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in
ZOOLOGY
(M.Sc. Programme)

SEMESTER-III

Parasitology and Immunology

ZDSE(MJ)T-302

Self-Learning Material

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Development of printed SLMs for students admitted to the DODL within a limited time to cater to the academic requirements of the Course as per standards set by Distance Education Bureau of the University Grants Commission, New Delhi, India under Open and Distance Mode UGC Regulations, 2020 had been our endeavour. We are happy to have achieved our goal.

Utmost care and precision have been ensured in the development of the SLMs, making them useful to the learners, besides avoiding errors as far as practicable. Further suggestions from the stakeholders in this would be welcome.

During the production-process of the SLMs, the team continuously received positive stimulations and feedback from Professor (Dr.) Amalendu Bhunia, Hon'ble Vice- Chancellor, University of Kalyani, who kindly accorded directions, encouragements and suggestions, offered constructive criticism to develop it within proper requirements. We gracefully, acknowledge his inspiration and guidance.

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Their persistent and coordinated efforts have resulted in the compilation of comprehensive, learner-friendly, flexible texts that meet the curriculum requirements of the Post Graduate Programme through Distance Mode.

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Theory (Discipline Specific Elective – Major II) - [ZDSE(MJ)T-302]

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Unit-I

Classification of helminths

Objectives: In this section we will discuss on helminths classification.

Introduction:

The classification and identification of helminths are dependent on numerous factors including body shape, body cavity, body covering, digestive tubing, sex and type of attachment organs.

Platyhelminths (flatworms) include both trematodes (flukes) and cestodes (tapeworms). Specifically, tapeworms are characterized using the above criteria and areorganized in a segmented plane. They lack a body cavity and have a *tegument* body covering. Tapeworms lack a digestive tube and are hermaphroditic. They utilize suckers or *bothridia*, and *rostellum* with hooks for an attachment organ.

Trematodes are characterized by an unsegmented plane for body shape. They also lack a body cavity and have a tegument for body covering. However, the digestive tube for trematodes ends in the cecum. Trematodes are hermaphroditic and utilize oral suckers, ventral suckers or *acetabulum* for attachment organs.

Nematodes are characterized by a cylindrical body shape and do indeed have a body cavity. Its body covering is a cuticle and the digestive tube ends in the anus. The sex of nematodes is *dioecious* (distinct male and female organisms). Lastly, their attachment organs range from lips, teeth, filariform extremities and dentary plates.

General Characteristic Features of Platyhelminthes:

1. Bilaterally symmetrical, dorso-ventrally flat, triploblastic animals without true segmentation.
2. Body covered with epidermis which is soft and ciliated (Turbelleria) or covered by cuticle and provided with external suckers or hooks or both for attachment to the host (Trematoda, Cestoidea).
3. Digestive system incomplete, having a mouth but no anus and much branched (Absent in Cestoda).
4. No body cavity; space between internal organs and body wall filled with loose parenchyma; muscle layers well developed.
5. Excretory system characteristic; consisting of numerous flame cells distributed in the parenchyma and connected to excretory ducts opening to outside.
6. Apair of anterior nerve ganglia or a nerve ring connected with 1 to 3 pairs of longitudinal nerve cords with transverse commisures.
7. Sexes united; fertilization internal; eggs numerous, minute yolky, development

direct. (Turbellaria, Monogena) or indirect and complicated (Digena).

8. Circulatory, respiratory or skeletal systems absent.

Classification of Platyhelminthes:

The classification adopted here is from Rupart and Barnes, 1994 (6th edition). Phylum Platyhelminthes is divided into four classes on the basis of body shape, mouth position and habitat.

I. Class: Turbellaria (L., turbella = a little string)

i. Mostly free-living flatworms but some ectocommensals and endocommensals or parasitic.

ii. Body unsegmented.

iii. Body covered with a cellular or syncytial epidermis usually with mucous cellsand which is usually partly ciliated.

iv. Adhesive organs abundantly present.

v. Digestive system usually consists of mouth, pharynx and intestine, anus not found.

vi. Excretory system consists of protonephridia, the flame cells.

vii. Sense organs consist of tango receptors and chemoreceptors.

viii. Mostly hermaphrodite.

ix. Reproduction sexual, asexual and by regeneration.

x. Life cycle simple.

Example: *Ectocotyla, Planeria, Dugesia, Bipalium*

II. Class: Trematoda (Gr., trematodes = having pores)

i. Ectoparasitic or endoparasitic forms, commonly called flukes.

ii. Body shape usually leaf-like, dorsoventrally flattened.

iii. Body wall without epidermis and cilia.

iv. Body undivided and covered with a cuticle.

v. Well-developed suckers usually present.

vi. Digestive tract incomplete consisting of mouth, pharynx and two forked or many branched intestine; anus absent.

vii. Protonephridial excretory system consisting of flame cells.

viii. Mostly hermaphrodite (monoecious).

ix. Ovary single, testes two to many.

x. Life history simple or complicated.

Example: *Polystoma, Gryodactylus, Aspidogaster, Fasciola, Schistosoma, Bucephalus, Opisthorchis* (=Clanorchis).

III. Class: Monogenea

i. Monogeneans are mostly ectoparasites of aquatic vertebrates, particularlyfishes, but amphibians, reptiles and cephalopod molluscs are also hosts.

ii. Body dorsoventrally flattened and have a large, posterior attachment organ,the haptor, which bears hooks and suckers.

iii. Anterior end has also adhesive glands.

iv. A gut is present but mouth lacks a sucker. The pharynx secretes a protease that digests the host's skin.

v. They respire aerobically, being ectoparasites.

vi. Monogeneans have inconspicuous protonephridia for excretion

vii. All members are hermaphrodite.

viii. Life cycle simple and having no intermediate host.

ix. One egg by way of a ciliate larva (Oncomiracidium) gives rise to only oneadult worm and hence the name monogenea meaning "one generation"

Examples: *Polystoma* (bladder of frogs), *Polystomoidella* (Urinary bladder of turtles), *Dactylogyrus* (gills of freshwater fishes)

IV. Class: Cestoidea (Gr., kestos = girdle + eidos = form):

i. Endoparasites in the intestine of vertebrates.

ii. Commonly called tapeworms.

iii. Body without epidermis and cilia but covered with cuticle.

iv. Body usually divided into few to many segments (proglottids), rarely undivided.

v. Anterior end (scolex) is provided with adhesive structures (hooks, suckers)except in Cestodaria.

vi. Mouth and digestive tract totally absent.

vii. Excretory system consists of protonephridia with typical terminal flame bulbs.

viii. Nervous system usually comprises a pair of ganglia and two lateral longitudinal nerve cords.

ix. Each segment (proglottis) contains one or two sets of complete hermaphroditic reproductive system.

x. Life cycle complicated usually involving two or more hosts.

xi. Embryos possess hooks.

Example: *Amphilina, Gyrocotyle, Echinobothrium, Taenia, Echinococcus, Hymenolepis.*

General Characteristic of Aschelminthes:

1. Body of Phylum Nematoda is un-segmented, bilaterally symmetrical, elongated and tapering at both ends.
2. Triploblastic animals with perivisceral cavity are more extensive than that of Platyhelminthes.
3. Body of of Phylum Nematoda is generally covered with thick, flexible multi-layered collagenous cuticle and often bears cuticular setae (hairs), spines orannulations.
4. Cuticle moulted periodically.
5. Epidermis or hypodermis syncytial; i.e., the nuclei are not separated from each other by cell membranes.
6. Only longitudinal body-wall muscles; no circular body-wall muscles.
7. Body cavity of of Phylum Nematoda is pseudocoel filled with parenchyma inmost cases.
8. Alimentary canal provided with distinct mouth and anus (complete digestive tract). Muscular pharynx and the inner surface of the gut usually not lined by cilia. Extracellular digestion.
9. Mouth of of Phylum Nematoda is surrounded by six lips.
10. Blood vascular system and respiratory system are absent in of Phylum Nematoda.
11. Haemoglobin sometimes present in the pseudocoelomic fluid.
12. Excretory system without nephridia and flame cells. In the class Adenophorea glandular renette cells with a duct or in the class Secernentea excretory canal system without flame cells act as excretory system.
13. Dorsal and ventral nerve cords in the epidermis.
14. Chemosensory organs are small cuticular projections called amphids which are situated on the lips, derived from cilia and opening to the exterior through a small

pore, and lined with modified non-motile cilia called sensillae.

15. Sexes of of Phylum Nematoda are separate (gonochoristic).
16. Tubular gonads are present in of Phylum Nematoda.
17. Amoeboid sperm cells.
18. Fertilization is internal in of Phylum Nematoda.
19. They are free-living or phytoparasitic or zooparasitic.

Classification of Aschelminthes:

Following the scheme of Chitwood (1933), the phylum Nematoda or Aschelminthes is divided into twoclasses:

1. Adenophorea or Aphasmida and
2. Secernentea or phasmidea.

I. Class: Adenophorea or Aphasmida

i. Most species possess caudal adhesive glands and epidermal glands.

ii. Phasmids (caudal papillae bearing pores connecting with glandular pouch called phasmids which are thought to be chemosensory in function) are absent.

iii. Amphids are post labial and variously shaped such as pouch-like or tube-like, rarely pore-like.

iv. Coelomocytes well developed.

v. Excretory organs are only renette cells but without collecting tubules.

vi. Males usually without caudal alae.

vii. Usually two testes in males.

viii. Mostly marine, and include both free- living and parasitic species. The free-livingspecies include both terrestrial, freshwater, and major marine forms.

Examples: *Enoplus, Anticoma, Metonchdiamus, Xiphinema, Trichodoris, Desmoscolex, Odontophora.*

II. Class: Secernentea or Phasmida

i. Caudal phasmids present.

ii. Labial amphids pore-like.

iii. Excretory system canal-like and comparatively more complex.

iv. Epidermal and caudal adhesive glands absent.

v. Males with a single testis.

vi. Mostly parasitic.

vii. Free-living species are largely terrestrial.

Examples: *Rhabditis, Heterodera, Bunonema, Ancylostoma duodenale (Hookworm), Strongylus, Trichostrongylus (Hair worm), Ascaris (Ascaris lumbricoides, Ascaris megalocephala, Ascaris suillas), Parascari, Toxocara, Spirura, Wuchereria bancrofti (Filaria), Loa loa (Eye worm), Brugia, Onchocerca, Trichuris (Parasites of mammals), Trichinella spiralis (Trichinia worm), Camallanus, Dracunculus* etc.

Probable questions:

1. Discuss the characteristics of phylum platyhelminthes.
2. Discuss the systematic position of *Fasciola.*
3. Elaborate the schematic diagram of phylum Aschelminthes up to class with example.
4. Write down the characteristics of class phasmida with example.

Suggested reading:

1. Barnes: Invertebrate Zoology (Holt-Saunders International, 4th edition, 1980)
2. Barnes: The Invertebrates – A synthesis, 3rd edition, Blackwell, 2001
3. Hunter: Life of Invertebrates, Collier Macmillan Pub. 1979
4. Marshall: Parker & Haswell Text Book of Zoology, Vol. I, 7th edition, Macmillan,1972

Unit-II

Origin and evolution of parasitic helminth

Objectives: In this section we will discuss on Origin and evolution of parasitic helminth.

Introduction

The parasitic worms form an ecological group rather than a related series. Conventionally, we divide them into the Flatworms and the Roundworms. These two "phyla" are not zoologically related and it is far from clear that a number of the minor groups classified under the term of Platyhelminthes are closely related either. However, there is little doubt that most of the Platyhelminthes-the Trematoda and the Cestoda particularly-are almost certainly monophyletic, having descended from different pre-turbellarian ancestors at different times and in different directions. The cestodes were the earliest and they originated from relatively simple beginnings to develop into a great variety of obviously quite closely related parasites.

A. THE CESTODA

To the zoologist, a cestode is an animal of ribbon-like appearance with a scolex, neck and a series of proglottids each of which contains the sexual organs. There is no digestive canal. There is no justification whatever for the statement that a cestode is degenerate. This is based on the assumption that it has somehow lost a gut and adopted a fundamentally different type of absorption of food. It is more probable that the proto-cestode never had a gut and was an early turbellarian. Some turbellarians still parasitize Crustacea, molluscs and echinoderms. The genus Fecampia when free, has a mouth and a pharynx; it loses them both when it becomes parasitic in a marine crustacean, retaining only an intestine. The free-living Convoluta, which also originally had a gut, loses it when it begins to feed on its symbiotic algae. The scolex contains the nerve ganglia and the connecting commissures which together constitute the brain. There are flame cells scattered throughout the entire worm, but here there is a more or less elaborate plexus. At the base of the scolex is the "neck" from which grow the genital segments serially. The scolex and neck together can be considered as the main body of the tapeworm. In vitro, they can often be kept alive for long periods (Webster and CaEeron, 1963) but in vivo, they serially bud off genital segments, which in all cestodes have basically the same genital plan-sometimes duplicatedof a single ovary and a number of testicles, some yolk glands (although these may be combined with the ovary) and the usual genital canals-sperm duct, vagina, and uterus. The uterus may have no opening to the exterior and become gravid or may have an opening through which eggs can be passed continuously. Tapeworms are classified mainly on the different kinds of adhesive organs on the scolex (Baer, 1950), but the organs of adhesion are obviously an adaptation to parasitism. The fact that they not only vary in different groups of cestodes but vary quite consistently

within host groups, suggests that the modern classification is in effect and in broad general lines, a phylogenetic one. The phylogenetic significance, however, lies in the fact that series of similar types tend to occur in series of related hosts (Yamaguti, 1959).

It is generally assumed that the function of the adhesive organs is to maintain the worm in situ in the small intestine. This can be true only when the tapeworm is small or immature. Once full grown, it maintains itself by muscular activity against the peristaltic action of the gut. Most adhesive organs are obviously quite inefficient except to hold a small worm in close contact with the villi, or a young one while it is growing up.

There is a tendency in the larger cestodes for the scolex to become simpliiied morphologically. In these large forms, its function as a hold-fast organ is obviously greatly reduced. In Dibothriocephalus, the bothria are rudimentary. In the large taenias of man and carnivores, the scolex is only used as an attachment organ during early growth and there is a tendency for the hooks to disappear. Thus, in the cysticercus of T. saginata, as Leuckart (1866) showed, embryonic hooks start to develop only to be reabsorbed. Lubinsky (1960) has demonstrated the labile characters in the hooks of larval Echinococcus. The variation in the organs of attachment in the different groups strongly suggests that not only are they not part of the original ancestor but that their appearance is relatively recent. This is probably true also of the segments in which the gonads appear, mature, and become detached to spread their eggs abroad. The scolex-neck with its. centra1 nervous system and its complex of canals is the basic animal. The suckers, appendages, hooks and so on, came laterand likewise the segments. Stunkard (1962) believes that elongation of the body and replication of reproductive organs are correlated with the parasitic habit and the production of enormous numbers of offspring. He is convinced that the cestode is an individual, not a colony, because of the organic unity of the nervous, muscular and excretory systems. Serial repetition of the gonads is a sexual phenomenon within an individual and modern tapeworm quite frequently show this tendency to reduplicate gonads within the same segment (Baer, 1951). The genital segments of tapeworms are based essentially on one or other of two plans-a closed or an open tubular uterus. The closed uterus is characteristic of tapeworms of terrestrial vertebrates, the open one of aquatic vertebrates. The open uterus has an egg containing a ciliated larva and is obviously the more primitive type. The closed uterus, which does not discharge eggs until the gravid segment containing it disintegrates, has an egg intended to be swallowed, in the great majority of cases, by an arthropod. In the forms in aquatic vertebrates a second intermediary-usually a fishis required. The embryo is identical in all cestodes and is known as the onchosphere or hexacanth larva. It develops in the intermediate host into a mobile procercoid which sheds the six larval hooks. The procercoid grows into what is in effect the scolex of the adult tapeworm. It may be a plerocercoid, a cysticercoid, an amphicyst or a cysticercus-or some modification of these; this takes place in an arthropod except in the case of the cysticercus which develops in a mammal. The plerocercoid has developed organs of attachment to the mucosasuch as suckers and sucking grooves. The cysticercoid has converted the lower middle of the plerocercoid into a capsule consisting of a double fold of cuticle, leaving a solid tail. The

amphicyst which is somewhat similar has the scolex invaginated (Spasski, 1951). The cysticercus has obtained its protection by an enlargement of an excretory vesicle into which the scolex is sunk; the scolex develops in an invaginated form. The hydatid cyst is the most complicated of all the larval stages and is a modification of the cysticercus with a great multiplicative potential in which a number of scoleces develops inside a mother cyst; it is probably the most recent of the cestode larvae (Webster and Cameron, 1961). In all cases, when the young tapeworm is swallowed by the definitive host, the accessory parts are digested away leaving the scolex and neck of the adult. In a recent review, Stunkard (1962) suggests that the progenitor of the cestodes before they became parasitic had a planula-like larva and that changes in physiology, morphology and life cycles proceeded step by step as evolutionary adaptations to parasitism. He regards the greatest hiatus in knowledge of cestodes as the gap between the original ciliated planula and the onchosphere with its gland cells and hooks-the loss of which and the development of plerocercoids, are correlated with the elaboration of the life history which resulted from the infection of vertebrate hosts. Cestodes, like trematodes, he believes, were originally parasites of invertebrates and the digestive tract was lost before the worms became parasites of vertebrates and strobilization provided the method of maintaining populations after the original hosts were eaten.

Baer (1947) believed that there is no justscation whatever for assuming that tapeworms were originally parasites of invertebrates that might today be the intermediate hosts. This is obviously true. However, there is no evidence to conclude that the original hosts of tapeworms could not have been invertebrates. As Stunkard (1957) has put in “There are of course no factual data concerning the origin of parasitism by the ancestor of the cestodes”. Nevertheless, the balance of probability favours a crustacean origin which may, however, have passed through an equally primitive echinoderm before reaching the early vertebrates. While it is still true that the origin of tapeworms is speculative, it is a reasonable assumption that there was a free-living turbellarian-like ancestor, probably with a considerably reduced digestive system. It was probably a freshwater form which became parasitic and matured in Crustacea. The hexacanth embryo shows that the young larva was designed to penetrate the gut wall and reach the body cavity in which it developed. The protocestode, with its single segment and no attachment organs, present in infected Crustacea which were ingested by primitive fish-like vertebrates, was able to survive in the intestine and produce eggs; ultimately the young parasite in the crustacean lost the ability to develop gonads and remained a procercoid larva.

Parasitism of the vertebrates probably began in the Palaeozoic-that of Crustacea much earlier. During the Mesozoic, the transition to land animals began and was well under way by the end of the era. Birds easily became infected and as a result of their own sudden species explosion, their tapeworms quickly became group specific and in consequence, the majority of cyclophyllidean tapeworms have avian hosts. The herbivorous and insectivorous mammals also became infected in the late Mesozoic or the very early Tertiary.

To become established a parasite must first get into a host and possess the ability to avoid destruction by digestion or otherwise and to obtain nourishment. It must not materially harm the host and it must develop the ability to remain in the host, either by developing mechanisms (such as suckers or muscles) to counteract peristalsis or by leaving the digestive tract to live elsewhere. It may have to become microaerobic or anaerobic in order to metabolize its food. It also loses certain useless senses and properties.

B. THE ACANTHOCEPHALA

The Acanthocephala is another small group of elusive a€Enities and puzzling origin. In spite of the efforts of numerous zoologists to classify them with the roundworms, they are not even remotely related to the Nematoda. In fact, van Cleave (1948) considered that they might even be Platyhelminthes of some sort, with a distant relationship to the Cestoda. If they are so related, the relationship is quite distant. They probably have no living relations. They are exclusively endoparasitic and like the Cestoda, have no digestive tract at any phase of their existence. The sexes are separate. The complex egg-shell contains a hooked embryo (the acanthor) which hatches only after ingestion by the intermediate host-an arthropod. The hooks, like those of the cestode larva, are used only to penetrate the gut of the intermediate host; they are then lost and replaced by the proboscis hooks of the adult. When the intermediate host is eaten, the young adult becomes sexually mature in the intestine. They are absent in elasmobranchs, rare in marine fishes but common in freshwater teleosts. They are equally absent from terrestrial reptiles but occur in Amphibia and freshwater turtles, birds and mammals. They probably originated in freshwater teleosts, after elasmobranchs became marine, from ancestors which cannot now even be suggested.

C. THE TREMATODA

The Trematoda are usually classilied in two groups on the basis of their life histories (Yamaguti, 1958). It is quite doubtful if the smaller of these groups, the Monogenea, have any even relatively close relationship with the Digenea. Most are ectoparasitic on elasmobranchs but as Baer (1951) has pointed out, the distinctive freshwater fishes of Australia are common hosts and these fish are regarded as of marine origin, having immigrated relatively recently from the sea. This, and the relatively straightforward type of life cycle, suggests a very early origin of the stock from a primitive rhabdocoelan turbellarian and their early diversification in marine and freshwater animals. As freshwater turtles are hosts but marine turtles are not, and as the marine turtles separated from the land about mid-Mesozoic, it would seem that the Monogenea had become quite host specific by that time and that their origin lies in the earliest Mesozoic-very much earlier than the probable appearance of the Digenea, which certainly did not evolve from them. Although the digenetic trematodes also probably arose from an early turbellarian ancestor, they are not closely related to the cestodes. They are less host

specific in their adult stages, although as van Cleave and Mueller (1934) pointed out "as a general rule, phylogenetically related fishes may be expected to carry similar parasitic populations. However other factors, such as food habits and habitat preferences frequently prove more important than the taxonomic relationships of the host in determining what kind of parasites occur". Stunkard (1937) agrees that while parallel evolution of hosts and parasites exist, "its uncritical application can result in grossly erroneous conclusions". If observations are confined to sexually mature helminths, phylogeny is liable to be misinterpreted and it is important to take life cycles and larval morphology into consideration. Adult trematodes are parasites of vertebrates and host specificity is far from strict. This is not true for the "larval" stages which occur in molluscs; they are usually very host specific. The first larval stage is a ciliated planula-like miracidium, cilia being present in this larva even if the egg does not hatch until it is eaten by a terrestrial snail. In the snail the larva becomes a sporocyst and the germinal cells contained in it multiply and as the result of what is probably polyembryony, produce cercariae. The cercariae are immature adults with some quite specialized larval organs developed to enable them ultimately to become mature adults in a vertebrate. These specialized larval characters are very varied in different groups and suggest a relatively recent evolution; the adult characters on the other hand are much more constant. It seems quite obvious that the digenetic trematodes were originally parasites of molluscs in which they became sexually mature, only subsequently deferring maturity until they left the mollusc. Three main methods of development evolved. The immature adult, i.e. the cercaria, after losing its tail and some larval characters, became encysted in a mucous coat either freely in water, on vegetation, or on fish scales. There it remained until the necessary stimuli to develop further were provided by the vertebrate host swallowing it. In the second case, the cercaria actually penetrated the substance of a fish and there became encysted in a mucous coat secreted by itself; its larval organs were lost during the penetration process but it commenced the development of its adult organs. Its subsequent development was in a carnivorous fish-eating vertebrate. The great variety of obviously related species in both birds and mammals, suggests that this is a comparatively old method of development. In the third kind, the cercaria itself penetrated the skin of the final host without encysting and became sexually mature in the bloodstream. Stunkard (1923) believed that the blood flukes are a very old group and that the ancestors of those of warm-blooded animals are derived from their reptilian ancestors. As birds and mammals originated from entirely different Mesozoic reptilian groups, it would seem that the common ancestors were at least very early Mesozoic, possibly even Palaeozoic. The blood flukes of reptiles and fishes are still hermaphroditic; those of warm-blooded forms are physiologically hermaphroditic even if physically dioecious.

D. THE NEMATODA

The phylum Nematoda contains a very large number of free-living speciesforms which live in almost every conceivable habitat-terrestrial, aquatic and marine-and is

comparable in numbers to the arthropods. All are relatively simple small animals with a pronounced basic similarity. All possess a digestive tube which, typically, has its anterior portion in the form of a triradiate, muscular sucking pharynx. By a rhythmical contraction of the radial fibres a negative pressure is produced against a food particle, a wave of contraction passes down the pharynx, and the particle enters the intestine through a valvular apparatus at the posterior end. This radial arrangement is further reinforced by a, posterior muscular bulb in front of the valve, which by contracting its fibres, increases the negative pressure. This is one of the most characteristic features of the phylum but it is generally modified in the parasitic species by the absence of the posterior bulb, except in some archaic forms living in the large bowel and in the free-living non-parasitic stages of species which feed on comparatively gross material. Its presence in these forms is of no phylogenetic significance. It is merely the most efficient mechanism for ingesting particulate food and almost certainly was present in the ancestors of all parasitic nematodes. It is quite impossible to say how parasitism began-it certainly occurred a number of times, giving rise to several distinct and unrelated groups of parasitic nematodes-a fact completely obscured by most of our modern systems of classification (Baer, 1946; Cameron, 1956). Undoubtedly, in most cases it was (as it still is) by ingestion; in some cases, however, the well-known thigmotaxis of so many small forms appear to have accounted for an entry through the skin.

Probable questions:

1. Discuss the origin and evolution of class trematoda.
2. Discuss the origin and evolution of class Acanthocephala.
3. Discuss the origin and evolution of class cestoda.
4. Discuss the origin and evolution of phylum nematoda.

Suggested reading:

1. Host Specificity and the Evolution of Helminthic Parasites THOMAS W. M. CAMERON Institute of Parasitology, McGill University, Montreal, Canada
2. Marshall: Parker & Haswell Text Book of Zoology, Vol. I, 7th edition, Macmillan,1972

Unit-III

Life cycle pattern in trematoda

Objectives:

In this section we will discuss on life cycle pattern in trematoda.

Introduction:

There are approximately 25,000 species of digenetic trematodes, or flukes, the adults of which are obligate parasites in a variety of vertebrate animals, ranging from fishes, to birds and mammals. All trematodes, regardless of their definitive hosts, are flat, they possess a proto-nephridial osmoregulatory system, an incomplete gut, and are covered by a syncitial tegument through which at least some nutrient resources are absorbed. Despite these, and a number of other morphological and physiological similarities, there is a huge diversity in their size, shape, and behavior, as well as the site of infection within their hosts.

The Life-Cycle Pattern:

Larval and Juvenile Development

- **Egg (Shelled Embryo)**

The structure referred to as an egg of trematodes is not an ovum but a developing (or developed) embryo enclosed by its shell, or capsule. The egg capsule of most flukes has an operculum at one end, through which the larva eventually will escape. It is not clear how the operculum is formed, but it appears that the embryo presses pseudopodium like processes against the inner surface of the shell while it is being formed, thereby forming a circular groove. An operculum is absent from eggshells of blood flukes. Considerable variation exists in shape, size, thickness, and coloration of fluke capsules.

In many species an egg contains a fully developed miracidium by the time it leaves the parent; in others development has advanced to only a few cell divisions by that time. In *Heronimus mollis* miracidia hatch while still in their parent's uterus. For eggs that embryonate in the external environment, certain factors influence rate of development. Water is necessary, since eggs desiccate rapidly in dry conditions. High oxygen tension accelerates development, although eggs can remain viable for long periods under conditions of low oxygen. Eggs of *Fasciola hepatica* will not develop outside a pH range of 4.2 to 9.0. Temperature is critical, as would be expected. Thus, *F. hepatica* requires 23 weeks to develop at 10°C, whereas it takes only eight days at 30°C. However, above 30°C development again slows, and it completely stops at 37°C. Eggs are killed rapidly at

freezing. Light may be a factor influencing development in some species, but this has not been thoroughly investigated.

Eggs of many species hatch freely in water, whereas others hatch only when eaten by a suitable intermediate host. Light and osmotic pressure are important in stimulation of hatching for species that hatch in water, and osmotic pressure, carbon dioxide tension, and probably host enzymes initiate hatching in those that must be eaten. Time of hatching is correlated with the time the snail intermediate host is nearby. Light is also required for hatching of *Echinostoma caproni* eggs, which likewise show a circadian hatching pattern.

The miracidium of *F. hepatica* within its capsule is surrounded by a thin vitelline membrane, which also encloses a pad-like viscous cushion between the anterior end of the miracidium and the operculum. Light stimulates hatching activity. Apparently, the miracidium releases some factor that alters the permeability of the membrane enclosing the viscous cushion. The latter structure contains a mucopolysaccharide that becomes hydrated and greatly expands the volume of the cushion. The considerable increase in pressure within the capsule causes the operculum to pop open, remaining attached at one point, and the miracidium rapidly escapes, propelled by its cilia. The nonoperculated capsules of *Schistosoma* spp. are fully embryonated when passed from the host, and they hatch spontaneously in fresh water. Miracidia release substantial quantities of leucine aminopeptidase, and this enzyme probably helps digest the capsule from the inside. Unlike leucine aminopeptidases from other sources, the enzyme produced by schistosome miracidia is inhibited by NaCl, which prevents hatching while in the host's body.

- **Miracidium**

A typical miracidium is a tiny, ciliated organism that could easily be mistaken for a protozoan by a casual observer. It is piriform, with a retractable apical papilla at the anterior end. The apical papilla has no cilia but bears five pairs of duct openings from glands and two pairs of sensory nerve endings (Fig. 1). The gland ducts connect with penetration glands inside the body. A prominent apical gland can be seen in the anterior third of the body. This gland probably secretes histolytic enzymes. An apical stylet is present on some species, and spines are found on others. Sensory nerve endings connect with nerve cell bodies that in turn communicate with a large ganglion. Miracidia have a variety of sensory organs and endings, including adaptations for photoreception, chemoreception, tangoreception, and statoreception.

The outer surface of a miracidium is covered by flat, ciliated epidermal cells, the number and shape of which are constant for a species. Underlying the surface are longitudinal and circular muscle fibers. Cilia are restricted to protruding ciliated bars in genus *Leucochloridiomorpha* (Brachylaimidae) and family Bucephalidae, and they are absent altogether from families Azygiidae and Hemiuridae. One or two pairs of protonephridia are connected to a pair of posterolateral excretory pores.

In the posterior half of a miracidium are found propagatory cells, or germ balls (embryos), which will be carried into the sporocyst stage to initiate further individuals.

Free-swimming miracidia are very active, swimming at a rate of about 2 mm per second, and they must find a suitable molluscan host rapidly, since they can survive as free-living organisms for only a few hours. In many cases mucus produced by the snail is a powerful attractant for miracidia.

On contacting an appropriate mollusc, the miracidium attaches to it with its apical papilla, which actively contracts and extends in an auger-like motion. Cytolysis of snail tissues can be seen as the miracidium embeds itself deeper and deeper. As penetration proceeds, the miracidium loses its ciliated epithelium, although this may be delayed until penetration is complete. A miracidium takes about 30 minutes to complete penetration. Miracidia of many species will not hatch until they are eaten by the appropriate snail, after which they penetrate the snail's gut.

- **Sporocyst**

Often miracidia undergo metamorphosis near their site of penetration, such as foot, antenna, or gill, but they may migrate to any tissue, depending on the species, before beginning metamorphosis. Metamorphosis of a miracidium into a sporocyst involves extensive changes. In addition to loss of ciliated epithelial cells, there is formation of new tegument with its microvilli. Sporocysts retain the sub-tegumental muscle layer and protonephridia of the miracidium, but all other miracidial structures generally disappear. A sporocyst has no mouth or digestive system; it absorbs nutrients from its host tissue, with which it is in intimate contact, and the entire structure serves only to nurture the developing embryos. The sporocyst (or other stage with embryos developing within it—that is, the miracidium or redia) may be referred to as a germinal sac. Sometimes sporocysts may become very slender and extended or branched or highly ramified.

Embryos in a sporocyst may develop into another sporocyst generation (daughter sporocysts), into a different form of germinal sac (redia), or directly into cercariae.

Leucochloridium paradoxum has a specialized sporocyst with a fascinating adaptation that evidently enhances transfer to its bird definitive host. The sporocyst is divided into three parts: a central body located in the snail's hepatopancreas, where the embryos are produced; a broodsac lying in the head-foot of the snail and entering its tentacles; and a tube connecting the broodsac to the central body. Embryos pass from the central body through the tube to the broodsac, where they mature into cercariae. The sporocyst within the snail's tentacles causes the tentacles to enlarge, become brightly colored, and pulsate rapidly.

- **Redia**

Rediae burst their way out of the sporocyst or leave through a terminal birth pore and usually migrate to the hepatopancreas or gonad of their molluscan host. They are commonly elongated and blunt at the posterior end and may have one or more stumpy

appendages called procrusculi. More active than most sporocysts, they crawl about within their host. They have a rudimentary but functional digestive system, consisting of a mouth, muscular pharynx, and short, unbranched gut. Rediae pump food into their gut by means of pharyngeal muscles, as previously described in adults. They not only feed on host tissue but also can prey on sporocysts of their own or other species. The luminal surface of their gut is greatly amplified by flattened lamelloid or ribbonlike processes. Gut cells are apparently capable of phagocytosis. The outer surface of their tegument also functions in absorption of food, and it is provided with microvilli or lamelloid processes.

Embryos in rediae develop into daughter rediae or into the next stage, cercariae, which emerge through a birth pore near the pharynx. The epithelial lining of the birth pore in *Cryptocotyle lingua* (and probably other species) is highly folded, making it able to withstand the extreme distortion produced by the exit of a cercaria. It appears that rediae must reach a certain population density before they stop producing more rediae and begin producing cercariae: Young rediae have been transplanted from one snail to another through more than 40 generations without cercariae being developed. This type of regulation is an interesting parallel to certain free-living invertebrates that reproduce parthenogenetically only as long as certain environmental conditions are maintained.

- **Cercaria**

Cercariae represent a juvenile stage of the vertebrate-inhabiting adult. There are many varieties of cercariae, and most have specializations that enable them to survive a brief free-living existence and make themselves available to their definitive or second intermediate hosts. Most have tails that aid them in swimming, but many have only rudimentary tails or none at all; these cercariae can only creep about, or they may remain within the sporocyst or redia that produced them until they are eaten by the next host.

Structure of a cercaria is easily studied, and cercarial morphology often has been considered a more reliable indication of phylogenetic relationships among families than has adult morphology. The name Cercaria can be used properly in a generic sense for a species in which the adult form is unknown, as is done with the term Microfilaria among some nematodes.

Most cercariae have a mouth near the anterior end, although it is midventral in Bucephalidae. The mouth is usually surrounded by an oral sucker, and a prepharynx, muscular pharynx, and forked intestine are normally present. Each branch of the intestine is simple, even those that are ramified in adults. Many cercariae have various glands opening near the anterior margin, often called penetration glands because of their assumed function. Cercariae of most trematodes probably have glands that serve several functions; schistosome cercariae have no fewer than four distinguishable types:

1. Escape glands. They are so-called because their contents are expelled during emergence of the cercaria from the snail, but their function is not known.

2. Head gland. The secretion is emitted into the matrix of the tegument and is thought to function in the post-penetration adjustment of the schistosomule.

3. Postacetabular glands. They produce mucus, help cercariae adhere to surfaces, and have other possible functions.

4. Preacetabular glands. The secretion contains calcium and a variety of enzymes including a protease. The function of these glands seems most important in actual penetration of host skin.

Secretory cystogenic cells are particularly prominent in cercariae that will encyst on vegetation or other objects.

Cercariae have many morphological variations that are constant within a species (or larger taxon); thus, certain descriptive terms are of value in categorizing the different varieties. Some of the more commonly used terms are xiphidiocercaria (with a stylet in the anterior margin of the oral sucker), ophthalmocercaria (with eyespots), cercariaeum (without a tail), microcercous cercaria (with a small, knoblike tail), and furcocercous cercaria (with a forked tail).

The excretory system is well developed in cercariae. In some cercariae the excretory vesicle empties through one or two pores in the tail. Because the number and arrangement of protonephridia are constant for a species, these are important taxonomic characters. Each flame cell has a tiny capillary duct that joins with others to form an accessory duct. The accessory ducts join the anterior or posterior collecting ducts, whose junction forms a common collecting duct on each side (Fig. 2). When the common collecting ducts extend to the region of the midbody and then fuse with the excretory vesicle, the cercaria is called mesostomate. If the tubules extend to near the anterior end and then pass posteriorly to join the vesicle, the cercaria is known as stenostomate. The number and arrangement of flame cells can be expressed conveniently by a flame cell formula. For example, 2[(3 + 3) (3 + 3)] means that both sides of the cercaria (2) have three flame cells on each of two accessory tubules (3 + 3) on the anterior collecting tubule, plus the same arrangement on the posterior collecting tubule (3 + 3). The flame cell formula for the cercaria in Figure 15.25 would be 2[(3 + 3 + 3) (3 + 3 + 3)].

Mature cercariae emerge from the mollusc and begin to seek their next host. Many remarkable adaptations facilitate host finding. Most cercariae are active swimmers, of course, and rely on chance to place them in contact with an appropriate organism. Some species are photopositive, dispersing themselves as they swim toward the surface of the water, but then become photonegative, returning to the bottom where the next host is. Some opisthorchiform cercariae remain quiescent on the bottom until a fish swim over them; the resulting shadow activates them to swim upward. Some plagiorchiform cercariae cease swimming when in a current; hence, when drawn over the gills of a crustacean host, they can attach and penetrate rather than swim on. Large, pigmented azygiids and bivesiculids are enticing to fish, which eat them and become infected. Some cercariae float; some unite in clusters; some creep at the bottom. Cercariae of *Schistosoma mansoni*, which directly penetrate a warm-blooded definitive host, concentrate in a thermal gradient near a heat source (34°C). In certain cystophorous hemiurid cercariae, their body is withdrawn into the tail, which becomes a complex injection device. The

second intermediate host of the trematode is a copepod crustacean, which attempts to eat the caudal cyst bearing the cercaria. When the narrow end of the cyst is broken by the mandibles of the copepod, a delivery tube everts rapidly into the mouth of the copepod, piercing its midgut. The cercaria then slips through the delivery tube into the hemocoel of the crustacean! These and many more adaptations help the trematode reach its next host.

- **Mesocercaria**

Species of strigeiform genus *Alaria* have a unique larval form called a mesocercaria, which is intermediate between a cercaria and metacercaria.

- **Metacercaria**

Between cercaria and adult is a quiescent stage, or metacercaria, although this stage is absent from blood flukes. Metacercariae are usually encysted, but in genera *Brachycoelium*, *Halipegus*, *Panopistus*, and others they are not. Most metacercariae are found in or on an intermediate host, but some (Fasciolidae, Notocotylidae, and Paramphistomidae) encyst on aquatic vegetation, sticks, and rocks or even freely in the water.

A cercaria's first step in encysting is to cast off its tail. Cyst formation is most elaborate in metacercariae encysting on inanimate objects or vegetation. The cystogenic cells of *Fasciola hepatica* are of four types, each with the precursors of a different cyst layer. Metacercariae encysting in intermediate hosts have thinner and simpler cyst walls, with some components contributed by the host.

The extent of development in metacercariae varies widely according to species, from those from which a metacercaria is absent (*Schistosoma* spp.) to those in which the gonads mature and viable eggs are produced (*Proterometra* spp.). Often some amount of development is necessary as a metacercaria before a trematode is infective for its definitive host. We can arrange metacercariae in three broad groups on this basis:

1. Species such as *Fasciola* spp., whose metacercariae encyst in the open on vegetation and inanimate objects and that can infect a definitive host almost immediately after encystment, in some cases within only a few hours, with no growth occurring.

2. Species that do not grow in an intermediate host but that require at least several days of physiological development to infect a definitive host, such as those in family Echinostomatidae.

3. Species whose metacercariae undergo growth and metamorphosis before they enter their resting stage in a second intermediate host and that usually require a period of weeks for this development; examples are found in family Diplostomidae.

These developmental groups are correlated with longevity of the metacercariae: Those in group 1 must live on stored food and can survive the shortest time before reaching a definitive host, whereas those in groups 2 and 3 obtain some nutrients from

their intermediate hosts and so can remain viable for the longest periods—in one case up to seven years. After the required development, metacercariae go into a quiescent stage and remain in readiness to excyst on reaching a definitive host. *Zoogonus lasius,* a typical example of group 2, has a high rate of metabolism for the first few days after infecting its second intermediate host, a nereid polychaete, and then drops to a low level, only to return to a high rate on excystation. Metacercariae of *Bucephalus haimeanus* remain active in the liver of their fish host and increase threefold in size. They take up nutrients from degenerating liver cells, including large molecules, by pinocytosis. Metacercariae of *Clinostomum marginatum* take up glucose both by facilitated diffusion and by active transport.

The metacercarial stage has a high selective value for most trematodes. It can provide a means for transmission to a definitive host that does not feed on the first intermediate host or is not in the environment of the mollusc, and it can permit survival over unfavorable periods, such as a season when definitive hosts are absent.

Development in a Definitive Host

Once a cercaria or metacercaria has reached its definitive host, it matures in a variety of ways: either by penetration (if a cercaria) or by excystation (if a metacercaria) and then by migration, growth, and morphogenesis to reach gamete production. If the species does not have a metacercaria and the cercaria penetrates the definitive host directly, as in schistosomes, the most extensive growth, differentiation, and migration will be necessary. At the other extreme some species acquire adult characters as metacercariae, the gonads may be almost mature, or some eggs may even be present in the uterus; and little more than excystation is needed before the production of progeny (*Bucephalopsis, Coitocaecum, Transversotrema*). A very few species (*Proctoeces maculatus* and *Proterometra dickermani*) reach sexual reproduction in the mollusc and apparently do not have a vertebrate definitive host. Others may mature in another invertebrate; for example, several species of Macroderoididae mature in leeches and *Allocorrigia filiformis* matures in the antennal gland of a crayfish. These are probably examples of neoteny.

Normally, development in a definitive host begins with excystation of the metacercaria, and species with the heaviest, most complex cysts, such as those with cysts on vegetation (for example, *Fasciola hepatica*), seem to require the most complex stimuli for excystation. Digestive enzymes largely remove the outer cyst of *F. hepatica,* but escape from the inner cyst requires presence of a temperature of about 39°C, a low oxidation-reduction potential, carbon dioxide, and bile. Such conditions enhance excystment of a number of other species. This combination of conditions is not likely to be present anywhere but in the intestine of an endothermic vertebrate; like the conditions required for the hatching or exsheathment of some nematodes, these requirements constitute an adaptation that avoids premature escape from protective coverings. This kind of adaptation is less important to metacercariae that are not subjected to the widely varying

physical conditions of the external environment, such as those encysted within a second intermediate host. These have thinner cysts and excyst on treatment with digestive enzymes. A number of species require presence of a bile salt(s) or excyst more rapidly in its presence. Some metacercariae may release enzymes that assist in excystment.

After excystation in the intestine, a more or less extensive migration is necessary if the final site is in some other organ. The main sites of such parasites are the liver, lungs, and circulatory system. Probably the most common way to reach the liver is by way of the bile duct (*Dicrocoelium dendriticum*), but *F. hepatica* burrows through the gut wall into the peritoneal cavity and finally, wandering through the tissues, reaches the liver. *Clonorchis sinensis* usually penetrates the gut wall and is carried to the liver by the hepatoportal system. *Paragonimus westermani* penetrates the gut wall, undergoes a developmental phase of about a week in the abdominal wall, and then reenters the abdominal cavity and makes its way through the diaphragm to the lungs.

Host hormones have significant effects on survival, growth, and maturation of schistosomes. There is little evidence that such effects are mediated by control of gene expression via nuclear receptors, but a number of apparent nuclear receptors ("orphan" receptors) have been described in schistosomes for which a ligand is currently unknown.

Trematode Transitions

A remarkable physiological aspect of trematode life cycles is the sequence of totally different habitats in which the various stages must survive, with physiological adjustments that must often be made extremely rapidly. As an egg passes from a vertebrate, it must be able to withstand rigors of the external environment in fresh water or seawater, if only for a period of hours, before it can reach haven in a mollusc. There conditions are quite different from those of both the water and the vertebrate. A trematode's physiological capacities must again be readjusted on escape from the intermediate host and again on reaching a second intermediate or definitive host. Environmental change may be somewhat less dramatic if the second intermediate host is a vertebrate, but often it is an invertebrate. Although adjustments must be extensive, the nature of these physiological adjustments made by trematodes during their life cycles has been little investigated, the most studied trematodes in this respect being *Schistosoma* spp.

Penetration of a definitive host is a hazardous phase of the life cycle of schistosomes, and it requires an enormous amount of energy. Hazards include a combination of dramatic changes in the physical environment, in physical and chemical nature of host skin through which a schistosome must penetrate, and in host defense mechanisms. Depending on the host species, losses at this barrier may be as high as 50%, and the glycogen content of newly penetrated schistosomules (schistosomule is the name given a young developing worm) is only 6% of that found in cercariae.

Among the most severe physical conditions the organism must survive is a sequence of changes in ambient osmotic pressure. Osmotic pressure of fresh water is considerably below that in a snail, and that in a vertebrate is twice as great as in a mollusc. Assuming that osmotic pressure of cercarial tissues approximates that in snails, the trematode must avoid taking up water after it leaves the snail and avoid a serious water loss after it penetrates a vertebrate. Aside from the possible role of the osmoregulatory organs (protonephridia), there appear to be major changes in the character and probably permeability of the cercarial surface. The cercarial surface is coated with a fibrillar layer, or glycocalyx, which is lost on penetration of a vertebrate, and with it is lost the ability to survive in fresh water; 90% of schistosomules recovered from mouse skin 30 minutes after penetration die rapidly if returned to fresh water.

Biochemical changes in the tegument occur after penetration: The schistosomule surface is much less easily dissolved by a number of chemical reagents, including 8 M urea, than is that of cercariae. Antigenic epitopes on the tegument are changed as well. When cercariae are incubated in immune serum, a thick envelope called the CHR (cercarienhüllenreaktion) forms around them, but schistosomules do not give this reaction.

In several cases cercarial attraction to the next host is mediated by substances different from those that stimulate actual penetration. Schistosome cercariae are apparently attracted to host skin by the amino acid arginine, whereas the most important stimulus for actual penetration is the skin lipid film, specifically essential fatty acids, such as linoleic and linolenic acids, and certain nonessential fatty acids. Human skin surface lipid applied to walls of their glass container will cause cercariae to attempt to penetrate it, lose their tails, evacuate their preacetabular glands, and become intolerant to water. The presence of the penetration-stimulating substances causes loss of osmotic protection and a reduction of the CHR, even in cercariae free in the water. Successful penetration and transformation have been correlated with cercarial production of eicosanoids, such as leukotrienes and prostaglandins (fatty acid derivatives with potent pharmacological activity). These eicosanoids may enable schistosomules to evade host defenses by inhibiting superoxide production by neutrophils.

After penetration the tegument of developing schistosomules undergoes a remarkable morphogenesis. Within 30 minutes numerous subtegumental cells connect with the distal cytoplasm to become the tegumental cytons. Abundant multilaminate vesicles pass from the cytons through the distal cytoplasm to fuse at the surface. These laminae coalesce to form two layers: an outer membranocalyx and an inner apical plasma membrane. The old cercarial outer membrane, along with its remaining glycocalyx, is cast off. These changes are almost entirely complete within three hours after penetration. During the next two weeks the main changes in the tegument are a considerable increase in thickness and development of many invaginations and deep pits. The pits increase the surface area fourfold between 7 and 14 days after penetration. It is likely that this change represents an adaptation for nutrient absorption through the tegument.

Summary of Life Cycle

In summary, the basic pattern of a digenetic trematode's life cycle is egg → miracidium → sporocyst → redia → cercaria→ metacercaria → adult. The most common variations are (1) more than one generation of sporocysts or rediae, (2) deletion of either sporocyst or redial generations, and (3) deletion of metacercaria. Much less common are cases in which miracidia are produced by sporocysts and forms with adult morphology in the mollusc that produce cercariae (these in turn lose their tails and produce another generation of cercariae).

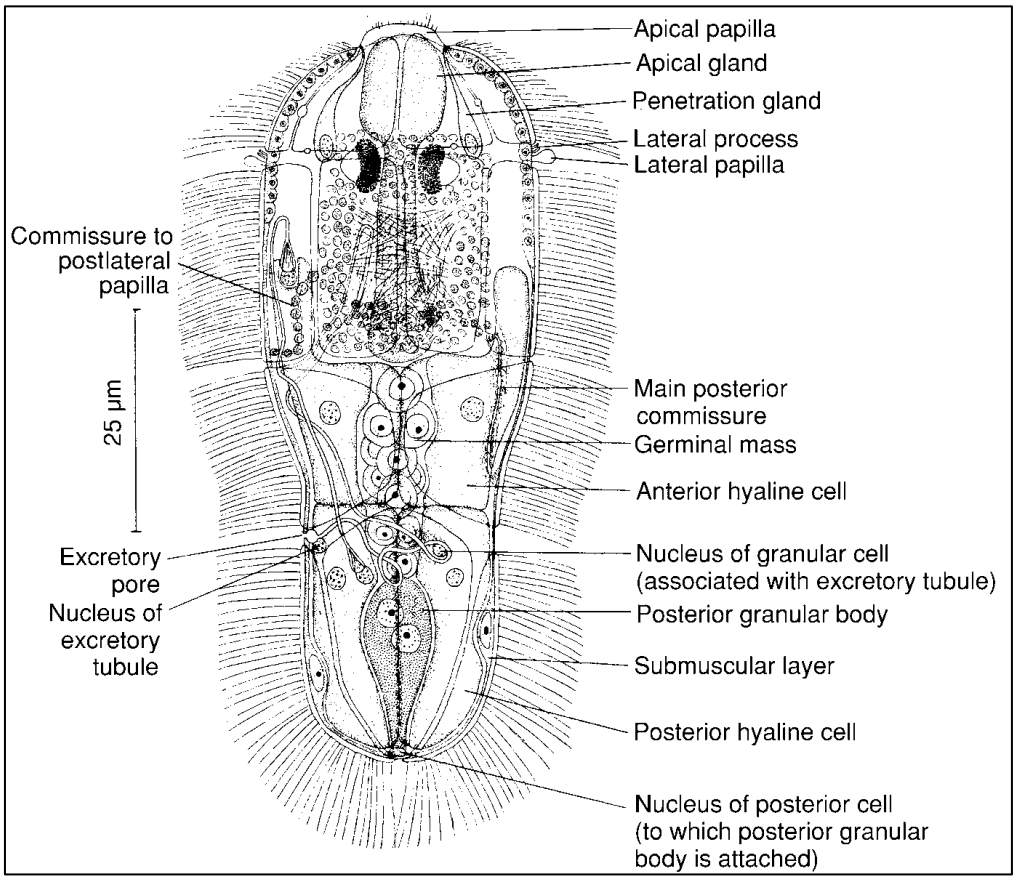

Figure 1 Miracidium of *Neodiplostomum intermedium*, dorsal view

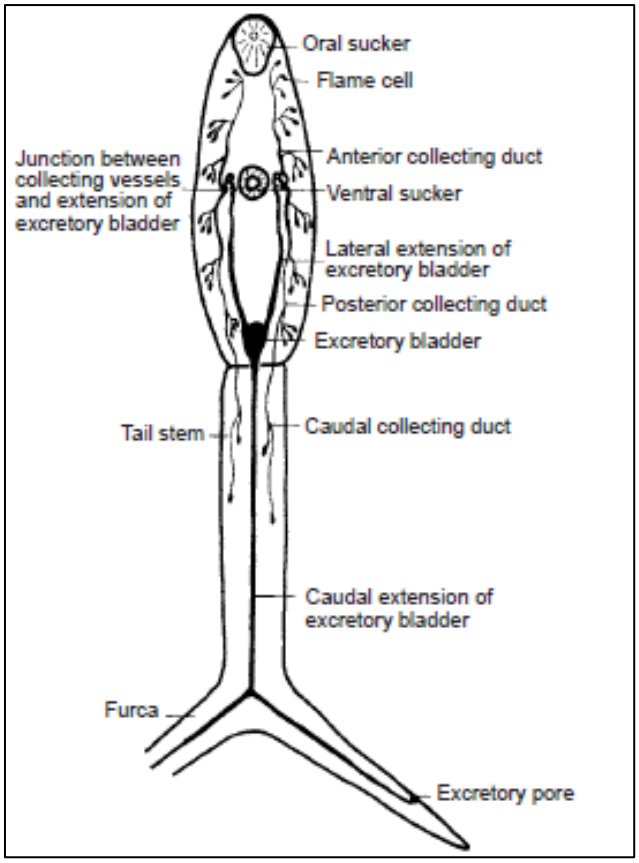

Figure 2 Diagrammatic representation of the excretory system of a fork-tailed cercaria

Probable questions:

1. State the function of operculum present in the egg of trematodes. Give an example of a trematode where egg shell is absent.
2. What are the factors responsible for the embryonation of egg in trematode?
3. Name the different glands found in cercariae. State the function of each gland.
4. Enumerate the types of cercaria based on their anatomical modification.
5. Which is the infective stage in the life cycle of trematodes? State its significance in propagation of a trematode.
6. How cercariae attaches to the body of host?

Suggested reading:

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Unit-IV
Life cycle pattern in cestode

Objectives:

In this section we will discuss on life cycle pattern in cestoda.

Introduction:

Cestoda is a class of parasitic worms in the flatworm phylum (Platyhelminthes). Most of the species—and the best-known—are those in the subclass Eucestoda; they are ribbon-like worms as adults, known as tapeworms. Their bodies consist of many similar units known as proglottids—essentially packages of eggs which are regularly shed into the environment to infect other organisms. Species of the other subclass, Cestodaria, are mainly fish infecting parasites. All cestodes are parasitic; many have complex life histories, including a stage in a definitive (main) host in which the adults grow and reproduce, often for years, and one or two intermediate stages in which the larvae develop in other hosts.

The Life-Cycle Pattern:

Nearly every life cycle known for tapeworms requires two hosts for its completion. One notable exception is *Hymenolepis nana,* a cyclophyllidean parasite of mice and humans, which can complete its juvenile stages within its definitive host. (*Hymenolepis nana* has been called *Vampirolepis nana* because the type species of *Hymenolepis, H. diminuta,* has an unarmed rostellum, and the rostellar hooks of *H. nana* were viewed as the basis for putting it in a separate genus. However, due to the case of *Taenia saginata* vs. *Taeniarhynchus saginatus*, we can no longer justify presence or absence of rostellar hooks as a generic distinction.) Complete life cycles are known for only a comparatively few species of tapeworms. In fact, there are some orders in which not a single life cycle has been determined. Among life cycles that are known, much variety exists in juvenile forms and patterns of development. Sexually mature tapeworms live in the intestine or its diverticula or rarely in the coelom of all classes of vertebrates. As mentioned, two genera are known that can mature in invertebrates. A mature tapeworm may live for a few days or up to many years, depending on species. During its reproductive life a single worm produces from a few to millions of eggs, each with the potential of developing into an adult. Because of the great hazards obstructing the course of transmission and development of each worm, mortality is high. Most tapeworms are hermaphroditic and are capable of fertilizing their own eggs. Sperm transfer is usually from the cirrus to the vagina of another proglottid in the same strobila or between adjacent strobilae, if the opportunity affords. A few species of tapeworms are dioecious. In these it is not clear what determines the gender of a given strobila, because it appears that each strobila has the potential of maturing as either male or female. Interaction between two or more strobilae is important in sex determination of dioecious forms. For example, in *Shipleya*

inermis (Cyclophyllidea, Dioecocestidae), if a single strobila is present in its shorebird host, it is usually female; if two are present, one is nearly always a male. In fact, most of the time the host intestine contains a single female and a single male worm. Both invertebrates and vertebrates serve as intermediate hosts of tapeworms. Nearly every group of invertebrates has been discovered harboring juvenile cestodes, but the most common are crustaceans, insects, molluscs, mites, and annelids. As a general rule, when a tapeworm occurs in an aquatic definitive host, the juvenile forms are found in aquatic intermediate hosts. A similar assumption can be made for terrestrial hosts. Vertebrate intermediate and paratenic hosts are found among fishes, amphibians, reptiles, and mammals. Tapeworms found in these hosts normally mature within predators whose diets include the intermediary.

Larval and Juvenile Development

Among life histories that are known, much variety exists in juvenile forms and details of development, but there seems to be a single basic theme: (1) embryogenesis within the egg to result in a larva, the oncosphere; (2) hatching of the oncosphere after or before being eaten by the next host, where it penetrates to a parenteral (extraintestinal) site; (3) metamorphosis of the larva in the parenteral site into a juvenile (metacestode) usually with a scolex; and (4) development of an adult from the metacestode in the intestine (enteral site) of the same or another host. Oncospheres of all Eucestoda have three pairs of hooks and thus also are referred to as hexacanths. Free-swimming oncospheres hatching from an egg of some Pseudophyllidea and a few Tetraphyllidea have a ciliated inner envelope (IE) and are called coracidia (Fig. 1). Larvae of gyrocotylideans and amphilinideans have 10 hooks (hence, they are decacanths), are also ciliated, and are called lycophoras.

In cestodes with free-swimming larvae a coracidium must be eaten by an intermediate host, usually an arthropod, within a short time. A coracidium sheds its ciliated IE and actively uses its six hooks to penetrate the gut of its host. In the hemocoel it metamorphoses into a procercoid (Fig. 2). During this reorganization the oncospheral hooks are relegated to the posterior end in a structure known as a cercomer. A procercoid is defined as the stage in which larval hooks are still present but the definitive holdfast has not developed. It is regarded by some authors as a differentiating metacestode. When the first intermediate host is consumed by a second intermediate host—often a fish—the procercoid penetrates the host gut into the peritoneal cavity and mesenteries and then commonly into skeletal muscles. Development of a scolex characterizes a plerocercoid (see Fig. 2), and there is commonly strobila formation at this stage, with or without concomitant proglottid formation.

In the pseudophyllideans *Ligula* and *Schistocephalus*, development as plerocercoids proceeds so far that little growth occurs when these worms reach a definitive host, and the gonads mature within 72 hours and start producing eggs within 36 hours thereafter. Proteocephalata develop a first-stage plerocercoid in an arthropod

intermediate host, with no intervening procercoid, and a second-stage plerocercoid in a parenteral site in a second intermediate host. In some species of this order, metacestode development (plerocercoid II) may be completed in the gut of a definitive host, or the metacestodes may develop through a sequence of sites: parenterally in an intermediate host, then parenterally in a definitive host, and finally enterally in the definitive host. Coracidia, procercoids, and plerocercoids of pseudophyllideans and plerocercoids of proteocephalatans are all plentifully supplied with penetration glands that aid in penetration of, and migration in, host tissues.

Life cycles of cyclophyllideans differ in that there is neither a procercoid nor a plerocercoid. Larvae are fully developed and infective when they pass from their definitive host, but they do not hatch until eaten by an intermediate host. The oncosphere penetrates the gut of its intermediate host to reach a parenteral site and metamorphoses to a cysticercoid or to a cysticercus type of metacestode. Cysticercoids (see Fig. 2) are solid-bodied organisms with a fully developed scolex invaginated into their body. It is surrounded by cystic layers, and the cercomer, which contains the larval hooks, is outside the cyst. If not displaced mechanically, the cercomer will be digested away, along with parts of the cyst, in the gut of the definitive host. A few cysticercoids have been described that undergo asexual reproduction by budding.

Members of cyclophyllidean family Taeniidae form a cysticercus metacestode (see Fig. 2), which differs from a cysticercoid in that the scolex is introverted as well as invaginated, and the scolex forms on a germinative membrane enclosing a fluid-filled bladder. Several variations from the simple cysticercus in the Taeniidae undergo asexual reproduction by budding. These juvenile stages are of considerable medical and veterinary importance.

Numerous other kinds of metacestodes can be distinguished from the typical forms just described, but they are, for the most part, simply modifications of the basic types:

1. Sparganum—a term originally proposed for any pseudophyllidean plerocercoid of unknown species but now usually used for some plerocercoids of genus *Diphyllobothrium* (formerly *Spirometra*).

2. Plerocercus—a modified plerocercoid found in some Trypanorhyncha, in which the posterior forms a bladder, the blastocyst, into which the rest of the body can withdraw (as in *Gilquinia* spp.). Also applied to plerocercoids of proteocephalatans with an invaginated scolex.

3. Strobilocercoid—a cysticercoid that undergoes some strobilation; found only in *Schistotaenia* spp.

4. Tetrathyridium—a fairly large, solid-bodied juvenile that can be regarded as a modified cysticercoid, developing in vertebrates that have ingested the cysticercoid encysted in the invertebrate host. It is known only in the atypical cyclophyllidean *Mesocestoides*.

5. Variations on cysticercus.

a. Strobilocercus—a simple cysticercus in which some strobilation occurs within the cyst (for example, *Taenia taeniaeformis*).

b. Coenurus—budding of a few to many scolices (called protoscolices) from the germinative membrane of the cyst, each on a simple stalk invaginated into the common bladder (as in *Taenia multiceps*).

c. Unilocular hydatid (see Fig. 2)—with up to several million protoscolices present; there are occasional sterile specimens. Usually there is inner, or endogenous, budding of brood cysts, each with many protoscolices inside. Exogenous budding rarely

occurs, resulting in two more hydatids called daughter cysts. Unilocular hydatids may grow very large, sometimes containing several quarts of fluid. Occasionally many protoscolices break free and sink to the bottom of the cyst, forming hydatid sand, but this is probably rare in the living, normal cyst. This metacestode form is known only for the cyclophyllidean genus *Echinococcus*.

d. Multilocular or alveolar hydatid— known only for *Echinococcus multilocularis*, exhibiting extensive exogenous budding, resulting in an infiltration of host tissues by numerous cysts. It forms a single mass with many little pockets that contain protoscolices when in a normal host.

Effects of Metacestodes on Hosts

Tapeworms present many examples of a phenomenon called parasite induced trophic transmission (PITT), in which parasite infection causes changes in the behavior, physiology, or morphology of one host that facilitates transmission to the next host. Because increased transmission generally increases parasite fitness, a selective value for a parasite from such host manipulation seems clear.

Life cycles of tapeworms in order Pseudophyllidea commonly include a procercoid stage in a crustacean first intermediate host and a plerocercoid in a second intermediate host (usually a fish but sometimes another vertebrate). Copepods infected with procercoids of *Triaenophorus crassus* swim near the water surface, where they are more likely to be preyed on by fish, their second intermediate host, whereas uninfected copepods remain near the bottom. Furthermore, infected copepods only show this behavior beginning 10 days after infection, when the procercoids have become mature enough to survive and continue development in a fish. Infected copepods are less motile and have reduced escape responses. Plerocercoids often develop in the skeletal muscle of second intermediate hosts, diverting energy from the muscle and degrading muscle function, so that a second intermediate host can be captured more easily by a predator. Plerocercoids such as those of *Ligula intestinalis* develop in the abdominal cavity of their fish host, growing rapidly and greatly distending the host belly. Not only is the swimming

ability of such a fish seriously impaired, but also it now prefers shallow water where it can be captured more easily by a piscivorous bird.

Many instances are known in which cyclophyllideans enhance transmission by affecting behavior or physiology of their intermediate hosts. Hydatids and coenuri directly disable hosts and facilitate predation by definitive hosts. Infections of mice with cysticerci of *Taenia crassiceps* can prevent adult males from becoming behaviorally dominant by influencing host endocrine function, leading to exclusion of an infected host from its group and increasing chance of predation. Infections of beetles (*Tenebrio molitor*) with cysticercoids of *Hymenolepis diminuta* extend the life of female beetles by reducing host fecundity and thus exposing beetles to more predation. These beetles become infected when they consume shelled larvae of *H. diminuta* in feces of infected rodents. Feces of infected hosts release a volatile attractant that causes hungry beetles (*Tribolium confusum*) to feed preferentially on feces containing larvae rather than feces from uninfected hosts. Whether the attractant originates from adult worms in the definitive host or from some induced modification in host physiology is not known.

Development in Definitive Hosts

When a juvenile tapeworm reaches the small intestine of its definitive host, certain stimuli cause it to excyst, evaginate, or both and begin growth and sexual maturation. In encysted forms action of digestive enzymes in the host's gut may be necessary to at least partially free the organism from its cyst. In *Hymenolepis diminuta* most of the cyst wall may be removed by treatment with pepsin and then with trypsin, but few worms will evaginate and emerge from the cyst unless bile salts are present.

In some pseudophyllideans with a well-developed strobila in the plerocercoid (for example, species of *Ligula* and *Schistocephalus*), an increase in temperature to that of their definitive host is all that is required for them to mature. The temperature "activation" of such plerocercoids is accompanied by a great increase in the rate of carbohydrate catabolism, excretion of organic acids, and levels of tricarboxylic acid cycle intermediates. A burst of neurosecretory activity occurs during activation of *Diphyllobothrium dendriticum* plerocercoids. Contact of the rostellum with a suitable protein substrate is necessary to induce strobilar growth in *Echinococcus granulosus*.

As strobilar development begins, subsequent events are influenced by a variety of conditions, including size of the infecting juvenile, species of the worm and host, size and diet of the host, presence of other worms, and the immune and/or inflammatory state of the host intestine. Under optimal conditions certain species have a burst of growth that must surely rival growth rates found anywhere in the animal kingdom. *Hymenolepis diminuta* can increase its weight by up to 1.8 million times within 15 to 16 days. Such rapid growth, accompanied by strictly organized differentiation, makes this worm a fascinating system for developmental studies, particularly since the course of the growth may be altered experimentally.

Worm growth is especially sensitive to composition of the host diet with respect to carbohydrates. The situation is best known for *H. diminuta*, but the findings can be extended to other tapeworms, to some extent at least. *Hymenolepis diminuta* apparently has a high carbohydrate requirement, but it can only absorb glucose and to a lesser degree galactose across its tegument. This is true for other cestodes tested, although some can absorb a limited number of other monosaccharides and disaccharides. For optimal growth carbohydrate must be supplied in the host diet in the form of a polysaccharide so that glucose will be released as digestion proceeds in the host gut. If glucose per se—or a disaccharide containing glucose, such as sucrose—is furnished in the host diet, worms are placed at a competitive disadvantage for glucose with respect to the gut mucosa, physiological conditions in the gut are altered, or both, so that the worm's growth is dramatically restrained.

Another important condition affecting worm growth is the increased presence of other tapeworms in the gut, the so-called crowding effect. This is an interesting adaptation by which parasite biomass adjusts to carrying capacity of a host. Again, evidence exists that, although best known in *H. diminuta*, the crowding effect occurs in at least several other species. Within certain limits, the weight of individual worms in a given infection is, on average, inversely proportional to number of worms present. In consequence, total worm biomass and number of eggs produced are the same and are maximal for that host, regardless of number of worms present.

The operational mechanism of the crowding effect is of considerable biological interest as a mode of developmental control. One view has been that the individual worms compete for available host dietary carbohydrate. However, the means by which such competition might be translated into lower rates of cell division and cell growth have not been elucidated, and the worms apparently secrete "crowding factors" that influence the development of other worms in the population.

As a worm approaches maximal size, growth rate decreases, and production of new proglottids is only sufficient to replace those lost by apolysis. Although some species, such as *H. nana*, characteristically become senescent and pass out of the host after a period, others may be limited only by length of their host's life. *Taenia saginata* may live in a human for more than 30 years, and *H. diminuta* may live as long as the rat it inhabits. In fact, Read reported an "immortal" worm that he kept alive for 14 years by periodically removing it from its host, severing the strobila in the region of the germinative area, and then surgically reimplanting the scolex in another rat.

Some tapeworms manage a surprising degree of mobility within their host's intestine. Cestodes may establish initially in one part of the gut and then move to another as they grow. *Hymenolepis diminuta* actually undergoes a diurnal migration in the rat's gut. This migration correlates with the nocturnal feeding habits of rats and can be reversed by giving food to the rat only in daytime. In fact, migration of the worms is apparently mediated by vagal nerve stimulation of gastrointestinal function rather than by the presence of food itself.

Wang and McKay (2005) found that *Hymenolepis diminuta* could modulate their host's immune response. Worm extracts and soluble products released by the cestodes suppressed immune cell proliferation and influenced cytokine production. IL-2 and IL-4 secretion was inhibited, and a cytokine with properties of IL12 was stimulated.

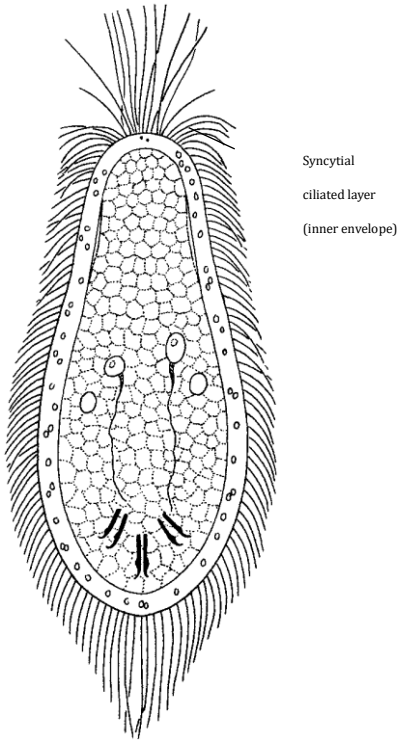

Figure 1 Coracidium of *Diphyllobothrium erinaceid*

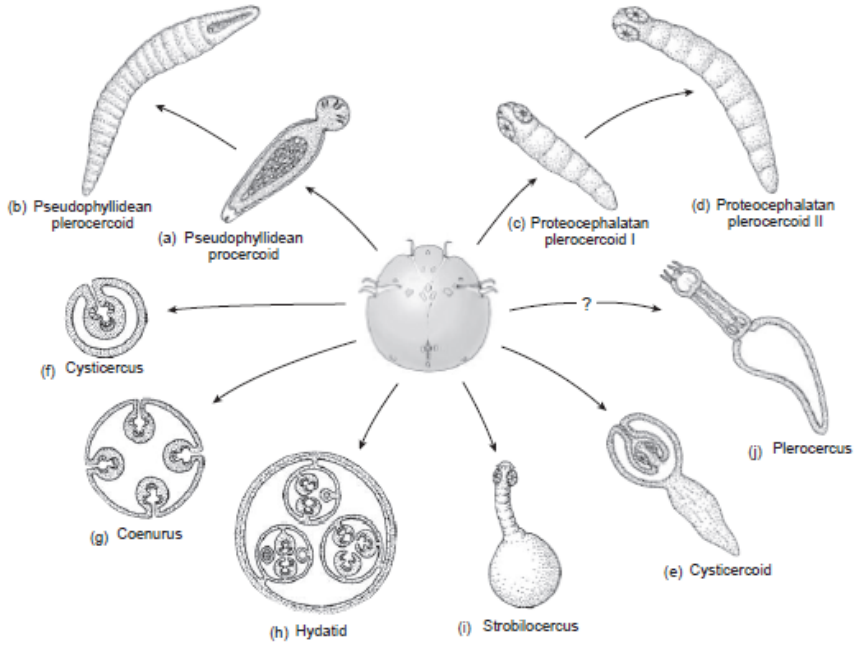

Figure 2 Types of cestodes metacestodes

Probable questions:

1. How strobilae act as determining factor of sex determination in dioecious form of cestodes?
2. What are the basic patterns of life cycle in cestodes?
3. What are the factors responsible for worm growth in cestodes?
4. What do you mean by parasite induced trophic transmission?
5. State the different types of metacestodes according to anatomical structure.

Suggested reading:

1. Noble, E. R. and Noble G. A. (1989). Parasitology. The biology of animal Parasites. 6th ed. Lea and Febiger, Philadelphia.
2. Roberts, L. S., Janovy, J. and Nadler S. (2013) Gerald D. Schmidt & Larry S. Roberts' Foundation of Parasitology. 9th ed. McGraw-Hill International.
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Unit-V
Life cycle pattern in nematode

Objectives:

In this section we will discuss on life cycle pattern in nematoda.

Introduction:

The nematodes, roundworms or eelworms constitute the phylum Nematoda. They are a diverse animal phylum inhabiting a broad range of environments. There are 28000 species of Nematoda identified till date. Most species are free-living, feeding on microorganisms, but there are many that are parasitic. The parasitic worms (helminths) are the cause of soil-transmitted helminthiases.

The Life-Cycle Pattern:

Hatching

In nematodes whose juveniles are free living before becoming parasitic, hatching occurs spontaneously. Some plant-parasitic species hatch in the presence of substances from their prospective hosts. Eggs of many species parasitic in animals, however, will hatch only after being swallowed by a prospective host. On reaching the infective stage, such eggs remain dormant until the proper stimulus is applied, and this requirement has the obvious adaptive value of preventing premature hatching. Ascarid eggs require a combination of conditions: a temperature of about 37°C, a moderately low oxidation-reduction potential (presence of an oxidizing agent reversibly inhibits hatching), a high carbon dioxide concentration, and a pH of about. These conditions are present in the gut of many warm-blooded vertebrates, and indeed *Ascaris suum* will hatch in a wide variety of mammals and even in some birds, but all four conditions are unlikely to be present simultaneously in the external environment. The lipid layer is impermeable to water, but fluid around a juvenile has a trehalose concentration of about 0.2 M, resulting in a high osmotic pressure surrounding the juvenile. The first change detectable on application of hatching stimuli is a rapid change in permeability; trehalose from the perivitelline fluid leaks from the eggs. Increase in water concentration apparently activates juveniles. The lipid layer also becomes permeable to enzymes secreted by the juvenile, such as chitinase, esterases, and proteinases, and these enzymes attack the hard shell, digesting it sufficiently for the worm to force a hole in it and escape. First-stage juveniles of some nematodes, such as *Trichuris* spp., possess a stylet on their anterior end. When juveniles are activated by a hatching stimulus, they penetrate the operculum (polar plug) with their stylet and emerge from the eggshell.

Growth and Ecdysis

- **Moulting**

There is growth in body dimensions of nematodes between moults of their cuticle. After the fourth molt in large nematodes such as *A. suum,* there is considerable increase in size, and the cuticle itself continues to grow after the last ecdysis. The moulting process has been studied in several species. First the epidermis detaches from the basement membrane of the old cuticle and starts to secrete a new one, beginning with the cortical zone. This process may continue until the new cuticle is substantially folded under the old cuticle, to be stretched out later after ecdysis. In some case old cuticle up to the cortical zone dissolves and new cuticle absorbs the resulting solutes. This conservation of resources is particularly important when materials and space are limited, such as in the first molt of *A. suum,* but less so when there is plenty of food and the old cuticle is very complex in structure, as in the fourth molt of *Nippostrongylus brasiliensis.* Escape from the old cuticle is facilitated by several enzymes, such as a collagenase like enzyme that attacks it.

- **Developmental Arrest**

A common adaptation in many nematodes is a resting stage at some point in their development (developmental arrest or hypobiosis), enabling them to survive adverse conditions while awaiting return of more congenial circumstances. A great deal has been learned about genetic control of development in nematodes using a free-living species, *Caenorhabditis elegans.* This species can undergo developmental arrest both as first stage juveniles (J1) or as third-stage (J3, dauer juveniles). Specific neurons in their amphids sense environmental signals, such as food supply, that result in either developmental arrest or continued development. Dauer arrest in *C. elegans* is controlled by three interacting signal transduction pathways, all of which also occur in parasitic *Strongyloides stercoralis.* Depending on conditions, *S. stercoralis* may have free-living or parasitic adults. Numerous examples in which developmental arrest is of survival value among free-living nematodes could be cited. A particularly interesting one was described by Hominick and Aston. Dauer juveniles of *Pelodera strongyloides* attach to mice, upon which they enter hair follicles on abdominal skin and molt to fourth-stage juveniles. They will develop no further at the body temperature of the mouse, and a mouse may accumulate hundreds or even thousands of nematode juveniles during its life. When the mouse dies and its body cools, nematodes rapidly emerge and, in the presence of a food source, molt to the adult stage. The mouse seems little inconvenienced by its passengers. Many parasitic nematodes produce infective third-stage juveniles comparable to dauer juveniles. They develop no further until a new host is available, remaining ensheathed in their second-stage cuticle. They live on stored food reserves and usually exhibit behavior patterns that enhance the likelihood of reaching a new host. For example, third-stage juveniles of *Haemonchus* and *Trichostrongylus* species migrate out of a fecal mass and onto vegetation that is eaten by the host. Third-stage juveniles of species that penetrate host skin, such as hookworms and *Nippostrongylus brasiliensis,* migrate onto small

objects (sand grains, leaves, and others) and move their anterior ends freely back and forth, in the same manner as some dauer juveniles. In both dauer juveniles and infective juveniles, a more or less specific stimulus is required for resumption of development and completion of ecdysis of the second-stage cuticle. Those that penetrate skin usually exsheath in the processes of penetration, but stimuli for exsheathment of swallowed juveniles (*Haemonchus* spp., *Trichostrongylus* spp., and others) are very similar to those required for hatching of *A. suum* eggs, including carbon dioxide, temperature, redox potential, and pH. In terms of developmental function, infective eggs (shelled juveniles) are fundamentally the same as dauer juveniles and infective juveniles. For most nematodes tested, carbon dioxide seems to be the most important stimulus for hatching or exsheathing. Nematodes with intermediate hosts normally undergo hypobiosis at the third stage and remain dormant until they reach a definitive host. Some species are astonishingly plastic in their capacities to sustain more than one developmental arrest if necessary. For example, if some species of hookworms and ascarids infect an unsuitable host, they enter another developmental arrest and lie dormant in host tissues until they receive another stimulus to migrate. In several of these, an older animal is an unsuitable host, and the worms lie dormant until they are stimulated by hormones of host pregnancy. They then migrate to the uterus or mammary glands and infect the infant by way of the placenta in utero or the milk after birth. Some species, such as *Strongyloides ratti*, may not undergo a second developmental arrest at this stage, but if a lactating female is infected, the juveniles are somehow diverted from completing their migration to the adult's intestine and migrate instead to the mammary glands and infect the suckling young. Females of many species of filaroids are parasitic in tissues of birds and mammals. Rather than eggs, they release relatively undeveloped J1s, termed microfilariae. Microfilariae do not develop further unless consumed by their invertebrate (commonly an insect) intermediate host, in which they undergo two molts and enter developmental arrest at J3. That arrest is broken when a definitive host, commonly a bird or mammal, is infected. Thus, in many species' initiation or cessation of developmental arrest is triggered by significant ambient temperature change, either up or down. In several cases initiation of such pathways includes heat shock factor (HSF) and one or more heat shock proteins (HSPs).

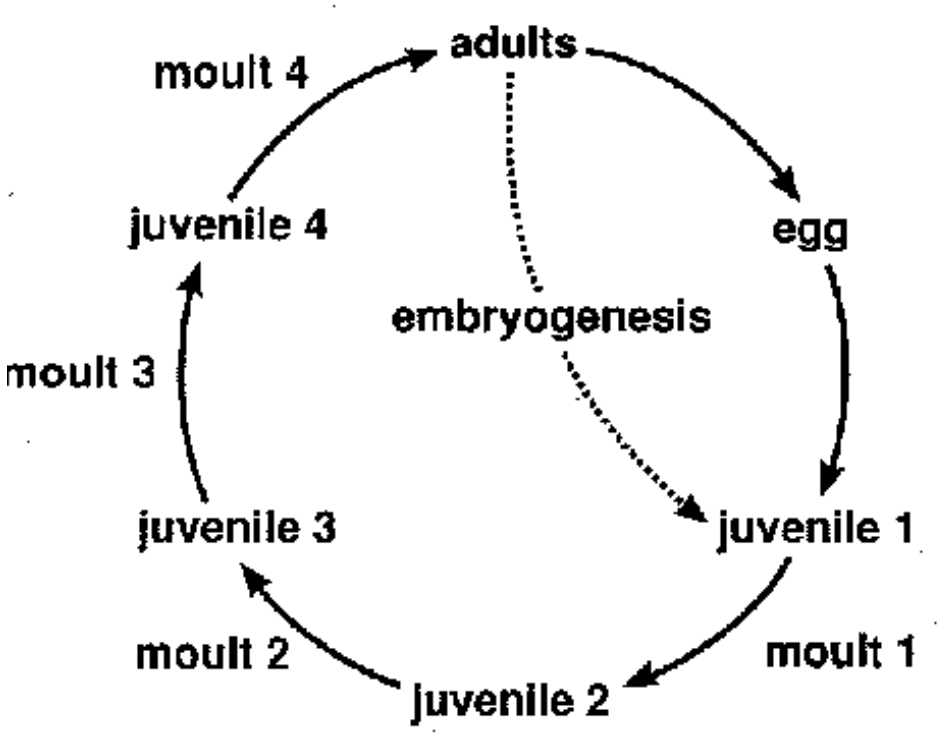

Figure 1 Generalised life cycle of a nematode

Probable questions:

1. State the hatching stimulus in case of eggs of *Ascaris*.
2. What is the function of stylet in the egg of *Trichuris*?
3. Why there are no nomenclature provided to the larval stages of nematodes unlike trematodes and cestodes?
4. Explain developmental arrest in the life cycle of nematode.
5. What are dauer juveniles?

Suggested reading:

1. Noble, E. R. and Noble G. A. (1989). Parasitology. The biology of animal Parasites. 6th ed. Lea and Febiger, Philadelphia.
2. Roberts, L. S., Janovy, J. and Nadler S. (2013) Gerald D. Schmidt & Larry S. Roberts' Foundation of Parasitology. 9th ed. McGraw-Hill International.
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Unit-VI

Biology, pathogenesis and control of *Fasciola hepatica*

Objectives:

In this section we will discuss on biology, pathogenesis and control of *Fasciola hepatica*.

Introduction:

Liver flukes are typical digenean trematodes and are commonly called “flatworms” or “flukes” on account of their flat, leaf-like structure. *Fasciola hepatica* is the common liver fluke of sheep. It is the first trematode whose life history was described by Thomas in 1883. It is of much importance as it causes fascioliasis—a disease that causes damage to liver tissues and bile ducts of sheep.

Etymology:

Latin: fasciola, small bandage

The species name hepatica stems from the Greek word hepar meaning liver.

Habit and Habitat of *Fasciola hepatica*:

The sheep liver fluke is an endoparasite which completes its life history in two hosts. The adult flukes are typical parasites of vertebrate animals but one stage of their life history is invariably spent in an invertebrate host — a mollusc. Thus, they have a digenetic life history for which the group to which they belong has been named as Digenea.

Sometimes the adult flukes invade other vertebrates like goat, horse, dog, ass, ox, rabbit, monkey and even man. They cause serious loss of human life and domestic animals. The disease caused by this parasite is known as liver rot. A single sheep may harbour as many as 200 liver flukes in its liver. *F. hepatica* has a cosmopolitan distribution and is common in areas where sheep and cattle are being reared.

Morphology of *Fasciola hepatica*:

F. hepatica is a soft-bodied, flattened leaf-like animal and exhibits bilateral symmetry. They measure about 1 to 2.5 cm in length and about 1 cm in width. The anterior end of the body (Fig. 1.50) is drawn out into a prominent conical projection, termed the oral cone or head lobe, bearing at its tip a somewhat triangular aperture, the mouth and surrounding it is the oral or anterior sucker.

On the ventral surface, a little behind the head lobe, is situated a much bigger sucker called the ventral or posterior sucker (also known as acetabulum). Between the two suckers and close to the posterior sucker is situated the genital opening through which the penis sometimes protrudes. At the extreme posteri-or tip of the body lies the excretory aperture, which is a single opening.

The canal of Laurer opens on the middle of the dorsal surface. The body surface is marked by the presence of a number of conical projections— the spinules or papillae which are exten-sions of the cuticle surrounding the body.

Life History of *Fasciola hepatica*:

Development in *F. hepatica* is indirect, involving four types of free-swimming and parasitic larval stages. *Fasciola* is digenetic and its life cycle always includes at least two infective stages. Two or more hosts are infected before its life cycle is completed. The definitive or primary host is a vertebrate (sheep), while the intermediate host is a snail (*Lymnaea*, *Planorbis* etc.).

Fertilization:

Although *Fasciola* is hermaphrodite, self-fertilization is uncommon. During copulation, sperm exchange is mutual and cross-fertilization is the general rule. Copulation takes place in the bile duct of the host. During copulation, the cirrus or penis of one worm is inserted, via the gono-pore, into the uterus of the other.

Copulation through Laurer's canal has also been reported. Sperms are thus ejaculated. The prostate gland supplies semen for sperm survival. Sperms then travel up the uterus, through the ootype, to be stored in the seminal recep-tacle.

Release of Fertilized Eggs from Primary Hosts (sheep) Body:

After being released from the ovary, the eggs are fertilized either in the oviduct or within the ootype. Each egg receives a fair amount of yolk from the yolk cells and vitelline secretions. It finally becomes enclosed in a proteinaceous shell or capsule secreted by the shell glands.

The shell becomes hard when it enters the uterus. The hardening is caused by the action of quinone. One pole of the egg shell bears a small lid or operculum for the exit of the future larva. The egg thus becomes complete and remains for a little time in the uterus.

Eventually the egg leaves the fluke's body through its gonopore and passes down the bile ducts of the sheep into the intestine, from where it is discharged to the exterior along with the faeces. The egg can survive if only it falls on damp soil.

- ### Miracidium Larva:

Active development within the zygote begins at this stage. After three to six weeks, depending upon the tem-perature, the egg shell opens at the opercu-lum and the miracidium larva emerges. The miracidium larva has a somewhat conical body, covered all over with vibratile cilia.

A distinct head lobe or apical papilla is situ-ated at the broad end. Behind the head lobe there are two spots of pigment, the eye spots. Within the body just below the epidermis lie delicate layers of circular and longitudinal muscle fibres, the mesenchyme.

There also lies one pair of flame cells with ducts and a sac-like intestine. The rest of the interior is filled with a mass of germ balls. The head lobe bears penetration glands. The miracidium larva swims freely in water or crawls over damp surface for some time and dies unless it happens to reach a particular water snail (preferably *Lymnaea truncatula*).

Miracidium (Fig. 1) is the first stage larva that comes out from the fertilized egg.

i) Miracidium is an oval, microscopic, flattened larva which appears conical in shape.

ii) Body is uniformly covered with epidermal plates. There are 21 plates arranged in 5 rows. First to fifth row contain 6,6,3,4,2 epidermal plates respectively.

iii) Anterior end of the larva is projected into a conical lobe called apical papilla which acts as a boring organ.

iv) Internal structures of the miracidium which can be seen include, the triangular sac called apical gland attached to the apical papilla, a pair of bag-like penetration gland located on each side of apical gland (also called cephalic glands), two eye spots, two flame cells, rudimentary gut and germ cells.

Miracidium larva comes out from the egg shell of the fertilized egg by eroding the operculum with the help of proteolytic enzyme. It is the first larval stage in the life cycle of *F. hepatica*. It is a free-swimming stage in fresh water. Miracidium larva swims freely in water for 4-30 hours in search of a suitable intermediate host which may belong to the genus *Lymnea* or *Planorbis*. If the larva does not come in contact with a suitable host it dies. Miracidium larva enters the snail and destroys its tissues. It penetrates through the pulmonary chamber; during this period, it loses its cilia, epidermal plates, brain, eye spots, apical and penetration glands as well as primitive gut.

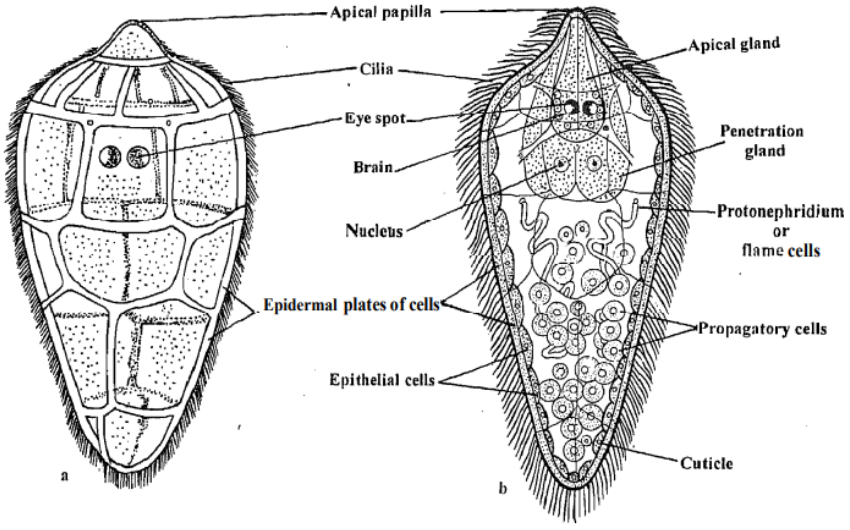


Figure 1 Miracidium larva of *Fasciola hepatica*. (a) External structure. (b) Internal structure

Infection to Secondary Host (Snail) of Fasciola Hepatica:

- **Sporocyst:**

The miracidium larva on encountering the snail, bores into it by means of the penetration glands and reaches the internal organs, especially the pulmonary sac. It metamorphoses into the sporocyst by casting off its ciliated covering.

The sporocyst is elongated, with an internal cavity containing germ ball and lined by a layer of cells, with remnants of the eye spots and flame cells. The germ balls, eventually, undergo a process of cleavage, resulting in the forma-tion of redia larva (first generation).

i. Sporocyst (Fig. 2) is the second larval stage in the life cycle of *F. hepatica*. It develops from the miracidium larva within the pulmonary chamber of its snail host. It shows extreme degree of parasitism. As a result, alimentary canal and locomotory organs are absent.
Sporocyst is an elongated sac-like structure, covered with cuticle.

ii. Body wall of the sporocyst consists of sub epithelial cells, mesenchyme and muscle layers.

iii. Body sac contains germ cells and flame cells.

iv. It is a non-feeding stage.

v. Germ cells within the sporocyst give rise to the next larval stage known as redia larva which develop within it. One sporocyst may give rise to 5-6 redia.

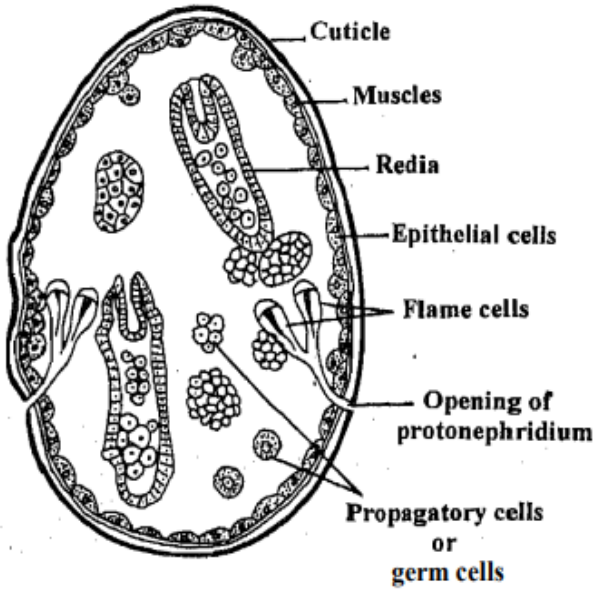


Figure 2 Sporocyst larva of *Fasciola hepatica*.

- **Redia Larva:**

Redia is provided with mouth, pharynx and a simple intestine and there is a system of excretory vessels. It bears a circular ridge or collar near the anterior end, formed by the bulging of body wall. The fully formed body of redia is elongated and bears a pair of short, muscular projections at the posterior side.

In the interior of the body are undifferentiated germ balls and these either develop into second generation of redia if it is winter, or if it is summer, it gives rise to cercaria larva. An opening near the collar, known as the birth pore is formed in the wall of redia, through which the cercaria escapes.

Redia (Fig. 3) is the third larval stage in the life cycle of F. hepatica. Redia develops from the germ cells of the sporocyst and comes out of the sporocyst by rupturing the sporocyst wall. Redia then migrates to the liver of the snail.

i. Each redia measures about 1.3- 1.6 mm in length.

ii. Body of redia is elongated, cylindrical and sac-like.

iii. Body-wall is composed of tegument, epithelial layer and delicate mesenchyme.

iv. Anterior end consists of mouth which leads into a muscular pharynx with pharyngeal glands and sac like intestine.

v. Just behind the pharynx is a muscular ringlike swelling called collar which helps the redia in locomotion.

vi. Just posterior to the collar is a permanent aperture called birth pore through which another generation of redia called second generation of redia or the next larval stage, the cercaria exits to the outside.

vii. Posterior region has two stumpy processes called lappets which help the redia in anchoring to the tissues of the snail and are also helpful in locomotion.

viii. The space between the body wall and intestine contains a few germ cells.

ix. Germ cells often give rise to second generation of daughter rediae.

x. The germ cells of redia as well as germ cells of daughter redia give rise to the next larval stage called cercaria.

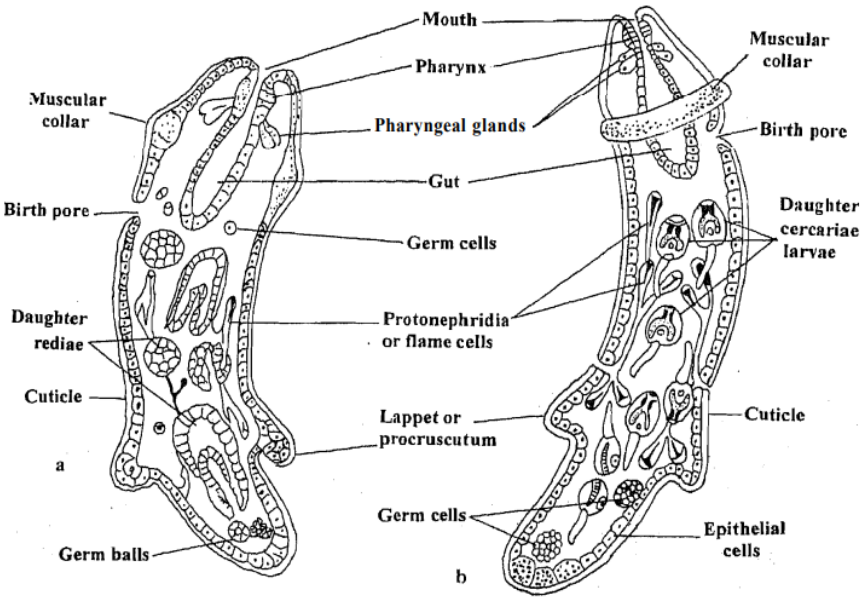


Figure 3 Redia larva of *Fasciola hepatica*. (a) with daughter rediae. (b) with cercaria

- **Cercaria Larva:**

The cercaria is provided with a long tail and with oral and ventral suckers. Alimentary canal is well developed and consists of mouth, pharynx and a bifid intestine. Paired excretory tubules with flame cells, germ balls and peripheral cyst-forming cells are also present.

They move actively by the help of their tail and forces their way out of the snail's body. Then losing their tail they become encysted. The cercaria in encysted condition is called metacercaria larva.

Cercaria larva (Fig. 4) is the fourth larval stage in the life-cycle of F. hepatica. It is a free-living stage produced by the redia larva.

i. It has a flat and oval body about 35 mm in length and a long tadpole like tail.

ii. Cercaria moves by muscular undulations of the tail.

iii. Cercaria has two suckers an anterior, oral sucker surrounding the mouth and a ventral sucker situated in the middle of the body.

iv. Body space is filled with parenchyma and contains a few cystogenous glands on each side which form the cyst of the future larva.

v. The alimentary canal consists of mouth, muscular pharynx, oesophagus and bifurcated and inverted Y-shaped intestine.

vi. It also possesses an excretory bladder with a pair of protonephridial canals and a number of flame cells.

vii. Cercaria also has two large non-functional penetration glands as well as rudiments of reproductive organs which have originated from germ cells.

viii. Cercaria is a young fluke of sexual generation.

ix. Cercaria larva first comes out of the redia through its birth pore and then also from the body of its snail host.

The free swimming cercaria larva swims for 2-3 days and then attaches to the aquatic plants where it gets enclosed in a cyst. The encysted larva is called metacercaria (Fig. 5) larva. The metacercaria larva is the infective stage for the final host. Metacercaria is the 5th larval stage of *F. hepatica*. It is 0.2 mm in size and is called a juvenile fluke. Its further development takes place in the final host the sheep. Metacercaria can also infect humans

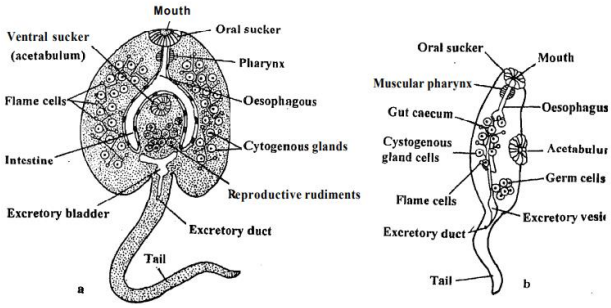


Figure 4 Cercaria larva of *Fasciola hepatica*. (a) Dorsal view. (b) Lateral view

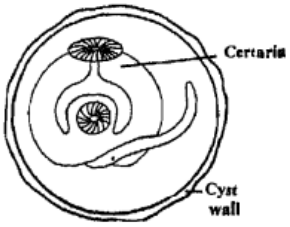


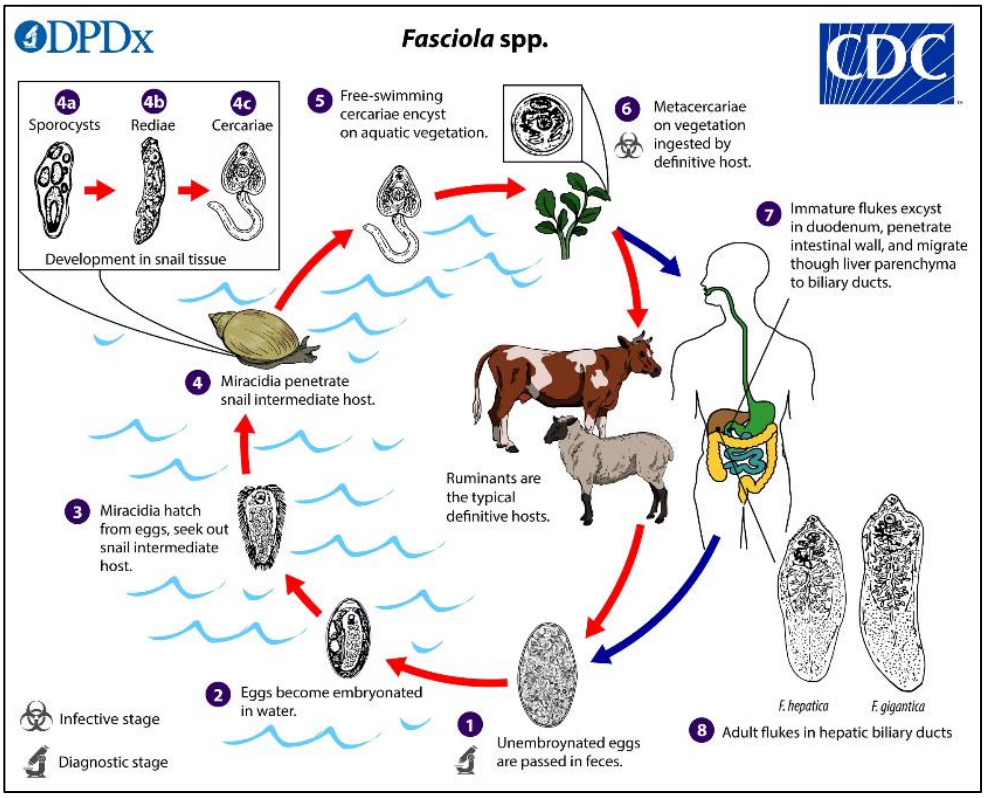
Figure 5 Metacercaria larva of *Fasciola hepatica*.

Infection to New Primary Host (Sheep) of *Fasciola hepatica*:

The metacercaria remains attached to blades of grass or leaves of other herbs. When the sheep feeds on these infected green leaves, metacercaria enters the gut. The young fluke emerges from the cyst and bores its way through the gut wall, passes over the viscera and penetrates into the liver and finally into the bile duct, where it grows rapidly and reaches sexual maturity.

From the life cycle of *F. hepatica* it can be seen that after having successfully invaded into the intermediate host (snail), Fasciola's developmental stages undergo two rounds of asexual divisions. Once through the formation of either one or two generations of many redia and then through the formation of numerous cercariae.

This greatly increases their number and thus, they have good chances to complete their life cycle. Thus, one egg has the capability to give rise to dozens of sexual adults in the definitive host.



Mode of transmission:

Ingestion of freshwater plants with metacercaria or by drinking water with floating metacercariae.

Pathogenicity and Clinical Features:

It causes zoonotic disease fascioliasis. After the larvae are ingested with contaminated food or water, a symptom-less incubation period starts, lasting for a few days to a few months. The disease may be in the following phases acute or invasive phase and Chronic or latent phase.

Asymptomatic phase

- It can cause fever.
- Right upper quadrant abdominal pain
- Hypereosinophilia

Acute or invasive phase

- The acute phase, lasting 2-4 months.
- Migration from the intestine to the liver
- Traumatic and necrotic lesions in liver parenchyma

- Typical symptoms include fever, nausea, a swollen liver, skin rashes, and extreme abdominal pain.

Chronic or latent phase

- The parasite has reached the bile ducts
- Obstruction
- Stimulates inflammation in the biliary epithelium leading to fibrosis
- Obstruction causes biliary sepsis
- Symptoms include intermittent pain, jaundice, and anemia. Pancreatitis, gallstones, and bacterial super-infections may also occur.
- Patients with chronic infections experience hardening of the liver (fibrosis) as a result of long-term inflammation.

The disease may be controlled by two methods:

1. Therapy:

It involves treatment with drugs. The drugs generally employed as anthelmintics are Carbon tetrachloride.

Emetine hydrochloride, Filicin, Hexachloroethane and Tetrachlorethane, etc.

2. Prophylaxis:

The preventive measures include:

i. Killing heavily infected sheep,

ii. Destroying eggs and manure of infected sheep,

iii. Feeding infected sheep with salt and little dry food, and

iv. Killing the larval stages and snails which serve as intermediate host.

Snails are killed by sprinkling copper sulphate solution in ponds and ditches or by bluestone in one hundred thousand parts of water. The breeding of snails can be checked by removing the vegetation from the ponds and the streams. Ducks feed on snails and can be helpful in the reduction of the snail population.

Snails cannot survive longer periods of drying and can be killed by draining their pastures. Human infection could be checked by eating adequately-cooked and properly-washed vegetables and water plants.

Probable questions:

1. What do you mean by digenetic trematode?
2. State the hosts of *Fasciola*.
3. Briefly enumerate the life cycle of *Fasciola*.
4. Which is the infective stage in *Fasciola* infection?
5. State the clinical features of *Fasciola* infection.
6. Which are the drugs of choice to treat *Fasciola* infection?

Suggested reading:

1. Noble, E. R. and Noble G. A. (1989). Parasitology. The biology of animal Parasites. 6th ed. Lea and Febiger, Philadelphia.
2. Roberts, L. S., Janovy, J. and Nadler S. (2013) Gerald D. Schmidt & Larry S. Roberts' Foundation of Parasitology. 9th ed. McGraw-Hill International.
3. Schmidt, G. D. and Roberts, L. S. (2001). Foundation of Parasitology. 3rd ed. McGraw Hill Publishers.
4. Schmidt, G. D. (1989). Essentials of Parasitology. Wm. C. Brown Publishers (Indian print;1990, Universal Book Stall).
5. Smyth, J. D. (1994). Animal Parasitology. 3rd ed. Cambridge University Press.

Unit-VII
Biology, pathogenesis and control of *Echinococcus granulosus*

Objectives:

In this section we will discuss on biology, pathogenesis and control of *Echinococcus granulosus*.

Introduction:

Echinococcus granulosus also termed as the hydatid worm or Hyper tapeworm or Dog Tapeworm. It is a cyclophyllid cestode that parasites the small intestine of canids as an adult but which has vital intermediate hosts as livestock and humans where it results cystic echinococcus, also termed as hydatid disease.

Habitat:

The adult tape worms are located affixed to the wall of intestinal mucosa of dogs and wild canines.

The larval stage (hydatid cyst) is present in humans and other herbivorous animals.

Morphology of *Echinococcus granulosus*:

Adult worm:

It is small tapeworm measuring 3-6 mm in length. It constitutes of scolex (head), neck and body or strobili.

- **Scolex:**

Scolex is pyriform, 300 mm in diameter. It bears four suckers and a protrusible rostellum with two circular rows of hooks.

- **Neck:**

It is short and thick.

- **Strobili or body:**
  - It consists of 3 segments (occasionally 4).
  - The segment is immature, the second one is mature.
  - The last one (as well as fourth one, when present) is gravid.
  - The terminal segment being the biggest, measures 2-3 mm in length and 0.6 mm in breadth.
  - The terminal gravid segment lacks uterine openings. The segment, thus always bursts open before or after passage into the stool, releasing hundreds of eggs.

Life History of *Fasciola hepatica*:

Eggs:

- The eggs are the infective stage of parasite.
- It is ovoid in shape and appears similar to other eggs of Taenia.
- It measures 32-36 mm in length by 25-32 mm in breadth.
- The egg has two layers- outer thin wall and inner embryophore.
- It consists a hexacanth embryo with 3 pairs of hooks.

Larva (Hydatid cyst):

- The larval stage of dog tapeworm is known as the hydatid cyst.
- It is present in various organs of man and other intermediate hosts.
- It depicts the scolex of the future adult worm and remains invaginated with a vesicular body.
- On entry to the definite host, the scolex with 4 suckers and rostellar hooklets, becomes evaginated and develops into an adult-worm.
- The hydatid cyst in man is particularly unilocular, subspherical in shape and filed with third.
- The mature cysts measure around 5 cm in diameter.
- The cyst wall is made up of two layers.
- The outer cuticular layer called the ectocyst is hyaline, milky, opaque, laminated and non-nucleated. It is tough elastic layer and is 1 mm thick.
- The inner or germinal layers, endocyst is cellular, nucleated and is extremely thin (22-25 mm in thickness). It is the vital layer of the cyst and gives rise to broad capsules with scolices. It secretes the specific hydatid fluid and gives rise to outer layers.

Hydatid fluid:

- The inner of the cyst is filled with a clear, colorless fluid, may be pale yellow in color known as the hydatid fluid.
- The third is nutritive and provides nourishment for growing brood capsule and scolices. The third is slightly acidic, pH 6.7.
- It consists of NaCl, Na_2SO_4, Na_3PO_4 and Na and Ca salts of succinic acid.
- A large number of brood capsule, free scolices and loose hooklets resembling sand grains float in the fluid called the hydatid sand.

- The hydatid fluid is antigenic and highly toxic when absorbed, it gives rise to anaphylactic symptoms.

Life cycle of *Echinococcus granulosus*:

The life cycle of *E. granulosus* is completed in two hosts:

Definitive host- dogs and wild canines

Intermediate hosts– man, sheep, cattle, goat etc.

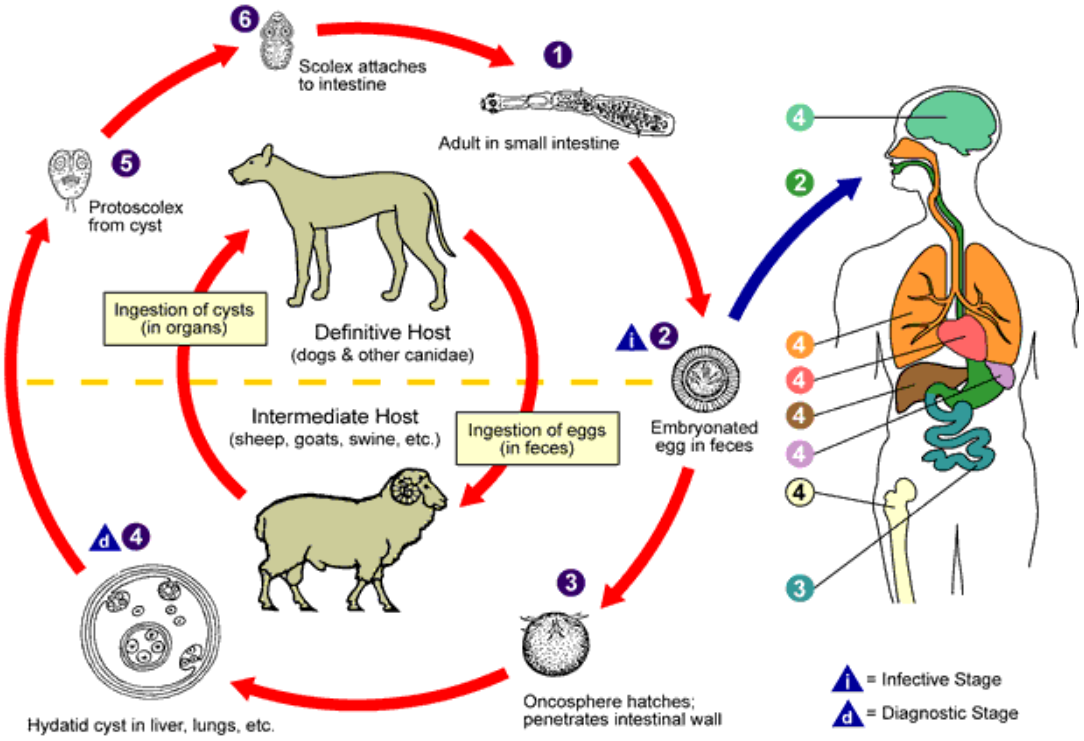
The eggs are discharged with the feces of the definitive hosts. These are swallowed by the intermediate hosts sheep and other domestic animals while grazing in the field and also by man, especially by children due to intimate handling of infected dogs.

In the duodenum, the hexacanth embryo are hatched out. In about 8 hours after ingestion the embryo bore their way through the intestinal wall and are carried by the blood stream to various internal organs especially the liver and lungs are the most common sites. In these organs, the embryos get through the initial inflammatory response of the host and undergo dramatic changes. It increases in size up to 5-8 cm in few months and is finally transformed into a fluid filled hollow bladder, hydatid cyst.

The cyst has an inner germinal layer that contains a large number of nuclei from which buds of tissue develop into hollow fluid filled brood capsules. These brood capsules may remain affixed to the cyst wall by means of their peduncle or may float in the hydatid fluid. Eventually many larvae are produced in large numbers from the germinal layer within the brood capsule. As the cyst grows older, a larger number of brood capsules and free protoscolices float free inside the hydatid fluid.

Dogs and other canid host gain infection by ingestion of hydatid cyst containing protoscolices (fertile cyst), present in the viscera of sheep, cattle or other intermediate hosts. In the small intestine, protoscolices evaginate, penetrate deep between villi and enter the cyst of Lieberkuhn. It develops into a mature adult worm and began to produce infective eggs within 41-76 days of ingestion of the hydatid cysts.

The life cycle of parasite comes to a dead end in man as dog have no access to infected viscera of man containing hydatid cyst. The natural cycle is maintained between dog and sheep.



Mode of transmission of *Echinococcus granulosus*:

The disease caused by *E. granulosus* is zoonotic. Human being an accidental host, acquires the infection through ingestion of the eggs in following ways:

- **Direct contact with infected dogs**

Indirectly through food, water and other materials contaminated with eggs of the parasite. Less commonly, by coprophagic flies which may aid as mechanical vector of eggs.

Pathogenesis of *Echinococcus granulosus*:

Mainly mechanical damage is produced. The young cyst that develops embryos lodged in vital centres may soon infected with functions of the organ with damaging, even fatal results. Benign cyst may be asymptomatic or it may produce physical burden to the patient. The severity relies on the type of tumor and organ or tissue where it first becomes implanted and Anaphylactic reactions develop.

Clinical manifestation of *Echinococcus granulosus*:

The parasite gives rise to cystic echinococcosis (CE) earlier known as hydatid disease or hydatidosis. The hydatid cyst is primarily responsible for the pathogenesis of the disease.

The condition persists as asymptomatic for a long period after infection in a majority of cases.

The clinical symptoms in CE in symptomatic cases, relies upon the organ involved, interaction between the expanding cyst and adjacent organ and complications occurred by rupture of cyst. The cyst in vital organs interfere with the function of affected organs with the risk of fatality in certain cases.

- **Hepatic hydatid:**

It is seen in 66% of cases. It may appear as hepatomegaly, with or without palpable abdominal man. The condition is linked with pain, nausea and vomiting, portal hypertension and biliary peritonitis. Cyst may rupture into bile ducts, leading to intermittent jaundice, fever and eosinophilia. Allergic exhibition up to anaphylactic shock may occur in case of sudden rupture.

- **Pulmonary Hydatid:**

Pulmonary cyst may be present in 22% of patients. Cyst occurring within the lung tissue cause hemoptysis transient thoracic pain and shortness of breath. In case the rupture is incomplete, cyst may transform into chronic pulmonary abscess. The patient reports of sudden attack of cough with sputum containing frothy blood, mucus and hydatid sand.

- **Brain cyst (up to 1%)**

A large cyst may lead to symptoms of increased intracranial tension (headache, vomiting and poor vision) up to epilepsy.

- **Renal cyst (about 3%)**

It causes intermittent pain and hematuria. The hydatid sand may be present in urine. Hydatid cyst of the spleen, heart may present as tumor like condition or an abscess.

- **Osseous cyst (about 2%)**

Osseous cyst wall has only 1 layer the germinal layer which develops first in the narrow cavity, then extend to the osseous tissue leading to:

- Erosion of large men of bone
- Destruction of bone trabecular
- Spontaneous (pathological) fractures

- **Complications:**

The cyst may rupture due to the trauma and during surgery into the pericardium, the bile ducts and the GI tract leads to severe clinical outcomes such as pleural effusion, pneumothorax and secondary echinococcosis of peritoneal or pleural cavity. A ruptured hydatid cyst leads to two risks:

The released hydatid fluid if absorbed in the circulation bronchi, peritoneum or pleura produces a sudden anaphylactic shock which may be fatal.

This may result in the formation of secondary echinococcosis in various parts of body due to dissemination of scolices by the circulation.

Treatment:

Albendazole is most effective than thiobendazole and mebendazole. Surgical technique is also helpful. Combination of praziquantel and albendazole is very effective.

Prophylaxis:

This consists of:

1. Avoidance of handling infected dogs
2. Avoidance of ingestion of raw vegetables polluted with eggs
3. Personal hygiene (cleaning hands before eating)
4. Preventing dogs from eating the carcasses of sheep; cattle and dogs in infected areas
5. Discarding all infected viscera; in slaughter houses by dumping them into pits inaccessible to dogs and
6. Educational propaganda in schools.

Probable questions:

1. Why *Echinococcus* is known as Dog tapeworm?
2. What is protoscolex?
3. Briefly enumerate the life cycle of *Echinococcus*.
4. State the symptoms of clinical echinococcosis.
5. What do you mean by hydatid cyst?
6. Which are the drugs of choice to treat *Echinococcus* infection?
7. How can *Echinococcus* infection be controlled?

Suggested reading:

1. Noble, E. R. and Noble G. A. (1989). Parasitology. The biology of animal Parasites. 6th ed. Lea and Febiger, Philadelphia.
2. Roberts, L. S., Janovy, J. and Nadler S. (2013) Gerald D. Schmidt & Larry S. Roberts' Foundation of Parasitology. 9th ed. McGraw-Hill International.
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Unit-VIII
Biology, pathogenesis and control of *Loa loa*

Objectives:

In this section we will discuss on biology, pathogenesis and control of *Loa loa*.

Introduction:

Loa loa is a blood dwelling nematode that is parasitic in humans. The adult worm wanders through the subcutaneous tissue but is most obvious as it crosses the conjunctiva of the eye, hence leading to its common name, the African eye worm. It causes loa loa filariasis (loiasis). It is one of three parasitic filarial nematode that causes subcutaneous filariasis in humans.

Habitat:

The adult worm inhibits the subcutaneous tissue of man, often in the sub conjuctival tissue of the eye. The microfilariae are found in blood.

Morphology:

Adult worm

The adult worms are thin, whitish and thread like. The anterior and tapers to a narrow head. Surface of the body is covered with small knobs. Microscopically the cuticula is found to have numerous rounded protuberances (cuticular bosses) which vary in number and arrangement in two sexes. The female worm is 4-7 cm in length and 0.5mm in diameter. The life span of worm is 4-12 years.

Microfilaria

They are found in peripheral blood during day time. Occasionally microfilaria have been demonstrated in the urine, sputum and even CSF. Microfilaria is sheathed and measures 250-300 mm in length and 6-8 mm in breadth. The column of nuclei extends upto the tail-tip. Sheath stains poorly with Geimsa stain but stains well with iron-haematoxylin.

Life cycle of *Loa loa*:

Loa loa completes its life cycle in two hosts:

Definitive host: Human

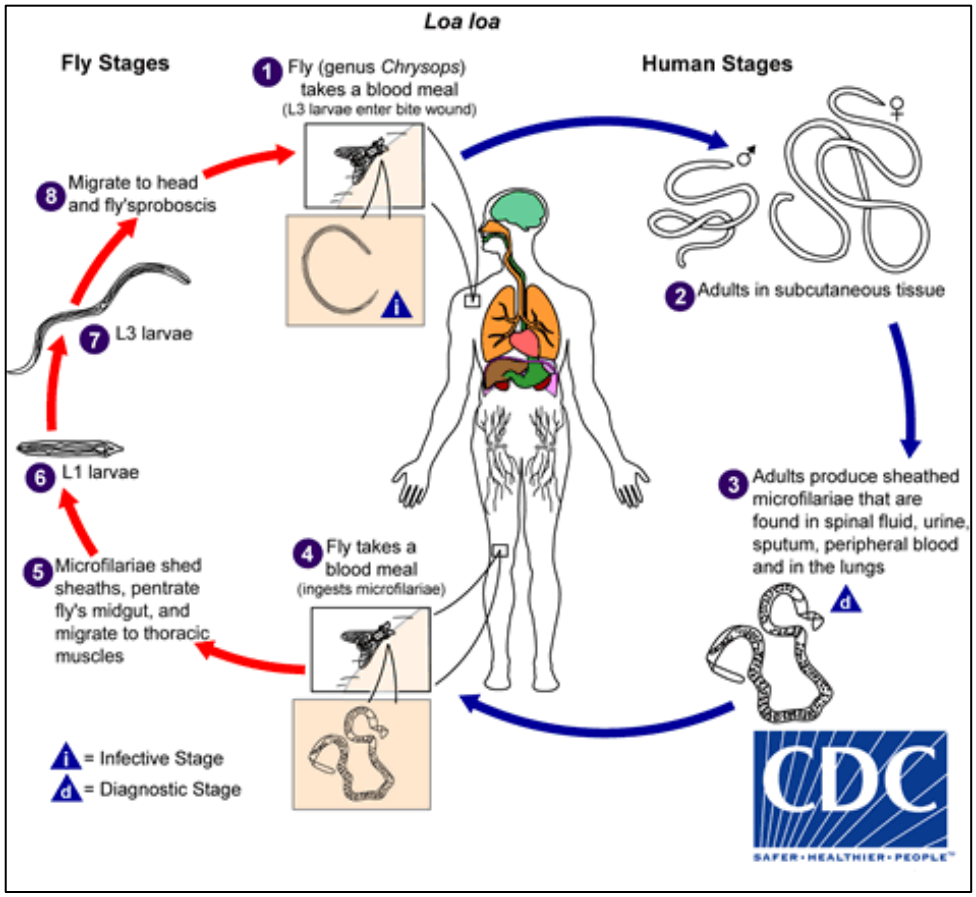
Man acquires infection by the bite of infected female *Chrysops*. During infection larva enter in large numbers through the punctured wound on the skin made by the fly, during the blood meal.

The larva enters the subcutaneous tissue and mount to develop into adult worms within a period of 6-12 months.

Adult worm occasionally migrates in the sub conjunctival tissue. The female worm after fertilizes by males produce microfilarial larva that circulate in the peripheral blood during the day time and also found in the sub-cutaneous tissue. When a female *Chrysops* bites the infected human to suck the blood, microfilaria is ingested that enter the fly's stomach. Microfilarial larvae lose their sheath, penetrate the wall of the stomach and invade thoracic muscles where they undergo changes to form the infective L3 larva.

Development in fly is completed in about 10 days.

The mature infective larva than migrate to the mouth parts of chrysops. When this fly bites a new host for blood meal, the cycle is again repeated.



Mode of transmission of *Loa loa*:

- Infected man is the only source and reservoir of infection for *Loa loa*.
- Transmission is acquired by the bite of female *Chrysops* species.

Pathogenesis and pathology of *Loa loa*:

- Microfilariae are not pathogenic.
- Adult *Loa loa* worms which live in the subcutaneous tissue are pathogenic.
- The migrating adult worms provoke an intense inflammatory reaction.

- Calabar swelling is the typical pathological feature of the *Loa loa* filariasis. It is formed as a result of an allergic response to adult worms migrating in the subcutaneous tissue.

Clinical manifestation of *Loa loa* filariasis:

- *Loa loa* filariasis (loasis) is the disease produced by adult worm in human.
- The incubation period is on an average 3-4 years.
- Loiasis is asymptomatics in many pcases.

Symptomatic manifestation of *Loa loa* filariasis:

- **Skin lesions**

Skin lesions consist of colabar swelling or fugitive swelling. During migration of the adult worm, it causes oedema of subcutaneous tissues, known as calebur swelling. They disappear in course of 2-3 days and are regarded as allergic reaction of the tissues to filarial toxins. Localized pain and itching for several hours usually precede the onset of the swelling. Usually, one swelling develops at a time. The swelling is non erythematous, measures 3-10 cm in diameter and last for few days to weeks. The wrist joints or knee joints are most frequently affected. Worms are not usually present in the swellings but are present below surface of the skin.

- **Ocular lesions**

These consists of;

Conjunctival granuloma: It is caused by the migration of adult worms in the sub-conjunctival tissues. These granulomas are present as solitary or multiple small nodules measuring 2 mm in diameter. These are found in the deeper layers of conjunctiva close to the sclera tissue.

Oedema of the eyelid: It is painless condition frequently accompanied by itching but not fever or any other constitutional symptoms.

Proptosis: This condition is known as 'bug eye' or 'bulge eye' caused by the edema of the orbital cellular tissue. It is a painless condition and of rapid onset frequently associated with itching.

Complications of loasis:

These include frequent recurrence of fugitive swellings, endomyocardial fibrosis, retinopathy, encephalopathy, neuropathy and arthritis. *Loa loa* meningoencephalopathy is a severe and often fatal complications of infection.

Treatment:

The surgical removal of adult worms may be possible. Microfilariae can be treated with diethylcarbamazine or ivermectin at standard doses of 150 µg/kg; however, there is

a risk of severe neurological reactions such as meningoencephalitis or encephalopathy due to dying microfilaria in patients with a high microfilaraemia load. These patients should therefore have their microfilaraemia load reduced with albendazole initially.

Prevention and control of *Loa loa*:

- Treatment of infected populations
- Using insect repellent
- Wearing protective clothing
- Avoiding visit to the place's endemic for the disease.

Probable questions:

1. Name the causative agents of subcutaneous filariasis in human.
2. State the habitat of *Loa loa*.
3. Briefly enumerate the life cycle of *Loa loa*.
4. What do you mean by calabar swelling?
5. State the different aspects of ocular lesions due to *Loa loa* infection.
6. What is the drug of choice due to *Loa loa* infection?

Suggested reading:

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Unit-IX

Leishmaniasis with reference to drug resistance

Objectives:

In this section we will discuss on leishmaniasis with reference to drug resistance.

Introduction:

The leishmaniases are complex diseases of (sub)tropical regions of the world caused by *Leishmania* spp. (Protozoa, Kinetoplastida, Trypanosomatidae) and spread by sand flies. The World Health Organization (WHO) considers the leishmaniases to be prominent among the global causes of death by infectious diseases.

Clinical manifestations produced by *Leishmania* comprise the visceral (VL) and tegumentary forms. The tegumentary forms of the disease include the cutaneous (CL), diffuse (DCL), and mucocutaneous (MCL) leishmaniases, but infections remain asymptomatic in many cases. *Leishmania* may also appear as an opportunistic parasite in immunosuppressed individuals. Chemotherapy constitutes the main approach to manage the disease although it is generally not applied to asymptomatic subjects. For a number of other neglected tropical diseases, mass drug administration—even without diagnosis—is possible, given the safety of particular drugs used in these circumstances (e.g., praziquantel in schistosomiasis and ivermectin for lymphatic filariasis). The combined problems of parenteral administration and toxicity of anti-leishmanials precludes such programs for leishmaniasis. Furthermore, the selection of resistant parasites carrying genetic mutations that lessen the parasite's response to drugs may emerge upon mass drug administration.

Leishmania has an intricate life cycle, and one of the developmental forms, the amastigote, dwells within immunological cells of the mammalian host, which adds to the challenge of accessing the parasites with specific drugs. Nevertheless, the aim of chemotherapy is to kill intracellular parasites; therefore, chemotherapy remains the best means available to cure the disease.

Antimonials (sodium stibogluconate [SSG]) are the primary drugs employed against leishmaniasis. They have been in use since the 1920s. These toxic compounds have a narrow therapeutic window, and their use has been largely superseded in the Indian subcontinent (ISC), where resistance has become widespread. However, they are still in use in other regions of the world, including Latin America and East Africa.

Miltefosin (MIL) has replaced SSG in the ISC in the context of the kala-azar elimination program, but efficacy of this drug as well had already dropped within a decade of its introduction. Initially, this decrease in MIL efficacy could not be related to increasing parasite resistance to the drug, although recently a few resistant clinical

isolates have been described, first in France in an HIV-coinfected patient and later in two Indian patients.

Amphotericin B (AmB) is highly efficacious but relatively toxic when injected in its free deoxycholate form. Administration in a liposomal formulation ameliorates the toxicity risk, although the high cost of this formulation has left its range of use restricted; this is the case even though it is provided free of charge to WHO for use in strategically important areas by its manufacturers (Gilead Sciences; with up to 350,000 vials over the next five years). Unfortunately, a risk of resistance is becoming apparent for AmB as well.

Paromomycin has a relatively restricted range of targeting *Leishmania* species, and the situation regarding resistance in the field is unclear, although laboratory-derived resistant isolates have been created.

Even combination therapies are not immune from selection of resistance, which poses potential challenges to the WHO's proposed next generation of combination therapies in the treatment of leishmaniasis.

It is critical to consider that treatment failure (TF) and drug resistance (DR) are not necessarily synonymous. TF goes far beyond DR, with numerous factors in the host (such as immunity or nutritional status), the parasite (e.g., whether or not the parasite resides in tissues not accessible to drugs), and the environment (e.g., global warming contributing to the expansion of the disease to new geographical areas) influencing treatment outcomes. Notwithstanding, DR is a fundamental determinant of treatment outcome, and understanding the mechanisms whereby parasites become resistant to drugs is essential. In this Review, we discuss the phenomenon of DR associated with SSG, AmB, and MIL and outline the molecular mechanisms associated with the selection of resistance in combination therapies, as well as the possible consequences of emergent resistance on the use of these drugs in the field.

What do we mean by the term "drug resistance"?

As mentioned previously, SSG were for a long time the mainstay treatment in the fight against leishmaniasis but were abandoned in 2005 in some areas, including the ISC, due to TF related to DR. SSG remain, nevertheless, the drugs of choice in many countries around the world. MIL, however, is an alternative drug that was developed in the mid-1980s and first became available in 2002 for VL in India, then in 2005 for CL in Latin America. Recent reports indicate that the effectiveness of MIL in India and Nepal is decreasing. In these areas, the drug has now been replaced by liposomal AmB for the kala-azar elimination program. Classically, resistance emerges as genetic mutations that lessen the parasite's response to a drug when the parasite is under drug pressure. This situation is easy to translate to selective conditions in the field. However, an intriguing point to consider is the description of *Leishmania* parasites resistant to SSG, even in cases in which parasites have not been exposed to the drug. Antimony is a heavy metal whose action against *Leishmania* shares characteristics with the related heavy metal arsenic.

Under experimental conditions, resistance selected to arsenic renders parasites cross-resistant to antimony. In North Eastern India, high levels of arsenic in drinking water may have led to selection of parasites with reduced sensitivity to both heavy metals, a plausible explanation for the spread of antimony resistance in this region. This has been evaluated in a retrospective epidemiological survey performed in Bihar, India, the conclusions of which suggest that arsenic-contaminated groundwater may well be associated with SSG TF. The failure rate with SSG was found to be 59%. Of note, patients living in areas with high mean local arsenic levels showed a higher risk (albeit statistically nonsignificant) to SSG TF than patients living in areas in which the wells had low arsenic concentrations.

Molecular mechanisms of DR to antimonials:

A significant amount of work has been devoted to understanding how SSG exert their selective action against *Leishmania* and how resistance emerges (see Fig 1).

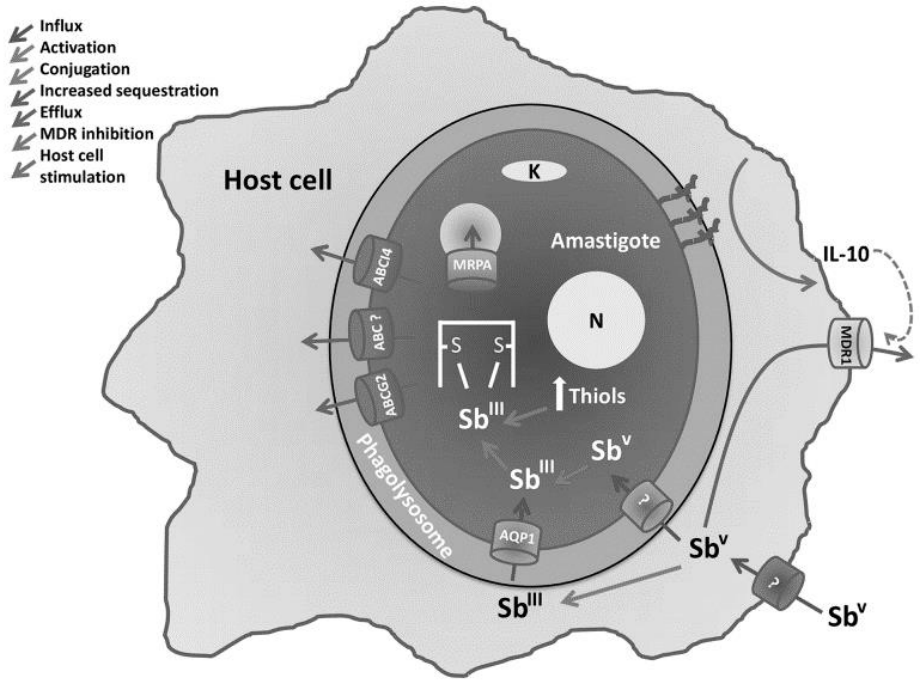

Figure 1 Molecular mechanisms of antimonial DR in *Leishmania*. The figure illustrates an amastigote inside a phagolysosome of the macrophage host cell. It explains the activation and influx methods used by antimonials to enter the parasite and the intracellular mechanisms used by the parasite to express the resistant phenotype. ABC, ATP-binding cassette; AQP, aquaporin; DR, drug resistance; IL-10, interleukin 10; MDR1, multidrug resistance protein 1.

To obtain the anti-leishmanial products SSG and potassium antimony tartrate, a chemical reaction must occur between various components; e.g., stibonic and gluconic acids are combined to produce SSG, i.e., it is a complex chemical mixture and not a single compound. Pentavalent antimony (SbV) must be reduced to its trivalent form (SbIII) for activity. Some of this reduction occurs within the host macrophage, and the resultant SbIII enters via the AQP1 membrane carrier. SbV also enters the parasite via another, as yet uncharacterized, carrier mechanism and is further reduced to SbIII within the cell.

Antimony accumulation is lower in resistant parasites when compared to sensitive parasites, although it is unclear whether or not actual levels of accumulation relate to sensitivity in wild-type cells. Overexpression of AQP1 renders the parasites hypersensitive to SbIII, whereas gene deletion renders them resistant, and reduced levels of AQP1 expression also relate to resistance. As described later, a mutation to AQP1 that renders the gene inactive is associated with a high level of antimony resistance in India.

Diminished biological reduction of SbV to SbIII, decreased internalization of the drug, and increased levels of trypanothione, which provides increased thiol redox potential, have also been implied in resistance. Overexpression of ATP-binding cassette (ABC) transporters involved in ATP-dependent transport of a variety of molecules across biological membranes has also been shown to influence the efflux of drugs and can play a role in antimony resistance in *Leishmania*. MRPA is an ABC transporter localized in membrane vesicles close to the flagellar pocket. Its overexpression confers antimonial resistance both in amastigotes and promastigotes by sequestering thiol–metal conjugates in intracellular vesicles.

The molecular epidemiological analysis of field isolates in the ISC also showed that an intrachromosomal amplification of MRPA that probably emerged in the mid-19th century predisposed *L. donovani* to develop resistance to SSG. Trypanothione is an unusual bis-glutathionyl spermidine adduct found uniquely in the phylogenetic group that includes *Leishmania*.

Trypanothione binds to SbIII, and the resultant metal–trypanothione conjugates are either sequestered into an intracellular organelle by MRPA or extruded from the cell by other efflux pumps. Overexpression of key enzymes in trypanothione synthesis (ornithine decarboxylase and gamma-glutamylcysteine synthase) has also been associated with resistance in conjunction with overexpression of MRPA.

PRP1 is another ABC transporter protein originally identified due to an ability to confer resistance to pentamidine. It has been postulated that it can also confer resistance to antimony, although its localization and mechanism of resistance have not yet been determined. Other ABC transporters in *Leishmania*, including ABCI4 and ABCG2, can also contribute to antimony resistance by the efflux of the drug as conjugated metal–thiol conjugates.

Finally, overexpression of tryparedoxin peroxidase has been associated with SbIII resistance through elevated levels of reduced intracellular thiols. Many resistant mutants exhibit significantly increased levels of intracellular thiols, including cysteine,

glutathione, and trypanothione, and lowering intracellular thiol levels in SSG-resistant (SSG-R) mutants can cause partial reversion of the resistance phenotype. Antimony induces oxidative stresses within the cells; therefore, an increased ability to deal with such stresses contributes to resistance. It is, then, clear that a variety of different mechanisms can all contribute—to varying degrees—to the ability of *Leishmania* parasites to resist antimony in a complex fashion.

Also of note is the fact that some changes to parasite physiology can also influence resistance by orchestrating changes to macrophage biochemistry, including increases in the macrophage's ability to extrude SbV- SSG. Complex glycans present on the surface of SSG-R parasites but not on SSG-sensitive parasites contribute through a cascade of cellular events to the up-regulation of the anti-inflammatory cytokine interleukin 10 (IL-10) levels in the host. This, in turn, provokes overexpression of multidrug resistance protein 1 (MDR1) efflux systems in the macrophage, which diminishes the total levels of antimony reaching the parasites. This also shows how coinfection status could influence treatment response; e.g., other agents that stimulate IL-10 levels could diminish the response of *Leishmania* to SSG.

The complexity of host and parasite factors that affect outcomes of *Leishmania* infections treated with SSG is becoming clear and, as outlined above, understanding of mechanisms that have been observed in laboratory analyses have been shown to be pertinent in explaining the emergence and spread of SSG resistance in the field.

Molecular mechanisms of DR to MIL:

MIL is the first and only oral drug available against leishmaniasis. Since its registration in India in 2002, it has replaced the use of SSG as first-line treatment in the ISC, with cure rates higher than 94%. MIL, a phosphorylcholine ester of hexadecanol, was originally developed as an anticancer drug. The exact mode of action of MIL is not well understood, although it has been described to have a direct effect on the parasites by interfering with biosynthesis of phospholipids and metabolism of alkyl-lipids, affecting mitochondrial cytochrome c oxidases and inducing mitochondrial depolarization and decrease of intracellular levels of ATP, and an apoptosis-like cell death.

The uptake of MIL and other alkyl-glycerophospholipids in *Leishmania* requires a translocation machinery that includes a P-type ATPase named the *Leishmania* miltefosine transporter (LMT), which is responsible for the translocation of phospholipids from the exoplasmic to the cytoplasmic leaflet of the plasma membrane of *Leishmania*. The function of LMT depends on its binding to a specific B subunit of LMT called LRos3, which belongs to the CDC50/ LEM3 protein family. Both proteins are mutually dependent for their function and their localization at the plasma membrane of *Leishmania*, being required for MIL uptake and susceptibility.

MIL has a long elimination half-life (approximately 120 h) that leads to subtherapeutic levels remaining for some weeks after a standard treatment course.

Following this observation, it was predicted that resistance to MIL would rapidly emerge in the regions where it was extensively used.

Ten years after the implementation of MIL in the ISC, its efficacy was shown to be decreasing with a relapse rate of 10% in India and up to 20% in Nepal upon 12-month follow-up. However, this increasing TF was not initially associated to drug resistance, with other factors invoked to explain the situation, including parasite virulence and host factors. Recently, however, two clinical isolates resistant to MIL were isolated in the ISC. Although slower to emerge in the field than some had feared, laboratory-based experimentation has demonstrated that in vitro selection of promastigotes resistant to MIL was easily achieved.

The main mechanism of experimental resistance observed is associated with a significant reduction in drug internalization due, mainly, to a reduced uptake or an increased efflux of MIL. The acquisition of inactivating mutations or deletions in MIL translocation machinery LMT and/or LRos3 in *L. donovani* was shown to drastically increase MIL resistance in both in vitro and in vivo assays. LMT and/or LRos3 have also been shown to represent MIL-resistant markers in clinical samples obtained from leishmaniasis patients showing therapeutic failure to MIL.

In addition, the overexpression of ABC transporters ABCB4(MDR1), ABCG4, and ABCG6 has also been described to be associated with an increased resistance to several alkyl-lysophospholipids analogues, including MIL in *Leishmania*, due to a reduced intracellular accumulation because of increased efflux of the drug across the plasma membrane.

Other cellular modifications have also been proposed to contribute to MIL resistance in *Leishmania*. These include changes in the length and levels of unsaturation of fatty acids, as well as a reduction in ergosterol levels; altered expression of genes involved in thiol metabolism, protein translation and folding, as well as DNA repair and replication machinery; and higher ability to resist reactive oxygen species (ROS) as well as a better tolerance towards, or reduced production of, oxidative stress. An increase in metacyclogenesis and infectivity has also been described in MIL-resistant promastigotes.

Recently, the use of omics techniques (whole-genome and RNA sequencing) in MIL experimental resistant *L. donovani* lines has revealed mutations in genes encoding proteins other than LMT. These include pyridoxal kinase and α-adaptin line protein as well as up- and down-regulation of specific genes associated with stress, membrane composition, and amino acid and folate metabolism. Specific roles in the drug's mode of action or resistance mechanisms are not known, but a picture of a multifactorial process contributing to resistance to MIL is emerging.

The molecular basis of AmB resistance in *Leishmania*:

AmB has been used as an antifungal agent for the last 70 years and as an anti-leishmanial since the 1960s. Its ability to bind to ergosterol-related sterols in cell membranes explains its specificity. Because *Leishmania* parasites, in common with fungi, use ergosterol as a primary membrane sterol, they too are sensitive to this drug. Mammalian cells use cholesterol instead and are accordingly less sensitive to the drug. AmB is a natural product produced by Streptomyces nodosus and has an amphipathic nature with hydrophilic and hydrophobic moieties. Once within the vicinity of the membrane, it spontaneously assembles with its hydrophobic surface in contact with membrane lipids while hydrophilic surfaces of adjacent molecules produce a pore. The exchange of ions across the surface via the pores contributes to cell death. However, the drug also induces oxidative stress, and binding to sterol per se irrespective of pore formation also contributes to the death of yeast cells.

WHO has been promoting the use of single-dose Ambisome, a relatively harmless liposomal formulation of the drug for VL patients, particularly on the ISC. This campaign, which enables the widespread use of the drug without needing patient hospitalization and repeated injections, has clear advantages from a public health perspective. However, the dose available in this single shot is not far from the minimum required to treat the disease. This poses the risk that this single shot, not being always curative, could select for parasites with reduced vulnerability to the drug. These may then transmit as a less sensitive population, itself then potentially capable of developing further resistance. As discussed below in the section on combination therapies, AmB is currently recommended as a potential partner drug in a number of regimens. If resistance genes to AmB are selected during a monotherapy phase, there is the risk that they will render ineffective the AmB part of any combination. In that case, the partner drug will be effectively used as monotherapy and—worse still—possibly be used against parasites for which that drug is used at doses that are suboptimal for monotherapy, while assuming that the AmB part is effective (i.e., in combination therapies, drugs are often given at lower doses than in monotherapy). Thus, selection of resistance to the second drug as well becomes increasingly likely.

Resistance, however, has been considered of low risk for AmB. This is partly because resistance has been relatively rare in fungal infections, in spite of 70 years of use. Moreover, reports of AmB resistance in leishmaniasis have also been rare.

Notwithstanding, there are multiple reports of AmB resistance in fungal infections. Moreover, the first cases of TF with AmB have already appeared in India, where resistant parasites were clearly associated with one case. In France, TF in HIV– *Leishmania* coinfections has been reported, and AmB unresponsiveness in an immunosuppressed patient in Switzerland was reported as well. In laboratory studies, it has been possible to select for resistance to AmB in several species of *Leishmania*, and both promastigote and amastigote forms of the parasite resistant to AmB have been selected. It seems, therefore,

that serious attention should be given to the risk of selecting resistance to AmB in *Leishmania*.

Several studies have started to elucidate the mode of action and resistance mechanisms to the drug. For example, it was shown that treatment with AmB permeabilized leishmanial lipid bilayers to ion and dye exchanges, indicating that the drug binds to the membrane as in yeast. A number of selected resistant lines revealed changes in the sterol content. Notably, ergosterol and related sterols were replaced by cholesta-related sterols. In one case, this was attributed to possible changes in sterol methyltransferase, causing disruption in the sterol pathway and accumulation of intermediates in the ergosterol synthetic pathway. Several other studies have shown that loss of ergosterol is associated with resistance, which links to the drug's dependency on binding this sterol to exert its mode of action. It has recently been demonstrated that mutations to the gene encoding sterol 14α-demethylase underlie resistance with an accumulation of that enzyme's product, which indicates that the demethylase is still active itself, but its product no longer enters the remainder of the pathway.

Other studies have also indicated separate changes associated with resistance. For example, a number of selected lines have increased parts of their oxidative defense mechanism, which indicates that part of the drug's mode of action is via the induction of oxidative stress and that prevention of this oxidative damage can yield a reduced sensitivity to the drug. In another resistant line, alterations to the MIL transporter were identified, and in this case, cross-resistance between AmB and MIL was detected, although the functional impacts of these mutations were unclear because the impact on MIL uptake was minimal. This may be attributed to changes in the lipid composition of the membrane because the MIL transporter plays a key role in arranging lipids within the membrane; indeed, extensive changes to lipid profile were observed in this resistant line and were partially restored by complementation with wild-type MIL transporter.

It appears, therefore, that resistance to AmB can be selected in *Leishmania*. Although it appears that this is more readily achieved in promastigote forms than in amastigote forms (possibly because a combination of changes in membrane sterol composition and response to oxidative stress are required), the latter form can indeed develop resistance to the drug, and this has been found already in cases of TF.

Probable questions:

1. What are the types of leishmaniasis occurring in man? What are the possible lines of treatment in such infection?
2. Elucidate drug resistance.
3. How resistance in *Leishmania* develops against antimonials?
4. How resistance in *Leishmania* develops against miltefosin?
5. State the molecular basis of amphotericin B resistance against *Leishmania*.

Suggested reading:

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Unit-X
Immunity in human trypanosomiasis

Objectives:

In this section we will discuss on immunity in human trypanosomiasis.

Introduction:

Sleeping sickness or human African trypanosomiasis (HAT) is an endemic parasitic disease exclusively located in intertropical Africa where it is transmitted by the tsetse fly or *Glossina*, its unique vector (Vickerman 1985). The new taxonomy tools used in African trypanosomes (isoenzyme characterisation, DNA analysis) have allowed scientists to separate the *Trypanosoma brucei* clade in several subspecies. Two are infective for humans: *T.b. gambiense*, and *T.b. rhodesiense*. These extracellular parasites are injected into humans by the bite of infected tsetse fly.

The inoculation of trypanosomes into their mammalian hosts triggers a series of events involving, at first, innate immunity and, secondarily, specific immunity. The latter requires an efficient presentation of parasitic antigens, activation of T and B cells implying specific antigen receptor recognition, and the development of effector cells and molecules. These mechanisms are highly regulated by multiple signals delivered through a large number of receptors transduced across the plasma membrane and processed. During co-evolution with their hosts, trypanosomes have learnt to cope with host immune systems, by penetrating, diverting, and altering the numerous steps leading to the generation of an effective immune response. Major modifications of immune systems have been observed in trypanosomiasis: lymphadenopathy, splenomegaly (up to thirty times the normal size) with destruction of lymphatic tissue architecture and hypergammaglobulinemia. However, their effectiveness is limited as, most of the time, parasites cannot be eliminated and immunopathological phenomena, which induce tissular alterations, appear.

One of the major characteristics of trypanosomes is the presence of the Variant Surface Glycoprotein (VSG) which covers nearly all the membrane of trypanosomes in mammals and is the predominant surface antigen of African trypanosomes. VSG constitutes an important molecular interface between trypanosomes and the host immune system. VSG prevents trypanosome lysis by complement alternative pathway, and, above all, enables them to avoid the specific immune response via the phenomenon of antigenic variation (trypanosomes sequentially express antigenically distinct VSG). VSG also has several effects on immune elements such as induction of autoantibodies and cytokines, in particular tumour necrosis factor (TNF)- α. Other trypanosome components and soluble factors, such as a trypanosome-released triggering factor (TLTF) which triggers interferon (IFN)-γ production by T cells, are also involved in modulation of the immune system by acting on the synthesis of immune elements. Furthermore, increased

levels of circulating endotoxins are a feature of human and experimental trypanosomiasis. These endotoxins, potent immunomodulatory molecules, participate to the immune disorders observed in trypanosomiasis. Elaboration of escape mechanisms to host immune defences and induction of parasite growth factor production are well developed by trypanosomes. In a recently discovered escape mechanism, host arginase induction, trypanosomes decrease immune response efficiency and increase the production of L-ornithine, an essential growth factor.

Innate immunity:

- **Natural immunity**

Normal human sera injected into *T.b. brucei*-infected mice caused a dramatic reduction in parasitemia. This phenomenon was not reproduced with the human trypanosome strains *T.b. gambiense* and *T.b. rhodesiense*. Trypanolytic factors (TLF) contained in normal human serum were identified as high-density lipoproteins. Recently, two TLFs have been characterised in human serum. The first one (TLF1) belongs to a subclass of high-density lipoproteins and is inhibited by haptoglobin. In contrast, the second factor, TLF2, has a much higher molecular weight and does not appear to be a lipoprotein. Probably, the main trypanolytic effect is due to TLF2, which is not inhibited by haptoglobin. The trypanocidal effect of cape buffalo serum has been attributed to xanthine oxidase. Recently a trypanosome lysosomal protein (SRA) was found to be associated with resistance to normal human serum. SRA is a truncated form of VSG and interacts with serum apolipoprotein L-I in the parasite lyzosome.

- **Chancre**

The local response in the skin corresponds to the first protection developed by the host. Following inoculation of *T. brucei* into mammalian hosts, by the tsetse fly, a local skin reaction is induced by trypanosome proliferation and appears a few days after inoculation. In efferent lymphatic vessels, trypanosomes have been detected in lymph 1-2 days before the chancre. Their number declined during development of the chancre (6 days) and later increased. They are detected in the blood 5 days after inoculation. In *T. congolense*-infected sheep, neutrophils predominate in the early days and then T and B lymphocytes infiltrate the chancre. Later, T lymphocytes predominate, especially CD8+ T cells. An early response due to an increase in $CD4^+$ and $CD8^+$ T cells was revealed by flow cytometry in the afferent lymph draining the chancre. As the chancres regressed there was an increase in lymphoblasts and surface immunoglobulin bearing cells. During this first stage, trypanosomes expressed Variable Antigen Types (VATs) found characteristically in the tsetse fly, which changed after few days. An antibody response specific to these VATs appeared in the lymph and then in the plasma.

- **Complement**

Both in humans and animals, complement activation by two pathways is detected in HAT. The alternative pathway, independent of specific antibodies, was studied by the induction of trypanosome lysis (*T. congolense* and *T.b. brucei*) observed after the addition

of fresh serum. Serum could induce trypanosome lysis only on uncoated VSG trypanosomes, as observed during the cycle of this parasite (procyclic forms). However, the appearance of VSG on parasites prevents trypanosome lysis by this alternative pathway. For another strain of *T.b. gambiense*, it was demonstrated that the alternative pathway was incompletely activated without generation of the terminal complex (C5-C9) able to induce membrane lysis. The classical pathway, mediated by specific antibodies against trypanosomes, was also described and could be involved in parasite clearance by antibody-mediated lysis and/or opsonisation. The coated stages of *T.b. brucei* are lysed by antibodies with activation of complement by the classical pathway. Nevertheless, during these complement activations, the appearance of soluble fragments, including C3a and C5a anaphylatoxins and the C567 complex, could induce, on the one hand, the chemotactism of neutrophils and monocytes and, on the other hand, the release of amines involved in vasoconstriction and an increase in vascular permeability participating in the initial inflammatory response in the chancre. Immune complexes can also activate the complement. These immune complexes are constituted by antibodies specific to trypanosomes (e.g. antiVSG antibody) leading to a rapid elimination of complement-fixing immune complexes or by autoantibodies, such as rheumatoid factor or anti-nucleic acid antibodies. These immune complexes with complement activation are also involved in some adverse effects, especially in tissue damage mediated by immune complex deposits, such as thrombosis and glomerular involvement.

- **Natural killer cells**

Natural killer (NK) cells have been identified as an important defence mechanism against tumour cells and intracellular pathogens, especially viruses. They are considered to belong to the lymphocyte lineage and have functions in both innate and acquired immune responses. NK cells lyse extracellular parasites. NK cells from *T. cruzi*-infected mice have been shown to exhibit significant activity against trypomastigotes of *T. cruzi*.

NK cells secrete cytokines and especially IFNγ and TNF-α, which play major roles in trypanosomiasis and are regulated by cytokines which can activate or inhibit NK cell functions. NK cells also participate in the initiation of the inflammatory response, through the synthesis of chemokines.

In *T. brucei*-infected mice, NK activity was not modified in the early stages of infection, but was severely reduced from day 9 onwards. By contrast, NK cells were activated in mice infected with a natural extracellular trypanosome (*T. musculi*) and their critical role was demonstrated by the effects of their depletion by antiserum against asialo GM1 or their activation by polycytidylic copolymer.

Acquired immunity:

- **T Cells**

Initial studies have evidenced alterations in T cell functions in trypanosomiasis, both in vivo and in vitro. Histological examination revealed a massive B cell expansion in the lymph nodes and spleen, which replaced the thymus-dependant area in *T.b. brucei* TREU 667-infected mice. These changes were seen within 7 days post-infection and persisted for at least 70 days. Moreover, the role of T cells in controlling infection was not clear.

Trypanosome antigen-specific T cell response was difficult to identify. In several studies, a transient proliferative T cell response to trypanosome antigens was noted in the first days of the infection followed by an absence of response. The kinetoplastid membrane protein 11 of African trypanosomes is a potent stimulator of T lymphocyte proliferation.

In *T. b. brucei* - infected mice, an increased proliferation of T cells was noted in the first days of infection in spleen and bone marrow, T blasts disappeared very rapidly. In *T. congolense*-infected cattle, antigen-specific proliferation of T cells was obtained with more or less difficulty according to the antigen, the T cell population and the time used. However, a strong trypanosome-specific T cell proliferation occurred in infected cattle following treatment.

Most T cells in humans and mice bear Tαβ antigen receptors. These cells possess surface markers, which allow the discrimination of $CD4^+$ T cells (helper T cells) and $CD8^+$ T cells (cytotoxic T cells). The knowledge of T cell subsets has been deeply modified by the discovery of two subsets of T helper cells, Th1 and Th2 cells. Th1 cells expressing a functional T cell response directed to VSG are generated in *T.b. rhodesiense*-infected mice. VSG specific T cells were found predominantly in the peritoneum. These cells did not proliferate but made a substantial IFN-γ and IL-2 cytokine response. The cellular phenotype of VSG-responsive T cells ($CD4^+$ $CD3^+$) indicates that the VSG appear to preferentially stimulate a Th1 cell subset during infection.

Analysis of lymphocyte subsets in regional lymph nodes of *T. congolense*-infected N'Dama (trypanotolerant) and Boran (trypanosusceptible) were performed by flow cytometry. In both breeds, a significant decrease in the percentage of $CD2^+$ and $CD4^+$ T cells was observed, associated with an increase in the percentage of $CD8^+$ T cells, B cells and γ δT cells. VSG and two invariant antigens (33 kDa cysteine protease and 66 kDa antigen homologous to immunoglobulin heavy chain binding protein hsp70/Bip) induced in vitro proliferation and synthesis of IL-2 and IFN-γ. No significant differences in the in vitro proliferation of lymph node cells to VSG, Concanavalin A (Con A) or hsp 70/Bip were observed between the two breeds. However, IFN-γ production in response to Con A was higher in Boran at 35 days post infection.

Human and mouse immune systems contain few γ δT cells, in marked contrast to those of ruminants. Functions of γ δT cells remain largely unknown. Involvement of γ δT cells in malaria and leishmaniasis has been observed. A proliferative response of CD8+ T

cells and γ δT cells from trypanotolerant N'Dama to an antigen complex containing immunodominant epitopes was observed whereas a quasi-absence of response was observed in trypanosusceptible Boran. The role of this γ δT cell response in parasite resistance remains unclear. So, γ δT cells, as $CD4^+$ or $CD8^+$, do not proliferate when stimulated with soluble VSG in vitro. It would be interesting to determine the role of cytokines synthesised by γ δT cells.

Indeed, although specific T cells do not act on trypanosomes in the same way as the cytotoxic T cells in several infectious diseases such as viral infections, they markedly modify immune responses, especially by the secretion of cytokines. They greatly modify functions of B cells (antibody synthesis, isotype switch) and macrophages (antigen presentation, effector mechanisms).

- **B Cells**

In African trypanosomiasis, the main feature is a dramatic increase in immunoglobulin (Ig) levels (especially IgM), including trypanosome-specific antibodies and non-specific Ig production induced by cytokine activation of B cells. Some of these antibodies are also raised against autoantigens, corresponding to non-specific polyclonal activation of B-cells producing natural autoantibodies and also to antigen-driven antibodies induced by molecular mimicry. DNA from *T.b. brucei* stimulated B cell proliferation. In *T.b. brucei*-infected mice, B lymphocytes display an aberrant activation phenotype.

Antibodies specific to trypanosomes are induced by several parasite antigens, including variant and invariant VSG epitopes, as well as membrane, cytoplasmic and nuclear antigens, through T-dependent and T-independent pathways. Antibodies directed against trypanosome VSG components appeared in sera and their binding to the surface coat of the trypanosomes was able to induce a decrease in parasitemia, both in the blood and extravascular spaces, specifically by immune lysis of parasites and their destruction by the Kupffer cells in the liver. Only heterologous antigenic variants (<0.1%) remain to repopulate the blood and tissues. Parasites are eliminated due to VSG-specific IgM (appearing at high levels, 3-4 days after infection). In contrast, VSG specific IgG does not seem to be involved in the destruction of trypanosomes, as they appeared after the disappearance of this VAT population. Another induction of antibodies, linked to the new VSG epitopes, appeared in sera and also contributed to decrease the new VAT-specific population. The VAT specific antibodies therefore decreased to low levels, whereas antibodies, belonging predominantly to the IgM class specific to invariant epitopes, remained at high levels. During infection, B cell nonspecific stimulation was enhanced as T-independent B cell responses to the VSG successive parasitemias. In contrast, specific trypanosome B cell response, depending on T cell regulation, was depressed. Several factors may contribute to this immunosuppression. Macrophages may become unable to present antigens to T cells (by defects in antigen processing and association of epitopes with MHC Class II) and produce immunosuppressive factors as nitric oxide (NO), prostaglandins (PG), and cytokines. An increase in immunosuppressive cytokines, such as INF-γ and transforming growth factor (TGF)-β, was also detected during infection.

However, TGF-β is known to inhibit the production of IL-4, IL-5, IL-6, the major cytokines implied in B cell proliferation and differentiation

Probable questions:

1. Name the two infective species of *Trypanosoma*.
2. Briefly explain the predominant surface antigen in *Trypanosoma*.
3. How *Trypanosoma* infection affect the innate immunity of human body?
4. State the activation of B and T cells due to *Trypanosoma* infection.

Suggested reading:

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Unit-XI
Epidemiology: General and landscape Malaria

Objectives:

In this section we will discuss on general and landscape Malaria.

INTRODUCTION:

Malaria is a potentially life-threatening parasitic disease caused by parasites known as Plasmodium vivax, Plasmodium falciparum, Plasmodium malariae & Plasmodium ovale. It is transmitted by the infective bite of Anopheles mosquito. Man develops disease after 10 to 14 days of being bitten by an infective mosquito. While the disease is uncommon in temperate climates, malaria is still common in tropical and subtropical countries. Each year nearly 290 million people are infected with malaria, and more than 400,000 people die of the disease. To reduce malaria infections, world health programs distribute preventive drugs and insecticide-treated bed nets to protect people from mosquito bites. The World Health Organization has recommended a malaria vaccine for use in children who live in countries with high number of malaria cases. Protective clothing, bed nets and insecticides can protect while traveling. We also can take preventive medicine before, during and after a trip to a high-risk area. Many malaria parasites have developed resistance to common drugs used to treat the disease.

Fig- *Anopheles* sp.

Epidemiology: General and Landscape Malaria :-

- Malaria occurs primarily in tropical and some subtropical regions of Africa, Central and South America, Asia, and Oceania. In areas where malaria occurs, there is tremendous variation in the intensity of transmission and risk of infection. For example, over 90 percent of clinical malaria infections and deaths occur in sub-Saharan Africa (World Health Organization 1996a). However, even there the risk varies widely.

- Highland (>1,500 m) and arid areas (<1,000 mm rainfall/ year) typically have less malaria, although these areas are prone to epidemic malaria if climactic conditions become favourable to mosquito development.

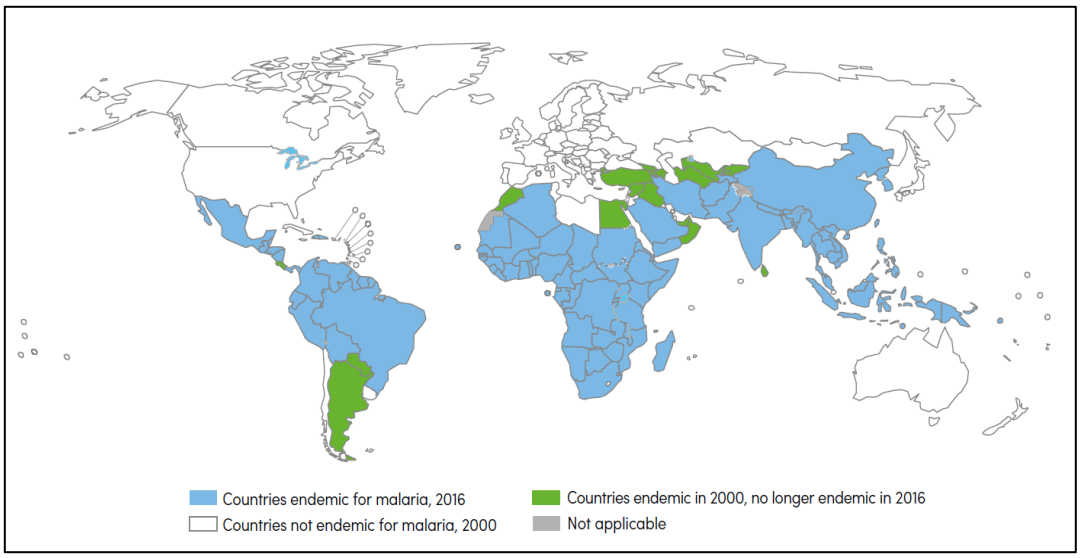

Fig- Epidemiology of malaria

- Although urban areas have typically been at lower risk, explosive unplanned population growth has been a major factor in making urban or peri-urban transmission an increasing problem.
- Human malaria is caused by one or more of four parasites: Plasmodium falciparum, P. vivax, P. ovale, and P. malariae. Distribution of these parasites varies geographically, and not all species of malaria are transmitted in all malarious areas.
- Plasmodium falciparum, the species most commonly associated with fatal malaria, is transmitted at some level in nearly all areas where malaria occurs. It accounts for over 90 percent of all malaria infections in sub-Saharan Africa, and causes two-thirds or more of the malaria cases in Southeast Asia.

- Plasmodium vivax is a relatively uncommon infection in sub-Saharan Africa. Duffy antigens, which are required by the parasite to invade red blood cells, are lacking in many ethnic groups, especially in West Africa.
- Vivax malaria, however, is the predominant species in Central America, most of malarious South America, and the Indian subcontinent.
- Affects about 515 million people per year.
- Kills between 1-3 million per year.
- Other Plasmodium species also infects birds, reptiles, rodents, monkeys and apes.

Probable questions:

1. State the epidemiology of Malaria?

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Unit-XII

Epidemiology: General and landscape Leishmania and Filaria

Objectives:

In this section we will discuss on epidemiology: General and landscape Leishmania and Filaria.

Introduction:

Leishmaniasis is widely distributed across 88 tropical, subtropical and temperate countries, with more than 350 million people at risk. An estimated 12 million patients suffer from leishmaniasis, with 0.2–0.4 million of new Visceral Leishmaniasis (VL) and 0.7–1.2 million of new Cutaneous Leishmaniasis (CL) cases per year worldwide. The disease mainly affects poor people in Africa, Asia and Latin America, and is associated with malnutrition, population migration, poor residency conditions, frail immune system and lack of resources. Specifically, more than 90% of VL cases globally occur in six countries: Bangladesh, Brazil, Ethiopia, India, South Sudan and Sudan. CL is more widely distributed throughout America, the Mediterranean basin and western Asia. The 10 countries with the highest number of affected cases of CL, which account for 75% of the global incidence, are Afghanistan, Algeria, Brazil, Colombia, Costa Rica, Ethiopia, Iran, Peru, North Sudan and Syria.

Overall, infection is caused by more than 20 species of *Leishmania* parasites, which are spread by approximately 30 species of phlebotomine sand flies. Female sand flies of the genus *Lutzomyia* in America (new world) and *Phlebotomus* in the rest of the world (old world) transmit *Leishmania* spp. The sand fly vectors generally are most active after evening, in the night-time hours ("from dusk to dawn"). Climate and other environmental changes have the potential to expand the geographic range of the sand fly vectors and the areas in the world where leishmaniasis is found. Typically, leishmaniasis is transmitted by the bite of an infected female sand fly to mammalian reservoirs, such as rodents, marsupials, edentates, monkeys and wild or domestic canines. Humans are infected incidentally in endemic areas. VL can also be transmitted via intravenous drug use, blood transfusion, organ transplantation, congenital infection and laboratory accidents, although these modes of transmission are relatively rare.

Anthroponotic transmission is characteristic of the *L. tropica* complex (old world) and *L. donovani* complex (especially in the Indian subcontinent). *L. donovani* occurs in South Asia (India, Bangladesh and Nepal) and East Africa (Sudan, Ethiopia, Kenya, Somalia). In areas with anthroponotic transmission, effective treatment of patients can help control the spread of the parasite. Clinical disease due to *L. donovani* can affect people of all ages, although in regions with constant endemic transmission the incidence

may fall with increasing age because of a high rate of acquired immunity in older adults. In East Africa, *L. donovani* is the cause of both anthroponotic and zoonotic disease.

On the contrary, *L. infantum*- VL (synonym to *L. chagasi*) occurs in the Mediterranean, the Middle East, Afghanistan, Iran, Pakistan and Brazil, although sporadic cases have been reported in Central Asia, China, Mexico and Central and Latin America. Notably, children and immunosuppressed adults are at higher risk of clinical disease due to *L. infantum* than immunocompetent adults. Transmission of *L. infantum* infection is considered predominantly zoonotic, with domestic dog being the major reservoir.

The high prevalence of asymptomatic human carriers of *L. infantum* in southern Europe suggests that this parasite is a latent public health menace. Whereas most immunocompetent individuals will not develop clinical disease after this parasitic infection, potential administration or acquired development of immunosuppression would result in the emergence of clinically severe disease. In this context, an increase of co-infections with human immunodeficiency virus (HIV) and Leishmania has been observed during the last 30 years. Cases of co-infection have been reported in the Mediterranean region, mainly in France, Italy, Portugal and Spain. Moreover, VL is an emerging condition affecting HIV-infected patients living in many Asian (especially India) and African countries as well as in Latin America, particularly in Brazil. The majority of non-HIV-related immunosuppressive conditions that are associated with VL emergence have been reported in the field of transplantation medicine, rheumatology, oncology and hematology. In the last 20 years, the increasing frequency of organ transplantations and the improvement on immunosuppressive treatments have led to the recognition of several cases of VL after organ transplantation. In addition, introduction of tumor necrosis factor-alfa (TNF-alfa) blockade into the clinical practice has been associated with increasing reports of leishmaniasis in patients with autoimmune rheumatic diseases who live in endemic areas. Moreover, several cases have been reported in patients under treatment with various immunomodulatory drugs, like azathioprine, methotrexate, steroids and cyclosporine. Thus, it is recommended that patients living in or returning from areas endemic for leishmaniasis should undergo serological screening before immunosuppressive treatment.

The South Asian region has suffered from the devastating epidemics of leishmaniasis since the early 1980s. Nonetheless, substantial progress has been made in controlling leishmaniasis in this region through successful diagnosis *via* active detection, complete case management, effective vector control measures, community mobilization and operational use of research generated knowledge. In Sri Lanka, leishmaniasis has received little attention from the authorities despite regular increases in the number of cases every year since 2011. One of the main limitations in the control of this disease in Sri Lanka is the lack of research at the regional level that would highlight the important risk factors that need to be addressed through control programmes. In this regard, the present study revealed valuable information.

The demographic factors associated with CL differ regionally, in some areas the elderly population is mostly affected, or in other areas the younger population. The younger population was the most affected group according to the univariate analysis. However, this did not significantly predict the disease incidence. Although gender and marital status showed statistically significant associations according to the univariate analysis, it did not significantly predict the disease incidence as indicated by the adjusted odds ratios. Hence, the observed significant relationships in the univariate analysis are likely to be a result of confounding effects. Therefore, demographic factors may not significantly affect the odds of having the disease.

In general, outdoor occupations increase the risk of leishmaniasis incidence due to a greater risk of being bitten by sand flies. Housewives were found to be at a risk of receiving infection. This may be due to their outside routine activities such as picking firewood, cleaning home gardens, washing clothes and bathing at outdoor water sources. Furthermore, women of farming families often denominate themselves as housewives, but they tend to assist their husbands in farming activities, which make them equally prone to vector sand fly bites.

Leishmaniasis is considered a neglected tropical disease, where the poorest of the population is affected. Those who had a monthly income of < Rs. 10,000 (56.76 USD) were potentially at risk of infection 9.5 times higher compared to those who had a monthly income of > Rs. 30, 000 (170.27 USD). Generally, neglected tropical diseases affect poor populations due to poor sanitation, poor housing conditions, and lack of access to essential nutrition. In this case, housing condition was not a significant factor. Therefore, house condition can be excluded as a major risk factor for the disease. However, with this knowledge, further studies would provide a better insight into other associated socioeconomical risk factors.

In any vector-borne disease, the presence of potential breeding/resting places of the vectors and reservoirs are associated with an increase in disease incidence. Presence of potential breeding places for sand flies (decaying garbage, termite hills and areas with wet soil) and potential adult resting places (gardening areas and unclear areas) were associated with increased odds of leishmaniasis incidence. This is consistent with previous studies which indicated a higher risk of acquiring leishmaniasis with the presence of suitable resting and breeding sites in close proximity. Although reservoir hosts of the parasite in Sri Lanka is unclear, dogs are suspected to be a reservoir host. Nevertheless, the presence or absence of dogs, other pets (cats), livestock (cattle and chicken), stray animals (wild boar, rodents and monkeys) commonly visiting home gardens did not differ significantly. The same scenario has been observed from another study conducted in a different region of Sri Lanka. The absence of increased exposure to potential reservoirs by patients is not adequate in making a strong conclusion about the role of reservoir hosts of the disease. The parasite *L. donovani* is known to have an anthroponotic transmission cycle, according to studies from other countries. A study suggested the possibility of cattle being a reservoir host for *L. donovani* in Bangladesh. However, more evidence indicates that the transmission may not involve an animal

reservoir. However, further studies on the interactions between the reservoir host, vector, parasite and human hosts are essential for a better understanding of the involvement of a reservoir in disease transmission.

Awareness about the disease was very poor. No one without a history of leishmaniasis infection knew about the causative organism, while few individuals knew that the disease is caused by a parasite. Some of the infected individuals mentioned the name of the vector correctly. But, none of them were aware what kind of insect it was. Therefore, inhabitants in these disease-endemic areas often misinterpret sand flea as the vector of leishmaniasis. Hence, the majority of the incorrect responses during the study was sand flea as the disease vector. In two cases, inhabitants believed that the disease can be transmitted by the fruit fly and the lesion is due to the eggs laid by the fruit fly on the skin. This assumption, which they believed to be true, was based on their experience in vegetable and fruit cultivations. Knowledge of protective measures was also very poor among the study population. One of the most common responses was the use of mosquito nets, which was considered as a wrong response, as sand flies can crawl through the mesh of mosquito nets. Another incorrect response was staying away from sand to avoid sand fly biting, and thereby to prevent from the infection.

Lymphatic filariasis is a parasitic infection caused by the filarial nematodes *Wuchereria bancrofti*, *Brugia malayi*, and *Brugia timori*. These parasites are transmitted by members of several mosquito genera, particularly *Anopheles*, *Aedes*, *Culex*, and *Mansonia*, with geographic variation in the identity of dominant vectors. Long-term infection can cause deterioration of the lymphatic system, characterised by severe swelling of the limbs (lymphoedema) and later elephantiasis or lymphoedema of the scrotum (hydrocele). Community-level transmission of infection can be interrupted by mass treatment with recommended oral regimens of the antihelmintic medicines albendazole, either alone or with ivermectin, or diethylcarbamazine citrate and albendazole, or a combination of all three, depending on the setting. These medicines are given in mass public health campaigns or, in certain settings, through salt fortification with diethylcarbamazine citrate. Treatment of at least 65% of the total population in endemic areas for at least 5–7 consecutive years through annual or biannual mass drug administration (MDA) is recommended by WHO, to reduce the reservoir of microfilaraemia and antigenaemia among humans, with the ultimate goal of interrupting transmission to eliminate lymphatic filariasis as a public health problem. WHO has recommended guidelines by which national elimination of lymphatic filariasis as a public health problem can be validated, and national programmes are requested to submit dossiers to document baseline prevalence, programme interventions and monitoring activities, prevalence during surveillance after MDA, and availability of care for people with lymphatic filariasis.

Lymphatic filariasis transmission has been documented throughout Africa, southeast Asia, and the Pacific, as well as in focal areas in the Caribbean, South America, and the Middle East. Use of population-level vector control or MDA began in the 1950s in India, China, Egypt, and Brazil, followed by implementation across Oceania from the

1960s to the 1990s. In 1997, the World Health Assembly recognised the goal of global elimination of lymphatic filariasis as a public health problem by 2020 under resolution WHA50.29, in which national programmes would aim to interrupt transmission and control morbidity. Elimination of lymphatic filariasis as a public health problem was first achieved in China in 2007 and South Korea in 2006. Coordinated efforts between ministries of health, international partners, and the research community under the auspices of the Global Programme to Eliminate Lymphatic Filariasis (GPELF) have been ongoing since WHO launched the programme in 2000. With the adoption of the London Declaration in 2012, the global community reinforced its commitment to elimination. New milestones and targets for elimination of lymphatic filariasis as a public health problem have been proposed by WHO in line with 2030 objectives for Sustainable Development Goals.

From the late 1990s onwards, most national lymphatic filariasis elimination programmes implemented some form of baseline mapping to identify implementation units eligible for MDA, such as districts or counties. Eligibility for MDA was generally determined by infection prevalence of more than 1%, measured by night blood smears to detect microfilaraemia, detection of circulating filarial antigen, or presence of known or suspected filarial lymphoedema and hydrocele cases. Global guidelines for monitoring and evaluation of these programmes were first adopted in 2000, followed by updates in 2005, and 2011. Monitoring of MDA is conducted through periodic sentinel site and spot check surveillance, and current guidelines recommend the Transmission Assessment Survey to determine if implementation units can enter the post-MDA surveillance phase. As of 2018, 21 lymphatic filariasis elimination programmes have begun post-MDA surveillance for all implementation units considered endemic, including 15 that have met validation criteria for having eliminated lymphatic filariasis as a public health problem. 51 countries or territories with ongoing lymphatic filariasis elimination programmes remain, 15 of which have yet to reach full geographic coverage with MDA as of 2018.

Despite the broad scale of lymphatic filariasis data collection since the inception of the GPELF, previous global infection prevalence estimates relied on older data; estimates for 1996, 2000, and 2013 were based on data extracted from 118 studies published between 1953 and 1991 for national-level analysis. Although other studies have employed geostatistical methods in lymphatic filariasis-related research, including estimates of population at risk and pre-control prevalence, tests for spatial clustering, country-level prevalence, and forecasted future prevalence in Africa, no previous analysis has used geospatial methods to estimate time trends in global infection prevalence accounting for subnational variation in covariates associated with lymphatic filariasis transmission. We therefore aimed to estimate the global prevalence of lymphatic filariasis to reflect the progress achieved after two decades of the GPELF and identify areas that might warrant additional programme investment to reach elimination goals by 2030.

Probable questions:

1. What are the socio-economic factors responsible for the spread of Leishmaniasis?
2. Name the New-World and Old-World vector and causative agents of Leishmaniasis.
3. Write a short note on the epidemiology and global prevalence of leishmaniasis.
4. How community level spread of lymphatic filariasis can be controlled? What is MDA?
5. State the resolution of London Declaration 2012 by WHO in line with 2030 objectives for Sustainable Development Goals.

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Unit XIII

Nosology in Relation to Protozoa

Objectives:

In this section we will discuss on nosology in relation to protozoa.

Introduction

Nosology [from Ancient Greek word*(nosos)* 'disease' and *(-logia)* 'study-of'] the systematic classification and naming of diseases, is fundamental to the field of medicine. It provides a structured framework for identifying, categorizing, and understanding diseases, facilitating diagnosis, treatment, and research. When it comes to protozoa—single-celled eukaryotic organisms—nosology becomes particularly significant due to the diverse and complex nature of protozoal infections.

Protozoa are a diverse group of organisms that can cause a wide range of diseases in humans and other animals. These diseases can affect various organ systems, present with a variety of clinical manifestations, and have different epidemiological patterns. Effective nosology in relation to protozoa involves categorizing diseases based on the causative protozoan species, the affected organs, clinical symptoms, and epidemiological characteristics.

Nosology is used extensively in public health, to allow epidemiological studies of public health issues. Analysis of death certificates requires nosological coding of causes of death.

Nosological classifications are used in medical administration, such as filing of health insurance claims, and patient records. The International Classification of Diseases (ICD) is a standard diagnostic tool for epidemiology, health management, and clinical purposes, developed and maintained by the World Health Organization (WHO). The ICD system classifies diseases and health conditions and provides codes for them, which can be used for statistical purposes and for health care management.

The most recent version is ICD-11, which was adopted by the WHO in 2019 and officially came into effect in 2022. ICD-11 includes improvements and updates from ICD-10, reflecting advances in medical science and health information management.

Here's a brief overview of how ICD relates to nosology:

Classification: ICD provides a comprehensive list of diseases and health conditions, organized in a hierarchical structure. Each disease is assigned a unique code, which helps in the systematic recording, analysis, interpretation, and comparison of mortality and morbidity data.

Standardization: ICD offers a standardized approach to disease classification, which is essential for international comparability of health data. This uniformity ensures that health professionals around the world use a common language when describing diseases.

ICD's role in nosology is crucial as it provides a structured framework that enables consistent and accurate identification and reporting of diseases and health conditions across the globe.

Key Protozoal Diseases and Their Nosology

1.Malaria

- Causative Agents:- Plasmodium species *(P. falciparum, P. vivax, P. ovale, P. malariae, and P. knowlesi).*

- Transmission:- Through the bite of infected Anopheles mosquitoes.

-Clinical Presentation:- Fever, chills, headache, muscle aches, and severe complications such as cerebral malaria and organ failure.

- Nosological Classification:- Based on the specific Plasmodium species, clinical severity (uncomplicated vs. severe malaria), and geographical distribution.

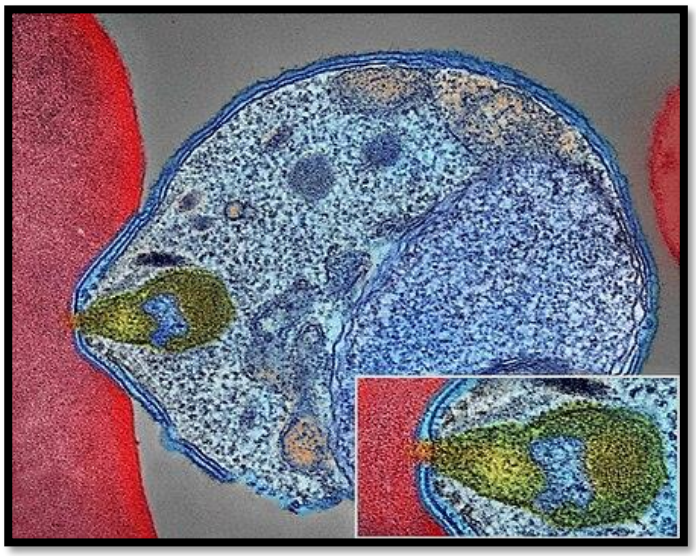

Fig: Malaria parasite connecting to a red blood cell

2. Leishmaniasis:

- Causative Agents: Leishmania *species (L. donovani, L. major, L. infantum, etc.)*.

- Transmission: Through the bite of infected sandflies.

-Clinical Presentation: Cutaneous leishmaniasis (skin ulcers), mucocutaneous leishmaniasis (mucosal damage), and visceral leishmaniasis (kala-azar, affecting internal organs).

-Nosological Classification: Based on the clinical form (cutaneous, mucocutaneous, visceral) and the Leishmania species involved.

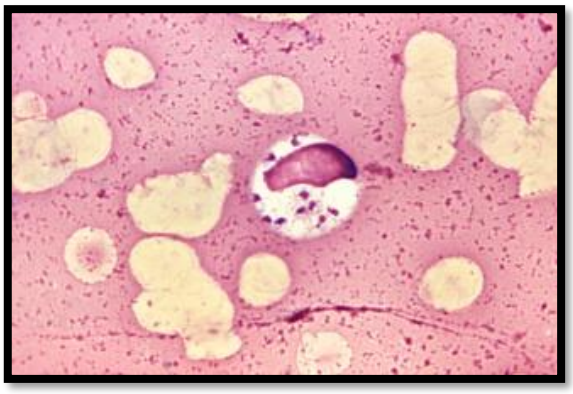

Fig: *Leishmania donovani* in bone marrow cell

3. Giardiasis:

- Causative Agent: *Giardia lamblia.*

- Transmission: Through ingestion of contaminated water or food.

- Clinical Presentation: Diarrhoea, abdominal cramps, nausea, and malabsorption.

- Nosological Classification: Based on the route of transmission and clinical symptoms.

Fig: Giardia cell viewed with scanning electron microscope.

4. Amoebiasis:

- Causative Agent: *Entamoeba histolytica.*

- Transmission: Through ingestion of cysts in contaminated water or food.

- Clinical Presentation: Intestinal amoebiasis (dysentery, diarrhoea) and extra-intestinal amoebiasis (liver abscess, pulmonary involvement).

-Nosological Classification: Based on the form of the disease (intestinal vs. extra-intestinal) and the severity of symptoms.

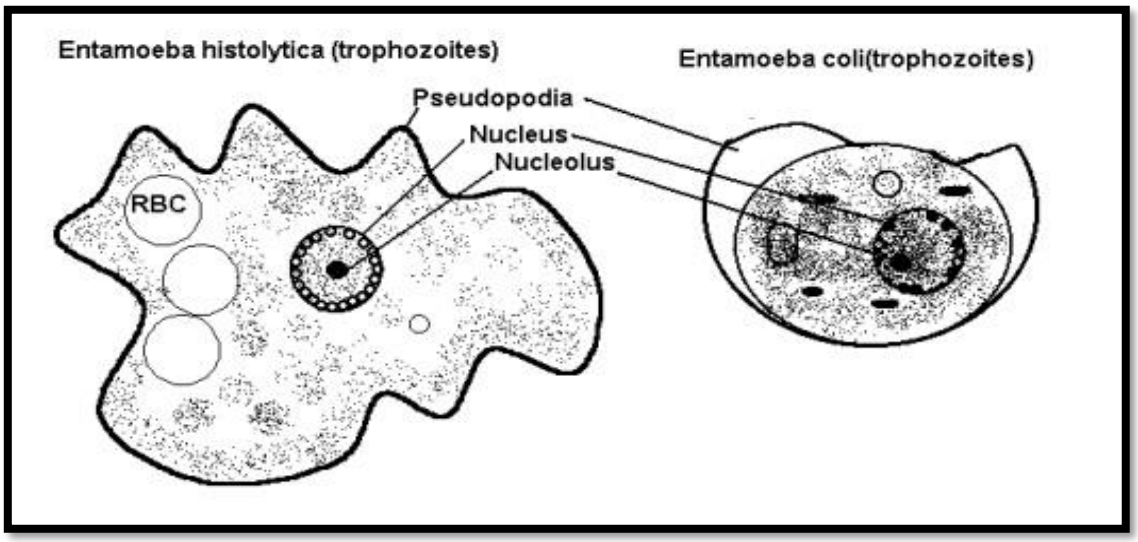

Fig: *Entamoeba histolytica*

5. Toxoplasmosis:

- Causative Agent: *Toxoplasma gondii.*

- Transmission: Through ingestion of undercooked meat, contaminated water, or exposure to infected cat feces; can also be congenital.

-Clinical Presentation: Asymptomatic in healthy individuals, severe complications in immunocompromised individuals (e.g., encephalitis) and congenital infections (e.g., ocular and neurological damage).

- Nosological Classification: Based on the mode of transmission (congenital vs. acquired) and the clinical presentation (asymptomatic, ocular, cerebral, congenital).

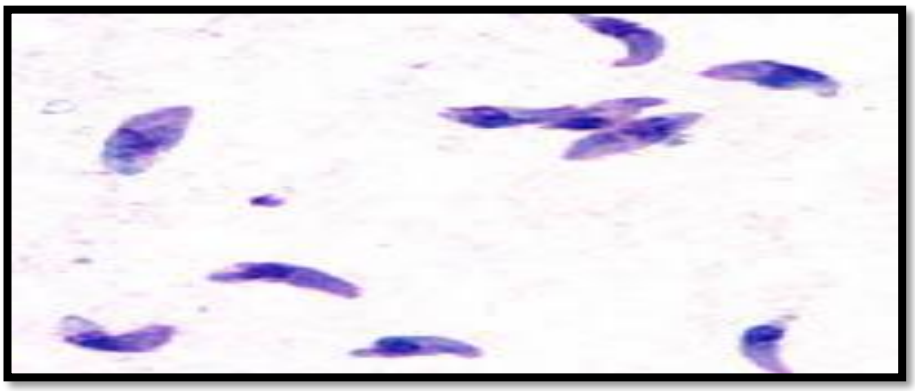

Fig: Giemsa stained *T. gondii* tachyzoites, 1000× magnification

Epidemiological Considerations

Nosology also incorporates epidemiological data, which is crucial for understanding the distribution and determinants of protozoal diseases. This involves analyzing the geographical distribution, population at risk, modes of transmission, and environmental factors influencing the spread of these diseases. For instance, malaria is endemic in tropical and subtropical regions, with factors such as climate, mosquito breeding sites, and human behavior affecting its transmission dynamics.

Challenges and Advances in Nosology of Protozoal Diseases

The nosology of protozoal diseases faces several challenges, including:

- Diagnostic Difficulties: Accurate diagnosis of protozoal infections can be challenging due to overlapping symptoms with other diseases and the need for specialized laboratory tests.

-Emerging and Re-emerging Infections: Changes in environmental conditions, human migration, and vector behavior can lead to the emergence or re-emergence of protozoal diseases in new areas.

- Drug Resistance: The development of resistance to antiprotozoal drugs complicates treatment and necessitates monitoring and updating of treatment guidelines.

However, advancements in molecular biology, genomics, and epidemiology have significantly enhanced the nosology of protozoal diseases. Techniques such as polymerase chain reaction (PCR) and next-generation sequencing (NGS) allow for precise identification and classification of protozoan species. Epidemiological modeling and Geographic Information Systems (GIS) help in mapping disease distribution and predicting outbreaks.

How to control?

Control measures for nosological diseases (diseases classified and studied within a specific framework) generally depend on the nature of the disease itself. However, some common control measures include:

Surveillance and Monitoring: Regular monitoring of disease incidence and prevalence to detect outbreaks early.

Vaccination Programs: Implementing vaccination campaigns to prevent the spread of infectious diseases.

Hygiene and Sanitation: Promoting good hygiene practices and ensuring access to clean water and sanitation facilities.

Public Health Education: Educating the public about disease prevention and control measures.

Isolation and Quarantine: Isolating infected individuals and quarantining those exposed to prevent disease spread.

Vector Control: Controlling vectors such as mosquitoes that transmit diseases.

Antimicrobial Stewardship: Promoting the appropriate use of antibiotics and antivirals to prevent resistance.

Health System Strengthening: Enhancing the capacity of health systems to respond to outbreaks and provide care.

Environmental Control: Reducing environmental risks that contribute to disease transmission, such as improving air quality and controlling waste.

Legislation and Policy: Implementing policies and regulations to support disease control efforts, such as vaccination mandates and food safety regulations.

Research and Development: Investing in research to develop new diagnostic tools, treatments, and vaccines.

Conclusion

Nosology in relation to protozoa is a dynamic and evolving field that is essential for the effective management of protozoal diseases. By providing a structured framework for classification, nosology aids in the accurate diagnosis, treatment, and prevention of these infections. Continued research and technological advancements will further refine our understanding of protozoal diseases, ultimately improving public health outcomes.

Probable questions:

1. What do you mean by Nosocomial disease?
2. Why atypical pneumonia is considered as a Nosocomial disease?
3. Write a note on Nosocomial disease in relation to protozoa.
4. Write about the control measure of Nosocomial disease.
5. Describe about the epidemiology of Nosocomial disease.

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Unit-XIV
Nutrition of parasites

Objectives:

In this section we will discuss on nutrition of parasites.

Introduction:

Parasitic nutrition is a mode of heterotrophic nutrition where a parasitic organism lives on the body surface or inside the body of another type of organism (a host) and gets nutrition directly from the body of the host. Since these parasites derive nourishment from their host, this symbiotic interaction is often harmful to the host. Parasites depend on their host for survival, since the host provides nutrition and protection. As a result of this dependence, parasites have considerable modifications to optimise parasitic nutrition and therefore their survival. Parasites are divided into two groups: endoparasites and ectoparasites. Endoparasites are parasites that live inside the body of the host, whereas ectoparasites are parasites that live on the outer surface of the host and generally attach themselves during feeding. Due to the different strategies of endoparasites and ectoparasites, they require different adaptations to derive nutrients from their host. Parasites require nutrients to carry out essential functions including reproduction and growth. Essentially, the nutrients required from the host are carbohydrates, amino acids and lipids. Carbohydrates are utilised to generate energy, whilst amino acids and fatty acids are involved in the synthesis of macromolecules and the production of eggs. Most parasites are heterotrophs, so they therefore are unable to synthesise their own 'food' i.e. organic compounds and must acquire these from their host.

Protozoa:

- **Acquisition of Nutrients**

Some protozoa are photosynthetic and synthesize carbohydrates in chloroplasts, the organelles of “typical” plants. Such organisms are often considered algae and claimed by the phycologists, but a few participate in symbiotic relationships of interest to parasitologists. Zooxanthellae (dinoflagellates) are very important mutuals living in cells of reef-forming corals and other invertebrates (including some other protozoa), contributing significant amounts of carbohydrates to their hosts.

Protozoa lacking chloroplasts are all heterotrophic, requiring their energy in the form of complex carbon molecules and their nitrogen in the form of a mixture of preformed amino acids. Protozoa are typically particle feeders— that is, grazers and predators—and many symbiotic species feed on host cells. Their mouth openings may be

temporary, as in amebas, or permanent cytostomes, as in ciliates. A submicroscopic micropore is present in *Eimeria* and *Plasmodium* species and, in certain stages, is involved in taking in nutrients.

Particulate food passes into a food vacuole, which is a digestive organelle that forms around any food thus ingested. Indigestible material is voided either through a temporary opening or through a permanent cytopyge, which is found in many ciliates. Pinocytosis is an important activity in many protozoa, as is phagocytosis. Both pinocytosis and phagocytosis are examples of endocytosis, differing only in that pinocytosis deals with droplets of fluid, whereas phagocytosis is the process of internalizing particulate matter.

Trematodes:

- **Acquisition of Nutrients**

Feeding and digestion in trematodes vary with nutrient type and habitat within their host. For example, two lung flukes of frogs, *Haematoloechus medioplexus* and *Haplometra cylindracea,* feed predominately on blood from the capillaries. Both species draw a plug of tissue into their oral sucker and then erode host tissue by a pumping action of their strong, muscular pharynx. Other trematode species characteristically found in the intestine, urinary bladder, rectum, and bile ducts feed more or less by the same mechanism, although their food may consist of less blood and more mucus and tissue from the wall of their habitat, and it may even include gut contents. In species without a pharynx that feed by this mechanism, the walls of their esophagus are quite muscular, and this apparently serves the function of a pharynx. In contrast *Schistosoma mansoni,* living in blood vessels of the hepatoportal system and immersed in its semifluid blood food, has no necessity to breach host tissues, and, not surprisingly, this species has neither pharynx nor muscular esophagus.

Digestion in most species studied is predominately extracellular in the ceca, but in *Fasciola hepatica* it occurs by a combination of intracellular and extracellular processes. A frog lung fluke, *Haplometra cylindracea,* has pear-shaped gland cells in its anterior end, and a nonspecific esterase is secreted from these cells through the tegument of the oral sucker, beginning the digestive process even before food is drawn into the ceca.

Those trematodes that feed on blood cope in various ways with the iron component of hemoglobin. In *F. hepatica,* in which final digestion of hemoglobin is intracellular, the iron is expelled through the excretory system and tegument. The fate of the iron in *H. cylindracea* is unclear, but apparently it is stored within the worm, tightly bound to protein. Extracellular digestion in *Haematoloechus medioplexus* and *S. mansoni* produces insoluble end products within the cecal lumen, and these wastes are periodically regurgitated.

In *S. mansoni* the end products are a heterogeneous population of molecules, but worms digest and incorporate some of both globin and heme moieties of hemoglobin.

Ceca of trematodes apparently do not bear any gland cells, but gastrodermal cells themselves may in certain species secrete some digestive enzymes: Proteases, dipeptidases, an aminopeptidase, lipases, acid phosphatase, and esterases have been detected. Alkaline phosphatase has not been found in most trematodes. *Fasciola hepatica* secretes a dipeptidyldipeptidase.

There are several peptidases in the intestine of *S. mansoni*, of which the most abundant are cathepsins (a family of cysteine peptidases), especially an enzyme designated SmCB1 (for *S. mansoni* cathepsin B1). *Trichobilharzia regent* are schistosomatid parasites that live in nasal cavaties of ducks, where they feed on blood. As in other schistosomatids, their cercariae penetrate their host's skin, but juvenile *T. regenti* (schistosomula) follow an unusual route to the nasal cavity: via their host's nervous system. Cathepsins in their gut are inefficient in digesting hemoglobin but readily degrade myelin basic protein, the major protein component of nervous tissue.

The gastrodermis of trematodes may be syncytial or cellular, according to species. Cytoplasmic processes, which vary from short (1 μm to 15 μm) and irregular to long (10 μm to 20 μm), extend into the lumen. Fujino (1997) distinguished three categories according to shape: (1) slender and ribbon shaped with narrow ends (for example, *Clonorchis sinensis, Eurytrema pancreaticum*); (2) broad and triangular with distal or marginal filamentous extensions (for example, *Fasciola hepatica, Echinostoma hortense*); and (3) broad, sheetlike, or triangular, with distal ends blunt or round (for example, *Haematoloechus lobatus, Schistosoma japonicum, Paragonimus* spp.). These processes greatly amplify the surface area of the gastrodermis for absorption of nutrients.

Within the gut cells of both *Gorgodera amplicava* and *Haematoloechus medioplexus* are abundant rough endoplasmic reticulum, many mitochondria, and frequent Golgi bodies and membrane-bound vesicular inclusions. High activity of acid phosphatase is found in vesicles of *H. medioplexus* and *Paragonimus kellicotti,* and after incubation in ferritin that material is found within the vesicles. No evidence of "transmembranosis" has been found, but the vesicles may be lysosomes that would function in degradation of nutritive materials after phagocytosis.

It is not surprising that trematodes can absorb small molecules through their tegument. Amino acids and hexoses can be absorbed, but various species differ in which molecules are absorbed by the tegument and which are absorbed by the gut. *Schistosoma mansoni* takes in glucose only through its tegument. Schistosomes absorb glucose both by diffusion and by a carrier-mediated system, and even brief exposure to a glucose-free medium disrupts uptake of a variety of other small molecules.

Megalodiscus temperatus cannot absorb glucose or galactose across its tegument, and this species, as well as several other paramphistomes, has no mitochondria in its tegumental cytoplasm. This could be a reason the tegument of these trematodes may have little or no absorptive capacity.

Cestodes:

- **Acquisition of Nutrients**

All nutrient molecules must be absorbed across the tegument. Mechanisms of absorption include active transport, mediated diffusion, and simple diffusion. Whether pinocytosis is possible at the cestode surface has been the subject of some dispute, but plerocercoids of *Schistocephalus solidus* and *Ligula intestinalis* are capable of this process. Cysticerci of *Taenia crassiceps* are capable of pinocytosis, and the process is stimulated by presence of glucose, yeast extract, or bovine serum albumin in the medium.

Glucose is the most important nutrient molecule to fuel energy processes in tapeworms. As noted, before, the only carbohydrates that most cestodes can absorb are glucose and galactose, and although some tapeworms can absorb other monosaccharides and disaccharides, we know of none other than glucose and galactose that can actually be metabolized. The primary fate of galactose seems to be incorporation into membranes or other structural components, such as glycocalyx. Galactose can be incorporated into glycogen but does not support net glycogen synthesis. Both glucose and galactose are actively transported and accumulate in the worm against a concentration gradient. Of the two sugars, glucose has been studied more extensively. Glucose influx in a number of species couples to a sodium pump mechanism; that is, the system of maintenance of a sodium concentration difference across the membrane. Accumulation of glucose, in *H. diminuta* at least, is also sodium dependent. At least two transport sites for glucose are kinetically distinct in the tegument of *H. diminuta,* and the relative proportion of these sites changes during development.

Fully developed larvae of *H. diminuta* with intact shells absorb very little glucose, but when the shell is removed, as it would be when eaten by a beetle intermediate host, they absorb much larger amounts.

Amino acids are also actively transported and accumulated, although less is known about them than about glucose. However, presence of other amino acids in the ambient medium stimulates efflux of amino acids from the worm; therefore, the worm pool of amino acids rapidly comes to equilibrium with amino acids in the intestinal milieu.

Purines and pyrimidines are absorbed by facilitated diffusion, and the transport locus is distinct from the amino acid and glucose loci.

The actual mechanism of lipid absorption has not been investigated, but it is likely to be a form of diffusion. Fatty acids, monoglycerides, and sterols are absorbed at a considerably greater rate when they are in a micellar solution with bile salts. *Hymenolepis diminuta* has a specific transporter for cholesterol.

Requirements for external supplies of vitamins are substantiated in only two cases. Investigations of vitamin requirements are difficult, as they often are in parasites, because of limitations in in vitro cultivation techniques, because the worm may be less sensitive than its host to a vitamin-deficient diet, or both. In any case, pathogenesis of a

vitamin deficiency in its host may have indirect effects on the worm. The necessity for an external supply of a vitamin has been demonstrated unequivocally in only one case—that of pyridoxine in *H. diminuta.* We can infer that *Diphyllobothrium latum* has a requirement for vitamin B12 because the worm accumulates unusually large amounts of it. In some cases, *D. latum* can compete so successfully with its host for the vitamin that the worm can cause pernicious anemia in persons genetically susceptible to its effects.

In a phenomenon possibly related to acquisition of nutrients, *H. diminuta* slows down intestinal transit. The worms cause myoelectrical alterations in the host intestine resulting in decreased luminal transit and increased non-propulsive contractility. Myoelectrical activity returns to normal when worms are expelled by drugs, and introduction of worm extracts by cannula mimics actual infection. A signal factor responsible for the myoelectrical alterations in host intestine is one of the molecules suggested as crowding factors, cyclic GMP. Interestingly, altered myoelectrical activity occurs only in ileum, not jejunum, and only at times when tapeworms are in the ileum, not when they are in the jejunum. Further experiments suggested that these observations may be related to a requirement of the worms for host bile salts.

Nematodes:

- **Acquisition of Nutrients**

Rogers and Lazarus (1949) found that *Nippostrongylus* rapidly acquired inorganic P^{32}-orthophosphate injected intramuscularly into the host, whereas *Ascaridia galli* failed to acquire significant amounts of phosphate given to the host intravenously. This is attributable to the very different feeding behavior of the two worms, *Nippostrongylus* being a feeder on host tissues and *Ascaridia* feeding on the gut contents of the host. Esserman and Sambell (1951) carried out similar experiments with *Haemonchus*, *Trichostrongylus*, and *Oesophagostomum* in the sheep. The rates at which these three nematodes acquired intravenously administered radiophosphate clearly indicated that they feed on the tissues of the host. However, when labeled phosphate was given to the host by intra-abomasal injection, *Haemonchus* and *Trichostrongylus* acquired the label more rapidly than the tissues of the abomasum or small intestine. This may indicate that these worms also feed on the gut content, but will bear further investigation.

Clark et al (1962) measured the loss of blood from sheep infected with *Haemonchus* by administering red cells tagged with Cr^{51} or Fe^{59}, and estimated an average blood-feeding rate of 0.049 ml/day/worm (range 0.005-0.173). This may be compared with an estimate of many years ago by Martin and Clunies Ross (1934), who determined the phosphorus content of eggs and egg-output of *Haemonchus*. From these data, they calculated the minimal amount of blood which would supply the phosphorus. Doubling their minimum estimate (to account for males and for waste), their data indicate a blood loss of 0.03 ml/day/ worm.

Rogers (1940) made an ingenious approach by determining the zinc in the tissues of *Strongylus vulgaris* and *S. edentatus* and in the gut tissues of horses. To account for the amount of zinc in the worms, Rogers estimated that 100 *S. edentatus* would have to eat from 400 to 2100 g of mucosa per year. In view of later appreciation that the sloughing of the intestinal mucosa occurs at a high rate, this figure may not be a real representation of the amount of living tissue devoured by *Strongylus*. Rogers concluded that the amount of blood taken from the host was small; after determining the amount of hematin in the gut of *S. edentatus*, he calculated that 0.0002-0.0009 ml of host blood would yield an amount of pigment equivalent to that found. Of course, as he recognized, such calculations are provisional since no time scale is available to judge the rate of acquisition and loss of hemoglobin. It may be emphasized that a visible blood pigment in the gut of a parasite may represent a very tiny amount of blood and may be an unreliable criterion as to whether blood constitutes a significant portion of the diet.

Food of nematodes parasitic in animals includes blood, tissue cells and fluids, intestinal contents, or some combination of these. Some species parasitic in the intestine feed only on tissue and not on blood or host ingesta. Hookworms, which feed solely on blood, accumulate granules of zinc sulfide in their intestinal cells, apparently as a waste product. Nematode parasites of vertebrates feed extravagantly and wastefully, and the thin-walled intestine with its brush border is an efficient absorptive mechanism.

Probable questions:

1. Briefly describe the acquisition of nutrients and digestion in
    i. Protozoa
    ii. Trematoda
    iii. Cestoda
    iv. Nematoda

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