can be demonstrated projecting from the cephalic space.
b. In the anterior half of the microfilaria, is an oblique area devoid of granules called the *nerve ring*.
c. Approximately midway along the length of the microfilaria is the *anterior V-spot* which represents the rudimentary excretory system.

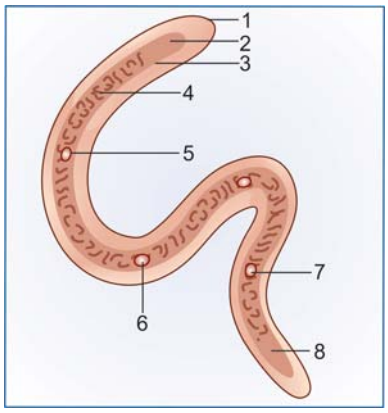


FIGURE 18.3: Morphology of *Microfilaria bancrofti*. 1. Sheath; 2. Stylet; 3. Cephalic space; 4. Nuclei; 5. Nerve ring; 6. Anterior V-spot; 7. Posterior V-spot; 8. Tail

d. The *posterior V-spot* (Tail-spot) represents the cloaca or anal pore.
e. The genital cells (G-cells) situated anterior to the anal pore.
f. The internal (central) body of Manson extending from the anterior V-spot to G-cell 1, representing the rudimentary ailmentary system.
g. The tail tip, devoid of nuclei in *Mf.bancrofti*, bears two distinct nuclei in *Mf. malayi*

The microfilariae circulate in the blood stream. In India, China and many other Asian countries. They show a nocturnal periodicity in peripheral circulation, being seen in large numbers in peripheral blood only at night, between 10 pm and 4 am. This correlates with the night biting habit of the vector mosquito. Periodicity may also be related to the sleeping habits of the hosts. It has been reported that if the sleeping habits of the hosts are reversed, over a period, the microfilariae change their periodicity from nocturnal to diurnal. Nocturnal periodic microfilariae are believed to spend the day time mainly in the capillaries of the lung and kidneys or in the heart and great vessels. In the Pacific islands and some parts of the Malaysian archipelago, the microfilariae are non-periodic or diurnal subperiodic, in that they occur in peripheral circulation at all times, with a slight peak during the late afternoon or evening. This is related to the day biting habits of the local vector mosquitoes. (Some authors separate the subperiodic Pacific type of *W. bancrofti* as a distinct species designated *W. pacifica*, but this is not widely accepted).

Humans are the definitive host. No animal host or reservoir is known for *W. bancrofti*. The intermediate host is the female mosquito, different species acting as vectors in different geographic areas. The major vector in India and most other parts of Asia is *Culex quinquefasciatus (C.fatigans).*

Microfilariae do not multiply or undergo any further development in the human body. If they are not taken up by a female vector mosquito, they die. Their lifespan is believed to be about 2 to 3 months. It is estimated that a microfilarial density of at least 15 per drop of blood is necessary for infecting mosquitoes. Densities of 20,000 microfilariae or more per ml of blood may be seen in some carriers.

When a vector mosquito feeds on a carrier, the microfilariae are taken in with the blood meal and reach the stomach of the mosquito. Within 2 to 6 hours, they

cast off their sheaths (*exsheathing*), penetrate the stomach wall and within 4 to 17 hours migrate to the thoracic muscles where they undergo further development. During the next 2 days, they metamorphose into the first-stage larva which is a sausage-shaped form with a spiky tail, measuring 125-250 × 10-15 µm. Within a week, it moults once or twice, increases in size and becomes the second-stage larvae, measuring 225-325 × 15-30 µm. In another week, it develops its internal structures and becomes the elongated third-stage filariform larva, measuring 1500-2000 × 15-25 µm. It is actively motile. This is the infective larva. It enters the proboscis sheath of the mosquito, awaiting opportunity for infecting humans on whom the mosquito feeds.

There is no multiplication of the microfilaria in the mosquito and one microfilaria develops into one infective larva only. The time taken from the entry of the microfilaria into the mosquito till the development of the infective third-stage larva located in its proboscis sheath, constitutes the *extrinsic incuation period*. Its duration varies with environmental factors such as temperature and humidity as well as with the vector species. Under optimal conditions, its duration is 10 to 20 days.

When a mosquito with infective larvae in its proboscis feeds on a person, the larvae get deposited, usually in pairs, on the skin near the puncture site.

The larvae enter through the puncture wound or penetrate the skin by themselves. The infective dose for man is not known, but many larvae fail to penetrate the skin by themselves and many more are destroyed in the tissues by immunological and other defence mechanisms. A very large number of infected mosquito bites are required to ensure transmission to man, perhaps as many as 15,000 infective bites per person.

After penetrating the skin, the third-stage larvae enter the lymphatic vessels and are carried usually to abdominal or inguinal lymph nodes, where they develop into adult forms. There is no multiplication at this stage and only one adult develops from one larva male or female. They become sexually mature in about 6 months and mate. The gravid female worm releases large numbers of microfilariae, as many as 50.000 per day. They pass through the thoracic duct and pulmonary capillaries to the peripheral circulation (Fig. 18.4).

The period from the entry of the infective third-stage larvae into the human host. till the first appearance of microfilariae in circulation is called the biological incubation period or the prepatent period. This is usually about 8 to 12 months. The period from the entry of the infective larvae, till the development of the earliest clinical manifestation is called the clinical incubation period. This is very variable, but is usually 8 to 16 months, though it may often be very much longer.

Pathogenesis

The outcome of filarial infection varies in different persons. In endemic areas, infection may be entirely asymptomatic in most persons. Carriers may have very high microfilarial density in peripheral blood (20,000 per ml or more) without any ill effects. Such persons appear to tolerate microfilariae, the immune response being

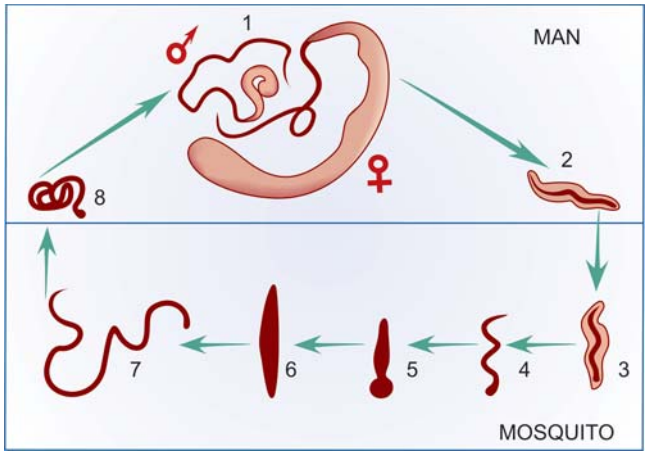


FIGURE 18.4: Life cycle of *W. bancrofti*. 1. Adult male and female in lymph node; 2. Microfilaria in peripheral capillaries at night. 3. Microfilaria ingested by mosquito reaches its stomach where it; 4. Sheds its sheath, penetrates gut wall and enters thoracic muscles where it develops into; 5. Short first-stage larva; 6. Second-stage larva and; 7. Infective third-stage larva which lies in the proboscis sheath. When the mosquito bites a person, it is deposited on the skin; 8. Penetrates, reaches lymphatics and develops into adult

inhibited by antigen-specific suppressor cells or other suppressor factors. On the other hand, in persons coming to endemic areas from places where filariasis is absent, infection may cause early clinical manifestations such as lymphangitis and lymphadenitis. They mount an immune response against the infection, so that microfilariae may not be demonstrable in them.

The infective larvae that enter the human body through mosquito bite migrate in the lymphatics and moult, during which they release their body proteins, secretions and other products. In some persons these may cause irritation, directly or due to hypersensitivity or other immunological inflammation.

Immune reactions are more common when the worms become adults. The typical manifestations of filariasis are caused by the adult worms blocking lymph nodes and vessels, either mechanically or more commonly due to allergic inflammatory reactions to worm antigens and secretions. The affected lymph nodes and vessels are infiltrated with macrophages, eosinophils, lymphocytes and plasma cells, and show endothelial hyperplasia. The vessel walls get thickened and the lumen narrowed or occluded, leading to lymph stasis and dilatation of lymph vessels. The worms inside lymph nodes and vessels may cause granuloma formation, with subsequent scarring and even calcifiation. Inflammatory changes damage the valves in lymph vessels, further aggravating lymph stasis. Increased permeability of lymph vessel walls leads to leakage of protein-rich lymph into the tissues. This produces the typical hard pitting or brawny oedema of filariasis. Fibroblasts invade the oedematous tissues, laying down fibrous tissue, producing the non-pitting gross oedema of elephantiasis. Recurrent secondary bacterial infections cause further damage.

Animal models have been developed, such as experimental filarial infection in cats with *Brugia pahangi* or *Br. malayi.* These have helped in understanding the pathogenesis of the disease, but in cats and other animals, filarial infection does not cause elephantiasis. Elephantiasis is a feature unique to human filariasis, apparently caused by human erect posture and consequent hydrodynamic factors affecting lymph flow.

In some persons, immune reactions to filarial antigens may produce clinical conditions unrelated to the lymphatic lesions described above. In these, microfilariae are not demonstrable in blood. These are known as *occult filariasis*.

Clinical Manifestations

Filariasis leads to a wide spectrum of clinical manifestations, ranging from carrier state with no evident disease to chronic incapacitating illness. Filariasis does not kill, but may cause great suffering, disfiguration and disability.

The earliest manifestations are seen during the stage of 'invasion', when the infective larvae enter the body and undergo development. In some persons, hypersensitivity to the antigens of the larvae causes constitutional symptoms such as malaise, headache, nausea, vomiting and low grade fever. Recurrent attacks of pruritus and urticaria may occur. Some develop 'fugitive swellings'—raised, painless, tender, diffuse, red areas on the skin, commonly seen on the limbs. These disappear spontaneously after a few days, but may reappear at the same or different sites.

The characteristic manifestations of filariasis are due to obstruction of lymph vessels and nodes. The essential features are lymphadenopathy, lymphangitis, lymphangiovarix, lymphorrhagia or chylorrhagia, hydrocoele, lymphoedema and elephantiasis. Depending on the sites affected, the clinical presentations vary.

Lymphadenitis

Repeated episodes of acute lymphadenitis with fever occur very frequently. The inguinal nodes are most often affected, and axillary nodes less commonly. The swollen nodes may be painful and tender

Lymphangitis

The acutely inflamed lymph vessels may be seen as red streaks underneath the skin. Lymphatics of the testes and spermatic cord are frequently involved, with epididymo-orchitis and funiculitis. Acute lymphangitis is usually caused by allergic or inflammatory reaction to filarial infection, but may often be associated with streptococcal infection also.

Filarial Fever

High fever of sudden onset, often with rigor, lasting for two or three days is the typical picture. This occurs repeatedly at intervals of weeks or months. It is accompanied by lymphangitis and lymphadenitis, with resultant lymphoedema. When

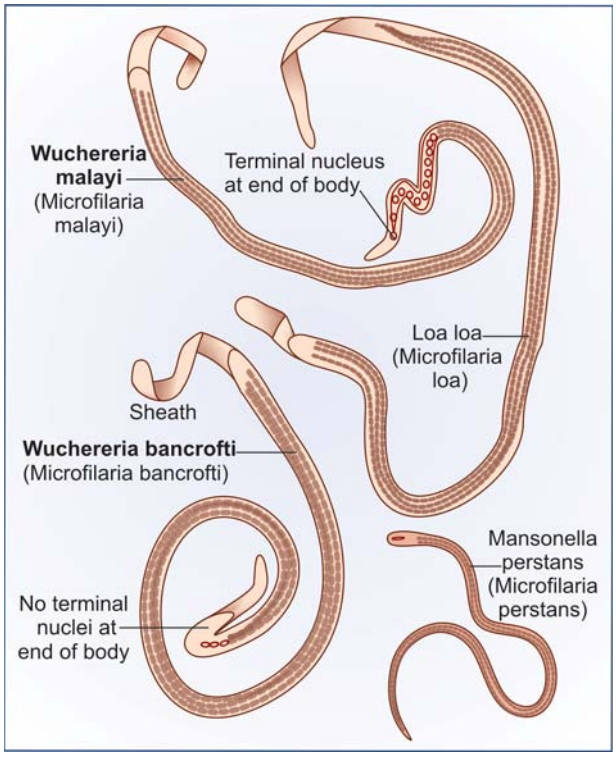


FIGURE 18.5: Microfilaria from human blood.
Thick drop, haematoxylin staining
Magn. x 700

the lymph nodes affected are intra-abdominal and hence not noticeable, the diagnosis may be difficult.

Lymphangiovarix

Dilatation of lymph vessels commonly occur in the inguinal, scrotal, testicular and abdominal sites.

Lymphorrhagia

Rupture of lymph varices leads to the release of lymph or chyle. The clinical picture depends on the sites involved and include lymph scrotum, lymphocoele, chyluria, chylous diarrhoea, chylous ascites and chylothorax.

Hydrocoele

This is a very common manifestation of filariasis. Accumulation of fluid occurs due to obstruction of lymph vessels of the spermatic cord and also by exudation from the inflamed testes and epididymis. The fluid is usually clear and straw coloured,

but may sometimes be cloudy, milky or haemorrhagic. The hydrocoele may be unilateral or bilateral and is generally small in size in the early stage, but may occasionally assume enormous proportions in association with elephantiasis of the scrotum. The largest reported hydrocoele weighed over 100 kilograms.

Lymphoedema

This follows successive attacks of lymphangitis and usually starts as swelling around the ankle, spreading to the back of the foot and leg. It may also affect the arms, breast, scrotum, vulva or any other part of the body. Initially the oedema is pitting in nature, but in course of time becomes hard and nonpitting.

Elephantiasis

This is a delayed sequel to repeated lymphangitis, obstruction and lymphoedema. Lymph exudate accumulating in the region stimulates connective tissue hypertrophy and hyperplasia. The part gets grossly enlarged and misshapen. The skin surface becomes coarse, with warty excrescences. Cracks and fissures develop with secondary bacterial infection. Elephantiasis is seen most commonly in the leg, but may also involve other parts of the body including the arm, breast, scrotum, penis and vulva.

Occult Filariasis

This term is applied to clinical conditions not directly due to lymphatic involvement, but to hypersensitivity reactions to filarial antigens. Here microfilariae are not seen in blood but may be present at the affected sites. The condition may be caused by Wuchereria, Brugia or by some animal filaria also.

The best studied syndrome of occult filariasis is *Tropical Pulmonary Eosinophilia*, which presents with low grade fever,loss of weight, anorexia and pulmonary symptoms such as dry nocturnal cough, dyspnoea and asthmatic wheezing. Blood eosinophil count is above 3000 per cmm and may even go up to 50,000 or more. IgG levels are elevated. Chest radiography shows mottled shadows resembling miliary tuberculosis. Young adults are more commonly affected. There is considerable geographical difference in its incidence, which is probably genetically conditioned. Microfilariae are not usually detectable in blood, but lung biopsies have shown microfilariae in some cases. It has been suggested that in these cases, there is a failure in the suppression of immune response to microfilarial antigens, so that microfilariae are filtered out and destroyed in the lungs, with allergic inflammatory reaction. Serological tests with filarial antigens are usually strongly positive. Nonspecific antibody production occurs and biological false-positive reactions are often seen in serological tests for syphilis. Prompt response to DEC confirms the diagnosis.

Occult filariasis has also been reported to cause arthritis, glomerulonephritis, thrombophlebitis, tenosynovitis and dermatoses. Endomyocardial fibrosis has been claimed to be associated with filariasis, but the relationship has not been proven.

Diagnosis

The diagnosis of filariasis depends on the clinical features, history of exposure in endemic areas and on laboratory findings.

The laboratory tests that can be used for diagnosis include the following:

a. Demonstration of microfilaria in peripheral blood. Microfilaria may also be detected in other specimens such as chylous urine or hydrocoele fluid. Sometimes it can be seen in biopsy specimens.
b. Demonstration of the adult worm in biopsy specimens.
c. Skin tests with filarial antigens.
d. Demonstration of antibody to filarial antigens by serological tests.
e. Demonstration of filarial antigens in blood by serological tests.
f. Indirect evidence such as eosinophilia.

Demonstration of microfilaria in the peripheral blood is the diagnostic test most commonly employed. It is also the method used for carrier surveys. It has also the advantage that the species of the infecting filaria can be identified from the morphology of the microfilaria seen. In India and other areas where the prevalent filarial species is nocturnal periodic, 'night blood' samples are collected between 10 pm and 4 am. Microfilaria can be demonstrated in unstained as well as stained preparations.

Unstained Film

From a finger prick, two or three drops of blood are collected on a clean glass slide, a cover slip applied and sealed with vaseline. Examination under the low power microscope will show the actively motile microfilariae lashing the blood cells around. The examination may be conveniently made the next morning as microfilariae retain their viability and motility for a day or two at room temperature.

Stained Film

A 'thick and thin' blood smear is prepared on a clean glass slide and dried, The thick part of the smear is dehaemoglobinised by applying distilled water. The smear is fixed in methanol and stained with Giemsa, Leishman or polychrome methylene blue stains. Microfilariae may be seen under the low power microscope in the thick film. Their morphology can be studied in the thin film.

By using a micropipette for taking a known quantity of blood (20 to 60 cu mm) for preparing the smear and counting the number of microfilariae in the entire stained smear, microfilaria counts can be obtained.

Concentration Techniques

When the microfilaria density is low, examination of large volumes of blood, 1 ml or more, gives more positive results. Concentration techniques are used for this purpose. In the *sedimentation* methods, blood is obtained by venepuncture, the red

cells lysed and the microfllariae concentrated by centrifugation. In the *filtration* methods used at present larger volumes of blood, up to 5 ml can be filtered through millipore or nucleopore membranes. The membranes may be examined as such or after staining, for microfilariae. The filter membrane technique is much more sensitive so that blood can be collected even during day time for screening. The disadvantages of the technique are the cost and the need for venepuncture.

DEC Provocation Test

A small dose of diethyl carbamazine (2 mg per kg body weight) induces microfilariae to appear in peripheral blood even during day time. For surveys, blood samples can be collected 20 to 50 minutes after the administration of one 100 mg tablet of DEC to adults.

Microfilaria may be demonstrated in centrifuged deposits of lymph, chylous urine or other appropriate specimens. Adult filarial worms can be seen in sections of biopsied lymph nodes, but this is not employed in routine diagnosis.

Intradermal injection of filarial antigens (extracts of microfilariae, adult worms and third-stage larvae of *Br.malayi* or of the dog filaria *Dirofilaria immitis* induces an immediate hypersensitivity reaction. But the diagnostic value of the skin test is very limited due to the high rate of false-positive and negative reactions.

Several serological tests, including complement fixation, indirect haemagglutination, indirect fluorescent antibody, immunodiffusion and immunoenzyme tests have been described. But the tests available now are not sufficiently sensitive or specific to be used either for individual diagnosis or surveys. Highly sensitive techniques are now being tried for detection of filarial antigens in blood. These hold promise.

Prevention and Control

The two major measures in prevention and control of filariasis are eradication of the vector mosquito and detection and treatment of carriers. The recommended teatment is diethyl carbamazine (DEC) 6 mg per kg body weight daily for 12 days, the drug being given for 2 weeks, 6 days in a week. The treatment may have to be repeated in endemic areas, every 2 years or so. Mass chemotherpay has been tried, but it may pose difficulties in large endemic areas such as India. As DEC is non-toxic, it can be safely administered in combination with food items such as common salt.

Treatment

DEC is the drug of choice. It is actively microfilaricidal, and in large enough doses may be fatal to adult worms also. Allergic reactions may occur due to the release of antigens from the large numbers of microfilariae which die on administration of the drug.

BRUGIA MALAYI

The genus Brugia was named after Brug, who in 1927 described a new type of microfilaria in the blood of natives in Sumatra. The adult worm of *B.malayi* was described by Rao and Maplestone in India (1940). Besides *B. malayi*, the genus includes *B. timori*, which parasitises humans in Timor, Indonesia and a number of animal species, such as *B. pahangi* and *B. patei* infecting dogs and cats.

The geographical distribution of *B.malayi* is much more restricted than that of *W. bancrofti*. It occurs in India and Far East, Indonesia, Philippines, Malaysia, Thailand, Vietnam, China, South Korea and Japan. In India, Kerala is the largest endemic area, particularly the districts of Quilon, Alleppey, Kottayam, Ernakulam and Trichur. Endemic pockets occur in Assam, Orissa, Madhya Pradesh and West Bengal. *B.malayi* and *W. bancrofti* may be present together in the same endemic area, as in Kerala. In such places, *B. malayi* tends to be predominantly rural and *W. bancrofti* urban in distribution (Fig. 18.6).

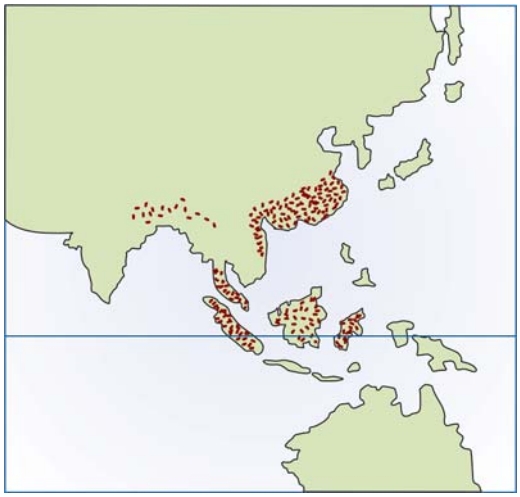


FIGURE 18.6: Geographical distribution of *Brugia malayi*

The adult worms of *B.malayi* are generally similar to those of *W.bancrofti*, though smaller in size. The microfilariae are, however, different in a number of respects, *Mf.malayi* is smaller in size; shows kinks and secondary curves; its cephalic space is longer; carries double stylets at the anterior end; the nuclear column appears blurred in Giemsa-stained films; and the tail tip carries two distinct nuclei, one terminal and the other subterminal (Table 18.2, Fig. 18.7).

BRUGIA TIMORI

Br. timori is limited to Timor and some other islands of eastern Indonesia. The vector is *Anopheles barbirostris* a night feeder. No animal reservoir is known. The microfilaria is larger than *Mf. malayi*. The sheath of *Mf. timori* fails to take Giemsa stain.

Table 18.2: Distinguishing features of *Mf. bancrofti* and *Mf. malayi*

Features	*Mf. bancrofti*	*Mf. malayi*
Length	250 to 300 µm	175 to 230 µm
Appearance	Graceful, sweeping curves	Kinky, with secondary curves
Cephalic space	Length and breadth equal	Almost twice as long as broad
Stylet at enterior end	Single	Double
Excretory pore	Not prominent	Prominent
Nuclear column	Discrete nuclei	Blurred
Tail tip	Pointed: free of nuclei	Two distinct nuclei, one at tip, the other subterminal
Sheath	Faintly stained	Well-stained

The lesions produced by *B.timori* are milder than those of bancroftian or malayan filariasis. A characteristic lesion is the development of draining abscesses caused by worms in lymph nodes and vessels along the saphenous vein, leading to scarring.

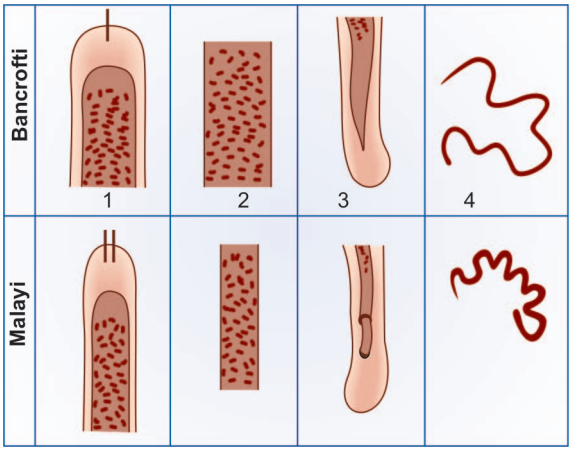


FIGURE 18.7: Differentiating features between *Mf. bancrofti* and *Mf. malayi*. 1. Cephalic space as long as broad and carries one stylet in bancrofti. It is longer than broad and carries two stylets in malayi. 2. Body nuclei round, distinct, well-separated in bancrofti. They are angular, blurred and squeezed together in malayi. 3. No nuclei in tail tip in bancrofti. Two widely spaced nuclei at tail tip in malayi. 4. Body curves large, regular, smooth in bancrofti. Several small, irregular, angular kinks in malayi .

SUBCUTANEOUS FILARIASIS

LOA LOA

Loa loa known also as the 'African eye worm' or the worm causing loiasis, 'fugitive swellings' or 'calabar swellings', was first detected in the eye of a patient in West Indies in 1770. But at present, it is limited to its primary endemic areas in the forests of West and Central Africa, where about 10 million people are affected.

The adult worm measures about 30 to 70 mm in length and 0.3 to 0.5 mm in thickness. In infected persons, they live in the subcutaneous tissues, through which they wander. The microfilariae are sheathed. They appear in peripheral circulation only during the day (diurnal periodic). The vectors are day biting flies of the genus *Chrysops*, in which the microfilariae develop into the infective third-stge larvae. Infection is transmitted through the bite of infected Chyrysops. Natural infection is seen in some African monkeys.

The pathogenesis of loiasis depends on the migratory habit of the adult worm. Their wanderings through subcutaneous tissues set up temporary foci of inflammation, which appear as swellings, of up to 3 cm in size. These are the *calabar swellings*. They are called *fugitive swellings*, because they disappear in a few days, only to reappear elsewhere. Ocular manifestations occur when the worm reaches the subconjunctival tissues during its wanderings. The ocular lesions include granulomata in the bulbar conjunctiva, painless oedema of the eyelids and proptosis.

Diagnosis rests on the appearance of fugitive swelling in persons exposed to infection in endemic area. The adult worm can be demonstrated by removal from the skin or conjunctiva. Microfilariae may be shown in peripheral blood collected during the day. High eosinophil count is common.

Treatment is by surgical removal of the adult worms when they come to accessible sites. DEC is active against the worm, but has to be used with caution as severe adverse reactions may develop following the sudden death of large numbers of microfilariae. Simultaneous administration of corticosteroids minimises such reactions.

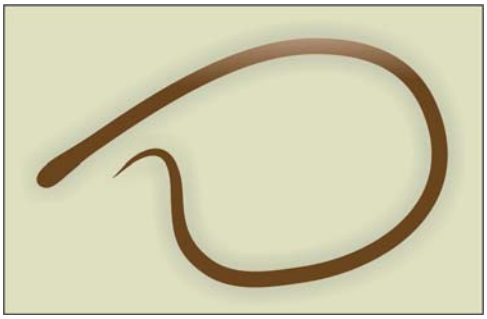


FIGURE 18.8: *Onchocerca volvulus*

ONCHOCERCA VOLVULUS

History and Distribution

Onchocerca volvulus, the 'convoluted filaria', or the 'blinding filaria' producing onchocerciasis or *'river blindness'* was first described by Leuckart in 1893. It affects about 40 million people, mainly in tropical Africa, but also in Central and South America. A small focus of infection exists in Yemen and south Arabia. Onchocerciasis is the second major cause of blindness in the world.

Morphology and Life Cycle

The adult worms are seen in nodules in subcutaneous connective tissues of infected persons. The worms are whitish, opalescent, with transverse striations on the cuticle. The posterior end is curved, hence the name Onchocerca, which means 'curved tail'. The male measures about 30 mm in length and 0.15 mm in thickness, and the female 50 cm by 0.4 mm. The microfilariae are unsheathed and non-periodic. They measure about 300 by 0.8 µm. The microfilaria are found typically in the skin and subcutaneous lymphatics in the vicinity of parent worms. They may also be found in the conjunctiva and rarely in peripheral blood (Fig. 18.8).

Humans are the only definitive host. Day-biting female black flies of the genus *Simulium* are the intermediate hosts. They are 'pool feeders' and suck in blood and tissue fluids. Microfilariae from the skin and lymphatics are ingested and develop within the vector, becoming the infective third-stage larvae, which migrate to its mouth parts. The extrinsic incubation period is about 6 days. Infection is transmitted when an infected Simulium bites a person. The prepatent period in man is 3 to 15 months. The adult worm lives in the human host for about 15 years and the microfilariae for about 1 year.

The vector Simulium species breed in 'fast-flowing rivers, and, therefore, the disease is most common along the course of rivers. Hence, the name 'river blindness'.

Pathogenicity

Pathogenesis depends on the host's allergic and inflammatory reactions to the adult worm and microfilariae. The infective larvae deposited in the skin by the bite of the vector develop at the site to adult worms. Adult worms are seen singly, in pairs or in tangled masses in subcutaneous tissues. They may occur in the subcutaneous nodules or free in the tissues. The subcutaneous nodule or *onchocercoma* is a circumscribed, firm, non-tender tumour formed as a result of fibroblastic reaction around the worms. Nodules vary in size from a few mm to about 10 cm. They tend to occur over anatomical sites where the bones are superficial, such as the scalp, scapulae, ribs, elbows, iliac crest, sacrum and knees. The nodules are painless and cause no trouble except for their unsightly appearance.

Microfilariae cause lesions in the skin and eyes. The skin lesion is a dermatitis with pruritus, pigmentation, atrophy and fibrosis. Ocular manifestations range from photophobia to gradual blurring of vision, progressing to total blindness. Ocular lesions include punctate or sclerosing keratitis, iridocyclitis, secondary glaucoma, choroidoretinitis and optic atrophy.

Diagnosis

The microfilariae may be demonstrated by slicing off a sliver of skin, which is placed on a slide in water or saline. The specimen is best collected around midday. Microfilariae may also be shown in aspirated material from subcutaneous nodules. In patients with ocular manifestations, microfilariae may be found in conjunctival biopsies.

Prevention

In 1974, WHO launched a control programme in West Africa using aerial larvicide for vector control and treatment of patients with ivermectin. This is believed to have prevented blindness in millions of children.

Treatment

Enucleation of nodules may reduce the worm burden, but cannot eliminate the infection. DEC and suramin have been used. DEC destroys microfilariae, but usually causes an intense reaction (*Mazzotti reaction*) consisting of pruritus, rash, lymphadenopathy, fever, hypotension and occasionally eye damage. Ivermectin is the drug of choice.

MANSONELLA STREPTOCERCA

Also known as *Acanthocheilonema, Dipetalonema* or *Tetrapetalonema streptocerca*, this worm is seen only in West Africa. The adult worms live in the dermis, just under the skin surface. The unsheathed microfiliariae are found in the skin. Culicoides species are the vectors. Chimpanzees may act as reservoir hosts. Infection may cause dermatitis with pruritus and hypopigmented macules. Diagnosis is made by demonstration of the microfilariae in skin clippings. DEC is effective in treatment.

SEROUS CAVITY FILARIASIS

MANSONELLA OZZARDI

Mansonella ozzardi is a New World filaria seen only in Central and South America and the West Indies. The adult worms are found in the peritoneal and pleural cavities of humans. The non-periodic unsheathed microfilariae are found in the blood. Culicoides species are the vectors. The infection is found mainly in isolated populations of Amerindians. Infection does not cause any illness. Diagnosis is made by demonstrating microfilariae in blood. No treatment is available.

MANSONELLA PERSTANS

Also known as *Acanthocheilonema, Dipetalonema* or *Tetrapetalonema perstans*, this worm is extensively distributed in tropical Africa and coastal South America. The adult worms live in the body cavities of humans, mainly in peritoneum, less often in pleura and rarely in pericardium. The microfilariae are unsheathed and subperiodic. Vectors are Culicoides species. African primates have been reported to act as reservoir hosts. Infection is generally asymptomatic, though it has been claimed that it causes transient abdominal pain, rashes and malaise. Diagnosis is by demonstration of the microfilariae in peripheral blood. Mebendazole has been reported to be more successful than DEC in treatment.

ZOONOTIC FILARIASIS

Filariae naturally parasitic in domestic and wild animals may rarely cause accidental infection in man, through the bite of their vectors. In such zoonotic filariasis, the infective larvae develop into adults, but do not mature to produce microfilariae. The worm dies and the inflammatory reaction around the dead worm usually causes clinical manifestations.

Brugia pahangi, a parasite of dogs and cats in Malaysia may infect man and cause lymphangitis and lymphadenitis.

Dirofilaria immitis the dog 'heart-worm' is a common parasite of dogs, widely distributed in the tropics and subtropics. When humans get infected, the worm lodges in the right heart or branches of the pulmonary artery .The dead worm becomes an embolus blocking a small branch of the pulmonary artery , producing a pulmonary infarct. The healed infarct may appear as a 'coin lesion' on chest radiography and can be mistaken for malignancy.

Dirofilaria repens a natural parasite of dogs may sometimes infect humans, causing subcutaneous and subconjunctival nodules. Many Dirofilaria species may form nodules in human conjunctiva and are collectively called , *'Dirofilaria conjunctivae.'*

CHAPTER 19

Guinea Worm

DRACUNCULUS MEDINENSIS

History

The guinea worm has been known from antiquity. It is believed to have been the 'fiery serpent' in the Bible, which tormented the Israelites on the banks of the Red Sea. The technique of extracting the worm by twisting it on a stick, still practised by patients in endemic areas is said to have been devised by Moses. The picture of the 'serpent worm' on a stick may have given rise to the physician's symbol of caduceus. Galen named the disease dracontiasis, (Greek *draco*-dragon or serpent). Avicenna called it the Medina worm as it was prevalent there. Hence, the name *Dracunculus medinensis* (Dracunculus being the diminutive of Draco).

Distribution

The worm was present in tropical Africa, the Middle East in Arabia, Iraq, Iran, and in Pakistan and India. In India, it was seen in the dry areas in Rajasthan, Gujarat, Madhya Pradesh, Andhra, Maharashtra, Tamil Nadu and Karnataka. About 50 million people were estimated to be infected with the worm (Fig. 19.1). The infection has been eradicated from India and all of South East Asia region by 2000.

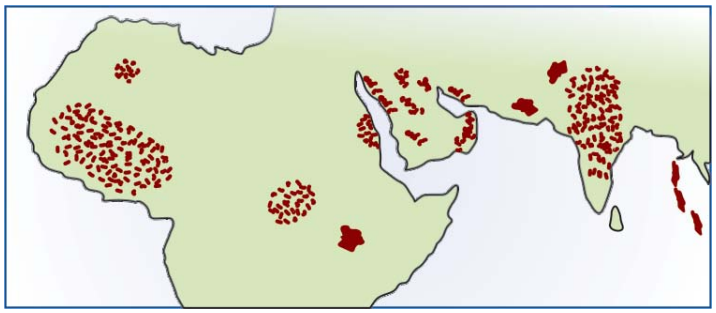


FIGURE 19.1: Geographical distribution of guinea worm infection (before its eradication)

Morphology

The adult female is a long, cylindrical worm with smooth cuticle resembling a long piece of white twine. It has a blunt anterior end and a tapering recurved tail. It measures about a metre (60 to 120 cm) in length and 1 to 2 mm in thickness. The body of the gravid female is virtualy filled with the branches of an enormous uterus containing some 3 million embryos. The male worm, which is but rarely seen, is much smaller, 10 to 40 mm long and 0.4 mm thick.

The larva measures 500 to 750 µm in length and 15 to 25 µm in breadth. It has a broad anterior end and a slender filiform tail which extends for a third of the entire body length. The cuticle shows prominent striations. The larva swims about with a coiling and uncoiling motion.

Biology and Life Cycle

Humans are the definitive host. There is no animal reservoir. The adult worm, which is viviparous discharges larvae, which are ingested by the fresh water crustacean *cyclops* the intermediate host. Humans get infected by drinking unfiltered water containing infective cyclops. The infective form is the third-stage larva present in the haemocoele of infected cyclops.

When water containing infective cyclops is swallowed, the cyclops is killed by the gastric acidity and the guinea worm larvae present in its haemocoele are released. The larvae penetrate the wall of the duodenum and reach the retroperitoneal and subcutaneous connective tissues. Here the larvae develop into male and female adults in about 3 to 4 months and mate. After mating, the male worms die in the tissues and sometimes become calcified. The fertilised female worm grows in size and migrates within the connective tissues and along fascial planes. After about 6 months it reaches a site where it is likely to come into contact with water. At this site, it sets up a local inflammatory reaction. The most common site involved is the leg, but other sites such as arms, shoulder, breast, buttocks or genitalia may be affected.

When the anterior end of the gravid female worm comes beneath the skin surface, it secretes a toxin which causes a blister formation. The blister ruptures, forming a shallow ulcer, in the base of which is a small hole through which the head of the worm protrudes. The hole is the front end of a tunnel in which the worm lives. When the ulcer comes into contact with water, the worm contracts, discharging a milky white fluid containing numerous larvae. This process continues for 2 to 3 weeks, till all the larvae are released. Afterwards the empty worm either extrudes spontaneously or gets absorbed.

The larvae swim about in water, where they survive for about a week. They are swallowed by the fresh water copepod cyclops, which is the intermediate host. The larvae penetrate the gut wall of the cyclops and enter its body cavity where they moult twice. In about 2 to 4 weeks, they develop into the infective third-stage larvae (Fig. 19.2).

The entire life cycle takes about a year, so that all the infected persons develop the blisters and attendent clinical manifestations at about the same time of the year.

In rural areas, where farm workers are affected, it seriously hampered agricultural operations and caused economic deprivation.

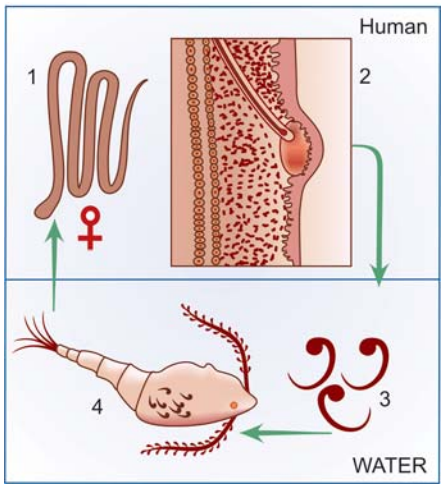


FIGURE 19.2: Life cycle of *Dracunculus medinensis:* 1. Adult female worm. 2. Gravid worm in subcutaneous tunnel, with anterior end protruding into blister, ready to discharge larvae on contact with water. 3. Larvae swim about in water and are 4. Ingested by cyclops in which they develop into the infective third-stage larvae. Human infection follows drinking water containing infective cyclops

Pathogenicity and Clinical Features

The incubation period is about a year. Infection induces no illness till the gravid female worm comes to lie under the skin, ready to discharge its embryos.

A few hours before the development of the blister there may be constitutional symptoms such as nausea, vomiting, intense pruritus and urticarial rash. The blister develops initially as a reddish papule with a vesicular centre and surrounding induration. The most common sites are on the feet between the metatarsal bones or on the ankles. The fluid in the blister is a sterile yellowish liquid with polymorphs, eosinophils and mononuclear cells. The local discomfort diminishes with the release of the embryos, but if the worm happens to break during attempted extraction, intense inflammation, with cellulitis and suppuration follows. Secondary bacterial infection is frequent. Sometimes it may lead to tetanus. The disability due to guinea worm disease (*dracunculosis, dracunculiasis* or *dracontiasis*) lasts usually for 1 to 3 months. Sometimes the worm travels to unusual sites such as the pericardium, the spinal canal or the eyes, with serious effects.

Diagnosis

Diagnosis is evident when the tip of the worm projects from the base of the ulcer. By bathing the ulcer with water, the worm can be induced to release the embryos, which can be examined under the microscope. Calcified worms can be seen by radiography. An intradermal test with guinea worm antigen elicits positive response.

Prevention and Control

Provision of protected piped water supply is the best method of prevention. Cyclops in water can be destroyed by chemical treatment. In an emergency water can be filtered through cloth before consumption. Boiled water is safe.

Control measures include the prevention of contamination of water sources with the larvae. Infected persons must not be allowed to bathe or wade in sources of drinking water. Step wells constituted a serious danger as people go down the steps to gather water from such wells and get their feet immersed in the water. Building parapet walls around such wells prevented contamination.

Because of its simple life cycle, localised distribution and the absence of animal reservoirs, guinea worm infection was eradicable. Measures to eliminate the infection have been successful. Global eradication of the infection is imminent.

Treatment

Antihistaminics and steroids were of help in the initial stage of allergic reaction. Metronidazole, niridazole and thiabendazole are useful in treatment. For removal of the worm, the best method was the ancient technique of patiently twisting it around a stick. It may take several days to extract the whole worm but if care is taken not to snap the worm, this method is safe and effective.

CHAPTER 20

Miscellaneous Nematodes

ANGIOSTRONGYLUS CANTONENSIS

Angiostrongylus cantonensis, the rat lung worm causes *eosinophilic meningoencephalitis (cerebral angiostrongyliasis)* in humans. This condition was first reported from Taiwan in 1945. Since then many hundreds of cases have occurred in Taiwan, Thailand. Indonesia and the Pacific Islands. Human infection has been recorded also in India, Egypt, Cuba and the USA.

Rats are the natural hosts, in which the adult worm is present in the branches of the pulmonary artery. It is about 20 mm long and 0.3 mm thick. Eggs resemble those of hookworms. They hatch in the lungs and the larvae which migrate up the trachea are swallowed and expelled in the feces. The larvae infect molluscs, slugs and snails which are the intermediate hosts. Crabs, fresh water prawns and frogs have also been found to be naturally infected. In about 2 weeks, the infective third-stage larvae develop, which can survive in the body of the intermediate host for about a year. Rats become infected when they eat the molluscs. In the rat, the larvae penetrate the gut wall to enter the venules, and are carried in circulation to the brain, where they develop into young adults in about a month. These penetrate the cerebral venules and reach the pulmonary artery, where they lodge, mature and start laying eggs.

Human infection is acquired by eating infected molluscs and other intermediate hosts containing the third-stage larva. Infection may also occur through raw vegetables or water contaminated with the larvae. The larvae penetrate the gut and are carried to the brain, but they are unable to develop further. The larvae die and induce an inflammatory reaction in the brain and meninges to produce meningoencephalitis. The incubation period is about 2 to 3 weeks. Patients present with intense headache, fever, neck stiffness, convulsions and various degrees of pareses. Peripheral eosinophilia and high CSF eosinophilia (up to 90%) are constant features. Larvae and adult worms may be seen in CSF. Most cases recover spontaneously, some with residual pareses. Fatality is rare. The worm may also cause ocular complications. Infection does not seem to confer immunity as second attacks have been recorded.

Anthelmintic treatment is not recommended as the disease is due to dead larvae. The drugs may even enhance the illness due to destruction of more larvae.

Angiostrongylus costaricensis,inhabiting the mesenteric arteries of wild rodents in Costa Rica in Central America may cause human infections. The disease presents as inflammation of the lower bowels and is known as *abdominal angiostrongyliasis*.

CAPILLARIA PHILIPPINENSIS

Capillaria philippinensis is a small nematode, about 3 to 4 mm long, which inhabits the jejunum. It belongs to the superfamily Trichuroidea. It has been responsible for several fatal cases of diarrhoeal illness in the Philippines from 1963. It has also been reported from Thailand, Japan, Iran and Egypt.

Its life cycle has not been worked out. Birds have been reported to be the natural definitive hosts. Human infection is believed to occur by eating infected fish, which are the intermediate hosts harbouring the infective larvae. Autoinfection is stated to be responsible for the high degree of infection in man. The clinical disease consists of a malabsorption syndrome with severe diarrhoea, borborygmi and abdominal pain. Serious cases may be fatal in 2 weeks to 2 months. Diagnosis is made by detection of the eggs, larvae and adults in stools. The eggs resemble those of *Trichuris trichiura*, but are smaller. Mebendazole is useful in treatment.

Capillaria hepatica is a common parasite of rats, which may occasionally infect man causing hepatitis, which may be fatal.

GNATHOSTOMA SPINIGERUM

Gnathostoma spinigerum, originally described from gastric tumours of a tiger, is a small spirurid nematode (female 25 to 55 mm; male 10 to 25 mm), which parasitises dogs, cats and their wild relatives. Eggs are passed in feces into water, where they hatch. Larvae are ingested by Cyclops in which the second-stage larvae develop. Cyclops are eaten by fishes, frogs and snakes, in which the third-stage larvae develop. When these are eaten by cats, dogs or other suitable hosts, the larvae develop into adults in them. When one eats undercooked fish containing third-stage larvae, the person gets infected, but further development of the worm cannot proceed normally. (Such hosts are called 'paratenic'). The larvae migrate in the tissues of infected persons, causing indurated nodules or abscesses and creeping eruption (larva migrans). When the nodules are superficial, they can be incised and the larvae removed. The wandering larvae may reach the brain or eyes causing severe damage. An intradermal test using the larval or adult antigens has been described.

Human infections have been reported from Thailand and other countries in the Far East. Cases of human infection with *G.spinigerum* and a related species *G.hispidium* have also been reported from India.

ANISAKIASIS

Anisakis species are nematode parasites of marine mammals like dolphins, seals and whales. The eggs passed in sea water hatch and infect marine crustacea (krill).Fish

eat these and the infective larvae remain in the fish viscera and flesh. When humans consume uncooked or improperly preserved fish containing the infective larvae, they penetrate the gut wall, at the level of the throat, stomach or intestine, leading to local inflammation and granuloma formation. The illness varies according to the site involved—such as throat irritation or acute gastric or bowel symptoms. Anisakiasis is common in Japan and other places where fresh or undertreated fish is a popular food.

LARVA MIGRANS

The life cycles of most nematodes parasitising humans include larval migration through various tissues and organs of the body. Sometimes the larvae appear to lose their way and wander around aimlessly. This condition is known as larva migrans. This is generally seen when human infection occurs with nonhuman species of nematodes. Such infections the worm being unable to undergo normal development and complete its life cycle. Abnormal or arrested larval migration may also sometimes occur when human parasitic nematodes infect immune persons. The immunity is sufficient to prevent the normal progression of infection. Larva migrans can be classified depending on whether the larval migration takes place in the skin or in deeper tissues, as follows.

Cutaneous Larva Migrans

This condition also known as *creeping eruption* is caused by nematode larvae that infect by skin penetration, most commonly by the nonhuman species of hookworms *Ancylostoma braziliense* and *A. caninum*. Infection with these hookworms of dogs and cats is acquired from soil contaminated with excreta of these animals. The larvae produce itching papules which develop into serpigenous tunnels in the epidermis. With the movements of the larva in the skin, the lesion also shifts, hence the name creeping eruption. Thiabendazole is useful in treatment. When the lesions are few, freezing the advancing part of the eruption with ethyl chloride is effective. Transient creeping eruptions may be produced sometimes by the human hookworm *Necator americanus*. *Gnathostomiasis* and *sparganosis* may produce larva migrans where the lesions are deeper, subcutaneous or in the muscles.

A rapidly moving lesion is produced by *Strongyloides stercoralis* particularly in immune persons. This is known as *larva currens*.

Creeping eruption also occurs in infections with *Loa loa* and *Dirofilaria*.

Ectopic infections with *Fasciola* and *Paragonimus* flukes may produce creeping lesions on the abdominal wall.

Creeping myiasis may be caused by flies of the genus Hypoderma and Gastrophilus.

Visceral Larva Migrans

This condition is caused by the migration of larvae of nonhuman species of nematodes that infect by the oral route. The most common cause is the dog ascarid *Toxocara canis* and less often the cat ascarid *T.cati*. When the infective eggs present in the

soil are ingested the larvae hatch in the small intestine, penetrate the gut wall and migrate to the liver. They may remain there or migrate to other organs such as lungs, brain or eyes. In humans they do not develop into adults, but induce granulomatous lesions which cause local damage. Clinical manifestations depend on the sites affected and the degree and duration of infection. As children are more likely to swallow dirt. This condition is much more frequent in them. Fever, hepatomegaly, pneumonitis, hyperglobulinaemia and pica are the common findings. Patients may develop neurological disturbances (*Neural larva migrans*) and endophthalmitis (*Ophthalmic larva migrans*). Marked leucocytosis occurs with high eosinophilia.

Serological tests, such as passive haemagglutination, bentonite flocculation, microprecipitation and more specifically ELISA have been developed for the diagnosis of toxocariasis. Thiabendazole may be useful in treatment. Deworming of household pets helps in prevention by limiting the contamination of soil.

Visceral larva migrans may also be caused by Anisakis which are large ascarid parasites of marine animals and also by Gnathostoma.

CHAPTER 21

Diagnostic Methods in Parasitology

Laboratory procedures play an important role in the diagnosis of parasitic infections, both for confirmation of clinical suspicion and for identifying unsuspected infections. The principles of laboratory diagnosis are the same as in bacterial and viral infections, but the relative importance of the different methods varies greatly. While isolation of the infecting agent and detection of specific antibodies are the major methods in bacteriology and virology, they are much less important in parasitology than morphological identification of the parasite by microscopy. Compared to bacteria and viruses, parasites are very large and possess distinctive shape and structure which enable their specific diagnosis on morphological grounds. Due to their complex antigenic structure and extensive cross-reactions, serological diagnosis is of limited value in parasitic infections. Though many pathogenic parasites can be grown in laboratory cultures this is not suitable for routine diagnosis because of its relative insensitivity and the delay involved.

Morphological diagnosis of parasites consists of two steps—detection of the parasite or its parts in clinical samples and its identification. Detection depends on collection of the appropriate samples and their examination by suitable techniques. Identification requires adequate skill and expertise in recognising the parasite in its various stages and its differentiation from morphologically similar artefacts.

A description of the common diagnostic techniques in parasitology is given below.

EXAMINATION OF FECES

Specimens should be collected in suitable clean containers, avoiding contamination with urine, water or disinfectants. Normally passed stools are preferable, though samples obtained after purgative (sodium sulphate) or high saline enema may also be used. Examination of fresh specimens is necessary for observing motility of protozoan parasites.

Feces should be examined for its consistency, colour, odour and presence of blood or mucus. In some instances, parasites may be seen on gross inspection as in the case of roundworm, pinworm or tapeworm proglottides.

Microscopy

The microscope should be equipped with a micrometer eyepiece, as it is often essential to measure the size of parasites. For example, the differentiation between cysts of the pathogenic *Entamoeba histolytica* and the non-pathogenic *E.hartmanni* is based entirely on their sizes.

Microscopy should also include contributory findings such as the presence of Charcot-Leyden crystals and cellular exudate.

For detection of parasites, it is best to employ a combination of methods, as different methods serve different purposes. The methods include examination of wet mounts, thick smears and permanent stained preparations. Various concentration methods can be used to increase the sensitivity of microscopic examination.

Wet Mounts

The unstained wet film is the standard preparation and is made by emulsifying a small quantity of feces in a drop of saline placed on a slide and applying a coverslip on top, avoiding air bubbles. A proper preparation should be just dense enough for newspaper print to be read through it. If the feces contains mucus, it is advisable to prepare films using the mucus part. Wet saline mounts are particularly useful for detecting live motile trophozoites of *E. histolytica, B. coli* and *G. lamblia*. Eggs of helminths are also readily seen.

Eosin, 1% aqueous solution can be used for staining wet films. Eosin stains everything except living protoplasm. Trophozoites and cysts of protozoa as well as helminth larvae and thin-walled eggs stand out as pearly white objects against a pink background and can be easily detected. Chromatoid bodies and nuclei of amoebic cysts can be seen prominently. Eosin also indicates the viability of cysts; live cysts are unstained and dead ones stained pink.

Iodine staining of wet mounts is another standard method of examination. Either Lugol's iodine diluted 1 to 5 or Dobell and O'Connor's iodine solution (1g iodine, 2 g potassium iodide, l00 ml distilled water) is used. Iodine helps to confirm the identity of cysts, as it stains prominently the glycogen vacuoles and nuclei.

Thick Smears

These are not useful for routine examination, but are valuable in surveys for intestinal helminth eggs. The method described by Kato and Miura in 1954 is known as the Kato thick smear technique. About 50 mg feces is taken on a slide and covered with a special wettable cellophane coverslip soaked in glycerine containing aqueous malachite green. The preparation is left for about an hour at room temperature, in which period the glycerine clears the feces enabling the helminth eggs to be seen distinctly under low power magnification. This method is however not useful for diagnosis of protozoa or helminth larvae.

Permanent Stained Smears

These are employed for identification of protozoa in feces and also as permanent records. The two methods commonly used are the iron-haematoxylin stain and Wheatley's trichrome stain. The iron-haematoxylin is the older method, but is more difficult.

Iron-Haematoxylin Stain

Fecal smear on a slide is fixed in Schaudinn's solution for 15 minutes and is immersed successively for 2-5 minutes in 70% alcohol, 70% alcohol containing a trace of iodine, 70% and 50% alcohol. It is washed in water for 5-10 minutes and immersed in 2% aqueous ferric ammonium sulphate solution for 5-15 minutes. It is then washed in water for 3-5 minutes and stained with 0.5% aqueous haematoxylin for 5-15 minutes. It is washed for 2-5 minutes and differentiated in saturated aqueous solution of picric acid for 10-15 minutes. It is then washed for 10-15 minutes and dehydrated by passing through increasing strengths of alcohol, cleared in toluene or xylol and mounted.

Wheatley Trichrome Stain

This is a simpler and quicker method. The smear is fixed in Schaudinn's solution and taken successively through alcohol, as above. Trichrome stain (chromotrope 2 R, light green SF and phosphotungstic acid in glacial acetic acid and distilled water) is then applied for 5-10 minutes, differentiated in acid alcohol for 2-3 seconds, dehydrated, cleared and mounted.

Other staining techniques are used for special purpose. For example, modified acid-fast or Giemsa stain is employed for detection of oocysts of cryptosporidium and isospora.

Concentration Methods

When the parasites are scanty in stools, routine microscopic examination may fail to detect them. It is then necessary to selectively concentrate the protozoan cysts and helminth eggs and larvae. Concentration may be done using fresh or preserved feces. Several concentration techniques have been described. They can be classified as the floatation or sedimentation methods. In floatation method, the feces is suspended in a solution of high specific gravity so that parasitic eggs and cysts float up and get concentrated at the surface. In sedimentation method, the feces is suspended in a solution with low specific gravity so that the eggs and cysts get sedimented at the bottom, either spontaneously or by centrifugation.

Floatation Methods

A simple and popular method is salt floatation using a saturated solution of sodium chloride, having a specific gravity of 1.2. About 2 ml of the salt solution is taken.

in a flat bottomed tube (or 'penicillin bottle') and 1 g of feces is emulsified in it. The container is then filled completely to the brim with the salt solution and a slide is placed on the container so that it is in contact with the surface of the solution, without any intervening air bubbles after standing for 20-30 minutes, the slide is removed, without jerking, reversed to bring the wet surface on top and examined under the microscope. A coverslip need not be applied if examination is done immediately. Any delay in examination may cause salt crystals to develop, interfering with clarity of vision.

This simple method is quite useful for detecting the eggs of the common nematodes such as roundworm (except unfertilised eggs), hookworms and whipworm, but is not applicable for eggs of tapeworms, trematodes and for protozoan cysts.

Zinc Sulphate Centrifugal Floatation

Make a fine suspension of about 1 g of feces in 10 ml of water and strain through gauze to remove coarse particles. Collect the liquid in a small test tube and centrifuge for 1 minute at 2500 RPM. Pour off the supernatant, add water, resuspend and centrifuge in the same manner, repeating the process, till the supernatant is clear. Pour off the clear supernatant, add a small quantity of zinc sulphate solution (specific gravity 1.18 to 1.2) and resuspend the sediment well. Add zinc sulphate solution to a little below the brim and centrifuge at 2500 RPM for 1 minute. Take samples carefully from the surface, using a wire loop, transfer to slide and examine under the microscope. A drop of dilute iodine helps to bring out protozoan cysts better.

This technique is useful for protozoan cysts and eggs of nematodes and small tapeworms. But it does not detect unfertilised roundworm eggs, nematode larvae and eggs of most trematodes and of large tapeworms.

Sedimentation Methods

Formol-ether concentration method has been the most widely used sedimentation method. Emulsify 1-2 g. feces in 10 ml water and let large particles sediment. Take supernatant and spin at 2500 RPM for 2-3 minutes. Discard supernatant. Add 10% formol-saline, mix well and stand for 10 minutes. Add 3 ml ether. Shake well. Spin at 2500 RPM for 2-3 minutes. Four layers will form—a top layer of ether, a plug of debris at the interface, the formol-saline layer, and the sediment at the bottom. Carefully detach the debris from the sides of the tube and discard the top three layers. Suspend the sediment in a few drops of fluid and examine wet mount and iodine preparation.

As ether is inflammable and explosive, its use can be hazardous. Ethyl acetate can be conveniently used in its place, with equally good results. The method is useful for all helminth eggs and protozoan cysts.

Egg Counts

A semiquantitative assessment of the worm burden can be made by estimating the number of eggs passed in stools. This is done by egg counts and by relating the

counts to the number of worms present by assuming the number of eggs passed per worm per day. However, these are at best approximations and only a rough indication of worm burden can be obtained. Egg counts help to classify helminth infections as heavy, moderate or light. Egg counts can be done by different methods.

The standard wet mount gives rough indication of the number of eggs. Ordinarily 1-2 mg of feces is used for proparing a wet film and if all the eggs in the film are counted. The numbers of eggs per gram of feces can be assessed.

The modified Kato thick smear technique using 50 mg of stool cleared by glycerine-soaked cellophane coverslip can be used for egg counting.

McMaster's egg counting chamber can be used. Here eggs in 20 mg of stool are concentrated by salt floatation on the squared grid on the roof of the chamber, which can be counted.

In Stoll's dilution technique, 4g of feces is mixed thoroughly with 56 ml of N/10 NaOH, using beads in a rubber stoppered glass tube. Using a pipette, transfer exactly 0.75 ml of the sample to a slide, apply coverglass and count all the eggs present. The number multiplied by 200 gives the number of eggs per gram of feces. This figure requires to be corrected for the consistency of feces, by multiplying by 1 for hard formed feces, by 2 for mushy formed feces, by 3 for loose stools and by 4 for liquid stools. Watery stools are unfit for counting.

Special techniques have been described for particular purposes as for example, Bell's dilution-filtration count for schistosome eggs.

Fecal Culture

Fecal culture is not used for routine diagnosis, but for species identification, as for example in differentiation between Ancylostoma and Necator. The Harada-Mori culture method uses strips of filter paper on which feces is smeared in the middle third. The paper strips are kept in conical centrifuge tubes with water at the bottom in which the stips dip. The tubes are kept at room temperature in the dark for 7-10 days during which time the larvae develop and fall into the water at the bottom, from which they can be collected.

Charcoal cultures are simple and efficient. Soft or softened feces is mixed with 5-10 parts of moistened charcoal granules and packed into a suitable container and kept covered. In 7-10 days, the larvae hatch and come to the surface. They can be collected by applying on to the surface a pad of soft cotton cloth for half an hour. The cloth is removed and kept upside down on a sedimentation flask filled to the brim with warm water . The larvae fall to the bottom of the flask while the charcoal particles remain on the cloth.

EXAMINATION OF BLOOD

Next to feces, the largest number of parasites are found in blood. Blood examination is the routine diagnostic method in malaria, filariasis, African trypanosomiasis and babesiosis. It is sometimes positive in Chagas' disease and rarely in kala-azar and toxoplasmosis. Blood examination is done in the following ways.

Examination for Malarial Parasites

The standard diagnostic method in malaria is the examination of stained blood films-both thin and thick smears.

Thin Smear

A thin smear is prepared from finger prick, or in infants from heel prick blood. A small drop is spread on a clean grease-free slide with a spreader, to give a uniform smear, ideally a single cell thick. The margins of the smear should be well short of the sides of the slide, and the tail should end a little beyond the centre of its length. The thin smear displays blood cells and parasites clearly. Its only disadvantage is that only a small volume of blood can be surveyed.

After drying, the smear is stained with Giemsa or Leishman stain. For Giemsa stain, the smear is fixed in methanol for 3-5 minutes. After drying, Giemsa stain diluted 1 drop in 1 ml of buffered water pH 7-7.2 is applied for 30-45 minutes. The slide is then flushed gently with tap water, dried and examined under the oil immersion objective. The cytoplasm of malarial parasites is stained blue and the chromatin dot red.

For Leishman stain, prior fixation is not necessary as the stain is an alcoholic solution which fixes as it stains. Leishman stain is applied for 30 seconds and diluted with twice its volume of buffered water, pH 7-7.2 and kept for 10 to 15 minutes. The smear is then dried and examined.

For demonstration of malarial parasites, blood should be collected not during the peak of fever, but optimally several hours after it. Bouts of fever follow the synchronous rupture of large number of parasitised erythrocytes releasing their membrane shreds and contents. The emerging merozoites parasites other erythrocytes and initiate a fresh cycle of erythrocytic schizogony. The timing is particularly important in *P.falciparum* infections as here the late stages of schizogony are not seen in peripheral circulation. In practice, the rule is to take a blood smear when a suspected malaria patient is first seen and then again subsequently after a bout of fever. Smears should invariably be collected before starting antimalarial treatment. In MT malaria, only the ring stage and gametocytes are seen in peripheral smear, while in BT malaria, all stages of schizogony and gametocytes can be seen. Thin smear examination enables the appreciation of changes in the erythrocytes, such as enlargement, alteration of shape, fimbriation, presence of Schuffner's dots or Maurer's clefts. Parasitised erythrocytes are seen most often in the upper and lower margins of the tail of the smear. A minimum of 100 fields should be examined before a negative report is given.

Thick Smear

Thick smears have the advantage that a larger quantity of blood can be tested. The disadvantages are that the red cells are lysed and the morphology of the parasites is distorted so that identification becomes difficult. A big drop of blood from finger

or heel prick is collected on a clean grease-free slide and spread with the corner of another clean slide to form a uniformly thick smear about 1 cm square. The thickness of the smear should be such that the hands of a wristwatch can be seen through it, but not the figures on the dial. The smear is dried in a horizontal position, kept covered from dust. Thick smears have to be dehaemoglobinised before staining. They can be stained with Giemsa or Leishman stains as described above. Wright stain and JSB stain (so called because it was devised by J Singh and Bhattacharjee in 1944) are very useful for staining large numbers of thick films, as in malaria surveys.

Wright's stain consists of two solutions—Solution A contains methylene blue and azure B in phosphate buffer. Solution B contains eosin in phosphate buffer. The film is immersed in solution A for 5 seconds, washed in tap water, immersed in solution B for 5 seconds, washed, dried and examined. Staining times may need adjustment. If the smear is too blue, stain longer in solution B, if too pink, in solution A.

JSB stain also consists of two solutions. The first contains methylene blue, potassium dichromate, suphuric acid, potassium hydroxide and water. The second solution is aqueous eosin. For staining, the smear is immersed in solution I for 10 seconds, washed for 2 seconds in acidulated water pH 6.2-6.6, stained in solution II for 1 second, washed in acidulated water, immersed again in solution I and washed.

Combined thick and thin smears can be taken on the same slide. Draw a thick line with a glass-marking pencil on a slide, dividing it into two unequal parts. The thick smear is made on the smaller part and the thin smear drawn on the larger. Thick smear is first dehaemoglobinised and the two then stained together. An easy method is to add undiluted Leishman stain over the thin smear and then the diluted stain flooded over to the thick smear also. The stained thin smear is examined first. If the thin smear is negative, the thick smear should be searched for parasites.

When a slide is positive for malarial parasites, the report should indicate the species, the developmental stages found and the density of parasites in the smear.

Examination for Microfilaria

Microfilariae may be detected in peripheral blood, both in unstained mounts and in stained smear. In case of nocturnal periodic microfilariae, blood should be collected between 10 PM and 2 AM.

Wet Mount

Two or three drops of blood are collected on a clean glass slide and a coverslip applied and sealed. The preparation is examined under the low power microscope for the motile microfilariae which can be seen wriggling about, swirling the blood cells in their neighbourhood. The examination may conveniently be deferred till next morning as microfilariae retain their viability and motility for one or two days at room temperature. By using a simple counting chamber, microfiliariae in the wet mount can be counted.

Staind Smears

A thick smear is prepared as for malaria, dehaemoglobinised and stained with Leishman, Giemsa or Delafield's haematoxylin stains. Stained smears have the advantage that the morphology of microfilariae can be studied and species. identification made. Thus, for differentiation between *Mf. bancrofti* and *Mf. malayi* stained smears are necessary .Sometimes microfilariae may be seen in thin smears also.

By using a measured quantity of blood for preparing smears, as for example with a 20 cu mm pipette and counting the total number of microfilariae in the smear, microfilaria counts can be obtained. Multiplying the number of microfilariae in a 20 cu mm smear by 50 gives the count per ml of blood.

Concentration Methods

These employ venous blood. Two methods are used for concentration of microfilariae-sedimentation and filtration.

In *sedimentation method* the sample of blood is first lysed with acetic acid, saponin or other lytic substance, or by freeze thawing and then centrifuged. The sediment is stained and the microfilariae counted.

In *filtration method*, a measured quantity (1-5 ml) of blood is collected into an anticoagulant solution and passed through membrane filters fixed on syringes with Swinney filter holder. Blood cells and proteins sticking on to the filter are washed away by repeatedly passing saline through it. The filter is removed, placed on a slide, stained with dilute Giemsa stain and examined under low power microscope for microfilariae. Millipore and Nuclepore membrane filters are available for this purpose, the latter being more sensitive as it can screen larger volumes of blood.

The membrane filter method is so much more sensitive than the finger prick method that blood samples taken during day also give reliable results even with nocturnal periodic microfilariae. However, the method has the disadvantages that venepuncture is necessary, membranes are costly and microfilariae may not be in a satisfactory condition for detailed morphological study.

DEC Provocation Test

Oral administration of diethyl carbamazine (l00mg, or 2 mg/kg body weight) brings about mobilisation of microfilariae into peripheral blood. Blood collected 20-50 minutes after the drug is given will show microfilariae so that blood collection can be done during day time. This is a great advantage for surveys. But the drug may cause febrile reactions, particularly in brugiasis. It cannot be used in areas endemic for onchocerciasis because of the danger of provoking severe reactions.

CULTURE METHODS

Many parasites can now be grown in culture, but this has not become a routine diagnostic method in parasitic infections. It is sometimes employed for accurate

identification of the parasite species. It is more often employed for obtaining large yields of the parasite as a source of antigen, for animal inoculation, drug sensitivity testing, for experimental or physiological studies and for teaching purposes. Some of the culture methods used for different parasites are indicated below.

Amoeba

E. histolytica and other intestinal amoebae can be grown in diphasic or monophasic media, in media containing other microorganisms or in axenic cultures.

Boeck and Drbohlav's diphasic medium, the classical culture medium for amoeba has been modified by various workers. The medium as used now is basically an egg slant, with an overlay of sterile serum or liver extract in buffered saline. A loopful of sterile rice powder is added to the medium just before inoculation with fresh feces or its saline centrifugal sediment. Cultures can be obtained from feces containing cysts or trophozoites. The cultures are incubated at 37° C and subcultured at 48 hour intervals. Amoebae can be demonstrated in the liquid phase in unstained mounts or stained smears.

Balamuth's monophasic liquid medium is also used commonly for cultivation of amoebae and other intestinal protozoa. This is an egg yolk-liver extract infusion medium.

Both protozoa and bacteria present in stools grow in the above media. Bacterial growth can be reduced by addition of penicillin or other antibiotics that do not inhibit protozoa. Axenic cultures (pure cultures without bacteria or other micro-organisms) were first developed by Diamond in 1961. Axenic cultivation has enabled precise antigenic and biochemical studies on amoebae.

Balantidium coli grows well in Balamuth's' medium. *Giardia lamblia* had been established in association with candida and saccharomyces, but axenic cultures were developed in 1970. *Trichomonas vaginalis* grows very well in several commercially available media such as trypticase serum media. Naegleria and Acanthamoeba from CSF can be grown on agar plates heavily seeded with *Escherichia coli*.

Leishmania and Trypanosomes

The classical Nicolle, Novy and Macneal (NNM) medium first described in 1904 for cultivation of leishmania is equally satisfactory for trypanosomes also. This is a defibrinated rabbit blood agar medium. Several modifications of this medium have been introduced.

Malaria Parasites

Cultivation of malaria parasites was first obtained by Bass and Jones in 1912. A simple method of cultivation is as follows. About 10-12 ml of defibrinated or heparinised blood rich in ring forms of malaria parasite, mixed with 0.2 ml of 50% dextrose solution are incubated at 37° C in a sterile test tube in an upright position. The blood separates into the erythrocytes below, plasma above and the buffy coat

in between. Malaria parasites grow in the erythrocyte layer immediately below the buffy coat. Smears are collected from this layer at intervals, without tilting the tube. Segmented schizonts are usually observed after incubation for 24 to 36 hours.

The breakthrough in cultivation of malarial parasites came in 1976 when Trager and Jensen successfully maintained *P.falciparum* in continuous cultures in human erythrocytes using RPMI 1640 medium. The cultures are incubated at 38°C with 10% human serum at pH 6.8-7.2 under an atmosphere with 7% CO_2 and 1-5% oxygen. A continuous flow system is used in which the medium flows slowly and continuously over the layer of erythrocytes. The method has been applied to various species of plasmodia. It has been employed for preparation of antigens, for drug sensitivity studies, vaccine tests and many other purposes.

ANIMAL INOCULATION

Animal inoculation is not a routine diagnostic procedure in parasitic infections, but can be used in some instances because of its sensitivity .

Animal inoculation can be used for isolating *Toxoplasma gondii* from infected persons. Lymph node or other biopsy materials are inoculated intraperitoneally into immunosuppressed mice. Peritoneal fluid obtained 7-10 days later may show the parasite in Giemsa-stained smears. However, serial passages may be necessary for its isolation. Brain smears may be examined for cysts after sacrificing the mice 3-4 weeks after inoculation. Seroconversion of the animal also indicates a positive result.

Bone marrow, liver, spleen or lymph node aspirates from kala-azar patients injected intraperitoneally into hamsters is a very sensitive method. Even a single amastigote can establish the infection in the animal. Spleen smears taken 4-6 weeks later show LD bodies.

Blood from patients with trypanosomiasis can be injected intraperitoneally or into the tail vein of mice or rats. Parasitaemia can be demonstrated in 2 weeks.

XENODIAGNOSIS

This method involves the diagnostic infection of a vector in which the parasite multiplies and can be demonstrated. In *T. cruzi,* diagnosis may be established by letting the vector reduviid bug feed on suspected patients. In 4-5 weeks, live flagellate forms can be seen in the feces of the bugs.

IMMUNOLOGICAL DIAGNOSIS

Serology

Several serological tests have been developed for detection of antibodies to parasites using antigens from cultured parasites or from natural or experimental infections in animals or humans. In some cases antigens are obtained from related parasites or even sometimes from bacteria. Advances in cultivation of parasites have made

parasitic antigens more readily available. Cloning of parasitic antigens promises to be a new source.

In some instances, diagnosis is attempted by serological demonstration of parasitic antigens in blood, tissues or secretions of suspected patients.

Virtually all types of serological reactions have been used. However, serodiagnosis in parasitic infections has only limited value due to various factors. Parasites are complex antigenically and exhibit wide ranging cross-reactions so that serological tests are not sufficiently specific. Another difficulty is in distinguishing between past and current infection. This has been solved partly by looking for IgM antibody, as in amoebiasis and toxoplasmosis.

In general, indirect haemagglutination (IHA), ELISA and counter immune electrophoresis (CIE) are most sensitive; indirect immunofluorescence (IF) and CF moderately sensitive; and simple precipitation in gel and coated particle aggutination least sensitive. Serology has not been very useful in the diagnosis of individual cases, but has been valuable as a screening method in epidemiological surveys. In some infections however where parasites are seldom demonstrable in patients, for example in toxoplasmosis and hydatidoss, serology is of great help. Listed below are some of the applications of serology:

Amoebiasis

Serology is of no value in the diagnosis of acute amoebic dysentery or luminal amoebiasis. But in invasive amoebiasis, particularly in liver abscess, serology is very useful. IHA is most widely employed. Titres of 128 or more are seen in cases of liver abscess.

Leishmaniasis

IHA, IF and CF with leishmania antigen are usually positive in kala-azar. Tests using the acid-fast Kedrowsky bacillus are relatively less sensitive.

Malaria

IF, ELISA and IHA are sensitive and specific, but are not useful for diagnosis of acute malaria because antibodies persist for some years after cure. A negative test may however help to exclude malaria. Serological tests are useful in epidemiological surveys for malaria. Molecular assays such as antigen capture have been applied for developing rapid dip-stick tests (e.g. ParaSight-F in MT malaria).

Toxoplasmosis

Serological tests offer the most useful diagnostic method in toxoplasmosis. The original Sabin-Feldman dye test, though very specific and sensitive, is no longer in use. IF, IHA and CF were other useful tests. The dye test remains positive for life, while CF tests become negative soon after active infection. At present ELISA is routinely

used in toxoplasma serology. It is very informative as it provides titres of IgM and IgG antibody separately for better interpretation of the results.

Intestinal Helminths

Antibodies can be demonstrated in most intestinal helminthiases, but extensive cross-reactions limit their use in diagnosis.

Trichinosis

Serology is very useful in diagnosis of trichinosis. Bentonite flocculation slide tests and CF become positive 3-4 weeks after infection. IF becomes positive even earlier. ELISA is also available. Demonstration of seroconversion is diagnostic.

Toxocariasis

High titres in serological tests are obtained in visceral larva migrans, but specificity is low due to cross-reactions with intestinal nematode antigens.

Filariasis

IHA and bentonite flocculation tests with antigen from *Dirofilaria immitis* gives positive reaction in patients, and high titres in tropical pulmonary eosinophilia. But cross-reactions are frequent.

Echinococcosis

Several serological tests have been developed using hydatid fluid or scolex antigens from hydatid cysts in sheep. IHA, IF and ELISA are very sensitive. Cross-reactions occur with cysticercosis.

Skin Tests

Intradermal tests have been used in many parasitic infections. They are sensitive and persist for many years, sometimes even for life. But specificity is relatively low.

Casoni's test had been used widely in the diagnosis of hydatid disease since its original description in 1911. The antigen is sterile hydatid fluid drawn from hydatid cysts from cattle, sheep, pig or humans, filtered and tested for sterility. Intradermal injection of 0.2 ml of the antigen induces a wheal and flare reaction within 20 minutes in positive cases. A saline control is used. False-positive tests are seen in schistosomiasis and some other conditions. Casoni's test is now largely replaced by serological tests.

Leishmanin test is sensitive and relatively specific. The antigen is obtained from cultured leishmania and consists of killed promastigotes in phenol saline. Intradermal injection of 0.1 ml induces a papule 5 mm or more in diameter in 48-72 hours. This delayed hypersensitivity test is positive in cutaneous leishmaniasis and negative in diffuse cutaneous and visceral leishmaniases.

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