Post-Graduate Degree Programme (CBCS)
in
ZOOLOGY
(M.Sc. Programme)

SEMESTER-IV

Theory Paper-Minor Elective

Parasites and Diseases
ZDSE(MN)T 412

Self-Learning Material

DIRECTORATE OF OPEN AND DISTANCE LEARNING
UNIVERSITY OF KALYANI
Kalyani, Nadia
West Bengal, India

CONTENT WRITER:

Sl. No.	Name of Content Writer	Designation	Unit
1.	Dr. Sudeshna Banerjee	Assistant Professor of Zoology, Directorate of Open and Distance Learning, University of Kalyani	Unit II, III, IV
2.	Dr. Dipanjan Dutta	Assistant Professor PG Department of Zoology, Hooghly Mohsin College, Chinsurah, Hooghly 712101	Unit I, V, VI

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Director's Message

Satisfying the varied needs of distance learners, overcoming the obstacle of distance and reaching the unreached students are the threefold functions catered by Open and Distance Learning (ODL) systems. The onus lies on writers, editors, production professionals and other personnel involved in the process to overcome the challenges inherent to curriculum design and production of relevant Self Learning Materials (SLMs). At the University of Kalyani, a dedicated team under the able guidance of the Hon'ble Vice-Chancellor has invested its best efforts, professionally and in keeping with the demands of Post Graduate CBCS Programmes in Distance Mode to devise a self-sufficient curriculum for each course offered by the Directorate of Open and Distance Learning (DODL), University of Kalyani.

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.) Kallol Paul, 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.

Sincere gratitude is due to the respective chairpersons as well as each and every member of PGBOS (DODL), University of Kalyani. Heartfelt thanks are also due to the Course Writers-faculty members at the DODL, subject-experts serving at University Post Graduate departments and also to the authors and academicians whose academic contributions have enriched the SLMs. We humbly acknowledge their valuable academic contributions. I would especially like to convey gratitude to all other University dignitaries and personnel involved either at the conceptual or operational level of the DODL of University of Kalyani.

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.

All the Self Learning Materials are self-writing and collected from e-book, journals and websites.

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Theory (Discipline Specific Elective - Minor) –

ZDSE(MN)T 412 – Parasites and Disease

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III	Important genera of fish parasitic ciliates -*Icthyopthirius* sp.		
IV	common helminthes of freshwater fishes and their life cycle patterns: a) *Proteocephalus* sp., *b) Camallanus* sp.		
V	Structure, Pathobiology prophylaxis and diagnosis of causative agents of filariasis		
VI	Parasitic insects: *Cimex lectularius and Xenopsylla cheopis*.		
	Total counseling session 6hrs		

Unit- I

Primary amoebic meningoencephalitis

Objective:

In this unit you will know about details of Primary amoebic meningoencephalitis.

Introduction:

Primary amoebic meningoencephalitis (PAM) is a rare and severe disease caused by a single-celled amoeba called *Naegleria fowleri*. *N. fowleri* is known as a "brain eating amoeba" because the microorganism can destroy neurons. It causes inflammation and destruction of the brain and the linings of the brain, stiff neck, fever (38.5°C–41°C), altered mental status, seizures and coma, leading almost always to death.

PAM was first described in South Australia in the 1960s. It has since been identified in many countries throughout the world. Although *Naegleria fowleri* occurs commonly in the environment, it only rarely causes disease. There have been four confirmed cases and one probable case documented in Queensland since the year 2000.

Naegleria fowleri occurs naturally in untreated fresh water, and prefers temperatures between 25°C and 40°C. It can grow in warm, stagnant water bodies such as lakes and hot springs. It can also occur in untreated water piped long distances above ground and in other man-made environments such as poorly maintained swimming pools, wading pools and spas. Infection with *Naegleria fowleri* can occur if water containing the amoebae is pushed up the nose, through activities such as jumping, diving or falling into the water. The amoebae invade humans via intact or disrupted nasal mucosa, crosses the cribriform plate, migrates along the basilar brain from the olfactory bulbs and tracts to the cerebellum, deeply penetrates the cortex to the periventricular system, and incites meningoencephalitis with rapid cerebral oedema, resulting in cerebellar herniation. The olfactory bulbs and orbitofrontal cortices are necrotic and haemorrhagic. The disease cannot be contracted by drinking water or through person-to-person contact. *Naegleria fowleri* are not found in sea water.

Habit and Habitat:

Naegleria feed on yeast, algae and both Gram-negative and Gram-positive bacteria. Food selectivity is observed with findings that filamentous cyanobacteria (e.g., *Anabaena*, *Cylindrospermum*, *Gloeotrichia*, and *Phormidium*) are consumed, while tight threads (*Oscilltoria*) and aggregates (*Aphanizomenon*) are not ingested. Unicellular Chroococcaceae (e.g., *Synechococcus, Aphanocapsa,* and *Microcystis*) are excreted after ingestion, indicating that food selection takes place inside food vacuoles. Ingestion

depends on the satiation status of the amoebae, as starved amoebae feed at higher rates compared with satiated amoebae. *N. fowleri* can be grown simply on the surface of non-nutrient agar overlaid with living or dead *Enterobacter aerogenes* or *E. coli* or other Gram-negative bacilli. Live bacteria support optimal growth compared with heat-killed bacteria. Under these conditions, the amoebae feed upon the bacteria, and as growth enters stationary phase and the food supply is used up, *N. fowleri* begin to encyst. Cysts, if kept from drying out, will remain viable for months, possibly years.

Life cycle:

Naegleria fowleri is a protist pathogen that is extensively dispersed in the environment. *N. fowleri* is the only pathogenic species in this genus. Given the opportunity and access, *N. fowleri* can cause fatal primary amoebic meningoencephalitis (PAM). Worryingly, mortality rates concomitant with PAM remain substantially high, irrespective of modern improvements in antimicrobial chemotherapy or supportive medical care. Being a free-living amoeba, *N. fowleri* can switch phenotype depending on the environmental conditions (Fig. 1).

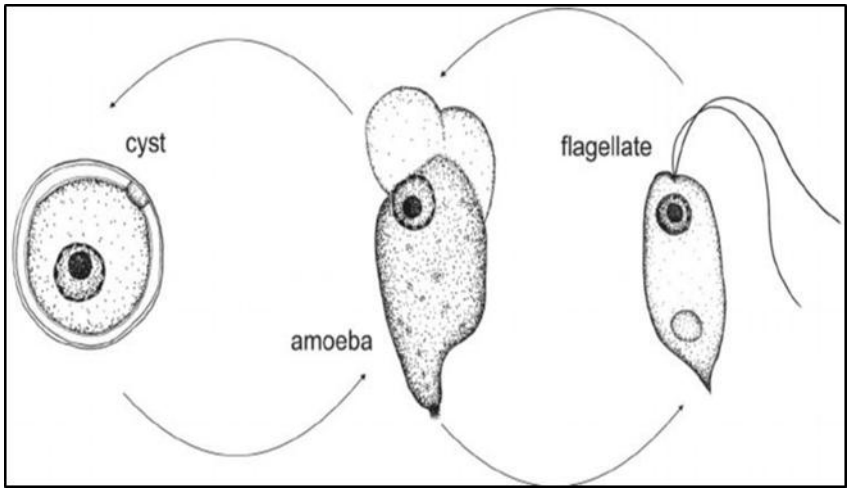

Fig 1: Different phenotypes of *N. fowleri*

Under favourable conditions, it exhibits a reproductively-active trophozoite stage. The trophozoite stage is considered as the infective stage. Under non-nutrient conditions; but presence of water, trophozoites switch to a transient flagellate stage allowing long distance movement, often in pursuit of nourishment. During this stage, *N. fowleri* does not reproduce or

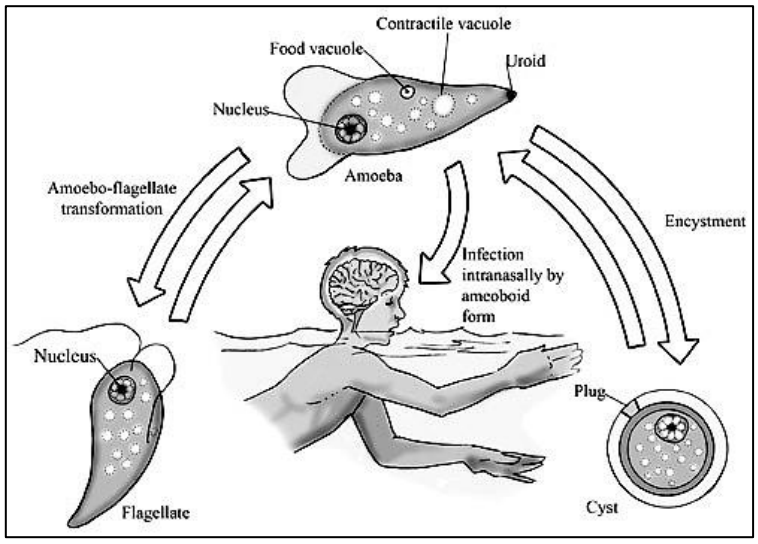

Fig 2: Life cycle of *N. fowleri*

form cyst. When the environment is adverse or unfavourable, trophozoites switch into a metabolically inactive or dormant form known as the cyst form.

Like the flagellate phase, the cysts are non-feeding, and non-reproductive. Only the trophozoites of N. fowleri can feed, reproduce, and/or become cysts. The parasites enter hosts through the nasal route, travelling via the olfactory neuroepithelial and thus gaining entry to the central nervous system with the production of PAM (Fig. 2).

CNS infection by *N. fowleri* occurs by the amoebae passing through the nasal cavity, penetrating the olfactory neuroepithelium, migrating through the olfactory nerves (Fig. 3) and crossing the cribriform plate until they reach the olfactory bulbs (OBs)

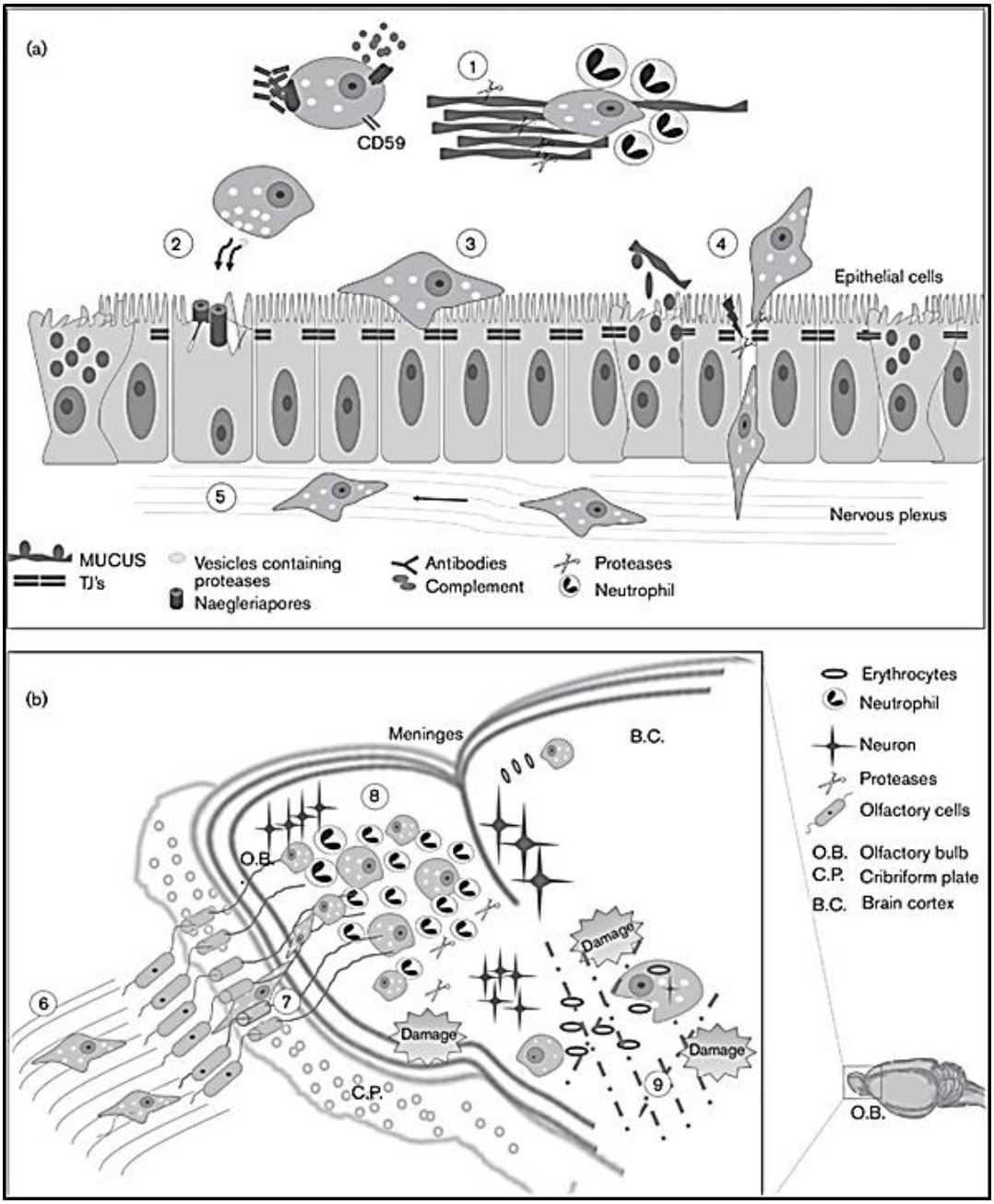

Fig 3: Schematic representation of *N. fowleri* infection.

(a) Initial stages of PAM. (1) Evasion of innate immune response, (2) independent-contact cytotoxicity (naegleriapores), (3) adhesion to epithelial cells, (4) invasion of the neuroepithelium, (5) migration to OBs.

(b) Late stages of PAM. (6) Contact with olfactory phyla, (7) amoeba crossing the cribriform plate, (8). *N fowleri* proliferation and inflammatory reaction in the OBs, (9) tissue damage (haemorrhage, phagocytosis and protease release).

Clinical and laboratory diagnosis:

Patients exhibiting CNS symptoms together with a history of swimming or use/exposure to contaminated water for nasal cleansing should be suspected of PAM.

- The computed tomographic scan (CT) reveals involvement of the CNS, around and above the midbrain and the subarachnoid spaces are eliminated on pre-contrast CT. Noticeable augmentation is seen after intravenous contrast medium administration. Whereas the ventricular size is usual, the sulci and adjacent grey matter are also intensely enhanced.
- The definitive diagnosis of PAM involves CSF (Cerebro-Spinal Fluid) findings, i.e., presence of amoebae in the CSF. In the majority of cases, motile trophozoites are observed in CSF by wet mount. Brief centrifugation of the CSF at 5,000 x g for 5 min is helpful to concentrate amoebae.
- In addition to microscopy, immunofluorescence assay (IF), enzyme-linked immunosorbent assay (ELISA), flow cytometry, and PCR-based assays have been developed. Assays should be employed on both CSF and nasal exudates.
- Apart from the presence of amoebae, CSF findings in PAM are comparable to bacterial meningitis. For example, the red blood cell count in CSF increases several fold from 250 cells per mm3 in the early stage to 25,000 cells per mm^3 in the late stage. Similarly, the white blood cell count is raised, with a polymorphonuclear leukocyte predominance, with a range of 300 cells per mm3 to as high as 26,000 cells per mm^3. The CSF pressure is typically raised (300 – 600 mm H2O). The protein concentration can range from 100 mg per 100 mL to 1000 mg per 100 mL, while glucose might be 10 mg per 100 mL or less.
- Endeavours ought to be made to culture the amoebae from the CSF. A few drops of CSF should be transferred to a non-nutrient agar plate seeded with bacteria and amoebae growth should observed daily for up to seven days. Amoebae appear as the trophozoite form within 1 – 2 days. *N. fowleri* can be differentiated from other pathogenic amoebae using enflagellation experiment by mixing one drop of amoebae culture or sedimented CSF and 1 mL of distilled water for 1 – 2 h with periodic observation for the presence of flagellates, however the molecular methods remain the method of choice.

Pathogenesis:

In vivo, ex vivo and *in vitro* models have been developed to study molecular mechanisms associated with N. fowleri pathogenesis. *In vivo,* mice are inoculated intranasally with *N. fowleri* that results in high mortality rate. The susceptibility of mice is influenced by weight (mice weighing less than 15 g are more sensitive), and age (younger mice are more sensitive). Following infection, *N. fowleri* are observed in mucous layer of the olfactory epithelium within 8 h post-infection and infected mice exhibit focal inflammation with the presence of *N. fowleri* in the submucosal nerve plexus, olfactory nerves penetrating the cribriform plate, and the olfactory bulb of the brain within 24 h post-infection. Following 96 h, the inflammatory response, primarily in the form of neutrophil polymorphs, is severe in the olfactory bulb and the brain, with tissue damage. Numerous amoebae are seen interspersed with the degenerating neurones, glial processes, and neutrophil polymorphs with major concentrations in the perivascular regions and in the lamina of blood vessels.

When *N. fowleri* are incubated with host cells *in vitro*, host cells show cell shrinkage, cell damage, invasion and destruction via phagocytic processes.

In *ex vivo* model, infection of organotypic slice cultures from rat brain with amoebae is comparable to findings with *in vivo* infection, suggesting its usefulness in the study of *N. fowleri* pathogenesis.

The pathogenicity of *N. fowleri* is discussed below-

N. fowleri is an amphizoic amoeba as it can survive in a free-living state in water, soil, or in the host which can be the human central nervous system (CNS). *N. fowleri* infections have been documented in healthy children and adults following recreational water activities including swimming, diving, and water skiing. *N. fowleri* has been thought to infect the human body by entering the host through the nose when water is splashed or forced into the nasal cavity. Infectivity occurs first through attachment to the nasal mucosa followed by locomotion along the olfactory nerve, through the cribriform plate (which is more porous in children and young adults), and finally reaching the olfactory bulbs within the CNS. Once *N. fowleri* reaches the olfactory bulbs, it elicits a significant immune response through activation of the innate immune system including macrophages and neutrophils. *N. fowleri* enters the human body in the trophozoite form. Structures on the surface of trophozoites known as food-cups enable the organism to ingest bacteria, fungi, and human tissue. In addition to tissue destruction by the food cup, the pathogenicity of *N. fowleri* is dependent upon the release of cytolytic molecules including, acid hydrolases, phospholipases, neuraminidases, and phospholipopolytic enzymes that play a role in host-cell and nerve destruction. The combination of the pathogenicity of *N. fowleri* and the intense immune response resulting from its presence results in significant nerve damage and subsequent CNS tissue damage, which often result in death.

Signs and Symptoms:

The typical symptoms of PAM appear during the first week after infection with *N. fowleri* trophozoites. There are no distinctive clinical features to differentiate PAM from other types of meningitis. Therefore, it is very important that physicians obtain a detailed clinical history of the patients. The earliest symptoms include

- severe headache,
- high fever and neck stiffness,
- anorexia,
- vomiting,
- irritability,
- photophobia and
- Neurological abnormalities, including diplopia, lethargy, seizures and coma.

Cranial nerve palsies may indicate brain oedema. Death occurs between the third and seventh days after symptom onset.

Autopsies of PAM patients have revealed brain inflammation with severe tissue damage throughout the area of invasion, with ulceration of the olfactory mucosa and necrosis of the olfactory nerves. Microscopically, the OBs were almost completely disorganized by fibrin-purulent exudates and by haemorrhaging from necrotic blood vessels, and the adjacent frontal cortex exhibited the invasion of a considerable number of amoebae.

Prevention:

Naegleria fowleri cannot survive in water that is clean, cool and chlorinated. To prevent infection:

- avoid jumping or diving into bodies of warm fresh water or thermal pools
- keep your head above water in spas, thermal pools and warm fresh water bodies
- empty and clean small collapsible wading pools daily
- ensure swimming pools and spas are adequately chlorinated and well maintained
- flush stagnant water from hoses before allowing children to play with hoses or sprinklers
- if you are using unchlorinated water:
    - don't allow water to go up your nose when bathing, showering or washing your face

    - supervise children playing with hoses or sprinklers and teach them not to squirt water up their nose
- potentially contaminated water should not be used for any form of nasal irrigation or nasal lavage including Neti (an Ayurvedic practice of nasal cleansing)

Treatment:

- It is important to highlight that an appropriate diagnosis is the key to choosing an appropriate treatment. However, PAM is not commonly confirmed during the early stages of infection, and most people infected with this organism die. Because of the high mortality rate, more effective drugs are urgently needed.
- Few people survive Naegleria infection, even with treatment. Early diagnosis and treatment are crucial for survival.
- The primary treatment for Naegleria infection is an antifungal drug, amphotericin B — usually injected into a vein (intravenously) or into the space around your spinal cord to kill the amoebas.
- An investigational drug called miltefosine (Impavido) is now available for emergency treatment of Naegleria infection. The medicine, when taken with other medications and along with aggressive management of brain swelling, may show promise for improved survival.

Control:

Chlorine kills Naegleria fowleri and is the most effective way to disinfect swimming pools and reticulated water supplies. In rural water supplies, chlorine may not reach areas where the amoeba may form colonies. In such circumstances, a process called *chloramination* is more effective to control *Naegleria fowleri*. Filtration and UV treatment systems may be effective in controlling *Naegleria fowleri*, but specialist advice should be sought.

Conclusion:

PAM is an acute and fatal disease that has recently become more common in both developed and underdeveloped countries. The number of PAM cases may increase due to global warming, global overpopulation and increased industrial activities. It is urgent that the health community, including medical and diagnostic laboratory technicians, be aware of this disease in order to make timely diagnosis that could save patients' lives. The knowledge of the biology and pathogenesis of *N. fowleri* in the past 50 years could be used

to make faster diagnosis and design new drugs against specific targets to eliminate the amoeba and increase the survival of the patients.

Probable questions:

1. Describe the life cycle of *Naegleria fowleri* with diagram
2. Briefly discuss different forms of *Naegleria fowleri* with diagram
3. Describe with diagram penetration and migration of *Naegleria fowleri* through olfactory nerves.
4. Write down the control measures and treatment of *Naegleria fowleri*
5. Describe pathogenicity and role in disease transmission of *Naegleria fowleri.*
6. Describe the morphology of *Naegleria fowleri.*

Suggested Reading:

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2. http://conditions.health.qld.gov.au/HealthCondition/condition/14/165/115/primary-amoebic-meningoencephalitis-pam
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Unit II

Important Myxozoan genera of fishes - Structure and life history of any *Myxobolus* sp.

Objective: In this unit you will know about important Myxozoan genera of fishes like*Myxobolus* sp.; emphasising on their structure and life history.

Introduction

Myxobolus cerebralis is a myxosporean parasite of salmonids (salmon, trout, and their allies) that causes *whirling disease* in farmed salmon and trout and also in wild fish populations. Whirling disease is so called because fish with the disease swim in circles when disturbed or feeding. It was first described from rainbow trout in Germany a century ago, but its range has spread and it has appeared in most of Europe (including Russia), the United States, South Africa and other countries. In the 1980s, it was discovered that *M. cerebralis* needs to infect a tubificid oligochaete (a kind of segmented worm) to complete its life-cycle. The parasite infects its hosts with its cells after piercing them with polar filaments ejected from nematocyst-like capsules.

Whirling disease afflicts juvenile fish (fingerlings and fry) and causes skeletal deformation and neurological damage. Fish "whirl" rather than swim forward, find feeding difficult, and are more vulnerable to predators. The mortality rate is high for fingerlings, up to 90% of infected populations, and those that do survive are deformed by the parasite residing in their cartilage and bone. They act as a reservoir for the parasite, which is released into water following the fish's death. *M. cerebralis* is one of the most economically important myxozoans in fish as well as one of the most pathogenic. It was the first myxosporean whose pathology and symptoms were described scientifically. The parasite is not transmissible to humans.

Systematic position

Phylum: Cnidaria
Subphylum: Myxozoa
Class: Myxosporea
Class: Myxobolus cerebralis
Order: Bivalvulida
Suborder: Platysporina
Family: Myxobolidae
Genus: *Myxobolus*
Species: *Myxobolus cerebralis*

Taxonomy

The taxonomy and naming of both *M. cerebralis* and of myxozoans in general have complicated histories. It was originally thought that this parasite infected fish brains (hence the specific epithet cerebralis), however it quickly became apparent that while it can be found in the nervous system, it primarily infects cartilage and skeletal tissue. Attempts to change the name to *Myxobolus chondrophagus*, which would more accurately describe the organism, failed because of nomenclature rules. Later, it became apparent that organisms previously called *Triactinomyxon dubium* and *T. gyrosalmo*(class Actinosporea) were in fact triactinomyxon stages of *M. cerebralis*, whose life cycle was expanded to include the triactinomyxon stage. Similarly, other actinosporeans were folded into the life cycles of various myxosporeans.

Today, the myxozoans, previously thought to be multicellular protozoans are considered animals by many scientists, though their status has not officially changed. Recent molecular studies suggest that they are related to Bilateria or Cnidaria, with Cnidaria being closer morphologically because both groups have extrusive filaments, but with Bilateria being somewhat closer in some genetic studies.

Morphology

M. cerebralis has many diverse stages ranging from single cells to relatively large spores, not all of which have been studied in detail.

- **Triactinomyxon stage**

The stages that infect fish, called triactinomyxon spores, are made of a single style that is about 150 micrometers (μm) long and three processes or "tails" that are each about 200 micrometers long (Fig 1). A sporoplasm packet at the end of the style contains 64 germ cells surrounded by a cellular envelope. There are also three polar capsules, each of which contains a coiled polar filament between 170 and 180 μm long. Polar filaments in both this stage and in the myxospore stage (see picture above) rapidly shoot into the body of the host, creating an opening through which the sporoplasm can enter.

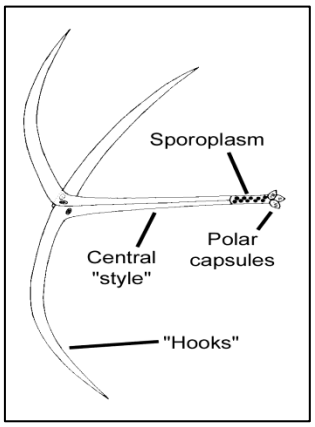

Fig 1: Diagram of the structure of a triactionmyxon stage spore of *Myxobolus cerebralis*

- **Sporoplasm stage**

Upon contact with fish hosts and firing of the polar capsules, the sporoplasm contained within the central style of the triactinomyxon migrates into the epithelium or gut lining. Firstly, this sporoplasm undergoes mitosis to produce more amoeboid cells, which migrate into deeper tissue layers, in order to reach the cerebral cartilage.

- **Myxosporean stage**

Myxospores, which develop from sporogonic cell stages inside fish hosts, are lenticular. Mature spores of *M. cerebralis* are broadly oval, with thick sutural ridges on the valve edges. They measure 7.4-9.7 µm long by 7-10 µm wide and are made of six cells. Spores are covered with a mucoid-like envelope. There are two polar capsules at the anterior end, each with a filament twisted into five or six coils. Twopolar capsules merge to form a binucleate sporoplasm, and two form protective valves. During development each polar capsule lies within a polar cell that also contains a nucleus, and nuclei of the two valvogenic cells may be seen lying adjacent to the inner surface of each valve. The sporoplasm contains two nuclei (presumably haploid), numerous ribosomes, mitochondria, and other typical organelles.

Myxospores are infective to oligochaetes, and are found among the remains of digested fish cartilage. They are often difficult to distinguish from related species because of morphological similarities across genera. Though *M. cerebralis* is the only myxosporean ever found in salmonid cartilage, other visually similar species may be present in the skin, nervous system, or muscle.

Life cycle

Myxobolus cerebralis has a two-host life cycle that involves the salmonid fish and an alternate host, the bottom-dwelling *tubifex* worm (*Tubifex tubifex*) (Fig 2).

First, myxospores are ingested by tubificid worms. In the gut lumen of the worm, the spores extrude their polar capsules and attach to the gut epithelium by polar filaments. The shell valves then open along the suture line and the binucleate germ cell penetrates between the intestinal epithelial cells of the worm. This cell multiplies, producing many amoeboid cells by an asexual cell fission process called merogony. As a result of the multiplication process, the intercellular space of the epithelial cells in more than 10 neighbouring worm segments may become infected.

Around 60–90 days post-infection, sexual cell stages of the parasite undergo sporogenesis, and develop into pansporocysts, each of which contains eight triactinomyxon-stage spores. These spores are released from the oligochaete anus into the water. Alternatively, a fish can become infected by eating an infected oligochaete. Infected tubificids can release triactinomyxons for at least 1 year.

The triactinomyxon spores swim through the water to infect a salmonid through the skin. Penetration of the fish by these spores takes only a few seconds. Within five minutes, a sac of germ cells called a sporoplasm has entered the fish epidermis, and within a few hours, the sporoplasm splits into individual cells that will spread through the fish.

Within the fish, there are both intracellular and extracellular stages that reproduce in its cartilage by asexual endogeny, meaning that new cells grow from within old cells. The final stage within fish is the myxospore, which is formed by sporogony. They are released into the environment when the fish decomposes or is eaten. Some recent research indicates that some fish may expel viable myxospores while still alive.

Myxospores are extremely tough: "it was shown that *Myxobolus cerebralis* spores can tolerate freezing at -20°C for at least 3 months, aging in mud at 13°C for at least 5 months, and passage through the guts of northern pike *Esox lucius* or mallards *Anas platyrhynchos* without loss of infectivity" to worms. Triactinomyxons are much shorter lived, surviving 34 days or less, depending on temperature.

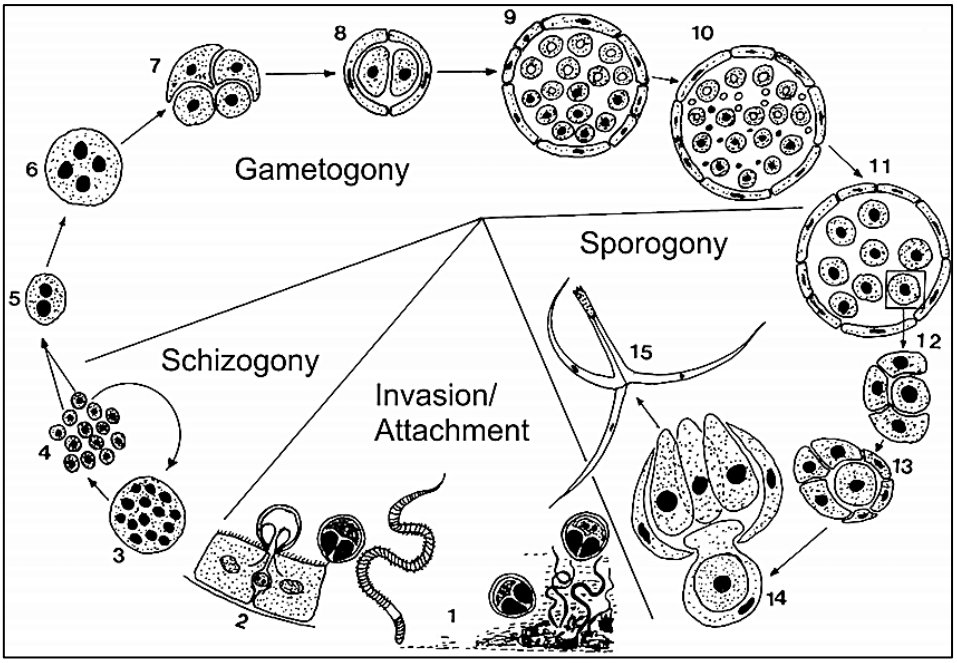

Fig 2: *Myxobolus cerebralis* life cycle: development of myxospore in oligocheate host *Tubifex tubifex*.

Stage 1: ingestion of M. cerebralis by T. tubifex.

Stage 2: extrusion of the polar filaments and anchorage of M. cerebralis spore into gut epithelium. After shell valves open, binucleate sporoplasm escapes and penetrates between epithelial cells.

Stage 3: interepithehal schizogonic multiplication of binucleate sporoplasm.

Stage 4: uninucleate 1-cell stages.

Stage 5: plasmogamy of 2 uninucleate cells to produce one binucleate cell-stage.

Stage 6: mitotic division of both nuclei to produce 4-nuclei stage.
Stage 7: formation of 4-cell stage by plasmotomy; 2 cells begin to envelop the other 2 cells.
Stage 8: formation of early pansporocyst with 2 somatic and 2 generative cells.
Stage 9: following 3 mitotic divisions of both generative cells and 2 mitotic divisions of the somatic cells, 16 gametocytes (8α and 8β) enveloped by 8 somatic cells are formed.
Stage 10: following meiotic division of the 16 diploid gametocytes, 16 haploid gametocytes and 16 polar bodies result.
Stage 11: production of 8 zygotes after copulation of each pair of α-and β-gametes.
Stage 12: sporoblast formation after 2 mitotic divisions of zygote, 3 pyramidally arranged cells and one inner cell are formed.
Stage 13: following mitotic division of the 3 peripheral cells, 3 capsulogenic and 3 valvogenic cells are produced.
Stage 14: the valvogenic cells extend around the capsulogenic cells, while internal cleavage of the developing sporoplasm cell produces one generative cell enveloped by one somatic cell. The sporoplasm remains naked in the pansporocyst until reaching the final number of germs through repeating mitotic divisions.
Stage 15: inflated mature triactinomyxon spore.

Pathogenesis

The main pathogenic effects of this disease are damage to cartilage in the axial skeleton of young fish, consequent interference with function of adjacent neural structures, and subsequent granuloma formation in healing of the lesions. Invasion of the cartilaginous capsule of the auditory-equilibrium organ behind the eye interferes with coordinated swimming. Thus, when an infected fish is disturbed or tries to feed, it begins to whirl frantically, as if chasing its tail. It may become so exhausted by this futile activity that it sinks to the bottom and lies on its side until it regains strength. Predation most likely occurs at this stage. Often the spine cartilage is invaded, especially posterior to the 26th vertebra. Function of sympathetic nerves controlling melanocytes is impaired, and an infected fish's posterior part becomes very dark, producing the "black tail." If the fish survives, granulomatous tissue infiltration of the skeleton may produce permanent deformities: misshapen head, permanently open or twisted lower jaw, or severe spinal curvature

Fig 3: Axial skeleton deformities in living rainbow trout that have recovered from whirling disease (*Myxobolus cerebralis*)
(*a*) Note bulging eyes, shortened operculum, and both dorsoventral and lateral curvature of the spinal column (lordosis and scoliosis).
(*b*) Note gaping, underslung jaw and grotesque cranial granuloma.

Probable questions:

1. Name the causative agent of whirling disease. Why this disease is named so?
2. Describe the life cycle of *Myxobolus cerebralis* with diagram
3. Describe triactinomyxon spores with diagram.
4. Describe the myxosporean stage of *Myxobolus cerebralis.*

References and Suggested readings:

1. http://www.columbia.edu/itc/cerc/danoff-burg/invasion_bio/inv_spp_summ/myxobolus_cerebralis.html

2. https://parasite.org.au/para-site/text/ichthyophthirius-text.html

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Unit III

Important genera of fish parasitic ciliates - *Icthyopthirius* sp.

Objective: In this unit you will know about important genera of fish parasitic ciliates *Icthyopthirius* sp. emphasising on their structure and life history.

Introduction

Ichthyophthirius multifiliis is a large, ciliated protozoan that causes "Ich" or "white spot disease." This disease is a major problem to freshwater aquarists and commercial fish producers worldwide. All species of freshwater fish are considered susceptible, and the parasite has been found in all areas of the world in both cultured and wild fish. These large parasites cause the characteristic white spots that are often seen on the skin and fins of infected fish. The disease is highly contagious and spreads rapidly from one fish to another without the need for additional hosts (direct life cycle). Although often considered a “warm water” disease, outbreaks often occur when water temperatures are changing, especially in the spring when water temperatures are increasing. The disease is particularly severe when fish are crowded. While many protozoans reproduce by simple division (one parasite “splits” into two), a single “Ich” organism can multiply into hundreds of new parasites in one generation, making early detection and treatment of this parasite crucial. The organism is unusual in that it is an obligate parasite, which means that it cannot survive unless live fish are present. “Ich” is capable of causing massive mortality within a short period of time. An outbreak of “Ich”is a true emergency situation and requires immediate treatment; if left untreated, this disease may result in 100% mortality.

Parasite morphology

The parasite forms three developmental stages: trophonts, tomonts and theronts (Fig 1). ***Trophonts*** variable in size (up to 1mm), horseshoe-shaped macronucleus encircling single micronucleus; subapical vestibulum with weakly developed buccal ciliature ***tomonts*** encysted on substrate, repeatedly divides to form numerous small tomites which break through cyst wall to become ***theronts***(25-70 x 15-22µm) covered with 36-48 meridional (longitudinal) kineties (ciliary rows) converging around the pre- and post-oral sutures. Ellipsoidal macronucleus and subspherical micronucleus.

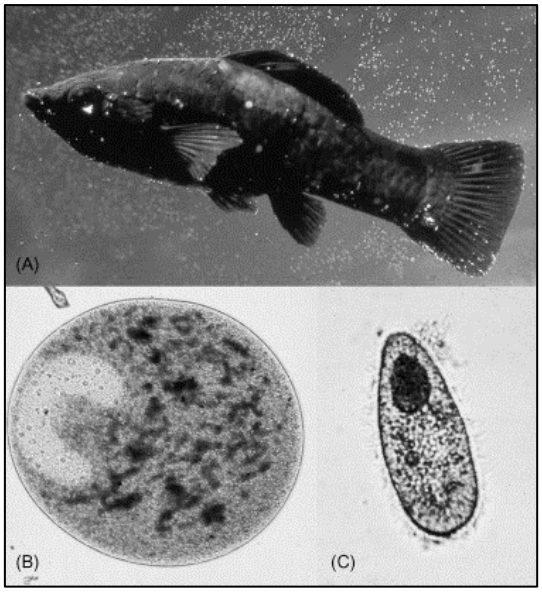

Fig 1: (A) Black molly with a few variably sized white spots caused by *Ichthyophthirius* cysts and the milky nature of the mucous coat that is seen in any condition where irritation causes excess mucus production. (B) *Ichthyophthirius* tomont with its characteristic horseshoe shaped nucleus (C) The much smaller free-swimming infective theront of *Ichthyophthirius*.

Host range

Infections have been detected in numerous species of aquarium and wild freshwater fish throughout the world. There is some conjecture about the existence of different parasite races, which may have different temperature tolerances, being adapted to hosts with specific temperature preferences, or they may be geographic races varying in virulence in introduced and/or endemic fish species.

Site of infection

Trophonts infect the epidermis, cornea and gill filaments.

Life Cycle

Although *Ichthyophthirius multifiliis* has a direct life cycle, it is fairly complex and has three distinct life stages:

1) The on-fish, feeding trophont;

2) The environmental, reproducing tomont; and

3) The infective, fish-seeking theront (Fig 2).

The trophont invades and encysts between the thin outer layers of the fish host's skin and gills in order to feed on those tissues. Because of the covering by this epithelial tissue and mucus, the trophont stage is protected from chemical treatment. Once the trophont is mature, it stops feeding, leaves the fish, and becomes a tomont. The tomont quickly secretes a gelatinous-walled outer cyst that allows it to stick to surfaces in the environment. The tomont begins to divide quickly, forming hundreds of new "daughter" parasites (tomites) within a single cyst. This can occur in a day or less at warmer water temperatures. The gelatinous wall of the tomont cyst protects it and the daughter tomites from chemical treatment.

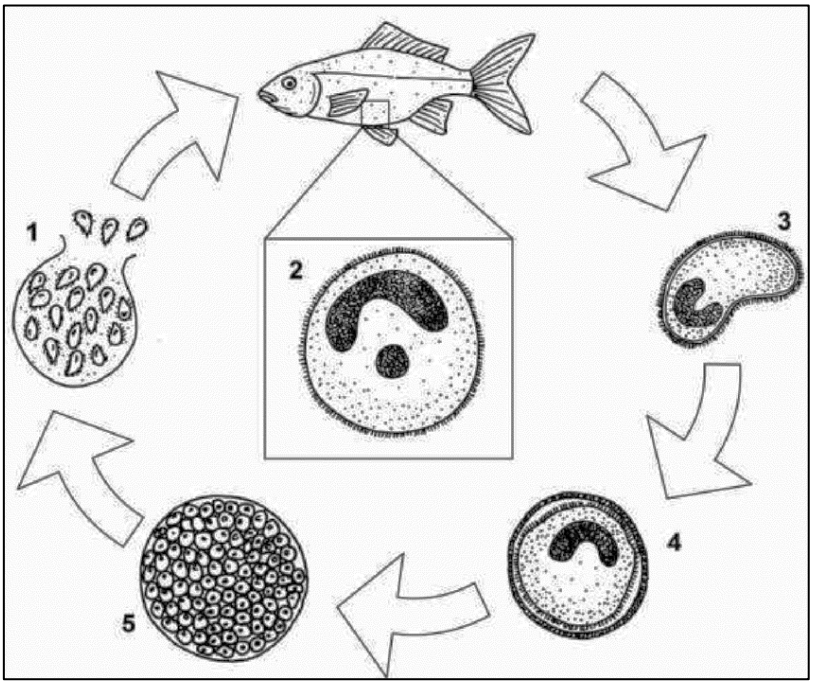

Fig 2: Life-cycle of *Ichthyophthirius multifiliis*. 1) Infective theronts released from cyst. 2) Parasitic trophont stage. 3) Exiting tomont. 4) Cyst. 5) Dividing tomites within cyst.

The tomites begin to develop and become theronts within the tomont cyst. Following a period of days (warm water temperatures) or weeks (cool water temperatures), the theronts bore out of the tomont cyst and become free-swimming, infective parasites in search of a fish host. These infective theronts must find a live fish to complete the parasite's life cycle. This free-swimming phase is unprotected and, therefore, highly susceptible to chemicals. Treatment protocols should be designed to target this theront stage.

Pathogenesis

Theronts use an elevated pointed ridge (perforatorium) to penetrate host tissues and they discharge their pellicular mucocysts to form a stick envelope glued to the host's epithelium. Within minutes, the parasites penetrate deeper into epithelial or epidermal

tissues where they feed and grow (increasing their volume up to 3,000 times). The trophonts form greyish pustules in the skin of their hosts where they feed by ingesting host cell debris. Infected fish produce excess mucus to combat the irritation but many epidermal cells are destroyed and are sloughed. Heavy infections of the gill filaments interfere with gas exchange and may prove fatal. Lesions containing engorging trophonts appear as visible white spots covering infected fish. Fish surviving infection exhibit some protective immunity against subsequent infections.

Mode of transmission

Engorged trophonts are liberated from ruptured pustules into the water column where they settle on convenient substrates or on the bottom. They form a gelatinous cyst and undergo a series of divisions producing from 250 to 2,000 tomites which are subsequently released and actively search for new hosts. The number, size and duration of the life-cycle stages depends prevailing environmental condition, particularly temperature (no development occurs below 2°C or above 30°C). The whole life-cycle may be completed in as little as 3-8 days at 23-24°C, but it progressively takes longer at lower temperatures (up to 3 months at 4-5°C).

Disease Signs

The classic sign of an “Ich” infection is the presence of small white spots on the skin or fins (Fig 3). These spots are caused as the adult parasite (trophont) penetrates and creates a space in the outer layers of the fish’s body surfaces (epithelium) in order to feed on the fish and move around. These lesions look like small white dots, blisters, or salt grains on the skin or fins of the fish. The white spots may not be as obvious on fish that are white or pale in color, or if the infection is limited to the gills. By the time the white spots are visible to the naked eye, the infected fish is very sick. Prior to the appearance of white spots, fish may have shown signs of irritation, flashing, weakness, loss of appetite, and decreased activity. A well-trained aquaculturist or aquarist will detect these changes before the fish’s condition worsens and mortalities occur. If the parasite is only present in the gills, white spots may not be seen at all but fish will die in large numbers. In these fish, gills will often be pale and very swollen. White spots should never be used as the only means of diagnosis because other diseases may have a similar appearance. Gill and skin biopsies should be collected and examined with a light microscope when the first signs of illness are observed. If even a single “Ich” parasite is seen, fish should be medicated immediately because as the infection advances fish may not survive, even with treatment.

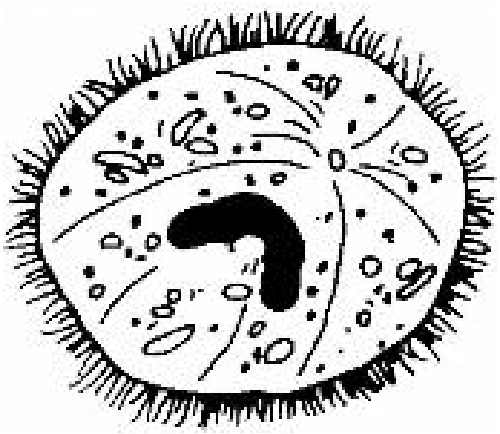

Fig 3: “Ich” trophont—feeding stage found on fish.

Prevention

Prophylactic treatments with saline baths or dilute copper are used on incoming fish during quarantine in some facilities. The immunocompromising impact of copper treatments is increasingly well documented. Maintenance of excellent water quality and minimization of stress are thought to reduce the likelihood of a clinical outbreak. Adequate water changes and cleaning of substrates are thought to help prevent accumulation of high numbers of infective tomonts.

Control

Heavy filtration with diatomaceous earth or membrane filters will reduce the number of circulating theronts. Transferring fish to clean aquaria every day for 7 days will limit the infection by keeping one step ahead of theront reinfestation. Removal of theronts from the water can also be accomplished by making large daily water changes. This method, while efficacious, may stress fish excessively unless attention is paid to makeup water temperature and pH. Alternatively, fish can be removed from a system, and the parasites will eventually die for lack of a host. Elevating the temperature several degrees Celsius over normal temperatures accelerates this process. To ensure that all theronts are eliminated in a system, at least one complete water change should be made, along with removing debris from the gravel before returning fish to the system after leaving a tank or system fallow.

Treatment

Currently available medications do not penetrate the encysted trophonts (Tomonts). All treatment is directed toward preventing reinfection of fish by killing free-swimming theronts. Formaldehyde at 25 ppm (1 ml/10 gallons) is effective if administered three times on alternate days. Water changes of up to 75% should be done 4–8 h after treatments. In addition to chemotherapy, management adjustments serve to control infestations. Elevating water temperatures several degrees Celsius over normal temperatures for 5–7 days will limit the

infection by adversely affecting the heat-sensitive theronts as well as enhancing the immune response of the host.

Probable questions:

1. Discuss the developmental stages of *Ichthyophthirius multifiliis*.
2. Which life cycle stage of *Ichthyophthirius multifiliis* ishighly susceptible to chemicals?
3. Briefly describe the life cycle of *Ichthyophthirius multifiliis*\ with diagram.
4. Write short note on white spot disease.

References and Suggested readings:

1. http://www.columbia.edu/itc/cerc/danoff-burg/invasion_bio/inv_spp_summ/myxobolus_cerebralis.html
2. https://parasite.org.au/para-site/text/ichthyophthirius-text.html
3. Roberts, L.S and Janovy, J. (2009). Smith & Robert's Foundation of Parasitology. 8th. Edn.McGraw Hill
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Unit IV

Some common helminthes of freshwater fishes and their life cycle patterns: A) *Proteocephalus* sp. B) *Camallanus* sp.

Objective: In this unit you will know about some common helminths of freshwater fishes and their life cycle patterns like *Proteocephalus* sp. and*Camallanus* sp.

Proteocephalus sp.

Introduction

Proteocephalid cestodes parasitize fish predominantly but occur also in amphibians and reptiles; their intermediate hosts are primarily planktonic crustaceans. They have distinct segmentation, variable scolex with four simple suckers, an apical sucker or occasionally an armed rostellum. Tapeworms of the genus *Proteocephalus* Weinland, 1858 are parasites of fishes, amphibians and reptiles. The systematics of this genus has not been sufficiently clarified and there are difficulties in the identification of individual taxa.

To date, the developmental cycles of only a few *Proteocephalus* tapeworms have been studied and, compared with other cestode groups such as the Pseudophyllidea and Cyclophyllidea, the biology of proteocephalidean tapeworms is much less well known. Life cycles of *Proteocephalus* species occurring in fishes in the Palearctic Region are reviewed with emphasis on the morphology of larval stages and development of parasites within the intermediate and definitive hosts.

Systematic position

Kingdom: Animalia

Phylum: Platyhelminthes

Class: Cestoda

Genus: *Proteocephalus*

Life cycle

The basic sequence of *Proteocephalus* development consists of an *adult*, which produces an *egg*, containing an oncosphere, i.e. a six-hooked (hexacanth) *larva*, which migrates to

a parenteral site (body cavity) of the intermediate host, where it metamorphoses and grows as a *metacestode,* and a sexually reproducing adult.

I. Egg

The egg results from oogenesis, fertilization of the oocyte and subsequent embryogenesis. This process, including the formation of sperm, i.e. spermatogenesis, will be briefly discussed, with emphasis on the morphology of formed eggs.

II. Spermatogenesis

The male reproductive plan of *Proteocephalus* species is typical of parasitic platyhelminths. Despite the fact that the first study on the ultra-structure of cestode sperm flagellum was that by Gresson (1962) in *P. pollanicola* (syn. of *P. longicollis* –Scholz *et al.*, 1998b), there is limited information about spermatogenesis and sperm ultra-structure of *Proteocephalus* species. It has been found that *P. longicollis* has a spermatozoon and spermiogenesis with the following chief features:

(i) A long thread-like body;

(ii) An elongated nucleus;

(iii) Cortical microtubules underlying the plasma membrane;

(iv)The absence of mitochondria; and

(v) The absence of a typical acrosome.

Because of the presence of two axonemes, *Proteocephalus* seems to belong to the 'two-axoneme' type of cestodes, found typically in the Pseudophyllidea and Tetraphyllidea. This type is considered primitive (plesiomorphic) because it is present in free-living platyhelminths

III. Oogenesis and insemination

There are few studies on oogenesis and insemination in cestodes, which is valid also for proteocephalideans. Although no data exist, it can be assumed that the chemical composition and ultra-structure of oocytes of *Proteocephalus* species resemble those of other cestode groups.

IV. Egg formation

Most studies on cestode egg formation have dealt with pseudophyllidean and cyclophyllidean tapeworms and practically nothing is known about the process of formation of egg envelopes of species of *Proteocephalus*. Four main types of egg-forming systems are recognized in cestodes and proteocephalidean tapeworms are considered to belong to the 'pseudophyllidean- type'. Cestodes with life cycles associated with water are placed in this group.

Many of them, in particular pseudophyllideans, have a thick, sclerotin capsule, produced by cestodes with well-developed vitellaria. In this feature, proteocephalideans distinctly differ from these groups in possessing a thin walled, transparent outer envelope. Eggs of proteocephalideans are named as 'egg-like oncospheres' to distinguish them from

coracidia of pseudophyllideans. In pregravid proglottides of *Proteocephalus* species, all eggs contain an unformed oncosphere, i.e. there are no embryonic hooks. The hooks appear simultaneously in most eggs in more developed proglottides, which are named gravid. The number of pregravid proglottides is highly variable in *Proteocephalus* species, ranging from a few to numerous.

V. Egg morphology

Eggs of *Proteocephalus* species are similar in their overall appearance and are composed of an oncosphere covered by membranes.

The oncosphere (hexacanth), already formed within the uterus of gravid worms contains three pairs of embryonic hooks with a straight, long and fine base, a short, slightly curved blade and a short, anteriorly directed guard. Hooks of the median pair are longer than those of lateral pairs. Two dark areas considered to be penetration glands, are situated on the opposite side of the oncosphere to the embryonic hooks. These are found in the morphology of the embryonic hooks of *P. exiguous, P. percae* and *P. thymalli* from Russia. They found significant differences in the size of the hooks of individual taxa studied. However, differences were also found between different populations of the same species.

VI. Egg size

The size of eggs has been used as an important feature in differentiating species of *Proteocephalus.* However, data taken from eggs in permanent preparations are of limited value because the eggs are generally deformed due to staining and dehydration; the capsule is collapsed so that most measurements provided in this study seems to relate to the outer envelope. It has been suggested that only measurements of eggs expelled from the uterus into the water should be measured. However, only ripe eggs, i.e. those containing fully formed and motile oncospheres with embryonic hooks, should be considered. It should also be pointed out that the capsule of most *Proteocephalus* tapeworms inflates in water which makes it difficult to compare with measurements of eggs present at different times in water. Therefore, it seems reasonable to provide measurements of a more stable structure, i.e. those of the outer envelope covering the rigid, granular layer, and of the oncospheres.

VII. Egg release

Ripe eggs, i.e. those containing oncospheres with embryonic hooks (hexacanth), are released through the uterine pores. In *Proteocephalus* species, the uterine pores are few (about 2–4), small, oval to spherical openings along the median line on the ventral side of gravid proglottids. To date, no data on the formation of uterine pores have been provided. Eggs are spontaneously released after the tapeworms are placed in water and it seems that this release corresponds with the strategy of egg release under natural conditions. It has been observed that eggs of some species, e.g. *P. osculatus* and *P. torulosus*, are released directly within the lumen of fishes. However, it is possible that this release is linked with the death of the host and it does not occur in nature. Outermembrane of egg is thin and apparently unable to prevent digestion by host enzymes in the anterior part of the

intestine, the site of location of adult tapeworms. The eggs, protected against unfavourable conditions of the intestinal lumen within the uterus of the worm, can reach the external environment with more safety by expulsion of the whole egg-containing parasite or by expulsion of the detached part of the strobili. Indeed, the body of gravid *Proteocephalus* is often fragmented within the intestinal lumen.

After the egg is laid in water, the external membrane quickly increases in size, becoming two or three times larger. It is presumed that this process is due to osmotic intake of water. It is also assumed that the swelling of the capsule helps in the floating of the egg because freshly laid eggs remained on the bottom whereas the eggs with inflated capsules may float in the water. Since intermediate hosts are mostly pelagic copepods, this is an adaption to facilitate transmission by selective ingestion. Eggs of *P. torulosus* are relatively large and they differ from those of congeners in possessing a somewhat thicker granular layer and a less inflated capsule. This is probably an adaptive character because *P. torulosus* is a riverine species, occurring even in rivers with strong current. In these localities, eggs which sink to the bottom are likely to survive for successful transmission to potential intermediate hosts, such as benthic copepods.

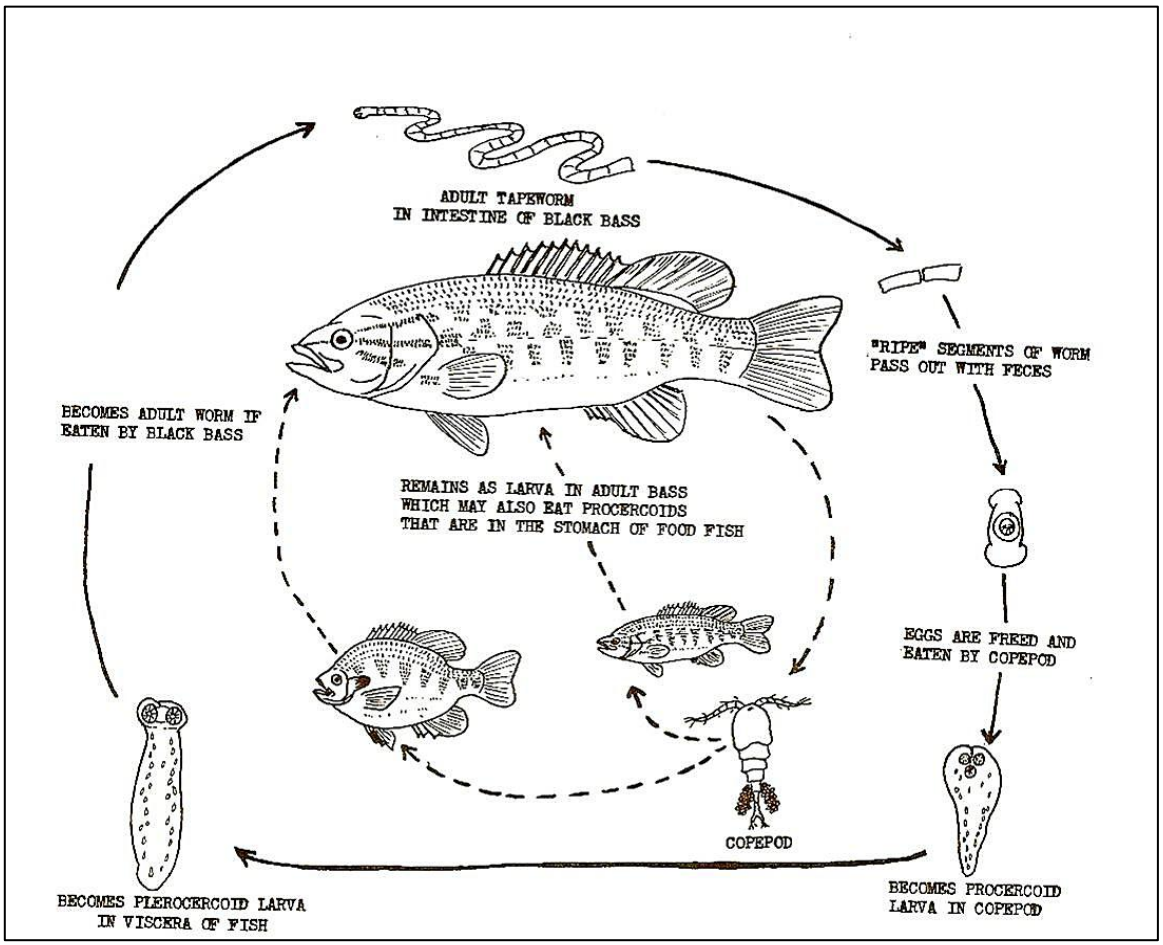

Fig : Life cycle of *Proteocephalus*sp.

Survival and infectivity

It has been observed that not all eggs spontaneously laid in water are ripe. Some eggs are apparently smaller than others and contain either unformed oncospheres. without embryonic hooks, or undifferentiated, granular tissue. The proportion of ripe eggs of *P. exiguus* was found to change during the year with the highest proportion in summer–autumn but only very few during the winter and spring.

Infectivity, i.e. the ability of oncospheres to infect intermediate hosts, was tested by experimental infections of copepods with eggs preserved in water of different temperatures. It has been demonstrated that eggs maintain their infectivity for a relatively long time and that infectivity depends mainly on the temperature. Only freshly released *P. torulosus* eggs are infective to copepods and that they lose their infectivity very quickly seem to be incorrect because they contradict successful experimental infections with *P. torulosus* eggs several days old.

Intermediate host

I. Range of intermediate hosts

Planktonic crustaceans of the order Copepoda (families Diaptomidae and Cyclopidae) serve as intermediate hosts (Fig) for *Proteocephalus* tapeworms in the Palearctic Region. An exception to this rule is the calanoid *Epischura baicalensis* (Temoridae), which is an intermediate host of *Proteocephalus* species in Baikal Lake, Russia. The finding of *Proteocephalus* larvae in cladocerans (*Bosmina coregoni*, *Bythotrephescederstroemi*, *Daphnia* spp.) should be considered as accidental or even doubtful. The eggs ingested by cladocerans survivedwithin the intestine for a short time (maximum 48 h) but oncospheres were unable to penetrate through the intestinal wall and quickly died. The suitability of individual copepod species as intermediate hosts of *Proteocephalus* species differs and is dependent upon the species and developmental stages of copepods as well as particular ecological conditions such as locality and season. Some copepods tend to be more susceptible to*Proteocephalus* infection than others but data which would explainthese differences are not available.

II. Infection of the intermediate host

Copepods are attracted by floating eggs and ingest them quickly but other authors suggest that copepods reject oncospheres and the consumption of eggs is occasional and accidental. After ingestion, oncospheres are liberated from surrounding membranes in the gut of copepod, and presumably the release of the larvae in the intestine is stimulated by the environment of the intestinal lumen. The process of liberation is rather rapid and oncospheres appear to be free of the egg membranes as early as 5 min after contact with the copepods. This liberation of the oncospheres has also been observed in eggs in water and it can be stimulated by applying slight pressure. However, those oncospheres which are liberated directly into water do not survive.

The success of oncosphere establishment within copepods and the proportion of intermediate hosts becoming infected are influenced by many factors, including physiological compatibility, ecological conditions and the geographic origin of the host and parasite, the time of contact of copepods with eggs, the density of copepods, and water temperature.

Following ingestion, oncospheres are liberated from egg membranes and, if in a suitable host, actively penetrate the gut of the copepod into the body cavity. Penetration of oncospheres though the gut wall is assisted by the mechanical action of embryonic hooks. The secretion of penetration glands, with presumably histolytic secretions, may play a crucial role in the process of penetration. The time of penetration of the gut wall is short, lasting 5–30 min.

Within the body cavity, **oncosphere** develops into a **metacestode**. A number of terms have been used to describe *Proteocephalus* metacestodes in the intermediate host. These include: plerocercoid, plerocercoid I, cercoscolex or procercoid. The term 'plerocercoid' has usually been restricted to pseudophyllidean metacestodes from the second intermediate host.

III. Morphogenesis of procercoids

Within the body cavity, i.e. in the parenteral site, the oncosphere quickly grows due to cellular proliferation and it metamorphoses into the developing **metacestode**. The metacestode becomes elongate 4–6 days post infection with one end more actively moving. On the opposite side, a small protuberance, ultimately becoming detached from the body and representing the primordium of a sarcomere. The sarcomere detaches from the body after 1–3 days or persists for three weeks. A detached sarcomere can remain viable within the body cavity of a copepod for as long as 60 days.

Embryonic hooks are normally located within the body, most often near its lateral margins in procercoids of the same species and those of other Proteocephalus taxa. Embryonic hooks are not present in a metacestode. it is suggested that they have no further role in the subsequent development of the cestode.

In some cestodes, a distinct cavity, the 'primitive lacuna', develops whereas other metacestodes grow as a compact mass of cells. The presence of a cavity or 'lacuna primitiva', it seems that in *Proteocephalus* a cavity normally does not develop and metacestodes are acystic and gymnosomic.

Development of the scolex may vary considerably; the appearance of a single structure at the extreme tip is often the first sign of scolex differentiation during the exogenous development.

Fully formed (infective) procercoids are elongate and highly mobile. The shape and size of procercoids vary considerably due to their high motility and there is also much individual variation in size. The procercoid possesses a well-developed anterior part (scolex) with four muscular suckers. Although the scolex does not reach the ultimate size

of that of the adult worm, its morphology generally does not differ from that of tapeworms from the definitive host.

IV. Localization of larvae

Larvae are freely moving within the body cavity of copepods but they are located most frequently in the first segments of the cephalothorax. At the beginning of development, larvae can also belocated in the antennulae, followed by exclusive development within the cephalothoracic or abdominal segments, apparently due to space limitation in the antennulae.

V. Occurrence in intermediate hosts

Values of prevalence reach up to 100% in experimentally infected copepods but in natural populations of conspecific copepods the prevalence values are considerably lower. Generally, the prevalence of infection of zooplankton with Proteocephalus procercoids (and other metacestodes) is extremely low, ranging between 0.001 and 1%. Although the prevalence values of copepod infection under natural conditions are very low, parasites can accumulate within fish hosts due to an intensive consumption of zooplankton by fishes. The absolute number of Proteocephalus procercoids in naturally infected copepods can reach 853–1193 specimens per m^3 with mean values 3–178 specimens per m3.

Definitive host

I. Range of definitive hosts

Host specificity of most *Proteocephalus* species from fishes has been considered to be quite narrow but there are marked differences in the range of fish hosts infected by individual species. Some species are specific to one host genus or one species of definitive host, e.g. *P. ambiguus* to the nine-spined stickleback (*Pungitius pungitius*), *P. filicollis* to the three-spined stickleback (*Gasterosteus aculeatus*), *P. macrocephalus*nto eels (*Anguilla* spp.), *P. osculatus* to wels (*Silurus*n*glanis*), and *P. thymalli* to graylings (*Thymallus* spp.).

II. Infection of the definitive host

Definitive hosts become infected after ingestion of copepods harbouring procercoids. Larvae continue to develop within the digestive tract of the fish host, with subsequent formation of attachment organs, nervous and excretory systems and musculature. The growth of these organs occurs within the definitive host but considerable changes in scolex morphology or structure of the osmoregulatory system do not occur.

Scolex of living Proteocephalus larvae becomes invaginated immediately after ingestion by the definitive host and this is related to the parasite being protected against unfavourable conditions such as the high acidity within the stomach of the fish definitive

host. Procercoids become more active and the scolex evaginates in alkaline conditions, so the role of chemical stimuli in the process of evagination requires further investigation.

Only a very small proportion of juvenile tapeworms are able to establish within the gut of the definitive host. Some time ('physiological maturation') after complete formation of internal organs is necessary for procercoids to become fully infective. Intraspecific competition between young worms within the pyloric caeca or in the intestine of infected fish is likely to occur but the very low establishment rate requires additional studies.

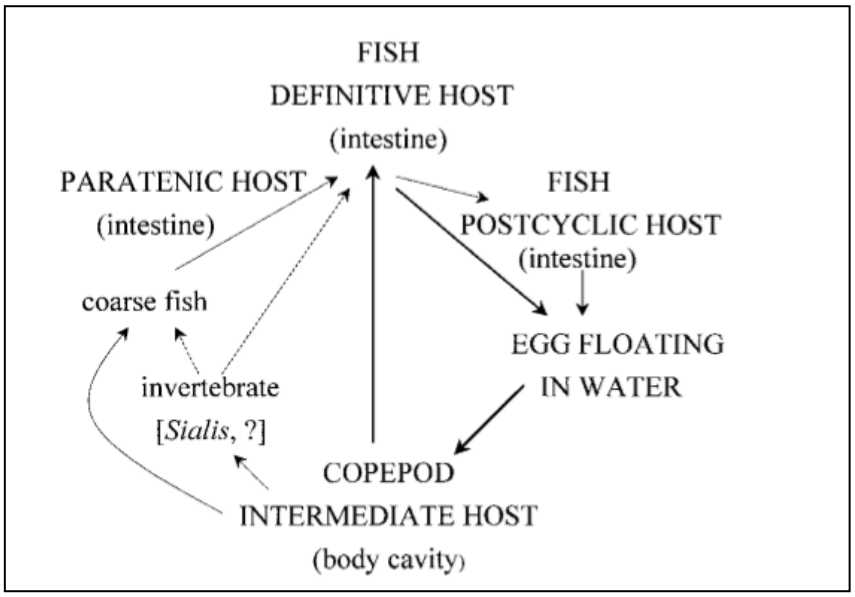

Fig: Flow diagram of the life cycles of Proteocephalus tapeworms. Dotted line indicates possible routes of transmission

Maturation dynamics

As in the case of the development of procercoids in copepods, the growth and maturation of tapeworms in the fish definitive host are controlled mainly by water temperature. The influence of host hormones, as observed in some pseudophyllideans, such as Triaenophorus spp. May also play some role. Although it is possible that one cycle may be completed in 1.5–2 months at water temperatures of 15–20° C. *Proteocephalus* have a one-year life span (i.e. the life cycle in total, including all developmental stages) with a pronounced seasonality in their maturation. The recruitment of new cestode generations takes place mainly in the summer or autumn. The tapeworms overwinter in fish and they start to grow rapidly and mature after the water temperature increases in the spring. Eggs are laid in late spring and summer. Such seasonal patterns inoccurrence and maturation have been observed in several taxa of Proteocephalus, e.g. *P. cernuae, P. exiguous*,*P. filicollis, P. osculates*.This general pattern is modified in each species, being dependent on its geographical position and particular ecological conditions, as the same species of cestode may show different patterns of maturation in distinct latitudes. Localization within the definitive host,as a rule, adult Proteocephalus tapeworms is located in the anterior part of the intestine. In fishes possessing pyloric appendages, adult

tapeworms are attached by their scoleces to the epithelium of these appendages with the strobilae lying within the intestinal lumen.

Conclusions

The most detailed information exists on the biology of *P. longicollis* but also in this species many aspects of its life history remain to be studied. There are still gaps in our knowledge of biology of Proteocephalus tapeworms parasitizing fishes in the Palearctic Region and further investigations into the life cycles are needed. It is difficult to list unsolved problems in the biology of Proteocephalus tapeworms, which should be addressed in future research, but the following deserve attention, namely the process of egg formation, including fertilization; ultra-structure of eggs and oncosphere, with special attention being paid to the penetration glands; comparative morphology of embryonic hooks; morphogenesis and ultra-structure of the procercoids, in particular those of *P. gobiorum* and *P. tetrastomus*; factors influencing the establishment of juvenile tapeworms and their morphogenesis within the definitive host; factors resulting in the host specificity of some taxa in the definitive host. Knowledge of tapeworm biology is still fairly limited despite the considerable progress being made in the past two to three decades.

Camallanus sp.

Introduction

The nematode *Camallanus anabantis* Pearse, 1933 is the common intestinal parasite of *Anabas testudineus* (Bloch) (type host) and also reported to be harboured by many other fishes such as *Clarias batrachus* (Linnaeus), *Channa gachua* (Hamilton), *C. punctatus* (Bloch), *Puntius filamentosus* (Valenciennes), *Bettaunimaculata* (Popta), *Mastacembelus armatus* (Lacepede) and *Trichogaster trichopterus* (Pallas). *Camallanus anabantis,* this species is common in various fishes in India, including the type host *Anabas testudineus* from freshwater swamps (Soota, 1984; De, 1993). Invasion of fish occurs in spring and summer and they grow in the fish during the monsoon and autumn. The proportion of males in the worm population increases in the early autumnal period and then decreases rapidly after they have fertilized the females. Larvae are released from females in late winter and early spring.

Systematic position

Phylum: Nematoda
Class: Secernentea
Subclass: Spiruria
Order: Camallanida
Family: Camallanidae
Genus: *Camallanus*

- **Life cycle**

I. Intermediate hosts

Larvae show considerable motility and become ingested by copepod, *Mesocyclops leuckarti*s, and migrate from its digestive tract to the haemocoel of the cephalothorax, where they develop further. The first-stage larvae increase in size, both the cuticle and wall of the oesophagus become thickened and consequently, the spacious oesophageal cavity narrows. The oesophageal gland cells with distinct nuclei appear at the posterior end of the oesophagus. The first moult occurred on day 4, at 21-26°C. The second moult, to attain the third infective-stage, occurred on day 11, at 21-26°C. The fully developed third-stage larvae were, however, first recovered from the copepod hosts on day 14. They remain in the haemocoel of the cephalothorax of the copepod intermediate host as coiled third-stage larvae and their further development ceases.

II. Definitive hosts

The nematodes were found located in the caecum (third- and fourth-stage only) and anterior and middle parts of the host's intestine. Morphological data of the nematodes recovered from the fishes revealed that the third moult occurred on day 15. The fourth i.e. final moult occurred at different times for the "male" and "female" larvae, on day 68 in "male" larva but on day 86 in "female" larva. A female with few larvae in the uteri was recovered on day 187.

Morphology and larval development

First-stage larvae

Body of the first-stage larvae (Fig A1) is translucent, 231-300 long and 13-15 in maximum width Body cuticle transversely striated. Head rounded, with a small dorsal cuticular tooth; oral papillae indistinct. Short buccal tube. Oesophagus thin-walled and with spacious lumen occupies 15-19% of body length. Nerve-ring encircles oesophagus at its posterior third. Excretory pore anterior to nerve cells. Tail conical, elongate with pointed tip, 13-19 % of body length. First-stage larvae (FigA2) after penetration into the haemocoel of the copepod, the dimensions of first-stage larvae changed only a littlebut the oesophagus differentiated into a thick-walled anterior moiety and a posterior glandular moietywith distinct cell nuclei.

Second-stage larvae

The first larval moult (Fig A3) occurred in the haemocoel of copepod, *Mesocyclops leuckarti* on day 4. At this stage larva measures 435µm long and bears no dorsal cephalic tooth. Oesophagus indistinctly divided into anterior muscular (58 long) and posterior glandular (21 long) parts. Intestine straight and wide, a short posterior part with large cuboidal cells and narrow lumen. Small oval ventral genital primordium near mid intestine. Tail relatively short, 14% of body length.

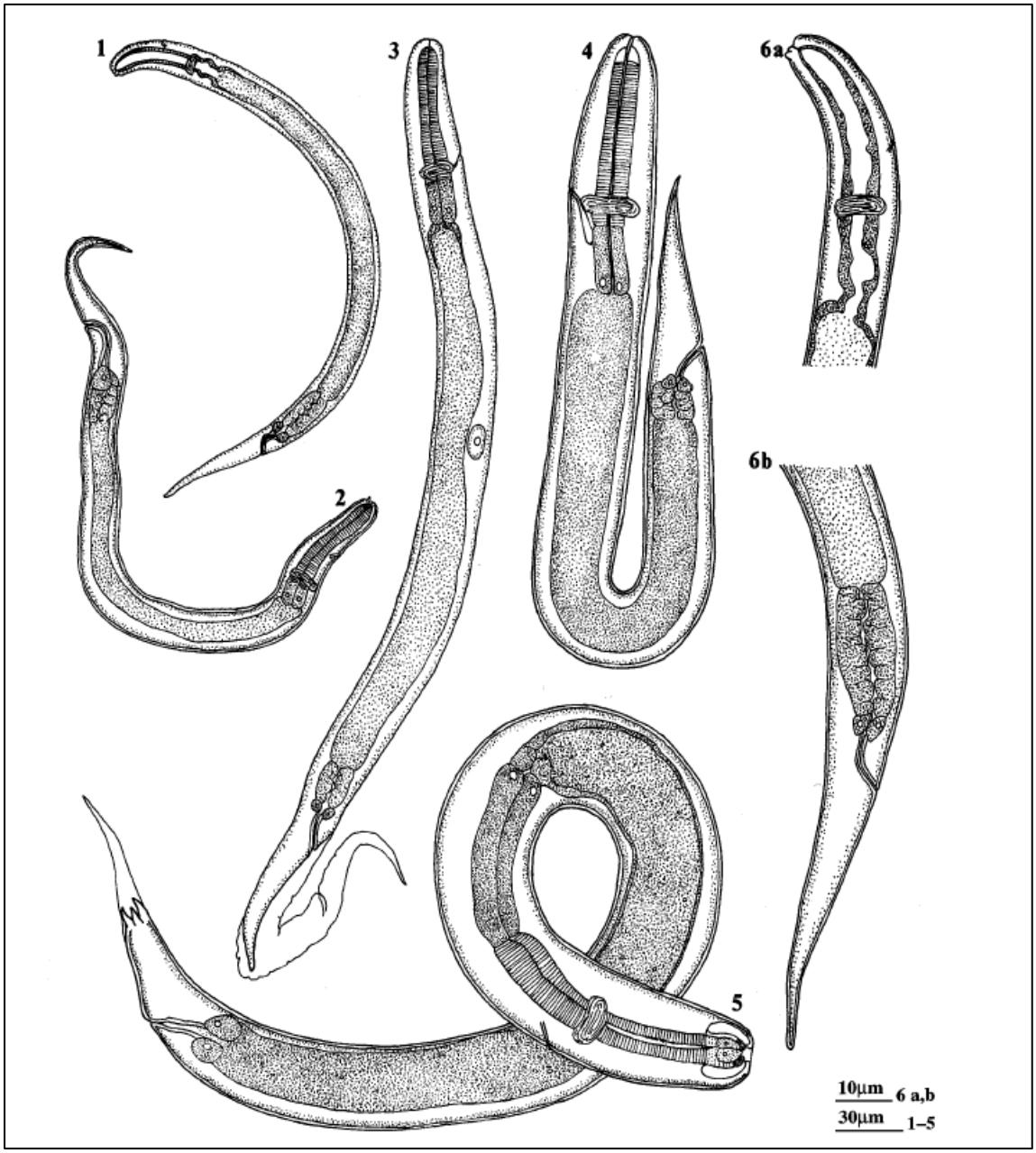

Fig A: Development of larva of *Camallanus anabantis*
(1) Free first stage larva
(2-6) Development of larva in copepod
(2) First stage larva on day 2
(3) Larva during first moult on day 4
(4) Second stage larva on day 7
(5) Late second stage larva on day 10
(6) Free first stage larva, enlarged view (a- anterior end of boby, b- tail end)

ring. Intestine wide, light-coloured and with fine granules; posteriorly with large cuboidal

Second-stage larvae (Fig A4, A5, B9) were first recovered on day 7. Body more stout, tail relatively short. Cuticle transversely striated. Cephalic end rounded and without dorsal cuticular tooth; buccal tube short. Oesophagus with anterior long muscular and posterior short glandular moieties. Straight, wide intestine with short posterior zone bearing large cuboidal cells.

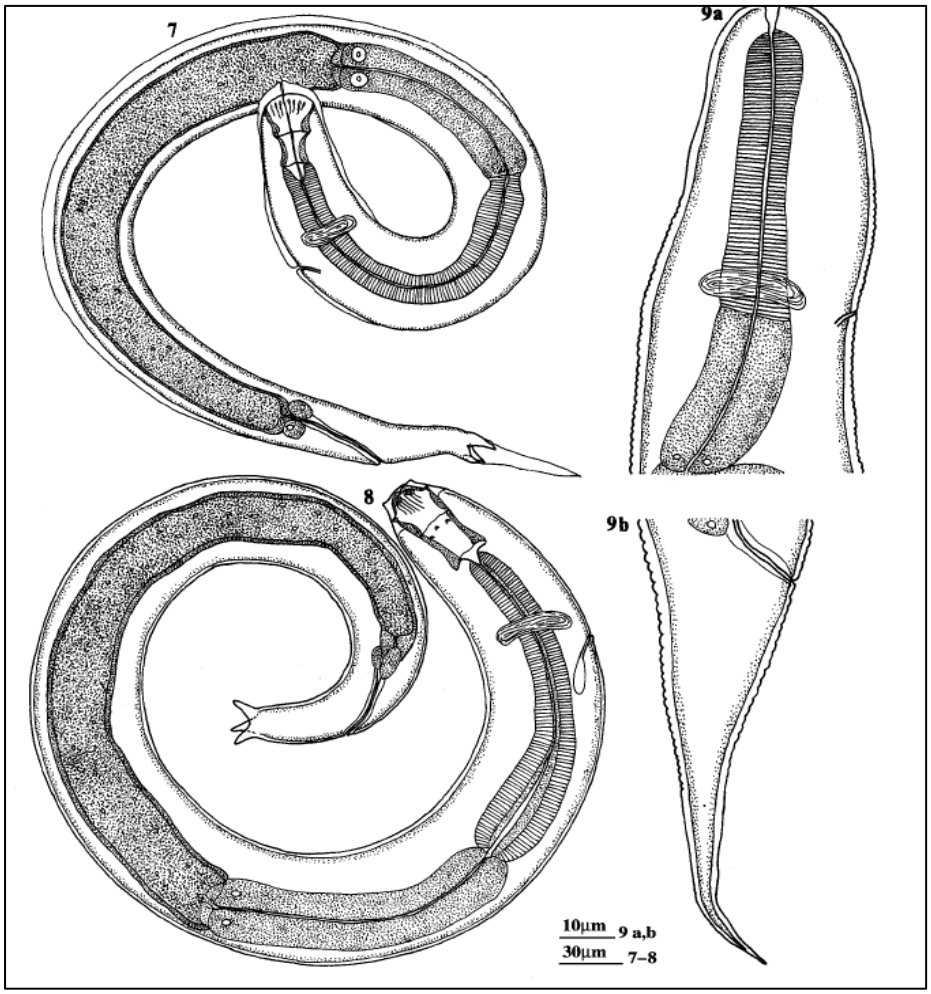

Fig B: Development of larva of Camallanus anabantis in copepod
(7) Larva undergoing second moult on day 11
(8) Third stage larva on day 15
(9) Second stage larva on day 6 (a - anterior end, b - posterior end)

The larva recovered on day 10 was found preparing for its second moult, best visible at tail end. The typical third-stage tail visible below larval cuticle. Anterior end of oesophagus with a hyaline bell-shaped structure, two small bead-like structures and two large cells with distinct nucleus also visible. Oesophagus with anterior muscular moiety longer than posterior glandular one. Nerve-ring near middle of muscular oesophagus. Excretory pore slightly posterior to nerve ring. Intestine straight, wide and with coarse granules.

Third-stage larvae from copepods

Larvae (Fig B7) undergoing the second moult wererecovered from the copepod,*Mesocyclops leuckarti,* haemocoel on day 11. During moulting, old cuticle becomes loosened at bothends, particularly on tail. Larval body light-colouredand covered with thin cuticle. **Oral openingdorsoventrally elongate.** Buccal capsule, typicalcamallanid type, already formed and contains two parts;anterior part bivalved, each valve reinforced with 7sclerotised longitudinal ridges on inner surface andposterior part simple leading posteriorly to anoesophageal cup. Sclerotised inner lining of anteriorpart of oesophagus seen to pass out through buccalcapsule. Oesophagus with longer anterior muscular andshort posterior glandular parts, Nerve-ring at about ¼ of oesophagus length. Excretory pore slightly posteriorto nerve-ring. Glandular oesophagus opens intointestine through valvular apparatus. Intestine wide,light orange in colour and posteriorly joins short,narrow rectum; three small unicellular rectal glandspresent at their junction. Tail ends in one dorsal and twosubventral mucrones.

Fourth-stage larvae

The single larva undergoing the third (first in thedefinitive host) moult was recovered from anexperimentally infected fish on day 15; its bodylonger than third-stage larvae from copepods. Cuticle thin and indistinctly striated. Apart from thebuccal capsule, typical of the third stage, a new buccalcapsule seen. Newly formed buccal capsule weaklysclerotised, wider and not divided into anterior andposterior chambers. Longitudinal ridges on innersurface of new buccal capsule not seen. Tail conicalwith three mucrones, one large dorsal and two smallersubventral.

Young adult, fifth stage

The fourth moult of "male" larvae first occurred onday 68 at water temperature 24-36°C. Body cuticle is thin. Both old, typical of fourth stage, and new widerand weakly sclerotised buccal capsule with faint beadedridges in each valve seen. Dorsal and ventral aggregationof large cells found below newly formed buccalcapsule. Muscular oesophagus shorter than posteriorglandular one. Nerve-ring at about 1/3 of muscularoesophagus. Excretory pore just posterior to nerve-ring.Intestine straight and wide. Tail conical, bears twomucrones. Single genital anlage differentiated into testisand vas deferens. Spicules indistinctly visible but caudalpapillae seen clearly.

The first two moults take place in the intermediate copepod host, and the next two, i.e. third and fourth moults, in the definitive host. The morphology of the first- and second-stage larvae of *C. anabantis* is found to be almost identical to that of corresponding larval stages. An interesting feature of the present first- and second-stage larvae is that the intestinal wall at its distal part is made up of large cuboidal cells and therefore, the lumen is narrow there. The larvae at their third stage, however, show some differences, particularly in the structure of the buccal capsule.

Probable questions:

1. Discuss about the egg of *Proteocephalus* sp.
2. Describe the egg morphology of *Proteocephalus* sp.
3. Name a species of *Proteocephalus* found in river water.
4. Name the infective stage of *Proteocephalus* sp.
5. Describe the morphology of procercoids of *Proteocephalus* sp.
6. Describe the development of *Proteocephalus* sp. in definitive host.
7. Describe the life cycle of *Proteocephalus* sp.with diagram.
8. Describe different stages of larval development in *Camallanus* sp.
9. Discuss the life cycle of *Camallanus* sp.

Suggested readings:

1. Scholz, T. (1999). Life cycles of species of *Proteocephalus*, parasites of fishes in the Palearctic Region: a review. *Journal of Helminthology*, **73**: 1–19.
2. Wagner, E. D. (1954). The life history of *Proteocephalus tumidocollus* Wagner, 1953, (Cestoda), in rainbow trout. *Journal of Parasitology*, **40:** 489–498.
3. De N.C (1999). On the development and life cycle of *Camallanus anabantis*(Nematoda: Camallanidae), a parasite of the climbing perch,*Anabas testudineus.Folia parasitological*, 46: 205-215.
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Unit- V

Structure, pathobiology, prophylaxis and diagnosis of causative agents of filariasis

Objective: In this unit you will know about Structure, pathobiology, prophylaxis and diagnosis of causative agents of filariasis.

Introduction:

Filariasis is caused by the worms *Wuchereria bancrofti, Brugia malayi*, and *Brugia timori*. These worms occupy the lymphatic system, including the lymph nodes; in chronic cases, these worms lead to the syndrome of elephantiasis.

- *Wuchereria bancrofti*, which is responsible for 90% of the cases
- *Brugia malayi*, which causes most of the remainder of the cases
- *Brugia timori*, which also causes the disease.

Due to high frequency here, we will discuss about *Wuchereria bancrofti.*

Geographic Distribution:

Wuchereria bancrofti is largely confined to the tropical and sub-tropical countries of the world. However, it occurs in India, West Indies, Puerto Rico, Southern China, Japan, Pacific Islands, West and Central Africa and South America. In India, the parasite is chiefly distributed along the sea coast and along the banks of big rivers (except Indus); it has also been reported from Rajasthan. Punjab, Delhi and from various vicinities of Uttar Pradesh.

Habit and Habitat:

Wuchereria bancrofti is a dreadful endoparasite of man; adults harbouring the lymphatic vessels and lymph nodes.Its life history is digenetic, as it involves a secondary host, the bloodsucking insects, i.e., the female mosquitoes of the genus *Culex*, *Aedes* or *Anopheles*; the secondary host for *W. bancrofti* in India and China is *Culex pipiens*, in Pacific Islands (except Fiji and New Caledonia) is *Anopheles punctatus* and in Polynesian Islands is *Aedes polynesiensis*.

Wuchereria bancrofti is viviparous or to say ovo-viviparous; its larvae are referred to as microfilariae which/harbour the blood of human beings.

Structure:

1. The adult filarial worms (*Wuchereria bancrofti*) are long and hair-like, often creamy-white in colour (Fig 1).

2. The sexes are separate and they show a distinct sexual dimorphism. The males measure 40 mm in length and 0.1 mm in diameter.

3. The females are about 80-100 mm in length and 0.24-0.3 mm in diameter.

4. The tail of the male is curved and there are two spicules of unequal length. It also contains a number of genital papillae and caudal alae.

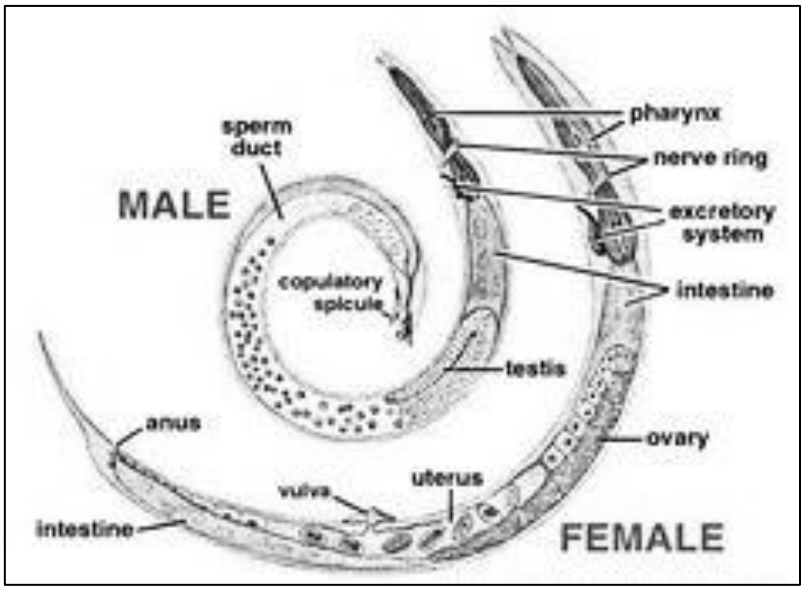

Fig 1: Structure of adult *Wuchereria bancrofti*

5. The body tapers to a slightly swollen head and bearing mouth as a simple pore without lips.

6. The oesophagus is partly muscular and partly glandular.

7. The vulva or genital pore of female opens a little behind the middle and is provided with a pyriform ovijector.

8. The female is ovoviviparous or lays eggs which contain embryos.

The embryo is known as microfilaria (Fig 2). Though they commonly referred to larvae (microfilaria larva) but they should appropriately be termed embryos because their internal organization represents an early developmental stage and they are also not comparable to other nematode larvae.

The microfilariae are very active and can move both with and against the blood stream. They have colourless and transparent bodies with blunt anterior ends and rather pointed tails. A microfilaria measures about 290 pm in length and 6 to 7 pm in diameter.

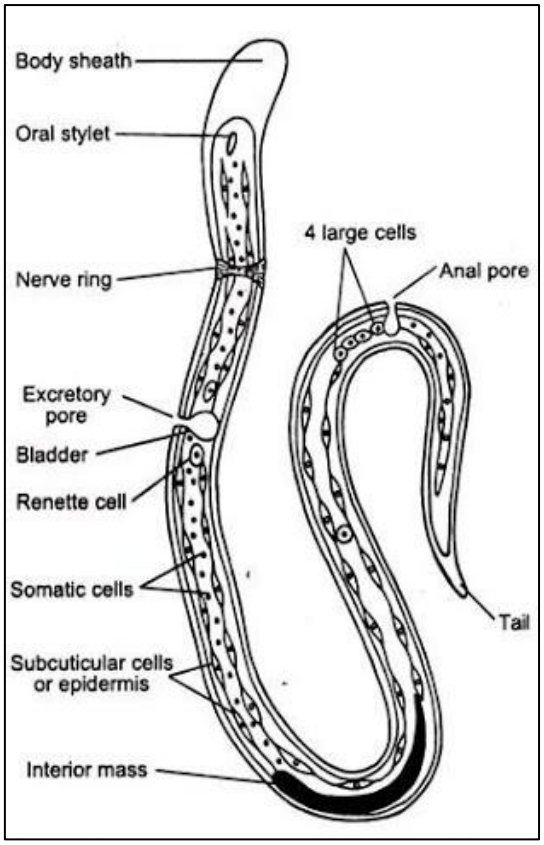

Fig 2: Larval stage (Microfilaria) of *Wuchereria bancrofti*

The body of a microfilaria is covered in a hyaline sheath followed by cuticula being lined by flattened subcuticular cells or epidermis and an inner column of cytoplasm containing nuclei. However, various structures from anterior end downwards are future mouth or oral stylet, nerve ring band, nephridiopore, renette cells and a dark coloured inner mass and four cells of future anus.

The microfilariae do not undergo further development in the human body unless they are taken up by their suitable secondary host (mosquito). If these microfilariae are not sucked up by the mosquito, they die in course of time. Their life span in human body is probably 70 days.

Life Cycle:

The life cycle of *Wuchereria bancrofti* (Fig 3) is completed through the two hosts (digenetic), man and mosquito. Man is the definitive host and the adult worms and the live embryos (microfilariae) inhabit in the lymphatic system and blood stream. Mosquito, the species belonging to genus *Culex, Aedes* and *Anopheles*, all act as intermediate host. *Culex quinquefasciatus*, *Culex fatigans* and in some places a closely related species, *C. pipiens* play a leading role as vectors of periodic form in different parts of the world.

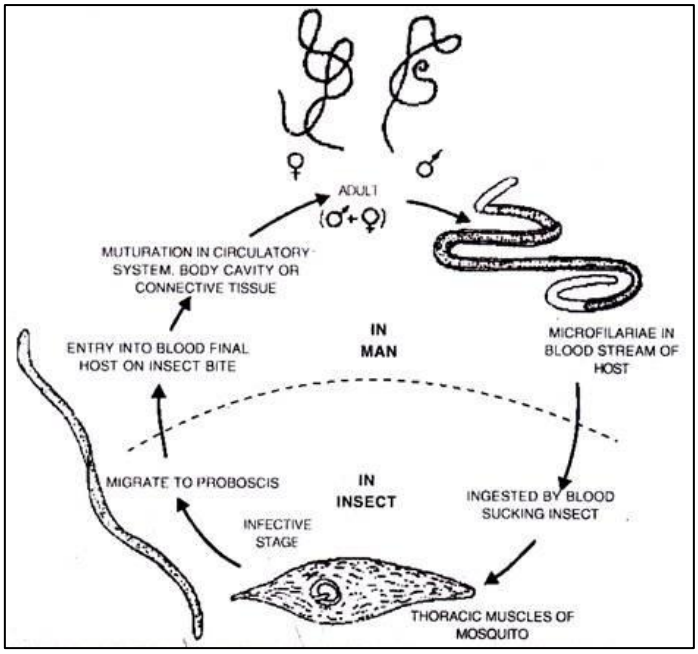

Fig 3: Life cycle of *Wuchereria bancrofti*

Life cycle in man:

When both sexes are found in the same afferent lymphatic vessel of human beings, the copulation takes place and each gravid female gives rise to 1000 minute sheathed larvae called microfilaria. These larvae are 127-320 μm long. They can move both with and against the blood stream.

Each larva is colourless, transparent and cylindrical with blunt head. The body is encased in a hyaline sheath and cuticle is thin, striated bearing underneath a single layer of flattened sub-cuticular cells or epidermal cells. The head bears a clear cephalic space and rudiments of adult buccal cavity with oral stylet. Various structures are seen towards backwards next to oral stylet.

These are nerve ring band, renette cells, a darkly stained interior mass, four large cells and future anus. The caudal end is pointed and without nuclei. The body column is provided with somatic cells being interrupted at intervals by cellular and nuclear land-marks.

There is an anterior shiny spot, called rudimentary excretory pore. The relative positions of these landmarks are definite in their body and they cause no trouble to man even if numerous.

The microfilariae are unable to develop further in the human host unless they are ingested by the mosquito. If not sucked up by the mosquito, the larvae will die.

Life cycle in mosquito:

When the microfilariae are sucked up by the vector mosquito (*Culex fatigans*) along with the blood meal, the larvae pass to the midgut of the insect and lose their sheaths within 2-6 hours. Then the larvae penetrate the gut wall and migrate to the thoracic muscles.

Inside these muscles the organisms become shortened into sausage-shaped forms measuring 124 to 250 μm in length and 10 to 17 μm in breadth.

This stage of development represents the first larval stage in which the first moult occurs. In the first larval stage the tail portion becomes atrophied and possesses the well-defined intestinal tract. Within three to seven days, the larvae grow rapidly, moult once or twice and attain the size which measure about 225-380 μm in length. These represent the second stage larvae.

On the tenth and eleventh day the metamorphosis of the larvae becomes complete inside muscles. The tail portion atrophies and the digestive system, body cavity and genital organs are fully developed. These forms are recognised as third stage filariform larva measuring 1500 to 2000 μm in length and 18-23 μm in breadth.

Then the larva migrates into the proboscis sheath of the mosquito's mouth parts and this stage is infective to man (third stage filariform larvae). It is to be noted that after the second moult third larval stage begins.

Infection to man and development into adult worms:

When the infected insect bites the man, the third stage larvae enter the human host through the site of puncture wound on the skin. Then the larvae reach to the lymph vessels via blood stream and settle down in inguinal, scrotal or abdominal lymphatics.

Within five to eighteen months the larvae undergo two additional moults to attain the sexual maturity and start new generation of microfilariae. In the life cycle of *W. bancrofti*, four moults and five stages are recorded of which fifth stage is the adult.

Conditions for development:

The development of the infective stage (3rd stage larvae) takes a minimum of 8-10 days but more frequently 2 weeks or more. The optimum conditions are 80 °F and 90% humidity.

Nocturnal and diurnal periodicity:

Nocturnal periodicity is the appearance of microfilariae at night in the peripheral circulation of the human host.In the common strain of *W. bancrofti* the nocturnal periodicity is the most common phenomenon but in Polynesian islands of South Pacific except Hawai the microfilariae appear in large numbers in the pulmonary vessels (i.e., blood vessels of the deep tissue of the body) during the day, so it shows a certain degree of diurnal periodicity.

It has been reported that *Aedes pseudoscutellaris*, a mosquito vector of the Pacific strain of *W. bancrofti*, sucks blood during daytime from the body of human's host. During night the microfilariae are found in the peripheral circulation between 10.00 pm to 2.00 am.

Reasons for periodicity:

The exact reasons for nocturnal and diurnal periodicity are not exactly known. In man, the decrease in body temperature, increased O_2 tension, decrease in CO_2 pressure, increased body acidity and relaxation of the host during sleep induce the microfilariae to migrate in the peripheral circulation during night. There is also chemotactic between the microfilariae and the saliva of mosquito hosts (vectors) which induces the microfilariae to be more plentiful in the peripheral circulation at night.

Transmission:

Adult worms' nest in the lymphatic vessels and disrupt the normal function of the lymphatic system. The worms can live for approximately 6–8 years and, during their life time, produce millions of microfilariae (immature larvae) that circulate in the blood.

Mosquitoes are infected with microfilariae by ingesting blood when biting an infected host. Microfilariae mature into infective larvae within the mosquito. When infected mosquitoes bite people, mature parasite larvae are deposited on the skin from where they can enter the body. The larvae then migrate to the lymphatic vessels where they develop into adult worms, thus continuing a cycle of transmission.

Symptoms:

The pathogenic effects seen during filariasis are caused by living or dead adult worms. A light infection does not produce serious effects; it causes filarial fever, headache and mental depression, etc. But, during heavy infection, the accumulated living or dead adult worms block the lymphatic vessels and glands causing lymphatic obstruction so that lymph cannot get back to the circulatory system; resulting the immense enlargement of the affected organs, such as limbs, scrotum, vulva, mammary glands. Ultimately elephantiasis or filariasis or Bancroft's filariasis is produced. It causes filarial fever, headache, mental depression and pain in the swollen area.

Hence, there occurs accumulation of lymph in the affected organs due to which they swell fantastically, a condition called lymphoedema. When they infect lymph nodes then they cause lymphadenitis, in lymph vessels they cause lymphangitis and after infecting epididymis and related areas they cause hydrocele.

However, the affected organs sometimes become enormously enlarged, producing a tumour-like ugly look, this condition is called elephantiasis; the elephantiasis of feet, hands, scrotum, etc., are of common occurrence in the areas where *W. bancrofti* is prevalent.

Prophylaxis:

No proper or satisfactory treatment is yet known.

(i) Oral administration of Hetrazan and compounds of antimony and arsenic may be effective by eliminating microfilariae from the blood circulation.

(ii) Administration of Diethylcarbamazine (DEC) mixed in common salts may be more effective under the National Filaria Control Programme.

(iii) There is no effective drug for elephantiasis.

(iv) Cortisone injection can reduce the swelling.

(v) Large swellings can be removed by surgery.

(vi) Protection from mosquito bites and control of mosquito vectors may be helpful in preliminary level.

Diagnosis:

The standard method for diagnosing active infection is the identification of microfilariae in a blood smear by microscopic examination. The microfilariae that cause lymphatic filariasis circulate in the blood at night (called nocturnal periodicity). Blood collection should be done at night to coincide with the appearance of the microfilariae, and a thick smear should be made and stained with Giemsa or hematoxylin and eosin. For increased sensitivity, concentration techniques can be used.

Serologic techniques provide an alternative to microscopic detection of microfilariae for the diagnosis of lymphatic filariasis. Patients with active filarial infection typically have elevated levels of antifilarial IgG_4 in the blood and these can be detected using routine assays.

Because lymphedema may develop many years after infection, lab tests are most likely to be negative with these patients.

Probable questions:

1. Name the causative agents of filariasis.
2. Name the secondary host of filariasis of India and Pacific Island.
3. Describe the structure if microfilaria with diagram.
4. Write short note on diagnosis of filariasis.
5.

Suggested readings:

1. Frishman A. 2000. Bed Bug basics and control measures. Pest Control 68: 24.
2. Snetsinger R. 1997. Bed bugs & other bugs, pp. 393-425. *In* Mallis A, Hedges SA [eds.], Handbook of Pest Control, 8th ed. Franzak & Foster Co., Cleveland, Ohio.
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Unit- VI

Parasitic insects: *Cimex lectularius* and *Xenopsylla cheopis*

Objective: In this unit you will know about parasitic insects: *Cimex lectularius and Xenopsylla cheopis*.

• *Cimex lectularius*

Introduction:

The common bed bug (*Cimex lectularius*) has long been a pest – feeding on blood, causing itchy bites and generally irritating their human hosts. The Environmental Protection Agency (EPA), the Centers for Disease Control and Prevention (CDC), and the United States Department of Agriculture (USDA) all consider bed bugs a public health pest. However, unlike most public health pests, bed bugs are not known to transmit or spread disease. They can, however, cause other public health issues, so it's important to pay close attention to preventing and controlling bed bugs.

Distribution:

Human dwellings, bird nests, and bat caves are the most suitable habitats for bed bugs because they offer warmth, areas to hide, and hosts on which to feed. Bed bugs are not evenly distributed throughout the environment but are concentrated in harbourages. Within human dwellings, harbourages include cracks and crevices in walls and furniture, behind wallpaper and wood panelling, or under carpeting. Bed bugs are usually only active during the night but will feed during the day when hungry. Bed bugs can be transported on clothing, and in luggage, bedding and furniture. Bed bugs lack appendages that allow them to cling to hair, fur, or feathers, so they are rarely found on hosts.

Habit and Habitat:

Bedbugs are found all over the world. They inhabit dark, damp human dwellings such as old houses, buildings, hotels, hostels, rest houses, barracks, carriages and almost anywhere else. They live in cracks in the walls and floor, in crevices in the beds and furniture, under mattresses, carpets and wall paper and in similar places. The thin bodies of bedbugs are well adapted to life in narrow spaces or crevices.

Bedbugs are nocturnal, but often come out during the day. They are gregarious insects. These are sanguinivorous ectoparasites. They are strongly attracted by the warmth and the odour of the body. They are incapable of flight but they migrate from one house to another along the walls, pipes or drains.

They are carried from one place to another on clothing, in luggage or furniture. If not allowed to reach the beds by placing their legs in water troughs or other barriers, they climb up the wall and move along the ceiling to drop down from there on the beds.

They usually suck human blood, but also attack warm blooded animals such as birds (domestic fowl) and mammals (mice, rats, rabbits, cats and dogs).

They suck human blood while the man is sleeping, after which they quickly run away. Their gregarious habit results in great discomfort to mankind. Bedbugs can survive without food for several months or a year and even longer. The starving bedbugs may feed upon birds, rats, mice and rabbits and may also resort to cannibalism.

Their retiring habits coupled with their power to fast for long periods make their eradication difficult. Bedbugs give out a peculiar kind of odour or foul smell due to the presence of secretion of stink gland. They are oviparous and undergo gradual metamorphosis.

Structure:

The morphology of bed bug is described below:

Adult bed bugs, in general (Fig 1,2) are:

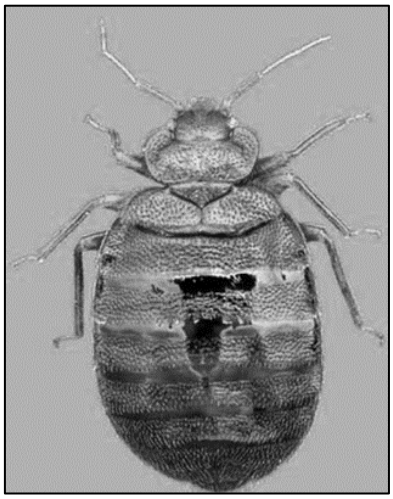

Fig 1: Dorsal view of an adult bed bug, *Cimex lectularius*

- about the size of an apple seed (average 5.5 mm and the width 2.5 mm);
- long and brown, with a flat, oval-shaped body (if not fed recently);
- balloon-like, reddish-brown, and more elongated (if fed recently);
- Females are larger than males.
- A “true bug” (characteristics of true bugs include a beak with three segments; antenna that have four parts; the first segment is shorter than other segments. The three segments closest to the antenna tip are thin and elongated; wings that are not used for flying; and short, golden-coloured hairs)
- The head is short, broad and pointed at the tip and had a pair of prominent red or black compound eyes resembling raspberries.
- Mouthparts or labium are three-segmented and specialized in piercing and sucking and are located on the ventral side of the head. The tip of the labium extends to the level of the first pair of legs.
- The thorax also is three-segmented.

- Smelly, with a “musty-sweetish” odour produced through glands on the lower side of the body.

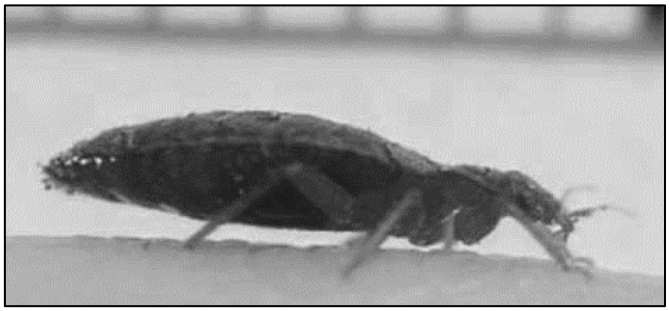

Fig 2: Lateral view of an adult bed bug, *Cimex lectularius*

Young bed bugs (also called nymphs), in general, are (Fig 3):

- smaller, translucent or whitish-yellow in colour; and
- If not recently fed, can be nearly invisible to the naked eye because of colouring and size.

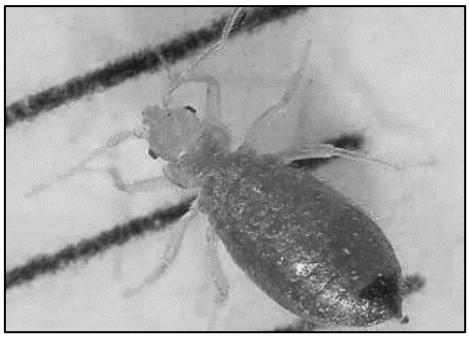

Fig 3: Nymph of the bed bug, *Cimex lectularius*

Bed bug eggs, in general, are:

- tiny, the size of a pinhead;
- pearl-white in colour; and
- Marked by an eye spot if more than five days old.

Life Cycle:

Before laying eggs, the female bug feeds on blood-meal and mates with the male bug. Life history of bedbug exhibits gradual metamorphosis and comprises three stages: egg, nymph and adult (Fig 4).

(i) Mating:

In bedbugs, the mating or copulation is quite interesting. While mating or copulating, the male bug takes up a position diagonally across the body of female bug and introduces its penis into the notch or cleft of the organ of Berlese to transfer the spermatophore. The spermatozoa bore through the wall of organ of Berlese and reach the ovary to fertilize the eggs. Thus, fertilisation is internal.

(ii) Eggs:

The female lays about 200 to 500 eggs, singly or in batches, 2 or 3 eggs per day, in cracks and crevices of cots and furniture, in holes, under mattresses and similar other places. The eggs of bedbugs are pearly white oval or cylindrical objects, furnished with a little cap-like lid at one end which is slightly curved to one side.

The end possessing the cap-like lid bears a micropyle. The eggs are about 1.00 mm in length and are laid singly or in small batches. The eggs are laid throughout the year in warm countries.

(iii) Nymph:

The eggs hatch in from 6 to 10 days during warm weather but take a longer period during cold weather as their development is retarded by cold. The young bugs or nymphs come out by pushing off the lids of the eggs. The nymphs are very small, about 1.00 to 1.50 mm long, flat, active, delicate, semi-transparent creatures and are pale in colour.

They resemble the parents in general appearance except being smaller and paler and possessing comparatively thicker antennae and stouter legs. After a few hours, the nymphs are able to pierce the skin of man and suck the blood, and if undisturbed they feed to repletion in about 3 or 4 minutes.

They may take a meal three or four times of their body weight and become globular and bright red. They need shelter but no more food till they moult into the second stage. After their first hearty meal they have a much more robust appearance, and grow rapidly. They feed on human blood and moult five times to become the adult.

After each moult, the nymphs become slightly larger and darker (Fig 7). If man is not available for feeding, they feed on blood from the older bugs. The nymphs can survive without feeding for 3 or 4 months during which period they do not moult. After five moults they become adult taking about 7 to 24 weeks in all. The entire life history takes about 2 months in warm weather and about 6 months during winter in cold regions.

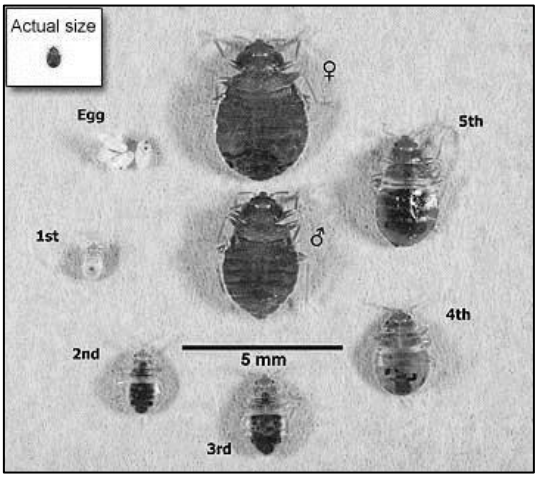

Fig 4: Life cycle and different nymph stages of *Cimex lectularius*

Symptoms:

Cimex lectularius, the bed bug has been suspected of being the cause of many human diseases, but this has not been proved. After identification of bedbug bites, skin and infectious transmissible diseases are the 2 main medical concerns of human contact with the bedbugs. Hosts are usually bitten at night. Small, flat, or raised bumps on the skin are the most common sign; redness, swelling, and itching commonly occur. If scratched, the bite areas can become infected. Bedbug saliva contains anaesthetic compounds, bites are painless and usually not felt until several hours later. Other compounds are also injected: anticoagulant factors (eg, factor-X inhibitor), vasodilatory compounds (such as nitric oxide), and proteolytic enzymes (eg, apyrase), which are all substances that participate in the ensuing local hypersensitivity reactions.

The typical skin lesion is a pruritic erythematous maculopapular, 5 mm to 2 cm in diameter, with a central haemorrhagic crust or vesicle at the bite site, similar to arthropod bites. Atypical forms vary from asymptomatic or pauci-symptomatic to purpuric, vesicular, and bullous lesions. The bedbug-bite distribution frequently follows a line or curve (Figure 8A and 8B). Lesion numbers range from several to many, depending on habitat-infestation intensity, and are preferentially located in unclothed zones (Figure 8*C*). Sometimes, the eruption mimics urticaria (Figure 5).

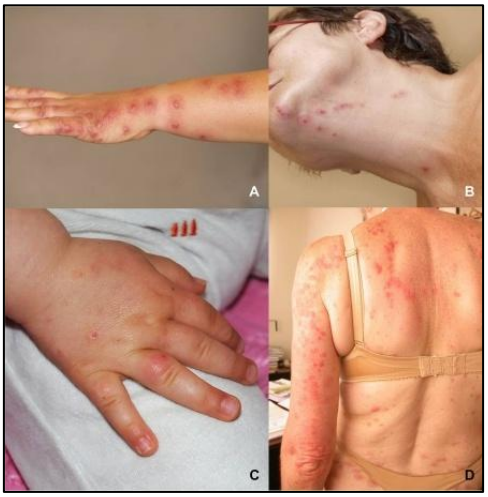

Fig 5: Presentation of bedbug (*Cimex lecturarius*) bites: forms vary from asymptomatic or pauci-symptomatic to purpuric, vesicular, and bullous lesions. The typical skin lesion is a pruritic erythematous maculopapule that is 5 mm to 2 cm in diameter with a central hemorrhagic crust or vesicle at the bite site, similar to other arthropod bites (*A*). A series of bites in a line is characteristic of bedbug bites (*B*). Lesion numbers range from a few to numerous, depending on habitat-infestation intensity, and are preferentially located in unclothed zones (*C*). In some cases, the eruption mimics urticaria (*D*).

In the gut of bed bugs are anti-bacterial substances which do not permit bacteria to live for long. *Cimex* may carry and transmit germs of plague and relapsing fever, this is only for short periods.

Prophylaxis:

1. Typically, no treatment is required for bedbug bites.
2. If itching is severe or if an allergic reaction to the bites occurs, topical steroid creams or oral antihistamines may be used for symptom relief.
3. Secondary bacterial infections that develop over heavily scratched areas may require antibiotics.
4. Anything that relieves and controls itching, such as cool compresses, oatmeal baths, or a paste made of baking soda and water.

Diagnosis:

If you suspect that you're being bitten by bedbugs, immediately inspect your home for the insects. Thoroughly examine crevices in walls, mattresses and furniture. You may need to perform your inspection at night when bedbugs are active.

Look for these signs:

Dark specks. Typically found along mattress seams, these specks are bedbug excrement.

Empty exoskeletons. Bedbugs molt five times before becoming adults. These empty skins are pale yellow.

Rusty or reddish stains. You may find small smears of blood on your bed sheets where you accidentally crushed a bedbug.

• *Xenopsylla cheopis*

Introduction:

The Oriental rat flea (*Xenopsylla cheopis*), also known as the tropical rat flea, is a parasite of rodents, primarily of the genus *Rattus,* and is a primary vector for bubonic plague and murine typhus. This occurs when the flea has fed on an infected rodent and bites a human, although this flea can live on any warm-blooded mammal.

Geographic distribution:

Xenopsylla cheopis usually inhabits tropical and subtropical habitats, although it has been reported in the temperate zone as well. *Xenopsylla cheopis* is rarely found in cold areas since it requires a tropical/subtropical climate to pupate. Fleas are prevalent in many major cities. Species of *Rattus* typically found in city sewer systems and other human related habitats are excellent hosts for *X. cheopis*. Seaports and other rat-infested areas are also common habitats for *X. cheopis*.

Fleas are nidiculous parasites; they live in the host's nest. Clothing, beds and couches make perfect homes for many of these fleas. Fleas only attach to the host while they are sucking blood; at other times they are free-living in the host's nest.

Habitat Regions: temperate tropical terrestrial
Terrestrial Biomes: desert or dune savannah or grassland chaparral forest rainforest scrub Forest Mountains
Other Habitat: Urban, Suburban, and Agricultural

Habit and habitat:

Adults of both sexes of *Xenopsylla cheopis* feed on blood. They bite *Rattus rattus* (Black Rat) and other mammals, including humans. *Xenopsylla cheopis* obtains the host's blood through a set of external mouthparts, which consist of the following maxillary lacunae and an epipharynx. The purpose of each structure is to aid in the sucking up of blood. After biting, the fleas suck blood from a pool (telmophagy), unlike some other insects like mosquitoes that feed directly from the blood vessel (solenophagy).

Piercing of the host's skin is achieved by the back-and-forth action of the maxillary laciniae. After the skin is cut the epipharynx enters the wound and injects salvia. Saliva contains special chemicals, which keep the host's blood from coagulating. A canal formed by the maxillary laciniae and the epipharnyx then sucks up blood. Further down the gut a specialized organ called the proventriclus then breaks down blood cells enabling the *X. cheopis* to digest the blood meal. The average capacity of *Xenopsylla cheopis* is 0.5 cubic millimeters.

The larvae of *X. cheopis* have mandibles, which they use to feed on detritus and the faeces of the adult fleas, which are found in the nests of hosts.

Structure:

Morphological structure of adult *Xenopsylla cheopis* is described below (Fig 6)

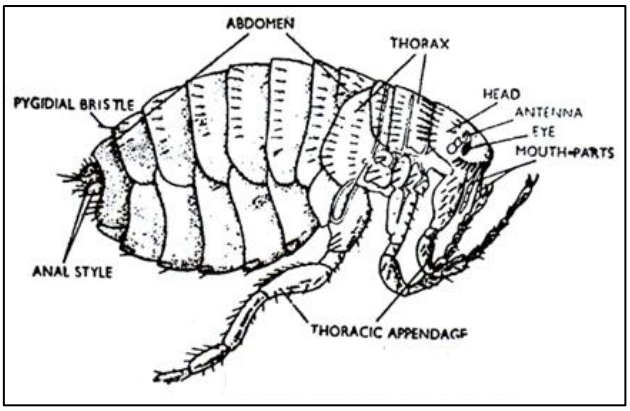

Fig 6: Morphology of *Xenopsylla cheopis*

1. It is commonly known as (Oriental rat flea or Pissu), and is common in tropics.
2. It is an ectoparasite on rats and other such mammals and acts as disease vector for plague.
3. Body is laterally compressed and maybe divided into head thorax and abdomen, which may not be demarcated clearly.

4. The head bears a small 3 to 4 jointed antennae a pair of simple eyes and piercing & sucking or siphoning mouthparts.
5. Thorax bears three pairs of long, jointed and clawed legs for hopping but no wings.
6. Abdomen is swollen in middle and has 8 segments and a pair of anal styles.
7. The whole body is covered over with bristles on the dorsal as well as lateral sides.

Life Cycle:

Fleas are holometabolous, which means they go through four life-cycle stages: egg (embryo), larva, pupa, and adult (imago) (Fig 7).

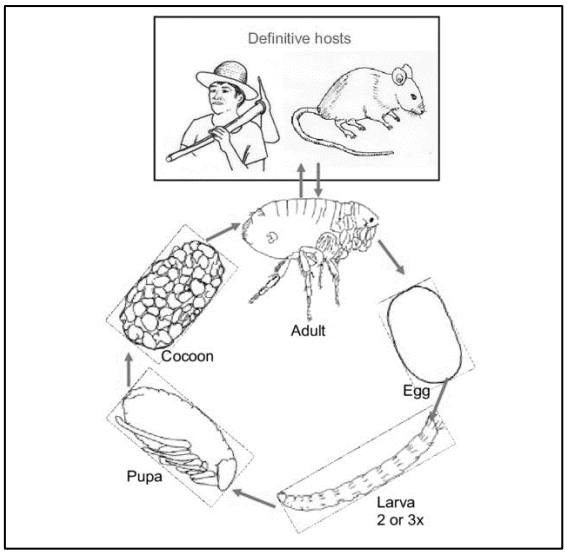

Fig 7: Life cycle of *Xenopsylla cheopis*

No information is available on the mating systems of these fleas.

After copulating with a male, the female is ready to lay her eggs. She does this at frequent intervals while feeding. *Xenopsylla cheopis* prefers temperatures of 65 to 80°F with about 70% humidity for egg laying. Higher or lower temperatures inhibit females from laying their eggs. Eggs usually do not hatch on the hosts, rather on their nests since fleas are nidiculous parasites (they live on host's nests). These fleas breed year-round, as long as the temperature and humidity favour egg-laying. *Xenopsylla cheopis* is distinct from other fleas in that it has a very large egg. Studies demonstrate that eggs of *X. cheopis* obtain extra nutrients from their mother, hence explaining the abnormally large egg. Once eggs are laid, however, they receive no further support from their parents.

They hatch into a larva that looks very similar to a worm and is about 2mm long. It only has a small body and a mouth part. At this stage, the flea does not drink blood; instead, it eats dead skin cells, flea droppings, and other smaller parasites lying around them in the dust. When the larva is mature it makes a silken cocoon around itself and pupates. The flea remains a pupa from one week to six months changing in a process called metamorphosis. When the flea emerges, it begins the final cycle, called the adult stage. A flea can now suck blood from host and mate with other fleas. A single female flea can mate once and lay eggs every day with up to 50 eggs per day.

Transmission:

This species can act as a vector for plague, *Yersinia pestis*. *Yersinia pestis* is transmitted in epidemics from rats to humans via the rat flea, *Xenopsylla cheopis*. Numerous rodents and other mammals serve as reservoirs of *Y. pestis*, some of which have been responsible for cases of human plague.

Naturally acquired plague in people occurs as a result of human intrusion into the zoonotic cycle during or following an epizootic, or by the entry of sylvatic rodents or their infected fleas into man's habitat with infection in commensal rodents and their fleas; this may result in the development of a domestic rat epizootic and epidemic plague. Domestic pets, particularly house cats and dogs, may carry plague infected wild rodent fleas into homes, and cats may occasionally transmit infection by their bites or scratches; cats develop plague abscesses that have been a source of infection to veterinarians.

The most frequent source of exposure that results in human disease worldwide has been the bite of infected fleas (especially *Xenopsylla cheopis*, the oriental rat flea). Other important sources include the handling of tissues of infected animals, especially rodents and rabbits but also carnivores; rarely airborne droplets from human patients or household cats with plague pharyngitis or pneumonia; or careless manipulation of laboratory cultures.

Symptom:

- Bubonic plague: enlarged, tender lymph nodes, fever, chills and prostration.
- Septicaemia plague: fever, chills, prostration, abdominal pain, shock and bleeding into skin and other organs.
- Pneumonic plague: fever, chills, cough and difficulty breathing; rapid shock and death if not treated early.

Prophylaxis:

- People with the plague need to be treated right away. If treatment is not received within 24 hours of when the first symptoms occur, the risk for death increases.
- Admitted to a hospital.
- Receive powerful antibiotics, such as: Gentamicin, Doxycycline (Monodox, Vibramycin, others), Ciprofloxacin (Cipro), Levofloxacin, Moxifloxacin (Avelox), Chloramphenicol
- Oxygen, intravenous fluids, and respiratory support are usually also needed.
- People with pneumonic plague must be kept away from caregivers and other patients. People who have had contact with anyone infected by pneumonic plague should be watched carefully and given antibiotics as a preventive measure.

Diagnosis:

If your doctor suspects plague, he or she may look for the *Yersinia pestis* bacteria in samples taken from your:

- **Buboes.** If you have the swollen lymph nodes (buboes) typical of bubonic plague, your doctor may use a needle to take a fluid sample from them (aspiration).
- **Blood.** You'll generally have *Yersinia pestis* bacteria present in your bloodstream only if you have septicemic plague.
- **Lungs.** To check for pneumonic plague, your doctor will take mucus (sputum) or fluid from your airways using a thin, flexible tube inserted through your nose or mouth and down your throat (endoscopy).

Probable questions:

1. State the morphology of *Cimex lecturarius* with diagram.
2. How can you identify bed bug bites?
3. How can you treat the bed bug bites?
4. Discuss the pathobiology and prophylaxis of *Cimex lecturarius*.
5. Describe the morphology of *Xenopsylla cheopis* with diagram.
6. Briefly discuss the pathobiology of *Xenopsylla cheopis.*

Suggested readings:

1. Frishman A. 2000. Bed Bug basics and control measures. Pest Control 68: 24.
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3. https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/cimex-lectularius
4. https://animaldiversity.org/accounts/Xenopsylla_cheopis/
5. https://www.cell.com/trends/parasitology/abstract/S1471-4922(22)00068-X
6. https://www.inaturalist.org/taxa/271312-Xenopsylla-cheopis
7. https://edis.ifas.ufl.edu/publication/IN1330

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