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

SEMESTER-IV

Parasitology and Immunology
ZDSE(MJ)T-403

Self-Learning Material

DIRECTORATE OF OPEN AND DISTANCE LEARNING
UNIVERSITY OF KALYANI
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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.

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

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

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Theory (Discipline Specific Elective – Major) - ZDSE(MJ)T-403-Parasitology & Immunology

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	V	General consideration of amoebae in man.		
	VI	Coccidia and coccidiosis in birds (with special reference to *Eimeria tenella*).		
	VII	Avian and simian malarial parasites		
	VIII	Comparative characterization of human malaria parasites		
	IX	Zoonoses with special reference to Japanese Encephalitis		
	X	Zoonoses with special reference to Toxoplasmosis		
	XI	Ultra structure of Trypanosomes		
	XII	Structure, biology and control of Reduvidbug		
	XIII	Structure, biology and control of Non biting dipterans		
	XIV	Structure, biology and control of lice.		
	Total counseling session 12hrs.			

Unit I

Structure and biology of *Trichomonas vaginalis*

Objective: In this unit we will discuss about Structure and biology of *Trichomonas vaginalis.*

Introduction:

Trichomonas vaginalis is a flagellate protozoan that parasitizes the human vagina, prostate gland, and urethra. Trichomoniasis caused by *T. vaginalis* is generally believed to be the most common sexually transmitted disease, and more than 170 million people are believed to be infected annually (WHO, 1995a). The parasite is able to colonize the host cells and cause damage to host cells. However, the pathogenesis of the parasite has not been fully understood, and the mechanisms of its transmission and transmission are not fully understood (Murkute et al, 2025).

Structure of *Trichomonas vaginalis*

i. Morphologically *T. vaginalis* has only the trophozoite stage, resembling other trichomonads. The trophozoites are 23 to 39 µm long (including a 8-13 µm body length and 8-15 µm flagella length) and 5 to 8 µm wide. There are 4 anterior flagella, and a fifth flagellum is incorporated within its undulating membrane.

ii. Motility is achieved by the presence of 5 flagella and is described as a "twitching" type of motility

iii. The shallow depression in the anterior end of the body is called the paraflagellar canal

iv. The characteristic undulating membrane is short, relatively speaking, extending only one half of the body length.

v. *T. vaginalis* trophozoites are equipped with an easily recognizable axostyle that often curves around the nucleus and extend posteriorly. The axostyle (3-14 µm in length) is usually extremely obvious, and the undulating membrane extends about

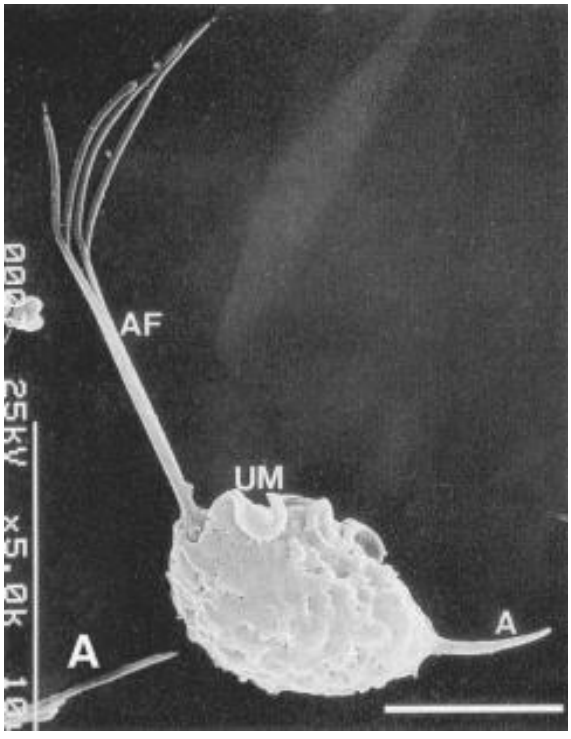

Fig 1. Normal trophozoites of *T. vaginalis* showing 4 anterior flagella (AF), an undulating membrane (UM) with posterior flagellum, and an axostyle

2/3 of the distance to the posterior end of the body, with no free flagellum (Fig. 1).

vi. Nuclear chromatin is uniformly distributed, and there are a large number of hydrogenosomes (formerly called siderophil granules) that are particularly evident around the axostyle.

vii. *T. vaginalis* does not have mitochondria in its cytoplasm. Instead, hydrogenosomes serve as mitochondria, and have been found to be important metabolic features that participate in energy production and drug activation. These hydrogenosomes are round to oval in shape and were so named because they produce molecular hydrogen as end product of metabolism (Ryu and Young Min, 2006).

viii. *T. vaginalis* only exists as a trophozoite; trichomonas has no cystic form.

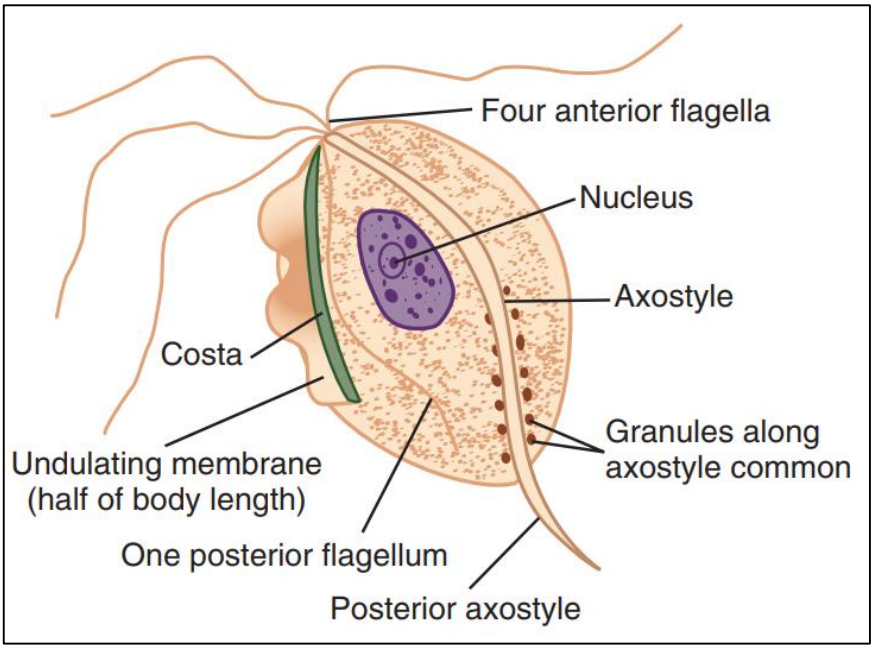

Fig: *Trichomonas vaginalis* trophozoite [Source: https://clinicalsci.info/trichomonas-vaginalis/]

Biology of *Trichomonas vaginalis*

Biology is the study of parasitic relationships, focusing on how parasites and their hosts interact and the biological aspects of these interactions.

Trichomonas vaginalis lives in the reproductive and urinary system of people (obligate parasite). It is an obligate parasite – cannot live without close association with vaginal, urethral or prostatic tissues. Squamous epithelium is affected by this parasite but not columnar epithelium. High incidence of symptomatic infection is seen in women. Zinc and other inhibitory substances probably inhibit their growth in men

Life cycle

- Life cycle of *T. vaginalis* is simple. It is completed in a single host either male or female.
- The infection is transmitted sexually from a woman acting as a reservoir of infection to man.
- In the female, the parasite gets nourishment from the mucosal surface of the vagina and from the ingested bacteria and erythrocytes.
- *T. vaginilis* reproduces by longitudinal binary fission.
- It begins by division of the neuromotor apparatus and finally separation of cytoplasm into two daughter trophozoites.
- Trophozoites are the infective stages. On sexual contact, trophozoites are transmitted to male and localize in the urethra and prostate gland.
- These trophozoites probably undergo replication in the same way as seen in the vagina in females.

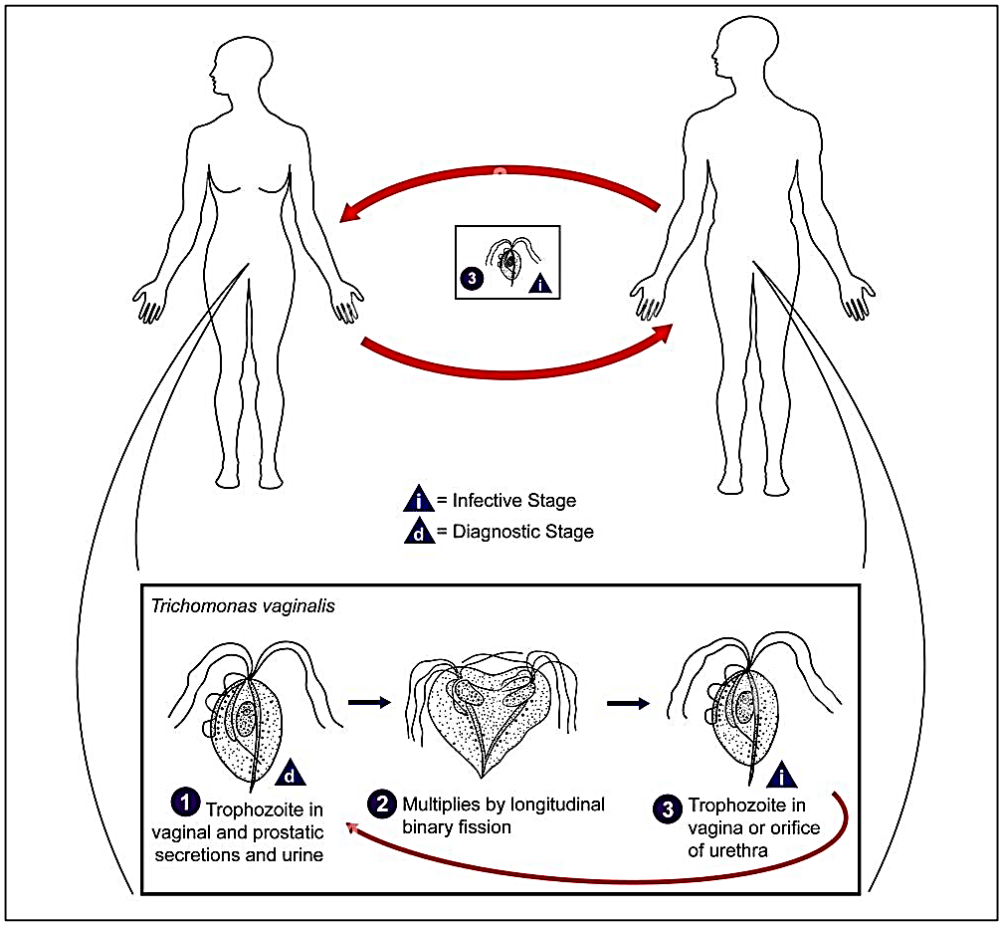

Fig: Life cycle of *Trichomonas vaginalis*

Trichomonas has following virulence factors:

- Protein liquids and proteases: They help in adherence of trophozoites to epithelial cells of the genitor-urinary tract.
- Lactic and acetic acid: it lower the pH of the vaginal fluid. The low pH of vaginal secretion is cytotoxic to epithelial cells
- Enzymes cysteine proteases: it is responsible for haemolytic activity of the parasite.

Mode of transmission;

- Venerally route by sexual contact with infected partners: It is the most common mode of transmission of infection in adolescents and adults.
- Occasionally non-venerally through fomites such as towels, toilet seats etc. and also from mud and water bath as well.
- By perinatal infection in some of female babies born to infected mothers during passage through the infected birth canal.

Pathogenesis and pathology of *Trichomonas vaginalis*

- *T. vaginalis* is an obligate parasite. It cannot survive outside the human body and needs close association of the vaginal, urethral or prostatic tissues for its survival.
- It is not an invasive parasite. It remains adherent to the mucosal epithelium of the vagina or urethra and causes super facial lesions. It infects squamous epithelium but not columnar epithelium.
- In pre-pubertal girls, the vaginal pH is more than 4.7, and the vaginal wall is thin and hypoestrogenic. As the girl attends puberty, the pH of the vagina lowers to less than 4.5. The vagina wall becomes thick and lactobacilli become the dominant flora of the vagina. The lactobacilli are important in protecting the vagina from infection by keeping the vaginal pH lower. The number polymorphonclear (PMN) leukocytes increase with an increase in the vaginal pH. These leukocytes are the key defense mechanisms of the host and they protect the host from the chomotactic substances produced by *Trichomonas*.
- Parasite causes degeneration and desquamation of the vaginal epithelium. Intra-cellular oedema and so called chicken-like epithelium is the most characteristic feature.
- Cellular atypia is a frequent finding. *T. vaginalis* destroys epithelial cells by direct cell contact and also by production of cytotoxic substance. The parasites combine with host plasma proteins, thus escaping from the lytic function of the alternative complement pathway and of the host proteinases.

Clinical features of Trichomoniasis

Although some people develop urethritis, epididymitis, or prostatitis, infection is mostly asymptomatic, particularly in men. It may cause serious pruritic vaginitis in women, accompanied by an offensive yellowish-green frothy discharge, dysuria, and dyspareunia. Erosion of the cervical spine is a natural occurrence. Complications such as endometritis and pyosalpingitis are rare. Trichomoniasis has a 4 days to a month incubation cycle.

I. **Persistent Urethritis**
The disease that symptomatic men develop as a result of a *T. vaginalis* infection is persistent or chronic urethritis. In extreme cases of infection, the seminal vesicles, higher parts of the urogenital tract, and the prostate can be affected. A swollen tender prostate, dysuria, nocturia, and epididymitis are also signs of a major infection.

II. **Persistent Vaginitis**
After a 4 to 28-day incubation cycle, persistent vaginitis causes a foul-smelling, greenish-yellow liquid vaginal discharge in infected women. The exacerbation of symptoms is more likely due to vaginal acidity present before and shortly after menstruation. There might even be burning, scratching, and chafing. Examining the vaginal mucosa of infected women can reveal red punctate lesions. The most frequent symptoms include urethral involvement, dysuria, and elevated frequency of urination. Cystitis is less common.

III. **Infant Infections**
T. vaginalis has been found in children with respiratory infections as well as conjunctivitis. *T. vaginalis* trophozoites migrates from an infectious mother to the child via the birth canal and/or during vaginal delivery.

IV. Other c**omplications:**

- In women, pelvic inflammatory disease is the most important complications.
- In Pregnant women infected with *T.vaginalis* are more likely to have premature rupture of membrane, premature birth and a pre-term or low birth weight baby.
- Prostatitis, epididymitis, urethral stricture and infertility are the common complications in men infected with *T. vaginalis.*
- *T. vaginalis* infection both in women and men is increasingly associated with the gonorrhea, Chlamydia and HIV infections.

Laboratory diagnosis of *Trichomonas vaginalis*

1. In women

- Specimens:

    - vaginal discharge
    - endocervical specimens
- Endocervical specimens are not used for wet mount examination, because small numbers of parasites are present in this specimens. However, the specimens can be collected for culture.

2. In men:

- Specimens: These includes
    - Urethral discharge
    - Prostatic fluid
    - Early morning first voided urine sediment
    - Urethral swab before voiding urine in the morning
    - Less commonly semen

Treatment of trichomoniasis

- Metronidazole is the drug of choice.
- It is given orally either in a single 2g dose or in a dose of 250mg three times daily for 7 days.
- This single dose therapy for trichomoniasis has the advantages of lower total dose, and good patient compliance. The drug produces a cure rate of 95%.
- Treatment of both sexual partners is recommended to prevent recurrence of infection and reduce the reservoir of infection.

Prevention and control of trichomoniasis

1. Detection and treatment of cases either male or female.
2. Avoidance of sexual contact with infected partners
3. Use of condoms

Probable questions:

1. Describe the morphology of *Trichomonas vaginalis*
2. Discuss the biology of *Trichomonas vaginalis* with proper diagram.
3. Describe the life cycle of *Trichomonas vaginalis* with proper diagram.
4. State the clinical features of *Trichomonas vaginalis* infection.
5. What is axostyle in *Trichomonas vaginalis.*
6. What is undulating membrane in *Trichomonas vaginalis*
7. Discuss the pathogenesis of *Trichomonas vaginalis.*

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UNIT II

Structure and biology of *Trypanosoma evansi* and Surra disease

Objective: In this unit we will discuss about Structure and biology of *Trypanosoma evansi* and Surra disease.

Introduction:

Trypanosoma evansi is a protozoan parasite that is the causative agent of the animal disease surra. It is a flagellated hemoprotozoan parasite. It kills thousands of animals every year and causes significant animal morbidity and loss of productivity. The disease occurs in a wide area from the northern part of Africa through the Middle East to Southeast Asia; it is thought to have been introduced to the Americas in the 16th century and is now found in much of Latin America except the southernmost parts. It is not known to occur in North America (except possibly Mexico), Australia, Europe (except for rare introductions into Spain and France), or northern Russia. It affects a very large range of domestic and wild animals; only two cases of human infection have been reported. It has a significant economic and animal health impact on horses, cattle, camels and other livestock in many countries. *T. evansi* is mechanically transmitted primarily by several species of haematophagous flies (mainly Tabanids and Stomoxes), but in Latin America the vampire bat (*Desmodus rotundus*) is a vector and reservoir host. Carnivores can become infected by eating infected meat. Clinical manifestations of disease include fever, anaemia, loss of appetite, weight loss, nervous signs, abortion, cachexia, and potentially death. No vaccine is available. Several chemotherapeutic drugs are used for the prophylaxis and treatment of surra; however, drug resistance is known to occur.

Structure of *Trypanosoma evansi*

i. *Slender in shape.* The mean length of the parasite is 24 ± 4 μm (min 15 μm, max 33 μm)

ii. Small size, compared with *Trypanosoma theileri*, but large compared to *T. Congolense*, thin posterior extremity, free flagellum, active movements

iii. Highly visible undulating membrane

iv. When observed on a Giemsa stained thin smear, *T. Evansi* has always been described as a monomorphic thin trypomastigote parasite. By comparison with *T. Brucei*, it shows mostly slender forms (long free flagellum and thin posterior extremity with subterminal small kinetoplast) (Figure **3**) and some intermediate

forms (shorter free flagellum and posterior extremity with almost terminal kinetoplast).

v. The kinetoplast is larger in *T. vivax* than in *T. evansi*.

Biology of *Trypanosoma evansi*

Biology is the study of parasitic relationships, focusing on how parasites and their hosts interact and the biological aspects of these interactions.

Distribution:

Widely distribution in India, Pakistan, Malaysia, Indonesia, China, Philippines, USA, USSR, Egypt. Sudan, Israel, Lebanon, Turkey, Iraq, Iran, Algeria etc. In India infection more common where insect vector Tabanus are common, where considerable rains & flood. Worse affected areas in northern India are Punjab, U.P., Gujarat & Rajasthan. In Eastern part of India – Bengal, Assam also in Maharashtra & M.P. In other state incidence is low. More common near the end of August to mid-winter

Host

Camel, Horse, Donkey, Mule, Cattle, Buffalo, Elephant, Goat, Sheep, Dog, Cat, Deer & other mammals. Tiger, Fox, Hyena etc. are also harbor the parasite.

Life Cycle

T. evansi transmitted mechanically by *Tabanus*, stomoxys and Haematopota, chrysops, Lyperosia and Hippobosca. Interrupted feeding of flies act as an essential factor as parasite do not survive more than 15 min in the proboscis of the fly. No cyclical development occurs. Non blood sucking fly also transmit by picking the infection from infected meat to open lesion or mucous membrane of susceptible animals. Vampire bat in America also can transmit the infection. The infection may be transmitted during mass vaccination. Dog may get infection by ingestion of infected meat

• Surra disease

Trypanosoma evansi causes a trypanosomosis known as 'surra'. It affects a large number of wild and domesticated animal species in Africa, Asia, and Central and South America. The principal host species varies geographically, but camels, horses, buffalos and cattle are particularly affected, although other animals, including wildlife, are also susceptible. It is an arthropod-borne disease; several species of haematophagous flies, including Tabanids and Stomoxes, are implicated in transferring infection from host to host, acting as mechanical vectors. In Brazil, vampire bats are also implicated in a unique type of biological transmission.

Symptoms & course of the infection:

I. **Horse:**
'Surra' is fatal to horses if treatment is not applied, death occurring in a few days to a few months depending on the virulence of the strain of organism. In horse the course of the disease is more serious than that seen in cattle and buffalo. Donkey and mule are resistant. Intermitted fever with temperature often arising to 44°C & anaemia are main symptoms. Transient local or general urtecarial eruptions may accompany or follow febrile paroxym. The plaques are usually seen on the neck and flanks, oedema of legs and lower parts of the body may be seen. The plaques may necrosed in the center & haemorrhage may occur at the junction of the skin and mucous membrane especially at the nostrils, eyes and anus. The animal becomes dull, listless and leg-weary. Gait becomes staggering & ultimately paraplagia supervenes. Respiration rapid and laboured, and pulse frequent and small. Nervous signs are common in horses.

II. **Cattle & Buffalo:**
Course of the disease varies from symptom less carrier to per acute infection. Cattle and Buffalo main reservoir of infection. Occasional outbreaks of acute disease may occur due to : (1) Introduction of the parasite as a new strain of it into a new area (2) Additional stress Vaccination with FMD or hard work In acute condition Dull and sleepy, staggering gait, eyes staring and wide open, breathing problem, encircling movement, nervous excitement, press head against hard object, apparent blindness, stamping of feet bellowing, groaning, frequent micturition, profuse salivation, shivering of body followed by coma & death in 6-12 hours. Temp rises to 39.40C - 40.60C. Abortions have been reported in buffalos and camels.

III. pyrexia directly associated with parasitaemia together with a progressive anaemia, loss of condition and lassitude are not sufficiently pathognomonic for diagnosis.

IV. Recurrent episodes of fever and parasitaemia occur during the course of the disease.

Pathogenesis

Pathogenesis depend on 3 main factor: Anaemia, tissue lesions like myocarditis & myositis and immuno suppression.

Progressive anaemia with reduction in the numbers of RBC & haemoglobin upto 25% of normal level. Anaemia due to:

1) Haemolysis produce by parasite (Toxin) – Haemolysis of RBC.

2) Immunoglobulin and antigen bound to RBC – erythrophagocytosis.

3) Haemodilution due to increase production of plasma.

4) Dishaemopoiesis

5) Disseminated intravascular coagulation - Leads to anaemia & death Muscle fiber degeneration, mononuclear cell infiltration, oedema & sarcolemma proliferation is responsible for significant myocardial damage and emaciation.

The infection in horse and dog is severe. The factors like progressive anaemia and intravascular coagulation, hypoglycaemia are suspected to be the cause of death of animals in trypanosomiasis. Increase in globulin (Immunoglobulin) and decrease in albumin fraction. Albumin Globulin ratio decreases by 14.49%.

Diagnosis Based on

(1) History of prevalence of infection and biting by Tabanid fly.

(2) Clinical symptoms.

(3) Laboratory examination:

i) Blood & body fluid by direct examination

ii) Chemical tests

iii) Animal Inoculation test

iv) Imminodiagnostic tests.

Prevention and Control

Control of surra can be difficult as there is no vector specificity and a wide range of hosts.

Sanitary prophylaxis

i. Control measures are aimed at the host rather than vector, unlike Nagana. Control measures include detection and treatment of infected animals, prophylactic treatment of susceptible animals, and protection of animals from biting flies and vampire bats.

Medical prophylaxis

i. Drugs such as suramin, prothridium and isometamidium chloride (as a prophylactic) and diminazene aceturate (curative) can be used although drug resistance has been reported.

ii. For camels melarsomine (cymelarsan) is very effective (curative) against *T. evansi*. So far this drug is only registered for use in camels.

iii. No vaccines are available nor likely in the near future because of the ability of trypanosomes to rapidly change their surface glycoproteins to avoid the immune response.

Probable questions?

i. Describe the morphology of *Trypanosoma evansi.*

ii. Describe the life cycle of *Trypanosoma evansi* with proper diagram

iii. What is Surra disease?

iv. Mention the causative agent of Surra disease.

v. Mention the symptoms of Surra disease in horses.

vi. Mention the symptoms of Surra disease in cattles.

vii. Mention the techniques by which Surra disease is diagonosed.

viii. How Transmission of Surra disease can be controlled?

Suggested Literature:

1. Bogitsh, B. J. and Cheng, T. C. (2000). *Human Parasitology*. 2nd Ed. Academic Press, New York.
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UNIT III

Structure, life-cycle, pathology and control of *Trichodina* in fishes

Objective: In this we will learn about Structure, life-cycle, pathology and control of *Trichodina* in fishes.

Introduction

Trichodinids are commensal ectoparasitic protozoa, having direct life cycle and may become pathogenic hindering with respiration and feeding of fishes. The infection rate was affected significantly by the season. The highest infection was recorded in autumn whereas the lowest infection was in summer (Khallaf et al. 2020).

Structure

The characteristic feature of *Trichodina* spp. is its skeletal ring with radially arranged denticles that are readily apparent when viewed dorsoventrally (Figure 1). These organisms have a saucer-to-bell shaped body that is about 84.5 ± 8.2 µm in diameter, numerous (48 to 64) denticulate rings, a highly developed basal adhesive disc, and an adoral zone of cilia arranged in a spiral and are motile. The number, arrangement, and shape of the teeth on the denticle have been used to identify *Trichodina* spp. at the species level by using silver-staining methods (Collymore et al. 2013).

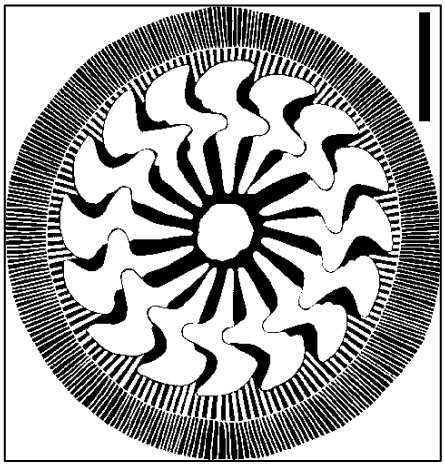

Fig: *Trichodina* sp.

Life cycle

They have a direct life cycle, and they reproduce asexually by binary fission and sexually by conjugation (Mitchell MA. 2007). Species of trichodinads inhabit freshwater, brackish water and salt water. They can colonize the skin, gills, gastrointestinal tract, oviduct, and urinary bladder of their host (Van der Bank et al, 1989). Although they have a sucking disc which they use to attach to their host, they do not feed on the host directly but on suspended organic material and bacteria. These protozoa are transmitted through several different mechanisms, the primary method being direct contact with another infected animal or contaminated water (Kent and Fournie, 2007).

Most species are host specific and presumably spread from fish to fish by incidental contact between susceptible host fish, as well as through contact with the organism in the water column.

Pathology is a branch of medical science that is focused on the study and diagnosis of disease. It includes cause, symptoms and diagnosis of a disease.

Symptoms of *Trichodina* sp. infection

i. *Trichodina* spp. cause damage by feeding on mucus and detritus covering the surface of the gills and skin of the fish causing irritation to the epithelial layer of cells.

ii. This can result in hyperplasia (proliferation) of the epithelial cells, clubbing of the gill filaments and even fusion of the gill filaments. This affects the ability of the gills to maintain optimal respiratory and excretory activities, and the ability of the skin to maintain proper homeostatic osmoregulatory properties.

iii. Massive infestations of these parasites on fish can also directly result in superficial to deep ulcerative skin lesions which then allow for secondary bacterial and fungal infections to develop at the affected site.

Diagnosis

Diagnostic pathology of Trichodina involves identifying the parasite in fish by examining skin scrapes or gill tissues under a microscope. Clinical signs include increased mucus, fin fraying, lethargy, and difficulty breathing if gills are affected. Histopathological changes in infected fish include gill tissue damage like hyperplasia, hypertrophy, and edema.

How can *Trichodina* spp. be controlled? (Smith and Schwarz 2009*)*

There are several methods by which *Trichodina* spp. may be controlled in the aquaculture of food fish. These include chemical treatments, freshwater baths, and flushing. UV is generally considered ineffective due to the high dosage rates required to kill the organism.

I. Chemical Treatment

The only FDA-approved chemical for the treatment of external parasites on food fish is aquaculture-approved formalin. This is probably the best method to date for controlling *Trichodina* spp. infestations in an aquaculture system.

A formalin bath of 170-250 ppm for 60 minutes is the FDA-approved recommendation. A single formalin bath may not completely remove all of the parasites from fish, especially marine fish, and long term or periodic treatments may be needed to keep this parasite under control.

Therefore, a continuous bath of 25 ppm formalin is also approved by the FDA for use on food fish.

In addition, sodium chloride (salt), regarded as a Compound of Low Regulatory Priority by the FDA, may be used at 1.5-3.0 ppt to treat *Trichodina* spp*.* infestations on freshwater fish.

II. Water Bath

Another common method for controlling *Trichodina* spp*.* on marine finfish is to utilize periodic fresh water dips. Though stressful on fish due to increased handling and the osmotic stress, this method can be very effective in reducing the overall number of parasites on fish. This is an effective method for treating individual fish such as broodstock, but may not be a viable option in a production facility due to the logistics associated with handling and treating large numbers of fish.

III. Flushing

Flushing of production systems (i.e., the removal of system water prior to treatment) is another means of reducing infestation levels of *Trichodina* spp. This method may be effective by physically removing any dislodged parasites in the water column from the system.

IV. UV Treatment

Recently, UV irradiation treatment of the water column has been examined as a potential control method for infections caused by ectoparasitic protozoans. A UV dose of 2.2 x 106 µW s/cm2 of the circulating rearing water succeeded in control of *Trichodina truttae* infection in juvenile salmon (*Oncorhynchus keta*).

Probable questions:

i. What is Trichodina?
ii. Describe the structure of *Trichodina sp.* with diagram.
iii. Discuss the pathology of *Trichodina* infection in fish.
iv. Write down the symptoms of *Trichodina* infection in fish.
v. How the *Trichodina* sp. infestation in fish can be controlled?
vi. Describe the diagnostic procedure of Trichodina infection?
vii. Describe the diagnosis and treatment procedure of Trichodina infection.

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Unit IV

Structure, life-cycle, pathology and control of Microspora in insects

Objective: In this unit we will learn about Structure, life-cycle, pathology and control of Microspora in insects.

Introduction

Microsporidia are unicellular, obligate intracellular, spore-forming eukaryotes classified among the protists. As parasites, they have been reported from every major group of animals from other protists to mammals and man. They are economically and medically important and can be found environmentally in terrestrial, marine, and freshwater ecosystems.

There is wide variation in the types of pathologies that can occur in insects as a result of infection with microsporidia. Microsporidian infections are classified as chronic and rarely as acute. Infected insects often exhibit external as well as internal changes as a result of development of the microsporidium. Development of all insect-parasitic microsporidia is restricted to the cytoplasm of the host cell. The chronic infections caused by microsporidia are a significant problem for all types of beneficial insects from honeybees to biological control agents such as parasitoids. The most common method of transmission is through direct oral ingestion of infectious spores found in food or liquids within the insect's immediate environment. The life cycles of entomogenous microsporidia range from relatively simple to the extremely complex (Becnel and Andreadis, 2014).

Structure

Microsporidia in insects are characterized by their spore structure, which includes an outer electron-dense exospore and an inner, thicker, electron-lucent endospore, separated by a unit membrane. These spores also contain a unique, coiled polar filament that extends to form a polar tube, aiding in spore invasion. Microsporidia are obligate intracellular pathogens, lacking mitochondria and instead possessing **mitosomes**.

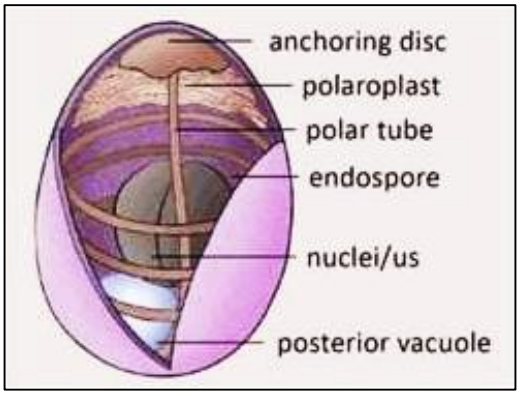

Fig: Morphology and diagram of a microsporidian spore

Microsporidia are obligate intracellular parasites with a unique mode of entering host cells via a polar tube. They are true eukaryotes with a nucleus, chromosome separation and an intracytoplasmic membrane system. However, they lack a classical stacked Golgi apparatus, centrioles, peroxisomes and the mitochondria have been substituted by a mitochondria-like organelle called mitosome. (Keeling 2011).

Microsporidia have a unique structure in nature, the polar tube or polar filament that is involved in host cell invasion. Their infective stage is the spore ranging 1-20µm long, but species infecting mammals are smaller (1-3 µm)

They are gram-positive and environmentally resistant, with a thick wall composed by three layers:

i) An electron dense proteinaceous outer layer, called exospore

ii) An electron lucent inner chitinous layer, called endospore and

iii) A plasma membrane enclosing an infective sporoplasm

The content of the spores is composed of two functionally different parts: the sporoplasm and the extrusion apparatus. The sporoplasm may be considered as the infectious material of microsporidia. It may contain a single nucleus, as it is the case of *Encephalitozoon* and *Enterocytozoon* or two nuclei in *Nosema* and *Vittaforma* with ribosomes and endoplasmic reticulum membranes.

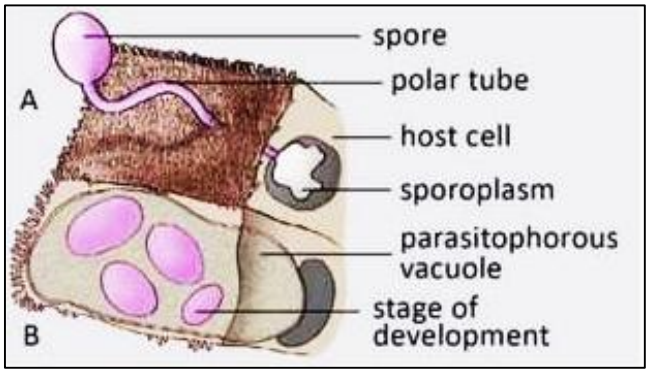

Fig: Life cycle of microsporidia.
Infection phase (A),
Sporogony phase (B)

The extrusion apparatus is composed of a polar tube, coiled around the posterior region of the spore. An anchoring disk, an anterior membrane-bounded organelle, termed polaroplast and a vacuole at the posterior end. The number and disposition of polar filament coils vary among microsporidia showing 5 – 7 coils in a single row in Encephalitozoon species and Enterocytozoon with double row. Under certain condition such as alkaline pH or increased concentrations of Na+, K+, Cl -, Ca ++ ions, the spore germinates and an inflow of water dramatically increases the pressure inside the spore.

Life cycle

the life cycle consists of three phases (Fig.): proliferative, sporogonic, and environmental.

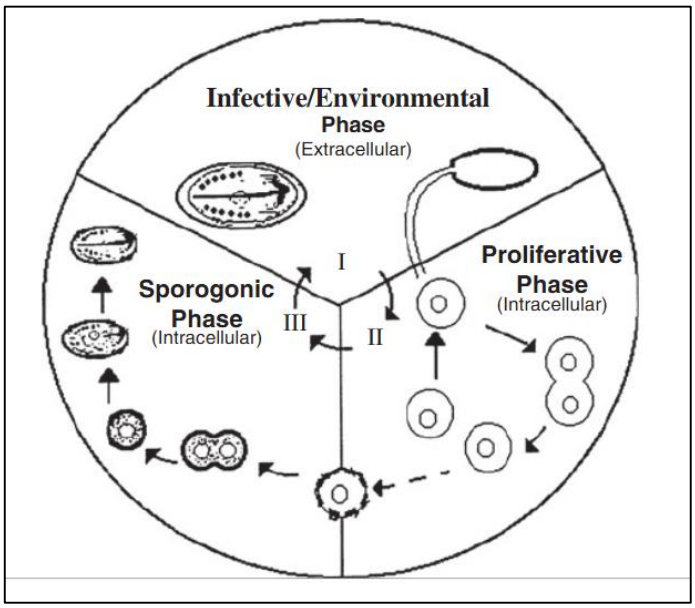

Fig: Developmental cycle of microsporidia

Phase I, the infective/environmental phase, is the only extracellular part of the cycle. It is represented by mature spores shed into the environment from previously infected hosts. Under appropriate conditions, the spores germinate (e.g., if the spores are ingested by an appropriate host, they are activated by the digestive tract environment), this results in the explosive expulsion of the polar filament (which everts becoming a hollow tube). If the polar tube pierces a host cell, the spore contents, the sporoplasm, is injected into it and phase II begins.

Phase II is the proliferative phase, the first phase of intracellular development. During this part of the microsporidian life cycle, organisms are usually in direct contact with the host cell cytoplasm or in a parasitophorous vacuole as they increase in number. The transition to Phase III, the sporogonic phase, represents the organisms' commitment to spore formation. In many life cycles this is morphologically indicated by parasite

secretions through the plasmalemma producing a “thickened” membrane (many also form a surrounding sporophorous vesicle, SPOV).

Phase III, the sporogonic phase is composed of a division sequence called sporogony (three completely different sequences in the polymorphic genera). Meiosis, when it occurs, is initiated in sporont cells, prior to spore formation. Spores fill host cells and may either auto infect after immediate germination within the infected host cell or they may require environmental exposure (environmental phase). These spores pass out into the environment during the life of the infected host, in its waste products or at its death, and are dispersed as a source of infections in new hosts.

Sporont cells are usually distinguished from proliferative stages (meronts) by the presence of an electron-dense surface coat secreted onto the plasma membrane during transition from proliferative cells to sporonts. In some species, an additional layer also develops around the sporonts. This layer forms the sporophorous vesicle which divides, forming groups of organisms that develop into mature spores. Sporophorous vesicle and the presence of the thickened surface coat indicate that the cells are irreversibly committed to spore production and are thus sporonts.

The products of sporont division are sporoblasts, which are cells that undergo morphogenesis, resulting in the formation of spores. The microsporidial spore, containing the unique polar filament complex is the diagnostic stage for the identification of organisms in this phylum.

Mechanism of infection:

The mechanism, by which Microsporidia actively infect host cells is unique. It involves penetrating the plasma membrane without its destruction or the formation of a phagosome. A spore organelle, the polar filament (called polar tube after germination), is only 0.1 μm in external diameter and often exceeding 100 μm in length, within the intact spore it is coiled and anchored by the anterior attachment complex. When the spore germinates, the activated polar structure is everted through the spore wall at this attachment. The sporoplasm (the infective agent) consisting of nucleus and cytoplasm bounded by a membrane passes through the tube and is inoculated into the host cell cytoplasm if the polar tube pierces a cell. This process ensures that the parasite initially lies directly within the host cell cytoplasm, not in a phagosome vacuole derived from host plasma membrane, as is generally the case with parasites internalized by phagocytic processes.

This provides protection against the lytic action of cells, but, even in cases where a vacuolar membrane is later formed around the dividing parasites, fusion of lysosomes does not occur.

Additionally, a more traditional means of entry, via phagocytosis, has been reported. The entire spore may be engulfed by the host cell with subsequent spore

activation and germination resulting in the polar tube extrusion and inoculation of another host cell.

As obligate intracellular parasites, the microsporidia were considered primitive but are now accepted as evolved and well-adapted specialized organisms, with alifestyle incorporating several unique features.

Pathology (Wei et al. 2022)

Microsporidiosis caused by *Nosema bombycis* in **silkworm**, i.e., pébrine, is one of the deadliest diseases seen in the sericulture industry. Transmission of this infection can occur both horizontally and vertically, causing heavy losses or even total crop failure.

Honey bees are key pollinators of both wild plant communities and agricultural crops, they are important to the environment as well as the food supply (Calderone, 2012). *Nosema ceranae* and *Nosema apis* are major causes of microsporidiosis in honey bees (Fries, 2010). *N. ceranae* is now the predominant microsporidium species seen in the western honey bee (*Apis mellifera*), which is the most important bee species for honey production and animal-mediated pollination (Williams et al., 2014).

Microsporidiosis is the most common and harmful eukaryotic pathogen to **shrimp**, and directly threatens shrimp aquaculture. *Enterocytozoon hepatopenaei* (EHP) and *Agmasoma penaei* are well-known species which cause economic losses in shrimp aquaculture. *A. penaei* can infect muscle and connective tissues of giant tiger shrimp *Penaeus monodon* and pacific white shrimp *Litopenaeus vannamei.* Infected shrimp are called “cotton shrimp” or “milk shrimp” due to the whitish or milky appearance seen on various parts of the body. Infected shrimp are not able to be sold leading to economic losses.

Control of Microspora in insects

Microsporidia infections in insects can be controlled through various methods, including heat therapy, chemical treatments, and by manipulating the host's environment. While some microsporidia can be useful for biological control, others cause chronic infections in beneficial insects, requiring targeted interventions.

- **Heat Therapy:**

  Exposing insects to specific temperatures (47°C for 30-45 minutes) can reduce the prevalence of microsporidiosis by exploiting the parasite's lower heat tolerance compared to the host. However, this method can negatively impact host survival.

- **Chemical Treatments:**

Some studies suggest that benomyl and its derivatives can effectively control microsporidiosis in insects. Albendazole and fumagillin, currently approved for human microsporidiosis, are also being explored for use in insect control.

- **Host Manipulation:**

  Manipulating the host's environment, such as reducing spore contamination in food or liquids, can help prevent infection.

- **Biological Control:**

  While not a direct control method, understanding the life cycle and host specificity of microsporidia can help identify potential pathogens for biological control of insect pests.

Probable questions:

1. Write short notes about Microsporidia
2. Describe the microsporidian spore structure with diagram.
3. What is mitosome?
4. What is polar tube?
5. Describe the life cycle of Microsporidia with diagram
6. Describe the proliferative phase of Microsporidian life cycle.
7. State the mechanism of infection of microspore in host body.
8. Discuss the pathology of Microsporidia infection.
9. How does the microsporidia infection can be controlled?

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Unit-V

General consideration of amoebae in man

Objectives:

In this section we will discuss on general consideration of amoebae in man.

Introduction:

Because of their small size, heterotrophic, eukaryotic microorganisms were not detected until Antony van Leeuwenhoek developed his microscopes in the 17th century. He recounted his discoveries to the Royal Society of London in a series of letters covering a period between 1674 and 1716. Among his observations were oocysts of a parasite in the livers of rabbits, the species known today as *Eimeria stiedai*. Another 154 years passed before a second apicomplexan was found, when in 1828 Delfour described gregarines from the intestine of beetles. Leeuwenhoek also observed *Giardia duodenalis* in his own diarrheic stools, and he discovered *Opalina* and *Nyctotherus* species in the intestines of frogs. By mid–18th century other species were being reported at a rapid rate, and such discoveries have continued unabated to the present. Parasitic protozoa still kill, mutilate, and debilitate more people in the world than do any other group of disease organisms. For this reason, studies on these parasites occupy a prominent place in the history of parasitology.

The word Protozoa was once a phylum name. Today, however, the term is used colloquially as a common noun to refer to a number of phyla. Several other nouns, such as Archaezoa, Protoctista, and Protista, have been used to refer to this highly diverse group of microscopic creatures. However, none of these terms, even when used as a taxon name, implies monophyly. Ultrastructural research and the accompanying life cycle and molecular work have shown that organisms once thought to be basically similar are in fact highly diverse and are organized structurally along a number of distinct lines. Thus, most current texts list at least seven phyla of protozoa, and some list over phyla.

- ## Form and Function:

Protozoa consist of a single cell, although many species contain more than one nucleus during all or portions of their life cycles. By mid–19th century, many protozoan genera had been described, and their enormous structural diversity, complexity, and even beauty were widely recognized. Early electron microscopists, as had the light microscopists, found unicellular eukaryotes fascinating subjects, and soon after World War II, researchers recognized that the group was a heterogenous assemblage whose members did not all conform to a single body plan. In 1980 a committee of the Society of Protozoologists revised the classification, recognizing seven phyla, and further revisions

were recommended by a similar group of experts in 1985. More recent classifications, incorporating molecular data, propose groupings that seem quite contrary to those of older systems. An example of the latter is the superphylum Alveolata, which includes dinoflagellates, phylum Apicomplexa, phylum Ciliophora, and Haplosporidia.

Ultrastructural studies have shown, however, that regardless of how elaborate or elaborately arranged they are, most components of protozoan organelles do not differ in any basic way from those of metazoan cells. Indeed, Pitelka concluded "that the fine structure of protozoa is directly and inescapably comparable with that of cells of multicellular organisms," and the "morphologist has to start out by admitting that protozoa are, at the least, cells." Much of the apparent upheaval in eukaryotic systematics is a result of such admission, with the distinctions between unicellular and multicellular organisms becoming quite blurred at the ultrastructural and molecular levels

- **Nucleus and Cytoplasm:**

Like all cells, the bodies of protozoa are covered by a plasma membrane, which is the lipid bilayer, fluid mosaic. Many protozoa have more than one such membrane as part of their pellicle. Additional membranes may be present as alveoli, or sacs, which in some ciliates are enlarged, producing ridges and craters on the cell surface. Protozoa may also possess a thick glycocalyx, or glycoprotein surface coat, which, in the case of parasitic forms, has immunological importance. Other membrane proteins may serve as binding sites that function during uptake of intracellular parasites by their host cells.

Pellicular microtubules may course just beneath the plasma membrane, the number and arrangement of such tubules being typical of a group. The pellicle may be thrown into more or less permanent folds, supported by microtubules, as in gregarine parasites of insects. Or such microtubules may underlie a flexible membrane, as in kinetoplastid flagellates. The structural elaboration of membranes, through folding and addition of electron-dense materials, also occurs in tissue-dwelling cysts. Adjoining membranes may have an electron dense or fibrous connection between them, such as that between the body and undulating membrane of trypanosomes and trichomonads.

Protozoa possess a great diversity of membranous organelles in their cytoplasm. Mitochondria, the organelles that bear enzymes of oxidative phosphorylation and the tricarboxylic acid cycle, often have tubular rather than lamellar cristae. In addition, some amebas have branched tubular cristae, but in other protozoan groups the cristae may be absent altogether. Mitochondria may be present as a single, large body, as in some flagellates, or arranged as elongated, sausage-shaped structures, as occur in pellicular ridges of some ciliates. The Golgi apparatus (dictyosome) is quite elaborate in some flagellates, occurring as large and/or multiple parabasal bodies in association with kinetosomes, the "basal bodies" of flagella, and is present, although not always as prominent, in amebas and ciliates. Dictyosomes can play diverse roles in the lives of

protozoa—for example, they can be the source of skeletal plates in some amebas and polar filaments in microsporidian parasites.

Microbodies are usually, but not always, spherical membrane-bound structures with a dense, granular matrix. In most animal and many plant cells, microbodies contain oxidases and catalase. The oxidases reduce oxygen to hydrogen peroxide, and catalase decomposes hydrogen peroxide to water and oxygen. Thus, microbodies in these cells are called peroxisomes because of their biochemical activity. Peroxisomes are found in many aerobic protozoa in which oxygen is a terminal electron acceptor in metabolism.27 In at least some anaerobes, such as parasitic *Trichomonas* spp., microbodies produce molecular hydrogen and are called hydrogenosomes. Microbodies may also contain enzymes of the glyoxylate cycle, a series of reactions that function in the synthesis of carbohydrate from fat. Microbodies of Kinetoplastida are called glycosomes and contain most of the glycolytic enzymes (which in other eukaryotic cells are found in the cytosol).

Other more unusual membrane-bound organelles include about a dozen kinds of extrusomes, which generally originate in the dictyosome and come to lie beneath the cell membrane. Upon proper stimulus extrusomes fuse with the cell membrane, releasing their contents to the exterior. Extrusomes astoxosomes may release toxic substances, evidently as a defensive mechanism, or function as kinetocysts in food capture, as haptocysts to paralyze prey, or as trichocysts in mechanical resistance to predators. The dark (electron-dense), elongated bodies perpendicular to the cell membrane are mucocysts of a parasitic ciliate, *Ichthyophthirius multifiliis*. Mucocysts are thought to provide a coating that protects the cell against osmotic shock. Not all extrusomes, however, have obvious functions.

The cytoplasmic matrix consists of very small granules and filaments suspended in a low-density medium with the physical properties of a colloid; that is, with the capability of existing in a relatively fluid (sol state) or relatively solid (gel state) condition. Central and peripheral zones of cytoplasm can often be distinguished as endoplasm and ectoplasm. Endoplasm is in the sol state, and it bears the nucleus, mitochondria, Golgi bodies, and so on. Ectoplasm is often in the gel state; under the light microscope it appears more transparent than sol, and in this physical state cytoplasm functions to maintain cell shape. The bases of flagella or cilia and their associated fibrillar structures, which may be very complex, are embedded in the ectoplasm.

Protozoa, like fungi, plants, and animals, are eukaryotes; that is, their genetic material—deoxyribonucleic acid (DNA)—is carried on well-defined chromosomes combined with basic proteins called histones, and the chromosomes are contained within a membrane-bound nucleus. At the light microscope level, protozoan nuclei are typically oval, discoid, or round, and they are usually vesicular, with an irregular distribution of chromatin material and “clear” areas in the nuclear sap. But in ciliates, which contain at least one micronucleus and one macronucleus, the latter may be dense, elongated, chainlike, or branched. Micronuclei are reproductive nuclei, undergoing meiosis prior to

sexual reproduction (conjugation). Macronuclei are considered “somatic”; they function in cell metabolism and growth but do not undergo meiosis.

In electron micrographs nucleoplasm appears finely granular, with aggregations of dense chromatin. Chromosomes may remain as recognizable bodies throughout the cell cycle. Nucleoli are usually present, but they typically disappear during nuclear division. Endosomes, conspicuous internal bodies, are nucleoli, although they do not disappear during mitosis. Parasitic amebas and trypanosomes have endosomes. The term endosome may also be used in reference to vesicles arising by endocytosis.

The nuclear envelope is similar to that of most eukaryotic cells, consisting of two membranes that fuse in the region of pores, but the envelope may be thickened by a fibrous layer or have strange honeycomblike tubes on the outer or inner face. The nuclear envelope may or may not persist during mitosis, again depending on the species, and mitotic spindles can be intra- or extranuclear.

- **Locomotor Organelles:**

Protozoa move by three basic types of organelles: pseudopodia, flagella, and cilia; flagella and cilia are also called undulipodia. Some amebas possess both flagella and pseudopodia, although transformation from flagellated to ameboid cell occurs in response to environmental conditions and is a recognized lifecycle event. Flagella may also occur in large numbers and in rows, thus superficially resembling cilia. In Ciliophora the cilia bases are connected by a complex fibrous network, or infraciliature.

Flagella (undulipodia) are slender, whiplike structures, each composed of a central axoneme and an outer sheath that is a continuation of the cell membrane. An axoneme consists of nine peripheral and one central pair of microtubules (the nine-plus-two arrangement found in cilia and flagella throughout the animal kingdom with a few exceptions). Central microtubules are singlets, but peripheral ones are often doublets or even doublets “with arms.” The central two microtubules are bilateral, and the peripheral ones can thus be numbered with reference to a plane perpendicular to the line between the central pair. The axoneme arises from a kinetosome (basal body), which is ultrastructurally indistinguishable from centrioles of other eukaryotic cells, being made up of the nine peripheral elements, typically microtubule triplets arranged in a cartwheel manner. Kinetosomes may lie at the bottom of flagellar pockets or reservoirs of differing depths, depending on the species. When a flagellate has at least two flagella with differing structures, the condition is termed heterokont.

The entire unit—flagellum, kinetosome, and associated organelles—is called a mastigont or a mastigont system. Kinetosomes are more or less fixed in position relative to other organelles; thus, flagella may be directed anteriorly, laterally, or posteriorly, independent of their movements. Most flagellates have more than one flagellum, and these may be inserted into the cell at different angles. The flagellum may also be bent back along and loosely attached to the lateral cell surface, forming a finlike undulating

membrane, which may be an adaptation to life in relatively viscous environments. Flagellar movements are generally helical waves that begin at either the base or tip, pushing fluids along the flagellar axis. The resulting body movement may be fast or slow, forward, backward, lateral, or spiral. In some cases, such as with trichomonad parasites, movement is highly characteristic and recognized instantly by most parasitologists who have previously studied these flagellates in fresh intestinal contents.

A mastigont system may also include a prominent, striated rod, or costa, that courses from one of the kinetosomes along the margin of the organism just beneath the recurrent flagellum and undulating membrane. A tubelike axostyle, formed by a sheet of microtubules, may run from the area of the kinetosomes to the posterior end, where it may protrude. In phylum Parabasalia, kinetosomes of the three anteriorly directed flagella are numbered 1, 2, and 3, and have lamina (sheets) of microtubules that in cross sections appear either as hooks (kinetosomes 1 and 3) or as sigmoid profiles (kinetosome 2). A Golgi body (dictyosome) may be present; if a periodic fibril, or parabasal filament, runs from the Golgi body to contact a kinetosome, the Golgi body is referred to as a parabasal body. A fibril running from a kinetosome to a point near the surface of the nuclear membrane is called a rhizoplast, and the entire complex of organelles and an associated nucleus is thus referred to as a karyomastigont.

In class Kinetoplastida, which includes the trypanosomes, a dark-staining body or kinetoplast is found near the kinetosome. The kinetoplast is actually a disc made of DNA circles, called kDNA, located within a single large mitochondrion. kDNA has different genetic properties from nuclear DNA. Kinetoplastids also have a paraxial (crystalline rod) that lies alongside the axoneme, within the flagellum. And, finally, many free-living flagellates possess fine fringes or hair like mastigonemes on their flagella, making them look like motile test-tube brushes in the electron microscope. Tubular flagellar hairs with three fine filaments at the tip are a structural character that unites the so-called “stramenopiles”

Cilia (also undulipodia) are structurally similar to flagella, with a kinetosome and an axoneme composed of two central and nine peripheral microtubules. Cilia typically appear to beat regularly, with a back-and-forth stroke in a two-dimensional plane, whereas flagella often appear to beat irregularly, turning and coiling in a three-dimensional space. However, cilia may beat in a helical movement, some flagella beat in a plane, and both types of undulipodia beat in metachronal waves, reminiscent of a field of waving grain, when they occur in large numbers.

Body cilia (somatic ciliature) are arranged in rows, known as kineties, which in turn are composed of kinetids, the basic units of ciliate pellicular organization. Monokinetids contain a single kinetosome and associated fibers; dikinetids contain a pair of kinetosomes; and so on. The pellicle of *Dexiotricha media*, a ciliate found in an Illinois pig wallow, is simple enough to serve as an introduction to ciliate organization. A kinetid consists of the kinetosome; a small membranous pocket, the parasomal sac; and a number of fibers or sheets, made from microtubules, that extend in various directions from the

kinetosome. A tapering banded fibre, the kinetodesma (plural kinetodesmata), arises from the clockwise side of each kinetosome (when viewed from the anterior end of the cell), courses anteriorly, and joins a similar fiber from the adjoining cilium in the same row. The resulting compound fiber of kinetodesmata is called a kinetodesmose. Flat sheets of microtubules, the post-ciliary microtubules, run posteriorly from each kinetosome, and similarly constructed bands, the transverse microtubules, lie perpendicular to kineties. Kinetosomes and associated fibrils constitute the infraciliature. Ciliates differ significantly in the structure of their infraciliature, and such differences are of major taxonomic importance. Obviously, the great diversity in structure is assumed to reflect an equal diversity in functional details.

Oral ciliature can be amazingly complex and is an outstanding example of the elaboration of familiar organelles. Oral membranes are actually polykinetids; that is, fields or rows of cilia and their kinetosomes linked by electron dense fibrous networks. The adoral zone of membranelles is a series of such oral membranes located to the "left" of or counter-clockwise from the side of the oral area of the more complex ciliates. Polykinetids may also be found on the body as cirri (singular cirrus), tufts of cilia that function together, usually in locomotion along a substrate. A group of kinetosomes forming a tuft of ciliary organelles in the aboral region of peritrich ciliates is called the scopula. It is involved in stalk formation.

Cilia beat with a powerful backstroke, pushing the surrounding fluid posteriorly, in metachronal waves. Membranelles have their own beat cycles that are usually independent of the somatic ciliature. When ciliates divide, the ciliature is usually reorganized according to a precise sequence of events. Reorganization of oral polykinetids is a complex process. This "embryological development" has been the basis for much of the class level taxonomy in the Ciliophora, but current classifications also rely heavily on ultrastructural details of body ciliature.

The mechanism by which flagella and cilia move requires ATP and involves the interaction of the arms of each microtubule pair with the neighbouring pair of microtubules. These interactions cause one member of a pair to slide lengthwise relative to the other microtubule in the pair (sliding microtubule model

Pseudopodia are temporary extensions of the cell membrane and are found in amoebas as well as in a variety of cell types in other organisms. Pseudopodia function in locomotion and feeding. In some amoebas, movement is by flow of the entire body, with no definite extensions. Such amoebas are called limax forms, after the slug genus *Limax*. Four general types of pseudopodia occur in amoebas. Lobopodia are finger-shaped, round-tipped pseudopodia that usually contain both ectoplasm and endoplasm. Most free-living soil and freshwater amoebas and all parasitic and commensal amoebas of humans have this kind of pseudopodium. Filopodia are slender, sharp-pointed organelles, composed only of ectoplasm. They are not branched like rhizopodia, which branch extensively and may fuse together to form netlike meshes. Axopodia are like filopodia, but each contains a slender axial filament composed of microtubules that extends into the

interior of the cell. Both pseudopod shape and the shapes of uroids (membranous extensions at the posterior end of the cell) are taxonomic characters in amoebas. Uroids may be bulbous, spiny, morulate (like a grape cluster), or papillate.

Movement by means of pseudopodia is a complex form of protoplasmic streaming involving protrusion of the cell, adhesion to substrate, and subsequent contraction. Evidence suggests that the mechanism requires coordinated structural modification, polymerization, and crosslinking of actin filaments, myosin-mediated filament sliding, adhesion, and deadhesion. Although protoplasmic streaming is well studied, the mechanisms that determine pseudopod shape are not known. Amoebas obviously have some characteristics that function to produce extensions of plasma membrane that are indeed temporary but are also consistent enough in structure so that they may be used in identification and classification. Pseudopod formation is certainly no less wondrous than polykinetid function.

In many apicomplexans (gregarines, coccidia, and malaria parasites), the merozoites, ookinetes, and sporozoites appear to glide through fluids with no subcellular motion whatever. Gregarines, for example, exhibit a variety of slow, sometimes almost snakelike movements, depending on the species and the kind of fresh tissue preparation that is examined. Electron microscope studies reveal longitudinal pellicular ridges (epicytic folds) on these cells, which often appear to have been fixed in the process of forming an undulatory wave. Subpellicular microtubules are found in the folds, and it has been proposed that these fibers function in the gliding locomotion. Experimental work, however, reveals that contact with a substrate is essential to gregarine movement and suggests that mucous secretion may also play a role in locomotion.

- **Reproduction and Life Cycles:**

Protozoan reproduction may be either asexual or sexual, although many species alternate the two types in their life cycles or perform one or the other reproductive functions in response to environmental conditions. Most often asexual reproduction is by binary fission, in which one individual divides into two. The plane of fission is random in amoebas, longitudinal in flagellates (between kinetosomes or flagellar rows; that is, symmetrogenic), and transverse in ciliates (across kineties, or homothetogenic). The sequence of division is (1) kinetosome(s), (2) kinetoplast (if present), (3) nucleus, and (4) cytokinesis.

Nuclear division during asexual reproduction is by mitosis, except in macronuclei of ciliates, which are highly polyploid and divide amitotically. However, patterns of mitosis are much more diverse among unicellular eukaryotes than among metazoa. Examples include nuclear membranes that persist through mitosis, spindle fibers that form within the nuclear membrane, missing centrioles, and chromosomes that may not go through a well-defined cycle of condensation and decondensation. Nevertheless, the

essential features of mitosis—replication of chromosomes and regular distribution of daughter chromosomes to daughter nuclei—are always present.

Multiple fission (merogony, schizogony) occurs in some amoebas and in Apicomplexa. In this type of division, the nucleus and other essential organelles divide repeatedly before cytokinesis. Thus, a large number of daughter cells are produced almost simultaneously and are, theoretically, in the same or similar physiological condition. Cells undergoing schizogony are called schizonts, meronts, or segmenters. Depending on the species, the schizont daughter nuclei may arrange themselves peripherally, with membranes of daughter cells forming beneath the cell surface of the mother cell. The daughter cells are merozoites, and they eventually break away from a small residual mass of protoplasm remaining from the mother cell to initiate another phase of merogony (schizogony producing more asexually reproducing merozoites) or to begin gametogony (gametocyte formation).

Another type of multiple fission often recognized is sporogony, which is meiosis immediately after the union of gametes, typically followed by mitosis. The products of merogony are additional parasites of the same life-cycle stage, such as those that invade red blood cells during a malarial infection. The products of sporogony, however, are usually of a completely different life-cycle stage, such as sporozoites in resistant oocysts ("spores") of gregarines. Several forms of budding can be distinguished. Plasmotomy, sometimes regarded as budding, is a phenomenon in which a multinucleate individual divides into two or more smaller but still multinucleate daughter cells. Plasmotomy itself is not accompanied by mitosis. External budding is found among some ciliates, such as suctorians. Here nuclear division is followed by unequal cytokinesis, resulting in a smaller daughter cell, which then swims away from the sessile parent and subsequently settles, metamorphoses, and grows to its adult size. Internal budding, or endopolyogeny, differs from schizogony only in the location where daughter cells are formed. In this process daughter cells begin forming within their own cell membranes, distributed throughout the mother cell's cytoplasm rather than at the periphery. The process occurs in schizonts of some coccidians. Endodyogeny is endopolyogeny in which only two daughter cells are formed. Protozoans, it seems, are as varied and elaborate in their asexual reproduction as they are in their structure.

Sexual reproduction involves reductional division in meiosis, resulting in a change from diploidy to haploidy, with a subsequent union of two cells to restore diploidy. Cells that join to restore diploidy are gametes, and the process of producing gametes is gametogony. Cells responsible for gamete production are gamonts. Reproduction may be amphimictic, involving the union of gametes from two parents, or automictic, in which one parent gives rise to both gametes. Uniting gametes may be entire cells or only nuclei. When gametes are whole cells, the union is called syngamy.

In syngamy, gametes may be outwardly similar (isogametes) or dissimilar (anisogametes). Although isogametes look similar, they will fuse only with isogametes of another "mating type." Anisogametes often differ in cytoplasmic contents, in size

(sometimes markedly), and in surface proteins that determine mating type. The larger, more quiescent of the pair is a macrogamete; the smaller, more active partner is a microgamete. It is tempting to call these forms female and male, respectively, but it is debatable whether gender, in the commonly used sense, can or even should be distinguished in protozoa. Fusion of a microgamete and macrogamete produces a zygote, which may be a resting stage that overwinters or forms spores that enable survival between hosts.

Conjugation, in which only nuclei unite, is found only among ciliates, whereas syngamy occurs in all other groups in which sexual reproduction is found. Two individuals ready for conjugation unite, and their pellicles fuse at the point of contact. The macronucleus in each disintegrates, and their micronuclei undergo meiotic divisions into four haploid pronuclei (of which two degenerate). A migratory pronucleus from each conjugant passes into the other to fuse with a stationary pronucleus, restoring the diploid condition. The cells separate, and subsequent nuclear divisions produce one or more macronuclei. The exconjugants, which are now genetic recombinants, then actively reproduce by fission. The details of conjugation, including exconjugants' relationships, extent of cytoplasmic sharing, and fate of exconjugants, vary widely among ciliates. Under natural conditions conjugating pairs are seen occasionally, especially when environmental conditions deteriorate. Clone cultures, descended from single individuals, can be prepared in the lab and stressed to produce cells that are ready to conjugate and will do so en masse when mixed with other clones (the mating type reaction). This technique has been useful in the study of mating specificity, genetics, and surface protein function in ciliates. Variations of conjugation are cytogamy, in which two individuals fuse but do not exchange pronuclei, with two pronuclei in each cell rejoining to restore diploidy, and autogamy, in which haploid pronuclei from the same cell fuse but there is no cytoplasmic fusion with another individual.

In Apicomplexa, meiosis occurs in the first division of the zygote (zygotic meiosis), and all other stages are haploid. Intermediary meiosis, which occurs only in the Foraminifera among protozoa but which is widespread in plants, exhibits a regular alternation of haploid and diploid generations.

- **Encystment:**

Many protozoa can secrete a resistant covering and enter a resting stage, or cyst. Cyst formation is particularly common among parasitic protozoa as well as among free-living protozoa found in temporary bodies of water that are subject to drying or other harsh conditions. In addition to providing protection against unfavorable conditions, cysts may serve as sites for reorganization and nuclear division, followed by multiplication after excystation. In a few forms, such as *Ichthyophthirius multifiliis*, a ciliate parasite of fish, cysts fall from the host to the substrate and stick there until excystation occurs. Cellulose has been found in the cyst walls of some amebas, and others contain chitin. Cysts can be highly complex and layered structures, as seen with an

electron microscope in the filamentous cysts of *Giardia* species. The outer layers may also react with immunodiagnostic reagents, although not always in a highly specific manner.

Conditions favouring encystment are not fully understood, but they are thought in most cases to involve some adverse environmental events such as food deficiency, desiccation, increased tonicity, decreased oxygen concentration, or pH or temperature change. It is vitally important for parasitologists to understand the elusive factors that induce cyst formation within the host, the role that cysts play in completion of a parasite's life cycle, and factors that work to disseminate cysts. For example, human amebiasis, caused by *Entamoeba histolytica*, is spread by persons who often have no clinical symptoms but who pass cysts in their feces.

During encystment a cyst wall is secreted, and some food reserves, such as starch or glycogen, are stored. Projecting portions of locomotor organelles are partially or wholly resorbed, and certain other structures, such as contractile vacuoles, may be dedifferentiated. During the process or following soon thereafter, one or more nuclear divisions can give a cyst morecytokinesis occurs in a characteristic division pattern after excystation. In coccidians the cystic form is an oocyst, which is formed after gamete union and in which multiple fission (sporogony) occurs to produce sporozoites. In eimerian coccidians, oocysts containing sporozoites serve as resistant stages for transmission to new hosts, whereas in haemosporidians (including the causative agents of malaria, *Plasmodium* spp.) oocysts serve as developmental capsules for sporozoites within their insect host.

In species in which the cyst is a resistant stage, a return of favorable conditions stimulates excystation. In parasitic forms some degree of specificity in the requisite stimuli provides that excystation will not take place except in the presence of conditions found in a host's gut. Mechanisms for excystation may include absorption of water with consequent swelling of the cyst, secretion of lytic enzymes by the protozoan, and action of host digestive enzymes on the cyst wall. Excystation must include reactivation of enzyme pathways that were "turned off" during the resting stage, internal reorganization, and redifferentiation of cytoplasmic and locomotor organelles.

- **Feeding and Metabolism:**

Some protozoa are photosynthetic and synthesize carbohydrates in chloroplasts, the organelles of "typical" plants. Zooxanthellae (dinoflagellates) are very important mutuals living in cells of reef-forming corals and other invertebrates (including some other protozoa), contributing significant amounts of carbohydrates to their hosts.

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

micropore is present in *Eimeria* and *Plasmodium* species and, in certain stages, is involved in taking in nutrients.

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

Like most other eukaryotic cells, protozoa generally carry out the many reactions of glycolysis, Krebs (citric acid) cycle, pentose-phosphate shunt, electron transport, transaminations, lipid oxidations and syntheses, nucleic acid metabolism, and the multitude of other metabolic events that make biochemical pathways look like printed circuits of high-tech electronic equipment. ATP is the most common form of immediately usable energy, although a few parasites use inorganic pyrophosphate in a similar role. Polysaccharides, especially glycogen or related molecules, function as deep energy storage. Genes are transcribed in the nucleus, and polypeptides are synthesized on ribosomes, as in other cells.

Comparative biochemical studies reveal that details of protozoan metabolism are as varied as the details of protozoan sex. Some important biological factors to consider are that many parasites occupy environments in which the oxygen supply is quite limited. Others live in tissues, such as blood, where neither oxygen nor glucose is limited. In the latter case, there is no energy advantage in completely oxidizing glucose. Organisms that are adapted to such environments, including many protozoan parasites, often derive all their energy from glycolysis and excrete the partially oxidized products as waste. The complete Krebs cycle and cytochrome system then become excess metabolic machinery, at least in terms of energy production. However, the problem of reoxidation of reduced NAD remains, because oxidized compounds must be available for continuous functioning of glycolysis. In some parasites the electrons are transferred to pyruvate, and the resulting ethanol or lactate is excreted, although many organisms excrete such compounds as succinate, acetate, and short-chain fatty acids as end products of glycolysis.

Metabolic flexibility is a feature of obligate heterotroph protozoa. For example, the Krebs cycle requires a continuous supply of the 4-carbon molecule oxaloacetate, one of the cycle's own end products, as an acceptor of 2-carbon units during formation of citric acid. Krebs cycle intermediates are routinely taken out of circulation and used in synthetic reactions such as transaminations. Thus, an alternate source of oxaloacetate is required, which in many protozoans is the glyoxylate cycle, a metabolic pathway especially important in those species that rely heavily on ethanol, fatty acids, and acetate for their energy and carbon skeletons. The glyoxylate cycle uses two acetyl-CoA molecules to make a single oxaloacetate molecule; the enzymes for this cycle are found in the glyoxysomes (peroxisomes).

Protozoa also may utilize a variety of hydrogen acceptors in the final oxidations coupled with ATP production. In aerobic metabolism of most animals, this final acceptor is molecular oxygen. Under anaerobic conditions protozoa may produce lactic acid or ethanol by using pyruvate as a hydrogen acceptor. Ciliates of genus *Loxodes* evidently use NO_3– as a terminal hydrogen acceptor in the mitochondria and contain enzymes more typical of bacteria than eukaryotes to carry out this feat. In parasitic protozoa without mitochondria—*Trichomonas vaginalis*, *T. foetus*, *Giardia duodenalis*, and *Entamoeba histolytica*—the final acceptor can be pyruvate, a key molecule in carbohydrate metabolism, in which case the end product is lactate or ethanol. These protozoa take up molecular oxygen, but availability of oxygen makes little or no difference in their energy metabolism. Absence of mitochondria has been variously interpreted as either a primitive character, reflecting an ancient evolutionary origin, or a derived character resulting from secondary loss.

Odd and parasite-specific metabolic pathways are, of course, inviting targets for chemotherapy. Some of the more effective antimalarial drugs interfere with the parasites' ability to metabolize 1-carbon units during nucleic acid synthesis. Intracellular stages of the flagellate genus Leishmania do not build their nucleic acid precursors but instead salvage them from their host cells. Allopurinol, a purine analog, cannot be metabolized by the parasites but can be taken up from the host cell and used to build nucleic acids that do not function properly in the parasite.

Many parasitic protozoa are intracellular. In some, entry into a host cell is by host phagocytosis of the parasite. An example is *Leishmania donovani*, which is eaten by free-roaming macrophages and reticuloendothelial cells. The host cell forms a membrane-bound parasitophorous vacuole around the parasite, but instead of killing the parasite with digestive enzymes, as might be expected from a macrophage, the host cell provides it with nutrients. Members of the important apicomplexan genera *Babesia*, *Eimeria*, *Plasmodium*, and *Toxoplasma* are all intracellular at least at some stages in their lives, and uptake is by active invasion of host cells by motile infective stages, probably aided by digestive secretions. Microsporidians employ a different mode of entry into host cells. These parasites' cyst stages contain a coiled, hollow filament that evidently is under great pressure. When eaten by a host, which is usually an arthropod, this tubule is forcibly extruded from the cyst and penetrates an adjoining host cell. The organism within the spore (sporoplasm) then crawls through the tube and enters its host. In this case the membrane of the parasite is in direct contact with the cytoplasm of the host, with no vacuole being formed around it.

- **Excretion and Osmoregulation:**

Most protozoa appear to be ammonotelic; that is, they excrete most of their nitrogen as ammonia, most of which readily diffuses directly through the cell membrane into the surrounding medium. Other sometimes unidentified waste products are also produced, at least by intracellular parasites. After these substances are secreted, they are

accumulated within their host cell and, on the death of the infected cell, have toxic effects on the host. Carbon dioxide, lactate, pyruvate, and short-chain fatty acids are also common waste products.

Contractile vacuoles are probably more involved with osmoregulation than with excretion per se. Because free-living, freshwater protozoa are hypertonic to their environment, they imbibe water continuously by osmosis. Contractile vacuoles effectively pump out the water. Marine species and most parasites do not form these vacuoles, probably because they are more isotonic to their environment. However, Balantidium species have contractile vacuoles.

Probable questions:

1. What do you mean by glycocalyx?
2. Briefly elaborate the structure of different cytoplasmic organelles in protozoa.
3. Comment on the locomotor organelles of protozoa.
4. What is mastigont system?
5. What are the different types of reproductive mechanisms found in protozoa?
6. Explain the mechanism of encystment in protozoa.
7. What are the different metabolic pathways prevalent in protozoa?
8. What is the function of contractile vacuole?

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References:

Unit- VI

Coccidia and coccidiosis in birds (with special reference to *Eimeria tenella*)

Objectives:

In this section we will discuss on Coccidia and coccidiosis in birds (with special reference to *Eimeria tenella*).

Introduction:

Chickens are major species globally adapted to a range of climatic conditions in which humans live and play an important role in supplying animal-derived proteins to improve human nutrition.

Coccidiosis is recognized as a pandemic disease of broilers caused by the intracellular protozoan parasites of the genus *Eimeria*. Although coccidiosis is known for many years, it is still considered as the major economical parasitic condition affecting poultry industries. The major part of these economic damages is due to losses in performance including mortality, weight loss and reduced nutrition digestibility resulting in malabsorption caused by gut damage. The minor part of these economic damages is due to the costs of therapy and prophylaxis.

For controlling coccidiosis in broilers, various preventive medications have been approved for use globally and approached, but resistance is increasingly important as no new anticoccidial compounds are known to be under development. Therefore, the purpose is to provide available information on coccidiosis in broilers and to highlight etiology, life cycle and preventive measures.

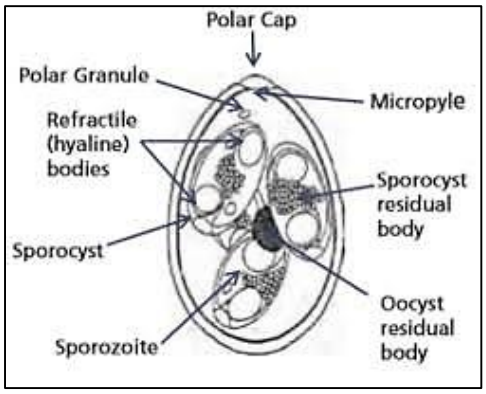
Etiology:

Coccidiosis in broilers is caused by the intracellular protozoan parasite in the genus *Eimeria* family Eimeridae order Eucoccidiorida and phylum Apicomplexa. Seven species of *Eimeria* (*E. acervulina*, *E. brunetti*, *E. maxima*, *E. mitis*, *E. necatrix*, *E. praecox*, and *E. tenella*) are recognized to infect chickens. All the *Eimeria* spp. are very host-specific and sites of development are certain sites of the intestinal tract in broilers with different pathogenicity. Concurrent infection with at least six species is prevalent in a single flock causing marked, independent, recognizable diseases leading to the subclinical intestinal infection to sub-acute mortality.

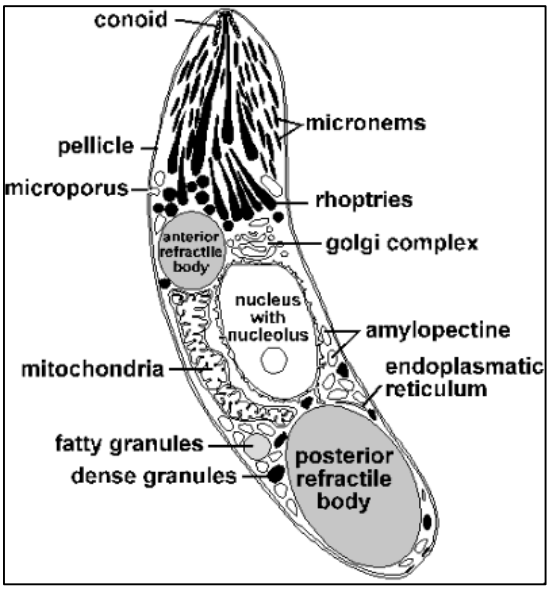
Morphology:

Oocyst of *Eimeria* spp. is a robust wall resistant to both mechanical, chemical and proteolytic degradation. Its wall has been associated with a bilayer structure composed of lipid and protein. The protein layer provides great stability against extreme cold and

heat while the lipid layer supplies a cushion against chemical damage. There are two types of oocysts depending on the infectious ability. Sporulated oocysts are infectious while unsporulated oocysts are non-infectious. The sporulated oocyst can survive up to 602 days in the external environment of its host whereas unsporulated oocysts can survive for seven months in the host caecum. The oocyst is unsporulated and non-infectious in the majority of cases whereas unsporulated oocysts can be converted into sporulated oocysts with appropriate temperatures and humidity. This process is known as sporulation and sporulated *Eimeria* oocyst is composed of four sporocysts and each with two sporozoites and the *lsospora* has two sporocysts, each with four sporozoites. Oocysts of distinct species show contrasts of size (area, diameter), contour (elliptic, ovoid, circular), thickness, internal structure and colour of the oocyst wall among other morphological variations.



Oocyst



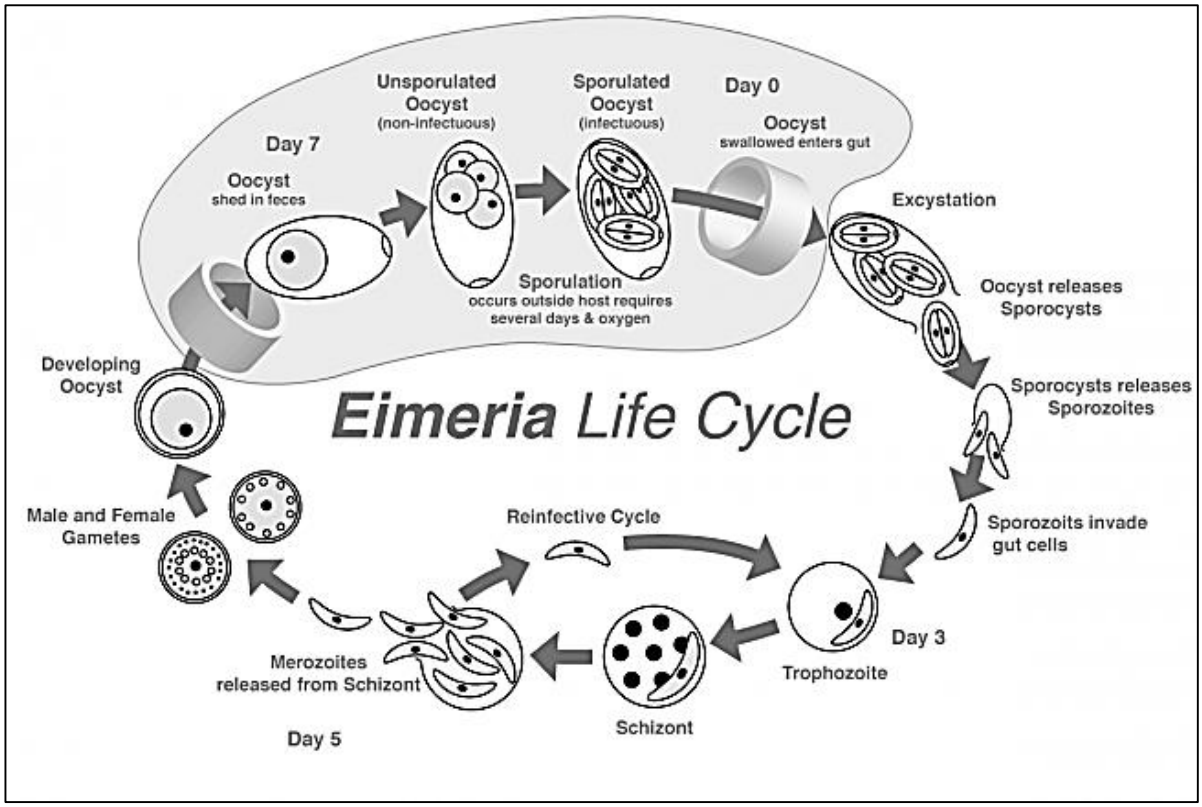
Sporozoite

Life Cycle:

Eimeria species follow a complex life cycle, consisting of two developmental stages namely the exogenous phase (sporogony; occurs in the outer environment) and the endogenous phase (schizogony/merogony and gametogony). The life cycle of all *Eimeria* species entails two or more generations of an asexual development (schizogony), then merogony and followed by a sexual phase known as gametogony which contributes to the formation of the oocyst. Under suitable conditions of temperature and moisture unsporulated oocysts develops within 24–48 hours to form a sporulated oocyst. The sporulated oocyst possesses four sporocysts, each containing two sporozoites (the infective agent). These oocysts can remain viable in the litter for a prolonged period (months to years) under conditions upon ingestion by a suitable host. Birds' intake the sporulated oocyst by pecking in the litter for bedding or on the ground. This process is the action of mechanical and chemical (i.e., trypsin, bile salt, carbon dioxide) factors that leads to the release of sporocysts and then sporozoites within the intestinal lumen of birds.

Excystation contributes to the opening of the anterior cap of sporocysts for releasing sporozoites. Interactions between the apical complex and the plasma membrane of epithelial cells permit sporozoites to penetrate the host cell. The released sporozoites penetrate directly into host intestinal epithelial cells in a specific site of the intestine determined by the species of *Eimeria*. After the invasion, sporozoites convert into a growing stage named trophozoites, which get enlarged and divide asexually to generate a large number of merozoites (known as merogony). Merozoites are separated from the original infected host epithelial cell to infect new cells in both the small and large intestine, developing on the second cycle of merogony. In the last merogonic cycle, the resulting merozoites invade adjacent epithelial cells undergoing the sexual phase of the life cycle known as gametogenesis. During gametogenesis, these merozoites develop into either microgametes or macrogametes. Microgametes give rise to the release of numerous minute biflagellate microgametes, that exit, seek, and fertilize the macrogamete then form a zygote. This stage is the immature oocyst.

The diploid zygote produced during fertilization forms the oocyst wall capacity strong resistance and invades the intestinal lumen. Ultimately, Sporulation arises within infective sporozoites that grow and pollute the environment to begin a new cycle. The life cycle of *Eimeria* generally takes 4–6 days.



Pathogenicity:

Pathogenicity in *Eimeria* deteriorates with boosting the number of sporulated oocysts ingested by the host. Infection of *Eimeria* impairs the intestinal epithelial cells

and tissues, destabilizing the gut of chickens. As a result, coccidiosis leads to disorder digestion of feed and nutrient absorption, dehydration, blood loss and loss of skin pigmentation. Moreover, *Eimeria* spp. change the overall morphology of the intestinal microbiota in the gut, truncating intestinal villi which result in a decreased ability for the digestion and absorption of nutrients.

Clinical Sign:

Coccidiosis of broilers affects two forms as sub-clinical and clinical. In sub-clinical coccidiosis, the symptom demonstrates poor weight gain and deteriorated feed efficiency, reduced growth rate and causes an enormous proportion of the economic loss. Clinical signs of coccidiosis are on account of the destruction of the intestinal epithelium and underlying connective tissue of the mucosa. It may go along with haemorrhage into the lumen of the intestine as symptoms advance. Accordingly, the clinical form of coccidiosis appears in weight loss, reduced feed intake, paleness, ruffled feathers, depression, pale colour comb, wattles, huddling, closed eyes, diarrhoea with bloody faeces, dehydration and increased number of mortalities may accompany.

Diagnosis:

Lesion scoring

Lesion scoring is the method to interpret poultry coccidiosis by observing each of the intestinal tracts for macroscopically visible lesions which are following a scoring system from 0 to 4 (i.e., 0 = healthy or no sign of infection, 1 = mild lesions, 2 = moderate lesions, 3 = severe lesions, 4 = extremely severe lesions) caused by *Eimeria*. This method is work-intensive by investigating post-mortem, occasionally subjective and only dependable when performed by veterinarians or highly trained individuals.

Detection of oocyst in faeces

Eimeria oocysts can be acquired easily from the faeces of infected birds hence using oocyst character is a non-invasive method to determine the causative species in chickens.

Qualitative techniques for faecal examinations

The most extensively used method for the concentration of parasite oocysts is flotation. A solution with a higher specific gravity (i.e., saturated NaCl + glucose) is used to float the relatively low specific gravity coccidial oocysts to the surface and remained other debris in faeces at the bottom of the solution.

Quantitative techniques for faecal examinations

This method is carried out to determine the number of oocysts per gram of faeces (OPG count) together with the percentage of sporulation and oocyst dimensions.

Typically, the McMaster chamber method is used for counting *Eimeria* oocysts in the faecal solution and for establishing the individual oocysts shedding pattern of an infected bird. The floatation method of oocysts is identical to the quantitative technique and uses the McMaster sliced to facilitate easy counting through the light microscope. Nevertheless, expertise is required to identify the different species based on the morphology of oocysts.

Molecular biological diagnosis

Although traditional methods such as lesion scoring and oocyst detection in feces are still being used, molecular biological methods are applicable in most research since the above-mentioned traditional methods are time-intensive and in the case of detection of oocyst, it is hard to distinguish what kind of oocysts are there.

Previous techniques distinguishing of different species were based on the isoenzyme patterns of oocysts and rRNA and rDNA proves. Polymerase chain reaction (PCR) is a rapid, accurate and highly sensitive molecular diagnostic technique to identify the *Eimeria* spp. in chickens by inspecting their variations of genomic DNA. This method amplifies the chicken coccidian species-specific DNA sequence for the detection and discrimination to hundreds of millions for a few hours and the new copies can be divided by electrophoresis to visualize it under UV light by a fluorescent dye.

Treatment, Control and Prevention:

Anticoccidial drugs

Chemoprophylaxis for the treatment and prevention of coccidiosis has been accomplished by the inclusion of a range of anticoccidial drugs in the feed or drinking water to the chickens. Anticoccidial drugs can be classified into three categories as follows: i) Synthetic compounds, ii) Ionophores/polyether antibiotics, iii) Mixed products of the synthetic compounds and ionophores. Unfortunately, most of the anticoccidial drugs became ineffective due to drug resistance.

Synthetic compounds

Synthetic compounds are manufactured by chemical synthesis and are often known as ‘chemicals. Synthetic drugs work by preventing different biochemical pathways of the developing parasite metabolism. Sulfonamides, nicarbazin, clopidol, quinolones, amprolium, halofuginone are chemical components’ examples of commonly used synthetic drugs.

Ionophores/polyether antibiotics

Ionophores/polyether antibiotics are manufactured by the fermentation of *Streptomyces* spp. or *Actinomadura* spp. and arrest the ion (i.e. sodium, potassium) transport channels and hinder the osmotic balance of the coccidian species. The groups of ionophores can be classified into three categories as follow: i) Monovalent ionophores (monensin, narasin,

salinomycin), ii) Monovalent glycosidic ionophores (maduramicin, semduramycin), iii) Divalent ionophores (lasalocid).

Vaccination

The intensive use of anticoccidial drugs which resulted in the development of resistance, chemical residues and environmental pollution of poultry products promoted research on alternative control methods such as early application of vaccines or the development of new drugs. There are two types of available live vaccines for the immunization of chickens as follows: i) virulent vaccines, ii) attenuated vaccines.

Virulent vaccines

Virulent (Unattenuated) vaccines are comprised of *Eimeria* species originating from laboratory or field strains, which have not been modified in any way to change their pathogenicity. Although these vaccines induce a healthy immune activity in the poultry, the exposure to the oocytes of *Eimeria* must arise again through the litter to maintain the immune status to the same level as birds saved previous induced immune activity. Furthermore, it is necessary to inoculate the recommended dosage of virulent vaccines to avoid clinical coccidiosis from the birds.

Attenuated vaccines

Attenuated vaccines are produced downgrading the virulence of *Eimeria* species artificially, making minimum damage to the intestinal epithelium after one passage through the gut. Notwithstanding these vaccines are safe, the price of their production is high because of the decreasing fecundity of precocious parasites, and 2–3 cycles of *Eimeria* are requisite for maximum acquired immunity.

Phytogenic compounds

Phytogenic compounds are a wide range of plant-derived products that find use as prophylactic agents in enhancing performance, productivity as well as alleviating harmful effects of coccidiosis. Furthermore, these compounds include antioxidant, antimicrobial, antiparasitic and antiviral properties. Currently, various studies related to phytochemicals are being conducted to replace the traditional methods (i.e., vaccination, use of anticoccidial drugs) for preventing poultry coccidiosis on account of safety, efficient price, drug resistance and drug residues.

Oregano extracts

Oregano extracts as phytogenic compounds are known to have positive effects on growth performance as well as the efficacy of cocci elimination of coccidiosis-infected broilers. Among them, phenols such as carvacrol and thymol have been analyzed as they exhibit plentiful biological properties, including antioxidant, antifungal, antibacterial, and antiprotozoal effects against *Eimeria* spp. The high lipid solubility and hydrophobicity of carvacrol and thymol led to interaction with the phospholipid bilayer in the *Eimeria* species cell membrane, resulting in a change in membrane permeability for cations such as H^+ and K^+. Consequently, the collapse of the parasitic cells occurs through energy losses

(i.e., ATPase inhibition) and ion leakage (particularly calcium), resulting in water imbalance.

3,4,5-Trihydroxybenzoic acid (THB)

3,4,5-Trihydroxybenzoic acid, also known as gallic acid, is found in several vegetables and fruits. Gallic acid is made up of a low molecular weight tri-phenolic compound. It possesses anti-inflammatory and anti-oxidative activities as well as several evident pharmacological effects including anti-tumour, anti-bacterial, anti-diabetes, anti-obesity, anti-microbial and anti-myocardial ischemia.

Probable questions:

1. Name the species of *Eimeria* known to infect chickens.
2. Briefly discuss the structure of sporozoite and oocyst of *Eimeria*.
3. Describe the life cycle of *Eimeria*.
4. What are the clinical signs manifested in chickens due to *Eimeria* infection?
5. How *Eimeria* infection is diagnosed in chickens?
6. State the drug of choice for *Eimeria* infection in chickens.
7. What are the different vaccines available for prevention of Eimeria infection in chickens?

Suggested reading:

1. Noble, E. R. and Noble G. A. (1989). Parasitology. The biology of animal Parasites. 6th ed. Lea and Febiger, Philadelphia.
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Unit-VII

Avian and simian malarial parasites

Objectives:

In this section we will discuss on avian and simian malarial parasites.

Introduction:

- **Avian malarial parasites**

Haemosporidians (Sporozoa: Haemosporida) are one of the most well-known groups of parasitic protists. They include the agents of malaria, one of the most lethal human diseases. But the systematic and ecological diversity of malaria parasites is much larger. Systematic parasitologists have erected more than 500 described species belonging to 15 genera within the order Haemosporidia (Phylum Apicomplexa) that infect squamate reptiles, turtles, birds, and mammals, and use at least seven families of dipteran vectors. These parasites are distributed in every terrestrial habitat on all the warm continents.

Bird haemosporidians are the largest group of haemosporidians by number of species. Avian malaria and related haemosporidians are widespread, abundant and diverse and are easily sampled without disrupting the host populations. In addition, experimental studies on bird malaria usually present bigger samples sizes than primate or human studies, achieving a high degree of precision and confidence in the outcome of the study. More than 200 parasite species of the genera *Plasmodium*, *Haemoproteus* and *Leucocytozoon* have been morphologically described among the 4.000 bird species investigated worldwide. All these characteristics turn bird blood parasites into an excellent model for the study of host–parasite interactions.

The term “malaria parasites” has been a controversial issue among parasitologists, ecologists and evolutionary researchers. The debate stems from the incomplete knowledge of the phylogenetic relationships and pathogenicity of non-human malaria parasites. The life cycles of *Plasmodium*, *Haemoproteus* and *Leucocytozoon* are similar, but they differ in important aspects. Looking at these differences in vectors, life cycles and epidemiology of these organisms, the traditional view accepts only *Plasmodium* species as being the true malaria parasites. The presence of both erythrocytic schizonts and gametocytes in infections with *Plasmodium* is a key difference from *Haemoproteus* and *Leucocytozoon* (these two latter undergo schizogony only in fixed non-circulating cells in the host) which is important for the identification, pathogenicity and experimental transmission of *Plasmodium*. Species of *Plasmodium* can be transmitted from infected to uninfected hosts by simple blood inoculation, while *Leucocytozoon* and *Haemoproteus* need an arthropod vector. These vectors are different among the three

genera: blood-sucking mosquitoes are the main vectors of avian *Plasmodium*, whereas biting midges and hippoboscid flies are the vectors of *Haemoproteus* and simuliid flies transmit *Leucocytozoon*. The number of merozoites produced in schizonts also differs among haemosporidians. Some species of *Leucocytozoon* and *Haemoproteus* form megalozhizonts in host tissues that yield millions of merozoites, species of *Plasmodium* form smaller tissue schizonts that produce tens to hundreds of merozoites.

However, based on recent molecular genetic studies describing the phylogeny of the group, other authors include other genera, particularly *Haemoproteus*, among the malaria parasites. Since the introduction of polymerase chain reaction-based methods for parasite identification, research in avian malaria has boosted. Also, the publication of genetic database of these parasites based on mitochondrial cytochrome b lineages has provided a valuable tool for cooperation between research groups and benefit the understanding of the ecology, evolution and taxonomy.

- **Importance of studies on avian malaria:**

Only five years later than Charles Louis Alphonse Laveran discovered the human malaria, in 1885 the Russian physiologist and protistologist Vassily Danilewsky found intraerythrocytic parasites in the blood of amphibians, birds and reptiles. He described in great detail the morphology of the various forms he observed, becoming aware of the fact that parasites from birds resembled the malaria parasites described by Laveran, Marchiafava and Celli. Intrigued and highly motivated with such similarities, he developed many ecological, anatomical and pathological investigations on infected birds, showing that the haemosporidiosis was accompanied by anemia, enlargement and whitening of the spleen and liver, and an accumulation of pigment. He also noticed some seasonality in parasitemia, where the presence of blood parasites in birds was higher during warm seasons. In 1888, he published a monograph in Russian on bird Haemosporidia, identifying and describing the main characters of the three main genera. But it was not until his three-volume book *La Parasitologie Comparée du Sang* had been published in French in 1889 that this information became widely available. This monograph drew Laveran's attention and he studied with interest Danielewsky's results. In 1891, Laveran urged physicians to enter the domain of naturalists and to research bird malaria. This example clearly illustrates that human and avian malaria researches are intimately linked.

As we have seen, from its origin research on bird malaria parasites has played an important role as human malaria research. Experiments with avian malaria contributed a great deal to our understanding of the life cycle of the human malaria parasite. In addition, the fundamental studies on chemotherapy of malaria were carried out with birds, and resulted in the discovery of two very well-known synthetic drugs, plasmochin and atebrin. The most remarkable scientific advance linking avian and human malaria was done by Sir Ronald Ross, a British officer in the Indian Medical Service. Because malaria was a devastating health problem in India, he began to study its cause in 1890.

For a long time, malaria was thought to be spread by odours, and vapours produced in swamps were blamed as the origin of malaria infection. But Laveran verified the presence of pigmented bodies of parasites in the blood of malaria infected patients, suggesting an alternative cause. In that time, Ross believed that malaria was caused by an intestinal infection, following numerous failed attempts to infect human "volunteers" with water contaminated with malarial mosquitoes and larvae. In 1894, Sir Patrick Manson informed to Ross about Laveran's observation and suggested that mosquitoes, and not the water, were responsible for transmitting these parasites to humans. Ross began to breed mosquitoes for experimental research on malaria inoculation in humans. Finally, in 1897, he managed to get mosquitoes to feed on malarial patients and found evidences of the parasite within the stomach cavities of *Anopheles* mosquitoes. But during these researches on human models, he found many frustrating obstacles, including a transfer within the Service prevented further work with human "volunteers", and his malaria research seemed to aim to an ending point. He still had, however, access to laboratory facilities. And he remembered that in 1894 Manson had suggested to him the idea of using malaria parasites of birds in his investigations. Ross then turned his attention to avian malaria parasites, giving him a control over his experiment subjects difficult to attain with human models. Working with caged birds, Ross confirmed the transmissive way of spreading of malaria, revealing the further development of the avian malaria parasite in the body of the mosquito. He followed the parasites *Plasmodium relictum* from an infected bird into the stomach of a *Culex* mosquito which had fed on the bird and from there to the mosquito's salivary glands. The bite of this mosquito then transmitted the malaria parasite to another bird. For this discover, Ross was awarded the Nobel Prize in 1902.

- **Pathogenicity of avian malaria parasites**

Malaria parasites are supposed to have strong negative effects on host fitness because this group of intra-cellular parasites causes dramatic reductions in the efficiency of metabolism. The infection begins with the bite of an insect inserting sporozoite stages from its saliva into the blood stream of the host. Then the development of extra-erthrocytic meronts starts by asexual division inside internal organs for several generations (a minimum of two generations) until penetration into erythrocytes gives rise to gametocytes. This extraerythrocytic step is very important in order to improve the initial infectious source. Once intensity of infection has increased, a few merozoites penetrate into red blood cells to initiate the erythrocytic cycle producing macro- and microgametocytes by sexual division. After a period of growth there is production of new erythrocytic meronts (schizonts). The infected cells then burst, releasing merozoites that will infect red blood cells, so that a high proportion of available erythrocytes may become infected. Erythrocytic cytoplasm and hemoglobin is digested by the parasite to obtain amino acids, but the haem is stored in the form of an insoluble pigment. When infected red blood cells burst, pigment and other metabolic products are released into the circulation, inducing the characteristic fever and other symptoms of illness. During the

exoerythrocytic meronts stage there are pathological changes such as blocking of brain capillaries and capillaries of other vital organs thereby producing anoxia, death of cells and necrosis of tissues (i.e. liver and spleen). The most severe pathology happens in the blood stage, when destructions of host blood cells provoke acute anemia. Other consequences of malarial parasite's infections include development of pneumonia-like symptoms and excessive enlargement of the spleen and liver that eventually causes rupture.

Previously blood parasites were considered low pathogenicity organisms in spite of them causing disease and death in captive birds. Other studies demonstrated subtle but important effects of hematozoan parasites on the life history of their avian hosts. However, some researchers did not find detrimental effect of these parasites. Therefore, there are no clear conclusions about parasite pathogenicity and about regulation of their host populations. The main problem of most of these studies is the lack of experimentation. The demonstration of effects of parasites requires an empirical approach, where experimental manipulation of natural blood parasite loads may reveal their harmful effects. In this sense, two have been the most successful methodologies employed for experimental approaches to test for fitness effects of avian malaria infection: i) direct inoculation of a parasite on uninfected individuals, and ii) experimental removal of parasites through medication.

The experimentally removal of parasites by anti-malaria medication has been a popular methodology to test the fitness consequences of avian malaria infection. Following this procedure, Merino et al. (2000) reduced through medication the intensity of infection by *Haemoproteus majoris* and the prevalence of infection by *Leucocytozoon majoris* in blue tits *Cyanistes caeruleus*. Medicated females then devoted more resources to parental care and, consequently, increased their reproductive success. This experimental reduction of parasite load revealed the causality in the association of natural infection levels with life-history variables.

But clutch size, one of the most important reproductive patterns in birds, could also be affected by malarial parasites. In addition, if malarial parasites have early effects on the reproductive cycle, then they could have disproportionately large effects on seasonal reproductive success. In this line, Marzal et al. (2005) studied the effect of *Haemoproteus* spp. on the reproductive success of migratory house martin *Delichon urbica*. At the beginning of the breeding season, they experimentally reduced levels and intensity of *Haemoproteus* infection, by randomly treating birds with an anti-malarial drug (primaquine). The results showed that clutch size was on average 18% larger in treated birds, while these differences increasing to 39% at hatching and 42% at fledging. These findings demonstrated that malarial parasites can have dramatic effects on clutch size and other demographic variables, potentially influencing the evolution of clutch size, but also the population dynamics of heavily infected populations of birds.

In the same year, Tomás et al. (2005) studied the role of blood parasites as a potential source of physiological stress for avian hosts in the wild. Through a medication,

they reduced the intensity of infection by *Haemoproteus majoris* and the prevalence of infection by *Leucocytozoon majoris* in female blue tits *Cyanistes caeruleus*. They showed an increase in stress proteins (heat shock proteins) in control females in compare to medicated ones, reporting the first experimental evidence relating blood parasite infection to the physiological stress response in a wild avian population.

Because intensity of blood parasite infection varies during infection, the dynamics of infection could have been the cause of difficulties for detecting their fitness effects in wild populations of birds. During the brief acute stage of a haemosporidian infection, parasites usually appear in the blood at high density and hosts can suffer marked mortality. However, in individuals that survive the acute stage, long-term chronic infections develop, in which parasites persist at low density and are thought to be controlled by acquired immunity. Recently, two studies have shown the negative effects of this malaria chronic infection to their avian hosts.

At the same time, however, in many of these above studies is quite frequent to find infected individuals that seem do not suffer from detrimental effect of blood parasites (e.g. no negative effect on survival and/or reproductive success). The question arising from here is why not all infected birds experience the pathological effect of malaria. Of course, these differences could be explained by a sampling bias in these studies. In this sense, only relatively healthy specimens (uninfected birds or with low intensity of infection) are active and can be caught in mist nets or stationary traps, whereas bird weak due to heavy parasitemias are under sampled because they are inactive. Nowadays ecologists and evolutionary biologists are investigating how host organisms do defend themselves against parasites, dividing host defences into two conceptually different components: resistance and tolerance. Following this line, once infected, hosts can resist the assault by minimizing the success of enemy harass directly attacking parasites and thereby reducing parasite loads. Or alternatively, they can tolerate the parasite limiting the injury caused by a given parasite burden and minimizing the fitness impact of enemy attacks. Recent evidence indicates that some native Hawaiian birds have developed some tolerance to malaria. This could lead to an increase of reservoirs of the disease, which in turn increases the risk of transmission to rarer species that are vulnerable to avian malaria.

- **Simian malarial parasites**

Simian malaria parasites were first reported in Malayan monkeys by Daniels in 1908. It had been assumed for a long time that transmission of simian malaria to humans would not be possible. However, an accidental infection of scientists in Atlanta, USA by mosquito bites in the laboratory proved that a simian malaria species– *Plasmodium cynomolgi* can be transmitted to humans. In 1965 the first natural infection in human was reported in an American surveyor who was infected in the jungles of Pahang, Malaysia. Fortunately, he returned to USA and was detected first as *Plasmodium falciparum* and later revised to

Plasmodium malariae due to the band form of the parasite. Further examination proved that it was actually *Plasmodium knowlesi*.

Plasmodium knowlesi was first found in *Macaca fascicularis* monkeys that were brought to India from Singapore. It was Sinton and Muligan who formally named the new species as *P. knowlesi* after Dr. Knowles. Studies that were carried out before the first human case was reported unveiled many new simian malaria parasites but no human cases. After the first human case was reported in 1965, blood samples were collected from about 1000 people from surrounding villages in West Malaysia where the case of *P. knowlesi* was found but none were positive for simian malaria. However, a presumptive case was reported from Johore, a southern state in peninsular Malaysia.

Mosquito surveys carried out in the area where the first case occurred did not reveal any sporozoite infections in the mosquitoes. However, studies in the coastal areas of Selangor in peninsular Malaysia found *Anopheles hackeri* to be a vector of *P. knowlesi* and this mosquito was attracted only to non-human primates and would not come to bite humans. Thus, at that time it was concluded that simian malaria parasites would not easily affect humans and if it did human malaria cases would occur at very low levels. In 2004 a large focus of *knowlesi* malaria among humans in Sarawak, Malaysian Borneo was reported. This significant finding stimulated many scientists who were interested in the field of simian malaria in humans and their vectors and hosts. Southeast Asia has now become a focal point for the distribution of *P. knowlesi* in humans.

- **Simian malaria parasites and their hosts**

In Southeast Asia, there are 13 species of *Plasmodium* affecting non-human primates. Of these *Plasmodium coatneyi*, *P. cynomolgi*, *P. fieldi*, *P. fragile*, *P. inui*, *P. knowlesi* and *P. simiovale* are known to occur in macaques and leaf monkeys. However, of the seven species, *P. fragile* has been reported in both India and Sri Lanka while *P. simiovale* is restricted only to Sri Lanka. *Plasmodium eylesi*, *P. jefferyi*, *P. youngi* and *P. hylobati* are found in gibbons while *P. pitheci* and *P. silvaticum* are found in orangutans in Borneo. These malaria parasites are found throughout mainland Southeast Asia and associated islands within the Wallace's line.

Information is currently available on the non-human primate malaria especially in Malaysia. Thus, so far five species of simian malaria parasites in non-human primates (macaques) have been reported from Malaysia. The simian malaria parasite *P. cynomolgi* is a species that had been experimentally transmitted to humans. *Plasmodium cynomolgi* in monkeys has many of the characteristics seen during infection of humans with *P. vivax*. It was always believed that monkey malaria was specific for monkeys and human malaria was specific for humans. However, in 1960 accidental infections in the laboratory of simian malaria to humans by mosquito bites led to investigative studies to be carried out in Malaysia and this resulted in the description of many new simian malaria parasites.

Simian malaria parasites have been detected in three main species of non-human primates. They are *Macaca fascicularis*, *Macaca nemestrina* and *Presbytis melalophos*. In the 1960's studies on malaria parasites of *M. nemestrina* revealed that this non-human primate can harbour the following simian malaria species: *P. cynomolgi*, *P. inui*, *P. knowlesi* and *P. fieldi*. Of these *P. fieldi* was a new species found in this macaque. Currently, *P. fieldi* has been found as mixed infection in longtailed macaques but less frequently compared to the other simian malaria parasites.

Plasmodium coatneyi was successfully established when sporozoites from *An. hacker* collected from Rantau Panjang Selangor, were inoculated into an uninfected rhesus monkey. The monkey exhibited infection after a prepatent period of 14 days. The young trophozoites were not easily distinguishable from those of *P. falciparum* and demonstrated a tertian cycle thus leading to a new species. This is the first instance of finding a new species of malaria in the vector before it was known from the primate host. Subsequently *P. coatneyi* was also isolated from *M. fascicularis* from the same area and also from the Philippines.

The pig-tailed macaque – *Macaca nemestrina* occurs in various sub-species from easternmost India and Bangladesh, through Myanmar and Thailand, Malaysia, Sumatra and Kalimantan. This animal is trained to harvest coconuts from tall trees and is kept as a pet by their owners. They coexist with long-tailed macaques-*M. fascicularis* but are ecologically less diverse in their choice of habitats. They are also less commonly seen compared to *M. fascicularis*. The parasites found in the pig-tailed macaques were *P. cynomolgi*, *P. inui*, *P. knowlesi*, *P. fieldi* and *Hepatocystis*.

- **History of natural infection of *P. knowlesi* in human host**

Scientists have always been curious as to the possibility of humans being infected with non-human primate malaria. This interest was intensified when two scientists working in the Memphis laboratory were infected with *P. cynomolgi*. They were conducting infection studies in the laboratory and they were dissecting a large number of mosquitoes heavily infected with malaria parasites two weeks prior to coming down with the illness. Following these infections, scientists decided to survey areas in peninsular Malaysia and search for natural transmission of simian malaria in humans. There were also attempts by scientists to probe into the natural transmission of monkey malaria to humans in the northernmost state of peninsular Malaysia. In the first survey they did not come across any human cases but described new species of monkey malaria parasites in macaques.

In 1965, an American surveyor working in Bukit Kertau in Pahang, Malaysia came down with malaria. Fortunately, he returned to USA where he was diagnosed as *P. knowlesi*. This was the first natural infection reported in humans. The surveyor was apparently working in the forested area at night. American scientists along with the scientists from the Institute for Medical Research carried out extensive surveys in that area where the surveyor was infected. Blood from 1117 persons from 17 villages were

examined for malaria parasites by microscopy using Giemsa-stained slides. Blood was also inoculated into rhesus monkeys to determine if there were natural infections of simian malaria in humans. Of these only 28 had malaria infection, 11 were *P. falciparum*, 13 *P. vivax* and four were not identifiable. None of the rhesus monkeys developed malaria parasites. Thus, it was concluded that simian malaria would not easily infect humans. *Plasmodium knowlesi* pathogenesis and parasite diversity

In malaria it is unclear why certain individuals develop severe disease while others present with uncomplicated infections. Studies can be equivocal but it is certain that both host and parasite variability contribute to severe malaria. For example, *P. falciparum* and *P. knowlesi* reach very high parasite counts in some patients and hyperparasitaemia is associated with severe disease in both of these types of malaria. However, it is not known if some hosts are particularly susceptible to *Plasmodium* infection or if some parasites grow more efficiently or are more virulent in the human host.

- **Hyperparasitaemia and invasion efficiency**

Patients with *P. knowlesi* malaria present with a range of parasitaemias from 1 to 764 720/μL, even though the duration of symptoms before presentation can be relatively short. A recent study examined the possibility that variation in *P. knowlesi* proteins involved in parasite invasion of host erythrocytes may confer an invasion advantage to particular parasite genotypes and lead to high parasite counts. Polymorphic regions of the two *P. knowlesi* members of an important invasion gene family in *Plasmodium* species (*P. knowlesi* normocyte binding protein Pknbpxa and Pknbpxb) were sequenced in patient isolates. Particular alleles of both genes clustered with clinical and laboratory markers of severe disease. Although this work is preliminary, the results suggest that certain genetic variants of *P. knowlesi* are more virulent in the human host.

- **Parasite sequestration in *P. knowlesi***

Severe malaria with coma in *P. falciparum* malaria is accompanied by sequestration of parasite-infected erythrocytes in the brain. Although incompletely understood, sequestration in *P. falciparum* is mediated by the expression of the *var* gene family of proteins, PfEMP1, on the surface of infected erythrocytes. *Plasmodium knowlesi* parasites express variant schizont-infected cell agglutination variant proteins on the surface of infected erythrocytes that are also encoded by a variant multigene family. PfEMP1 proteins predominantly bind to CD36, a receptor that is constitutively expressed on human endothelial cells, except on brain endothelium where they bind to up-regulated intercellular adhesion molecule 1 and to each other to form rosettes. Severe malaria with coma has not been reported in severe cases of *P. knowlesi* malaria even though sequestration of parasitized erythrocytes, similar to that found in severe falciparum malaria with coma, was observed in post mortem brain sections taken from a fatal case

of *P. knowlesi* malaria. A subsequent study found that *P. knowlesi*-infected erythrocytes, matured ex vivo, bound to intercellular adhesion molecule 1 and/or vascular cell adhesion molecule 1 but not CD36. The study was small but highlighted the potential not only for sequestration but also cytoadherence in *P. knowlesi* malaria. Further work on schizont-infected cell agglutination variants and their contribution to *P. knowlesi* virulence may well provide insight into the role of parasite sequestration, cytoadherence and disease severity, including severe disease with coma, in malaria.

- **Clinical presentation**

Plasmodium knowlesi infections are characterized by daily, symptomatic episodes because of the unique 24-h asexual (erythrocytic) life cycle of *P. knowlesi*. The asexual life cycle takes at least 48 h in all of the other *Plasmodium* species that infect humans and non-human primates. The predominant symptoms of *P. knowlesi* malaria are; fever and chills (100% of patients), headache (94.4%), rigors (89.7%), malaise (89.7%), myalgia (87.9%), cough (56.1%), nausea (56.1%), abdominal pain (52.3%), vomiting (33.6%) and diarrhoea (29%). Patients had a median axillary temperature of 37.6°C, median respiratory rate of 26 breaths/min, mean pulse rate of 95 beats per minute and mean arterial blood pressure of 89 mmHg.

In addition, parasite diversity and associated virulence at different study locations may affect the proportion of patients with severe disease. Parasitaemia is associated with disease severity in *P. knowlesi* infections. Thrombocytopenia is a characteristic feature of *P. knowlesi* infection. All studies that compared uncomplicated with complicated *P. knowlesi* malaria reported even lower platelet counts in patients with severe disease.

Patients with severe *knowlesi* malaria fulfil the WHO criteria for severe malaria apart from severe anaemia and coma. Four fatal cases of *P. knowlesi* malaria were reported in 2008. Since then, prospective and retrospective studies have recorded mortality rates of 1.8–10.7%. Importantly a recent study in Sabah reported no deaths in all patients with *P. knowlesi* who were ill enough to require referral to a specialist centre, when they received pre-referral intravenous artesunate. Intravenous artesunate had a faster parasite clearance time when compared with treatment with intravenous quinine and parasites responded uniformly well ex vivo to artesunate when compared with chloroquine, mefloquine and artemisinin.

Probable questions:

1. *Plasmodium* species are regarded as the only true malarial parasites-Justify.
2. Name the vectors of *Haemoproteus* and *Leucocytozoon*.
3. Describe the pathogenicity of avian malarial parasites.
4. Write a short note on host range of simian malarial parasites.
5. What do you mean by parasite sequestration of *Plasmodium knowlesi*?
6. What are the characteristic clinical features of *Plasmodium knowlesi* infection?

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Unit- VIII

Comparative characterization of human malaria parasites

Objectives:

In this section we will discuss on comparative characterization of human malaria parasites.

Introduction:

There are four species of *Plasmodium* which are known to infect humans; *Plasmodium falciparum*, *P. vivax*, *P. ovale* and *P. malariae*. Out of four species mentioned, *P. falciparum* is the major cause of morbidity and mortality. Recently, *P. knowlesi* has also been reported to cause infection in human beings which is known to infect monkeys.

i. Plasmodium vivax

P. vivax causes benign tertiary malaria. Primarily, the symptom includes headache, nausea, anorexia and vomiting. Other symptoms include perspiration, shivers and very high temperature.

ii. ***Plasmodium ovale***

P. ovale causes ovale malaria. The symptoms are comparable to benign tertiary malaria. If left untreated, it can last for about one year.

iii. *Plasmodium malariae*

P. malariae causes quaternary malaria. The bouts of temperature are of 72-hour periodicity. The symptoms are much similar to benign tertiary malaria. Untreated cases can last about 20 years.

iv. ***Plasmodium falciparum***

P. falciparum causes malignant tertiary malaria which is the lethal form of malaria. Symptoms are similar to flu with daily shivers, temperature, intense nausea, vomiting and diarrhoea. Crises reappear every 36 to 48 hours. In this, brain is more frequently affected. The unique property of causing agent is sequestration with endothelial wall of capillaries causing brain haemorrhage. This eventually leads to either coma or death of the infected individual. Renal lesions are found in infected individuals. Because of vomiting and diarrhoea liver is also affected which causes rapid dehydration.

Morphology of *Plasmodium*

The blood-stages of human *Plasmodium* species exhibit different morphology and modification in the host erythrocyte. These differences can be used to distinguish the four species (Table 1). *P. falciparum* blood stages are characterized by the presence of slightly smaller and numerous ring stages than the other species. Erythrocytes having multiple infections are seen more often in *P. falciparum* than in the other species. Distinct crescent-shaped gametocytes of *P. falciparum* appear late in the infection. *P. vivax* with enlarged infected erythrocytes and granules 'Schüffner's dots', over the erythrocyte cytoplasm, manifests at caveola-vesicle complexes that form on the erythrocyte membrane. The trophozoite of *P. vivax* has an ameboid appearance. The schizonts can have more than 20 merozoites. *P. ovale* also exhibits Schüffner's dots with an enlarged erythrocyte. It is difficult to distinguish the infection from *P. vivax*. In general, *P. ovale* is a more compact parasite than *P. vivax*. This insistence is most evident in the growing trophozite stage. Merozoites are fewer per schizont. Elongated host erythrocytes are found in case of *P. ovale*. *P. malariae* exhibit compact stages and does not modify the host erythrocyte except few elongated trophozoites which stretch across the erythrocyte to form a band like structure. Schizonts will typically have 8-10 merozoites, often arranged in a rosette pattern, with a clump of pigment in the centre.

A comparative account of various stages (Table 1) and the diagrammatic figures (Figure 1) clearly represents the disparity in the morphological appearance of the four *Plasmodium* species.

Table 1: Comparative account of the different stages of the *Plasmodium* spp. life cycle

Species	***P. falciparum***	***P. vivax***	***P. malariae***	***P. ovale***
❖ **Host cell**				
Size	Not enlarged	Enlarged	Not enlarged	Enlarged
Shape	Round	Round or oval	Round	Round or oval
Colour	Normal & may turn dark	Pale	Normal	Normal
Stippling	Large red spots Maurer's dots	Small red dots Schüffner's dots	few tiny dots Ziemann's dots	Numerous small red dots James's dots
Pigment	Usually black or dark brown	Golden brown Granules	Black or brown coarse granules	As of *P. malariae*
❖ **Parasite**				
General features	Small, compact dark, staining	Large light staining amoeboid	Regular shape Strong tendency to	Regular shape.

	parasite. Multiple infections of single RBC	parasite. Many trophozoites	form a band across the infected RBC	Size in between *P. vivax* and *P. malariae*
Common Stages found in smear	Only rings and gametocytes	Trophozoites, Schizonts, Gametocytes	As in *P. vivax*	As in *P. vivax*
Ring stage	Delicate, small, 1.5 µm Double chromatin and multiple rings common.	Large 2.5 µm, usually single. Prominent thicker chromatin	Similar to *P. vivax* but thicker	Similar to *P. vivax*, but compact
Trophozoite	Compact, small, vacuole inconspicuous, seldom seen in smear	Large, irregular vacuole prominent Chromatin as dots or threads	Characteristic band form, vacuole inconspicuous	Compact rough pigment, large irregular clumps of chromatin
Schizont	Small, compact rarely seen in blood smear	Large, filling the RBC, segmented, yellow brown pigment	Nearly fills RBC, segmented, pigment is dark brown	Fills three fourth of RBC, segmented, pigment dark yellow brown
Microgametocyte	Larger than RBC, kidney shaped with blunt round ends, cytoplasm reddish blue, many fine granules in smear	Fills enlarged RBC, round or oval, compact cytoplasm, pale blue, profuse brown granules	Smaller than RBC, very few in peripheral blood film, round compact. Pale blue cytoplasm. Pigment and chromatin as in *P. vivax*	Same of RBC, round, compact very few in peripheral blood film, cytoplasm pale blue, chromatin and pigment as in *P. vivax*
Macrogametocyte	Slender, nucleus small, compact,	Large, loose and ill-defined mass of	Same as *P. vivax*, low numbers	Same as *P. vivax*, low numbers

	pigment granules closely aggregated	chromatin and smaller mass	appear after 12–14 days.	appear after 12–14 days.

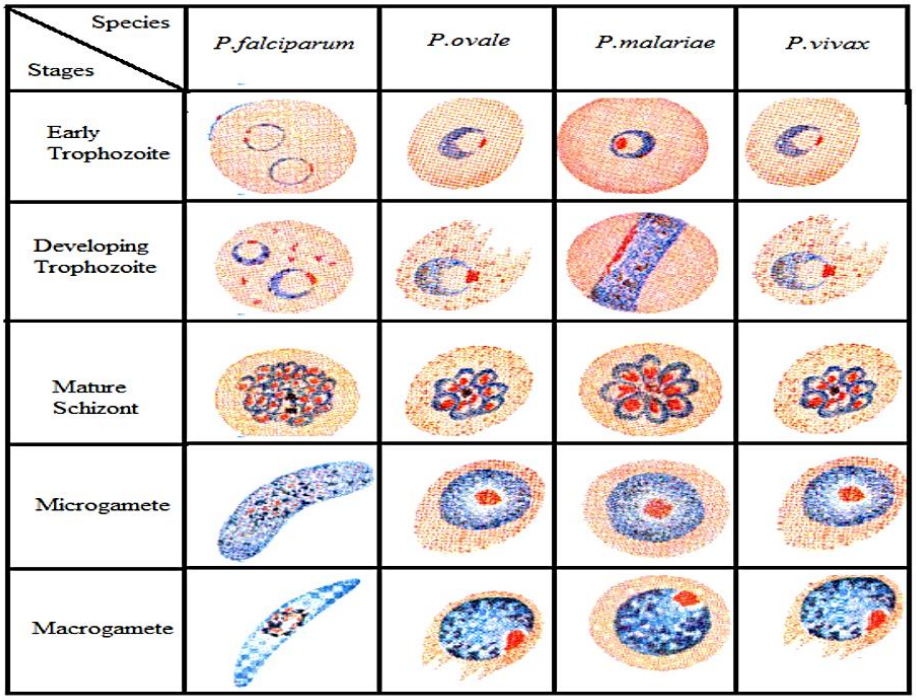

Figure 1: Diagrammatic illustration of the morphology of the different stages of the *Plasmodium* spp. life cycle in thin blood films

(Source: http://www.phsource.us/PH/PARA/Diagnosing_Medical_Parasites.pdf)

Probable questions:

1. Which malarial parasite is considered most fatal from a human perspective?
2. Write a short note on different types of malaria affecting humans with their causative organism.
3. Write a comparative account of different malarial parasites' structure and life cycle.

Suggested reading:

1. Noble, E. R. and Noble G. A. (1989). Parasitology. The biology of animal Parasites. 6th ed. Lea and Febiger, Philadelphia.

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Unit- IX

Zoonosis with special reference to Japanese Encephalitis

Objectives:

In this section we will discuss on zoonosis with special reference to Japanese Encephalitis.

Introduction:

Japanese encephalitis virus (JEV) is a mosquito–borne agent best known as a cause of encephalitis in humans and equids, though rare cases have been reported in other species, and reproductive losses may be seen sometimes in pigs. While most infections in people and horses are asymptomatic or mild, with < 1% developing neurological signs, human encephalitis is often severe and many survivors are left with neurological sequelae. Japanese encephalitis tends to be a childhood disease in endemic areas, where people are usually exposed to the virus by adulthood, though clinical cases are also seen occasionally in adults with waning immunity. At times, epidemics have resulted in hundreds or thousands of cases in people and/or horses when virus circulation was particularly high or population immunity low.

Definition of zoonosis:

Diseases and infections which are naturally transmitted between vertebrate animals and man.

Types:

- **Anthropozoonoses:** Diseases in animals that can be transmitted to man (eg. rabies).
- **Zooanthroponoses:** Diseases in humans that can be transmitted to animals (eg. tuberculosis in cats, monkeys).
- **Amphixenoses:** Diseases affecting humans and animals that can be occasionally transmitted from one to another (eg. staphyloccocal infection).
- **Euzoonoses:** Diseases in which humans are an obligatory host of the agent (eg. *Taenia solium* or *T. saginata*)

Some Major Bacterial Etiologic Agents of New Zoonoses

- *E. coli* O157:H7
- *Borrelia burgdorferi* (Lyme disease)

- *Helicobacter pylori* and other spp.
- *Ehrlichia chaffeensis* (Human Monocytic Ehrlichiosis)
- *Bartonella henselae* (Cat scratch Disease)
- *Rickettsia felis* (Murine typhus like)

Some Major Viral Etiologic Agents of New Zoonoses

- Guanarito virus (Venezuelan haemor fever)
- Sin nombre virus (Hantavirus Pulmonary Syndrome)
- Sabia virus (Brazilian hemorrhagic fever)
- Hendra virus (Equine morbillivirus)
- Australian bat Lyssavirus (Rhabdovirus)
- Menangle virus (paramyxovirus)
- Influenza virus H5N1 (Hong Kong)
- Nipah virus (Paramyxovirus)
- Influenza virus H9N2 (Hong Kong)
- Severe Acute Respiratory Syndrome SARS (Coronavirus)

Types on the basis of Epidemiological cycle/Modes of transmission:

- **Orthozoonoses:** Disease transmission cycle can be completed with only one vertebrate reservoir (eg. rabies).
- **Cyclozoonoses:** Diseases whose maintenance cycle requires more than one vertebrate species, but no invertebrate host (eg., hydatid disease, taeniasis).
- **Pherozoonoses (or Metazoonoses):** Diseases whose maintenance cycle requires both vertebrates and invertebrates to complete their transmission cycle (eg. arboviruses).
- **Saprozoonoses:** Diseases that depend upon inanimate reservoirs or development sites, as well as upon vertebrate hosts (eg. listeriosis)

According to the reservoir host:

- **Anthropozoonoses** Infections transmitted to man from lower vertebrate animals e.g. rabies, leptospirosis, plague, arboviral infections, brucellosis and Q-fever.
- **Zooanthroponoses** Infections transmitted from man to lower vertebrate animals e.g. streptococci, staphylococci, diphtheria, enterobacteriaceae, human tuberculosis in cattle and parrots.
- **Amphixenoses** Infections maintained in both man and lower vertebrate animals and transmitted in either direction e.g. salmonellosis, staphylococcosis

Depending on Clinical manifestations:

- **Phanerozoonoses:** Zoonoses for which symptoms are observed in animals and humans. They may be Iso-symptomatic (Symptoms are the same in humans and animals eg. Rabies, tuberculosis) or Aniso-symptomatic (Symptoms are different in humans and animals eg. Q fever, anthrax)
- **Cryptozoonoses:** Zoonoses for which there is only infection without symptoms in animals and/or humans. eg. Infection in animals/disease in humans: ornithosis, Infection in humans/disease in animals: Ebola

• Factors influencing prevalence of zoonoses:

Ecological changes in man's environment

With the expansion of human population, man is forced to exploit the virgin territories and natural resources like harnessing the power of rivers, constructing roads and pipelines through virgin or thinly populated areas, clearing, irrigating and cultivating new land, deforestation. All this would lead to entering of humans in the unaccustomed ecosystem in which potential pathogens form part of the biotic community (natural focus). Large scale expansion of agricultural and engineering resources, construction of dams, artificial lakes, irrigation schemes, clearing of forests -all these lead to changing of the biting habits of the blood sucking vectors and alteration in the population of reservoir animals which has led to the spread of leptospira, tuleraemia, helminthic infections etc. Handling animal by-products and wastes (occupational hazards)

There are significantly higher attack rates in workers during the course of their occupation than the rest of the population, e.g., anthrax in carpet weavers, live-stock raisers and workers with animal hair in the textile industry, leptospirosis in rice field workers, listeriosis in agricultural workers, erysipeloid in butchers and fish merchants, tularaemia and trypanosomiasis in hunters, creeping eruptions in plumbers, trench diggers etc. Other examples of zoonoses as occupational hazards are Q-fever in abattoir and rendering plant workers, jungle yellow fever and tick-borne diseases in wood cutters, salmonellosis in food processors, bovine tuberculosis in farmers etc.

Increased movements of man

Land development, engineering project work, pilgrimages, tourism, etc. expose the people to contaminated food and water leading to diseases like amoebiasis, colibacilliosis, giardiasis, salmonellosis, shigellosis, etc.

Increased trade in animal products

Countries which import hides, wool, bone meal, meat, etc. from an area where some of the zoonoses are endemic, are likely to introduce the disease into their territories, e.g., salmonellosis, foot and mouth disease, anthrax, Newcastle disease etc.

Increased density of animal population

Animals may carry potential risk of increased frequency of zoonotic agents in man e.g. dermatophytosis, tuberculosis, brucellosis etc.

Transportation of virus infected mosquitoes

Aircraft, ship, train, motor and other vehicles bring the viruses in to a new area, e.g. yellow fever Chikungunya fever, dengue fever etc.

Cultural anthropological norms

In Kenya, people allow the dogs and hyenas to eat human dead bodies infected with hydatidosis. This helps to perpetuate the transmission cycle of the disease.

Etiology:

Japanese encephalitis virus (JEV) is a member of the Japanese encephalitis serogroup in the genus Flavivirus, family Flaviviridae. There is only one serotype of JEV, but at least five genotypes, G-I to G-V. Some genotypes are more common than others, and the dominant genotypes in an area can change over time. Information on the genotypes circulating in some areas is still limited.

Species Affected:

JEV can infect a wide variety of vertebrates, though only those species that consistently develop viremia sufficient to infect mosquitoes can act as maintenance or amplifying hosts. Certain birds in the family Ardeidae (herons and egrets) appear to be important maintenance hosts, but antibodies to this virus have also been found in many other avian species. Laboratory experiments suggest that some non-ardeid birds, such as some ducks, passerines, gulls and pigeons, can develop significant viremia, while others (e.g., crows, American white pelicans, double-crested cormorants) have little or no virus in the blood. Among mammals, evidence for JEV infections has been demonstrated in pigs, wild boar, cattle, water buffalo, sheep, goats, alpacas, equids, rabbits, dogs, cats, raccoon dogs (*Nyctereutes procyonoides*), raccoons (*Procyon lotor*), seals, meerkats (*Suricata suricatta*), various nonhuman primates, bats, some captive cervids and other species. Most mammals seem to be dead-end hosts, and pigs are the only domestic animal thought to be important in virus amplification. While horse-to-horse transmission via mosquitoes appears possible in the laboratory, viremia in this species is low and there are usually too few susceptible horses nearby to maintain and propagate the virus. Research in wildlife is limited, but feral swine and wild boar could be amplifying hosts, there are reports that bats might participate in some cycles, and brush-tailed possums (*Trichosurus vulpecula*) can develop significant viremia after experimental inoculation. The virus also appears to infect some reptiles and amphibians.

Geographic Distribution:

Japanese encephalitis is widespread in temperate and tropical regions of eastern and southern Asia, with reports of the virus as far north as southern Russia. It also occurs in parts of the western Pacific. The precise distribution of JEV in some countries is unclear, due to limited surveillance and/or cross-reactivity with other flaviviruses in some serological tests. JEV isolates have been found regularly in the Torres Straits islands of Australia since 1995, and a different virus was detected in pigs and humans on the Australian mainland in 2021 and 2022. Serological evidence of widespread exposure in feral pigs after these outbreaks suggests the virus may have become established in this location. Countries where human cases are not reported, such as Singapore, sometimes have evidence of continuing subclinical JEV circulation in animals.

Rare reports have described infections that were apparently acquired outside Asia, though there is currently no definitive evidence that the virus has become established in any of these locations. In one instance, part of the JEV genome was identified in seven birds in Italy during dead bird surveillance in 1997-2000, gene segments and viral antigens were found in a few bone marrow samples collected from healthy birds around that time, and some PCR-positive mosquitoes were detected in 2010. Similarly, nucleic acids of both JEV and yellow fever virus were found in a yellow fever patient in Angola in 2016, though the patient had not travelled to regions where JEV is endemic.

Incubation Period:

The incubation period in humans is estimated to be 5 to 15 days.

Clinical Signs:

The initial signs of Japanese encephalitis are usually nonspecific and flu-like, and may include fever, chills, malaise, muscle aches and, in some cases, severe headache with vomiting. Children may appear to have a gastrointestinal illness, with nausea, vomiting and abdominal pain. Some patients also have thrombocytopenia, and coryza and diarrhea have been reported.

Most people recover after this initial stage, but a minority develops neurological signs that may include encephalitis, signs suggestive of benign aseptic meningitis, or atypical presentations such as flaccid paralysis with or without encephalitis. Encephalitis, the most common form, can appear either insidiously or as the sudden onset of fever and convulsions. Common symptoms include a reduced level of consciousness; focal neurological signs; quadriplegia, hemiplegia or cerebellar disorders; behavioural changes; painful stiffness of the neck; and mild to severe convulsions that range from subtle focal signs to generalized seizures. Movement disorders also occur frequently, and some people develop transient Parkinson's like signs (e.g., masking of the face, reduced

blinking, rigidity with or without tremor, akinesia). Various atypical presentations have been reported, and include isolated acute onset behavioural abnormalities that may be misdiagnosed as psychiatric illnesses. Apparent effects on other organs, such as pulmonary edema and upper gastrointestinal haemorrhage, have been described occasionally. Miscarriages can occur in pregnant women who are infected for the first-time during pregnancy; however, this is reported to be uncommon in endemic areas.

Convalescence from CNS signs can be prolonged, though some patients make a rapid, spontaneous recovery ("abortive encephalitis"). An estimated 30-50% of survivors have neurological sequelae such as epileptic seizures, deafness, cognitive, behavioural or language impairment, or a Parkinsonian syndrome with tremors and rigidity. Some survivors gradually improve, although this may take months or years.

Diagnostic Tests:

Serology is often used to diagnose Japanese encephalitis in endemic areas. IgM can be found in the CSF of most patients with neurological signs, and IgM in acute phase serum is suggestive of recent infection (or vaccination). A fourfold rise in neutralizing antibody titers can provide a retrospective diagnosis. Cross-reactivity with other flaviviruses is an issue in some other serological assays, as in animals. Viremia is usually transient and low level, but JEV or its nucleic acids may occasionally be found in blood, CSF or other samples (e.g., throat swabs) of encephalitic patients. Viral antigens may be detected in the post-mortem brain by immunohistochemistry. Neuroimaging and electroencephalographic analysis can also be helpful.

Treatment:

Treatment is supportive and symptomatic. While some therapies specific for the virus (e.g., antiserum) have been investigated, information about them is still limited.

Control:

Several different vaccines are in use for childhood vaccination in endemic areas. Vaccination may also be offered to some adults in these regions and, outside these areas, to some laboratory workers and travellers. Recommendations for travellers vary, depending on factors such as the season, duration of travel, activities and type of lodging. Mosquito bites can be discouraged with insect repellents, insecticide-impregnated bed nets, long-sleeved shirts and pants, and similar measures. Other interventions, such as environmental modifications to decrease mosquito populations (e.g., intermittent irrigation of rice fields, larvivorous fish, insecticide spraying) or relocation of pigs away from human population centres have occasionally been investigated or implemented.

Where Japanese encephalitis is seasonal, serological surveillance in pigs, and perhaps other species, can help predict epidemics in humans.

Probable questions:

1. Define zoonosis. Give an example.
2. Classify zoonosis citing examples in each case.
3. What are the factors responsible for the prevalence of zoonosis?
4. Name the animals susceptible for JE infection. Add a short note on the geographic distribution of JEV.
5. What are the clinical signs of JE?
6. Briefly describe the control methods of vector of JEV.

Suggested reading:

1. Noble, E. R. and Noble G. A. (1989). Parasitology. The biology of animal Parasites. 6th ed. Lea and Febiger, Philadelphia.
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Unit- X

Zoonosis with special reference to Toxoplasmosis

Objectives:

In this section we will discuss on zoonosis with special reference to Toxoplasmosis.

Introduction:

Toxoplasmosis, caused by the protozoan parasite *Toxoplasma gondii,* is one of the most common parasitic infections of man and other warm-blooded animals. It has been found world-wide from Alaska to Australia. Nearly one-third of humanity has been exposed to this parasite. In most adults it does not cause serious illness, but it can cause blindness and mental retardation in congenitally infected children and devastating disease in immunocompromised individuals.

Geographic Distribution:

Toxoplasma gondii infection is widespread in humans, although its prevalence varies widely from place to place. In the United States and the United Kingdom, it is estimated that 16–40% of the population are infected, whereas in Central and South America and continental Europe, estimates of infection range from 50 to 80%. Most infections in humans are asymptomatic but at times the parasite can produce devastating disease. Infection may be congenitally or postnatally acquired. Congenital infection occurs only when a woman becomes infected during pregnancy. Congenital infections acquired during the first trimester are more severe than those acquired in the second and third trimester. While the mother rarely has symptoms of infection, she does have a temporary parasitaemia. Focal lesions develop in the placenta and the foetus may become infected. At first there is generalized infection in the foetus. Later, infection is cleared from the visceral tissues and may localize in the central nervous system. A wide spectrum of clinical diseases occurs in congenitally infected children. Mild disease may consist of slightly diminished vision, whereas severely diseased children may have the full tetrad of signs: retinochoroiditis, hydrocephalus, convulsions and intracerebral calcification. Of these, hydrocephalus is the least common, but most dramatic, lesion of toxoplasmosis. By far the most common sequel of congenital toxoplasmosis is ocular disease.

The socio-economic impact of toxoplasmosis in human suffering and the cost of care of sick children, especially those with mental retardation and blindness, are enormous. The testing of all pregnant women for *T. gondii* infection is routine in some European countries, including France and Austria. The cost-benefit of such mass screening is being debated in many other countries.

Clinical Signs:

Postnatally acquired infection may be localized or generalized. Humans become infected by ingesting tissue cysts in undercooked or uncooked meat or by ingesting food and water contaminated with oocysts from infected cat faeces. Oocyst transmitted infections may be more severe than tissue cyst-induced infections. Enlarged lymph nodes are the most frequently observed clinical form of toxoplasmosis in humans. Lymphadenopathy may be associated with fever, fatigue, muscle pain, sore throat and headache. Although the condition may be benign, its diagnosis is vital in pregnant women because of the risk to the foetus. In an outbreak in British Columbia, of 100 people who were diagnosed with acute infection, 51 had lymphadenopathy and 20 had retinitis.

Encephalitis is the most important manifestation of toxoplasmosis in immunosuppressed patients as it causes the most severe damage to the patient. Infection may occur in any organ. Patients may have headache, disorientation, drowsiness, hemiparesis, reflex changes and convulsions, and many become comatose. Encephalitis caused by *T. gondii* is now recognized with great frequency in patients treated with immunosuppressive agents.

Toxoplasmosis ranks high on the list of diseases which lead to death in patients with acquired immunodeficiency syndrome (AIDS); approximately 10% of AIDS patients in the USA and up to 30% in Europe are estimated to die from toxoplasmosis. Most AIDS patients suffering from toxoplasmosis have bilateral, severe and persistent headache which responds poorly to analgesics. As the disease progresses, the headache may give way to a condition characterized by confusion, lethargy, ataxia and coma. The predominant lesion in the brain is necrosis, especially of the thalamus.

Life Cycle:

Contamination of the environment by oocysts is widespread as oocysts are shed by domestic cats and other members of the Felidae. Domestic cats are probably the major source of contamination since oocyst formation is greatest in domestic cats. Cats may excrete millions of oocysts after ingesting only one bradyzoite or one tissue cyst, and many tissue cysts may be present in one infected mouse. Generally, only about 1% of cats in a population are found to be shedding oocysts at any given time. Oocysts are shed for only a short period (1–2 weeks) in the life of the cat, however, the enormous numbers shed assure widespread contamination of the environment. Under experimental conditions, infected cats can shed oocysts after reinoculation with tissue cysts. It is not known whether repeated shedding of oocysts occurs in nature, but this would greatly facilitate oocyst spread. Sporulated oocysts survive for long periods under most ordinary environmental conditions. They can survive in moist soil, for example, for months and even years. Oocysts in soil do not always stay there, as invertebrates such as flies, cockroaches, dung beetles and earthworms can mechanically spread these oocysts and even carry them onto food. Congenital infection can occur in cats, and congenitally

infected kittens can excrete oocysts, providing another source of oocysts for contamination. Infection rates in cats reflect the rate of infection in local avian and rodent populations because cats are thought to become infected by eating these animals. The more oocysts there are in the environment, the more likely it is that prey animals will become infected, and this results in increased infection rates in cats. Oocysts can be detected by examination of cat faeces, though for epidemiological surveys, detection of *T. gondii* oocysts in cat faeces is not practical. Concentration methods (e.g. flotation in high-density sucrose solution) are often used because the number of *T. gondii* oocysts in cat faeces may be too few to be detected by direct smear. For definitive identification, *T. gondii* oocysts should be sporulated and then bio-assayed in mice to distinguish them from other related coccidians. Determining serological prevalence is a better measure of exposure of cats to *T. gondii* infection than detection of oocysts. It is a fair assumption that cats that are seropositive have already shed *T. gondii* oocysts.

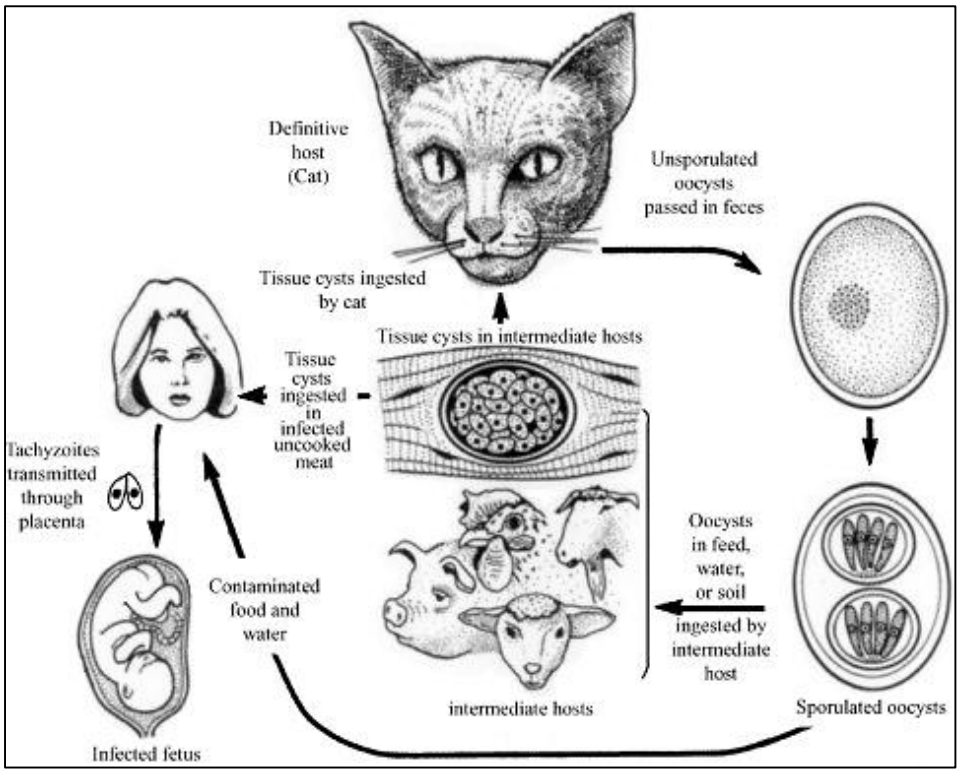

Figure: Life cycle of *Toxoplasma gondii*

Currently, there are no tests which can discriminate between oocyst ingestion and tissue cyst ingestion as the infection route. Available evidence for the oocyst infection route is based upon epidemiological surveys. For example, in certain areas of Brazil, approximately 60% of 6–8-year-old children have antibodies to *T. gondii* linked to the ingestion of oocysts from an environment heavily contaminated with *T. gondii* oocysts. Infections in aquatic mammals indicate contamination and survival of oocysts in sea water. The largest outbreak of clinical toxoplasmosis in humans was epidemiologically linked to drinking water from a municipal water reservoir in British Columbia, Canada.

This water reservoir was thought to be contaminated with *T. gondii* oocysts excreted by cougars (*Felis concolor*).

Mode of transmission:

Although *T. gondii* has been isolated from soil, there is no simple method for oocyst isolation from soil that is useful on an epidemiological scale. Although attempts to recover *T. gondii* oocysts from water samples in the British Columbia outbreak were unsuccessful, methods to detect oocysts were reported. At present, there are no commercial reagents available to detect *T. gondii* oocysts in the environment.

Infection in humans often results from ingestion of tissue cysts contained in undercooked meat. *T. gondii* infection is common in many animals used for food, including sheep, pigs and rabbits. Infection in cattle, horses and water buffaloes is less prevalent than infection in sheep or pigs. *Toxoplasma gondii* may survive in food animals for years in tissue cysts.

Virtually all edible portions of an animal can harbour viable *T. gondii*. *T. gondii* infection is also prevalent in game animals. Among wild game, *T. gondii* infection is most prevalent in black bears and in white-tailed deer. Approximately 80% of black bears are infected in the USA, and about 60% of raccoons have antibodies to *T. gondii*. Because raccoons and bears scavenge for their food, infection in these animals is a good indicator of the prevalence of *T. gondii* in the environment.

The number of *T. gondii* tissue cysts in meat from food animals is very low. It is estimated that as few as one tissue cyst may be present in 100 g of meat. Therefore, without using a concentration method, it is not practical to detect this low level of *T. gondii* infection. Therefore, digestion of meat samples in trypsin or pepsin is used to concentrate *T. gondii* in meat. Digestion in trypsin or pepsin ruptures the *T. gondii* tissue cyst wall, releasing hundreds of bradyzoites. The bradyzoites survive in the digests for several hours. Even in the digested samples, only a few *T. gondii* tissue cysts are present and their identification by direct microscopic examination is not practical. Therefore, the digested material is bio-assayed in mice. The mice inoculated with digested material have to be kept for 6–8 weeks before *T. gondii* infection can be detected reliably, and therefore this procedure is not practical for mass scale samples. The detection of *T. gondii* DNA in meat samples by polymerase chain reaction (PCR) has been reported, but there are no data on the specificity and sensitivity of this method to detect *T. gondii* in meat samples. A highly sensitive method using a real-time PCR and fluorogenic probe was found to detect *T. gondii* DNA from as few as four bradyzoites.

Cultural habits of a population may affect the acquisition of *T. gondii* infection from ingested tissue cysts in undercooked meat. For example, in France the prevalence of antibody to *T. gondii* is very high in humans. The high incidence of *T. gondii* infection in humans in France appears to be related in part to the French habit of eating some meat products raw or undercooked. In contrast, the high prevalence of *T. gondii* infection in

Central and South America is probably due to high levels of contamination of the environment by oocysts. As stated above, the relative frequency of acquisition of toxoplasmosis from eating raw meat and that due to ingestion of oocysts from cat feces is impossible to determine, and as a result, statements on the subject are at best controversial.

There is little, if any, danger of *T. gondii* infection by drinking cows' milk and, in any case, cows' milk is generally pasteurized or boiled, but infection has followed drinking unboiled goats' milk. Raw hens' eggs, although an important source of *Salmonella* infection, are extremely unlikely to transmit *T. gondii* infection. Transmission by sexual activity including kissing is probably rare and epidemiologically unimportant.

Transmission of *T. gondii* may also occur through blood transfusions and organ transplants. Of these routes, transmission by transplantation is most important. Toxoplasmosis may arise in two ways in people undergoing transplantation: (i) from implantation of an organ or bone marrow from an infected donor into a non-immune, immunocompromised recipient and (ii) from induction of disease in an immunocompromised, latently infected recipient. Tissue cysts in the transplanted tissue or in the latently infected transplant patient are probably the source of the infection. In both cases, the cytotoxic and immunosuppressive therapy given to the transplant recipient is the cause of the induction of the active infection and disease.

Diagnostic Tests:

Diagnosis of toxoplasmosis in humans is made by biological, serological, histological, or molecular methods, or by some combination of the above. Clinical signs of toxoplasmosis are non-specific and are not sufficiently characteristic for a definite diagnosis. Toxoplasmosis in fact mimics several other infectious diseases.

Detection of *T. gondii* antibody in patients may aid diagnosis. There are numerous serological procedures available for the detection of humoral antibodies; these include the Sabin–Feldman dye test, the indirect hemagglutination assay, the indirect fluorescent antibody assay (IFA), the direct agglutination test, the latex agglutination test (LAT), the enzyme-linked immunosorbent assay (ELISA), and the immunosorbent agglutination assay test (IAAT). The IFA, IAAT and ELISA have been modified to detect immunoglobulin M (IgM) antibodies. The IgM antibodies appear sooner after infection than the IgG antibodies and disappear faster than IgG antibodies after recovery.

The finding of antibodies to *T. gondii* in one serum sample only establishes that the host has been infected at some time in the past. It is best to collect two samples from the same individual, the second collected 2–4 weeks after the first. A 16-fold higher antibody titer in the second sample indicates an acute infection. A high antibody titer sometimes persists for months after infection. A rise in antibody titer may not be associated with clinical symptoms because, as indicated earlier, most infections in humans are asymptomatic. The fact that titers persist in infected people after clinical

recovery complicates the interpretation of the results of serological tests. Establishing recency of infection in pregnancy is of clinical importance with respect to medical intervention to minimize damage to the foetus, and there is not one test that can achieve this at the present time.

Toxoplasma gondii can be isolated from patients by inoculation of laboratory animals and tissue cultures with secretions, excretions, body fluids, tissues taken by biopsy, and tissues with macroscopic lesions taken post mortem. Using such specimens, one may not only attempt isolation of *T. gondii*, but one may search for *T. gondii* microscopically or for toxoplasmal DNA using PCR. Recent studies have shown that monoplex and multiplex PCR can be useful for specifically identifying *T. gondii* (using the B1 gene as the target sequence) from tissue biopsies, cerebrospinal fluid or vitreous body from patients with undiagnosed uveitis, fetal blood and amniotic fluid.

As just noted, diagnosis can be made by finding *T. gondii* in host tissue removed by biopsy or at necropsy. A rapid diagnosis may be made by microscopic examination of impression smears of lesions. After drying for 10–30 min, the smears are fixed in methyl alcohol and stained with one of the Romanowsky strains, the Giemsa stain being very satisfactory. Well-preserved *T. gondii* are crescent-shaped. In sections, the tachyzoites usually appear round to oval. Electron microscopy can aid diagnosis. *T. gondii* tachyzoites are always located in vacuoles. Tissue cysts are usually spherical, lack septa, and the cyst wall can be stained with a silver stain. The bradyzoites are strongly positive on periodic acid Schiff (PAS) staining. Immunohistochemical staining of parasites with fluorescent or other types of labelled *T. gondii* antisera can aid in diagnosis.

Treatment:

Sulphadiazine and pyrimethamine (Daraprim) are two drugs widely used for treatment of toxoplasmosis. While these drugs have a beneficial action when given in the acute stage of the disease process when there is active multiplication of the parasite, they will usually not eradicate infection. It is believed that these drugs have little effect on subclinical infections, but the growth of tissue cysts in mice has been restrained with sulphonamides. Certain other drugs, diaminodiphenylsulphone, atovaquone, spiramycin and clindamycin, are also used to treat toxoplasmosis in difficult cases.

Control:

To prevent infection of human beings by *T. gondii*, the hands of people handling meat should be washed thoroughly with soap and water before they begin other tasks. All cutting boards, sink tops, knives and other materials coming in contact with uncooked meat should be washed with soap and water also. Washing is effective because the stages of *T. gondii* in meat are killed by contact with soap and water.

T. gondii organisms in meat can be killed by exposure to extreme heat or cold. Tissue cysts in meat are killed by heating the meat throughout to 67˚C or by cooling to 13˚C. Toxoplasma in tissue cysts are also killed by exposure to 0.5 kilorads of gamma irradiation. Meat of any animal should be cooked to 67˚C before consumption, and tasting meat while cooking or while seasoning should be avoided.

Pregnant women, especially, should avoid contact with cats, soil and raw meat. Pet cats should be fed only dry, canned, or cooked food. The cat litter box should be emptied every day, a task to be avoided by pregnant women. Gloves should be worn while gardening. Vegetables should be washed thoroughly before eating because they may have been contaminated with cat faeces. Expectant mothers should be aware of the dangers of toxoplasmosis. At present there is no vaccine to prevent toxoplasmosis in humans.

Probable questions:

1. What are the clinical signs related to *Toxoplasma* infection?
2. Describe the life cycle of *Toxoplasma gondii*.
3. Discuss the transmission cycle of *Toxoplasma* with reference to zoonosis.
4. How *Toxoplasma* infection can be diagnosed in humans?
5. Name two drugs of choice for the treatment of toxoplasmosis. State the control measures of *Toxoplasma*.

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Unit XI

Ultrastructure of trypanosomes

Objective: In this unit we will learn about Ultrastructure of trypanosomes.

Introduction

Trypanosoma is a genus of protozoan parasites belonging to the class Kinetoplastea within the phylum Euglenozoa. These unicellular organisms are notorious for causing significant diseases in humans and animals, including African sleeping sickness and Chagas disease. The pathogenicity and life cycle of *Trypanosoma* are intimately connected to its unique and complex microscopic structure, which has adapted to facilitate its survival and transmission between hosts. Understanding the microscopic structure of *Trypanosoma* is crucial for comprehending its biology, pathogenic mechanisms, and for the development of therapeutic interventions.

General morphology

Trypanosoma species are by their elongated, spindle-shaped bodies, typically ranging from 15 to 30 micrometers in length. The cell is enclosed by a plasma membrane, beneath which lies the subpellicular microtubule corset that provides structural integrity and maintains the shape of the parasite. This microtubule array is one of the most distinguishing features of trypanosomes and is crucial for their motility.

I. Flagellum and undulating membrane

The flagellum originates from the basal body, located near the posterior end of the cell, and extends anteriorly. The undulating membrane is formed by the close association of the flagellum with the cell surface and is essential for the parasite's motility within the host's bloodstream or tissues. The flagellum itself is composed of a typical eukaryotic 9+2 arrangement of microtubules, which are powered by dynein arms that facilitate the whip-like motion characteristic of the parasite. The flagellar pocket, where the flagellum emerges from the cell body, is a crucial site for endocytosis and exocytosis.

II. Kinetoplast

A defining feature of *Trypanosoma* is the kinetoplast, a unique DNA-containing structure located within the single, large mitochondrion of the cell. The kinetoplast is found adjacent to the basal body of the flagellum and is composed of a dense network of circular DNA molecules, known as kinetoplast DNA (kDNA). This kDNA is organized into two types of circular molecules: minicircles and maxicircles. Minicircles are involved in the editing of mitochondrial mRNA transcripts, while maxicircles encode essential mitochondrial proteins. The kinetoplast is tightly

linked to the flagellum's basal body, ensuring that it is properly segregated during cell division.

III. Nucleus

Trypanosoma possesses a single, membrane-bound nucleus that contains the parasite's genomic DNA. The nucleus is typically located centrally within the cell and is responsible for the regulation of gene expression, DNA replication, and cell division. The nuclear envelope is continuous with the endoplasmic reticulum, and the nucleolus within the nucleus is the site of ribosomal RNA synthesis. Unlike many eukaryotes, trypanosomes exhibit polycistronic transcription, where multiple genes are transcribed as a single unit, and subsequent RNA processing steps are required to produce mature mRNA.

IV. Surface coat and glycosylphosphatidylinositol (GPI)anchors

The surface of *Trypanosoma* is covered by a dense coat of glycoproteins known as variant surface glycoproteins (VSGs). These VSGs are attached to the plasma membrane via glycosylphosphatidylinositol (GPI) anchors and play a crucial role in immune evasion. The parasite can rapidly switch the expression of different VSG genes, a process known as antigenic variation, which allows it to evade the host's immune system by altering its surface antigens. This ability to change its surface coat is one of the key factors that contribute to the persistence of *Trypanosoma* infections in the host.

V. Subpellicular microtubules and the cytoskeleton

The subpellicular microtubules, mentioned earlier, are a critical component of the trypanosome cytoskeleton. These microtubules are arranged in a highly organized array just beneath the plasma membrane, running parallel to the long axis of the cell. They provide structural support and help maintain the characteristic shape of the parasite. The cytoskeleton of *Trypanosoma* is not static; it is dynamic and undergoes rearrangements during the parasite's life cycle.

VI. Golgi apparatus and endomembrane system

Trypanosoma possesses a single Golgi apparatus, which is located near the flagellar pocket. The Golgi apparatus is involved in the modification, sorting, and trafficking of proteins and lipids, including the synthesis and addition of GPI anchors to surface proteins like VSGs. The endomembrane system of *Trypanosoma* is relatively simple compared to that of other eukaryotes, reflecting the parasite's streamlined cellular organization.

VII. Mitochondrion

In addition to the kinetoplast, the mitochondrion in *Trypanosoma* is an elongated, tubular structure that extends along the length of the cell. The mitochondrion plays a crucial role in energy metabolism, particularly in the insect vector stages

of the parasite, where oxidative phosphorylation is the primary source of ATP. In contrast, during the bloodstream stage in the mammalian host, the parasite relies on glycolysis for energy production, and the mitochondrion becomes less active.

The microscopic structure of *Trypanosoma* is a masterpiece of evolutionary adaptation, enabling the parasite to survive and thrive in diverse environments and hosts. From its flagellum and undulating membrane to the kinetoplast and VSG coat, each structural feature of *Trypanosoma* plays a vital role in its biology and pathogenicity. Understanding these structures not only sheds light on the intricate life cycle of the parasite but also provides valuable insights for the development of targeted treatments and vaccines against the diseases caused by *Trypanosoma*. As research continues to unravel the complexities of this parasite, new opportunities for combating trypanosomiasis and improving global health will emerge.

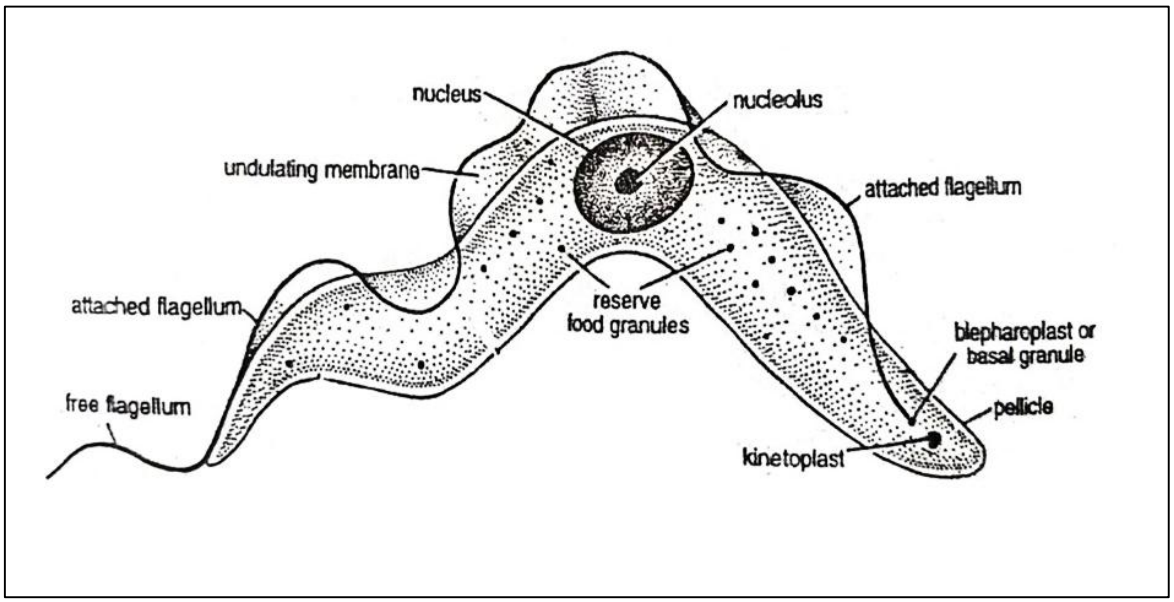

Fig- Ultrastructure of *Trypanosoma* sp.

Probable questions:

1. Describe the general morphology of *Trypanosoma* sp structure.
2. Write short notes on Kinetoplast structure of *Trypanosoma* sp.
3. Write short notes on undulating membrane structure of *Trypanosoma* sp.
4. Write short notes on about the mitochondrion structure of *Trypanosoma sp.*

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Unit XII

Structure, biology and control of reduviid bug

Objective: In this unit we will learn about Structure, biology and control of reduviid bug.

Classification: -

Kingdom: Animalia

Phylum: Arthropoda

Class: Insecta

Order: Hemiptera

Suborder: Heteroptera

Family: Reduviidae

Family reduviidae

Most reduviids are predators on other insects and are commonly called assassin bags for this reason. They are often valuable for their predation on pest species; *Reduvius personatus* may even enter houses and feed on bedbugs! Most of these can, but usually do not, bite humans, the bite is quite painful. The Reduviidae are a large, cosmopolitan, and morphologically diverse family of predatory true bugs. They include assassin bugs (genera include *Melanolestes*, *Psellipus*, *Rasahus*, *Reduvius*, *Rhiginia*, *Sinea*, and *Zelus*), wheel bugs (*Arilus cristatus*), kissing bugs (species of *Triatoma*, *Rhodnius* and *Panstrongylus*), ambush bugs (genera *Apiomerus* and *Phymata*), and thread-legged bugs (the subfamily Emesinae, including the genus *Emesaya*).

Fig- *Reduvius personatus*

One subfamily of the Reduviidae, the Triatominae, is of great public health significance because its members are the vectors for *Trypanosoma cruzi*, the causative agent of Chagas' disease. The prevalence of Chagas' disease is currently estimated at more than 10 million cases in South and Central America. The Triatominae characteristically feed on blood of various vertebrates. They are called kissing bugs because they often bite the lips of sleeping persons.

Epidemiology

The insects are nocturnal and hide in cracks, crevices, and roof thatching during day times. Poorly constructed houses are thus a significant epidemiological factor. *Triatoma infestans* does not have to be alive to transmit T. *cruzi* infections. Live trypomastigotes infective for mice were found in dead bugs for up to two weeks after one spraying campaign.

Dogs, cats, and rats are important reservoir hosts around human habitations, and there is a wide variety of sylvatic reservoirs, the most important of which is the opossum, *Didelphis marsupialis*. The opossum is a common and successful marsupial occurring from the northern United States to Argentina. Other important reservoirs include armadillos, bats, squirrels, wild rats and mice, guinea pigs, and sloths.

The Triatominae is divided into 5 tribes and 14 genera; 106 species are known only from the New World, 5 species (*Linshcosteus*) are found only in India, and 7 species (*Triatoma*) are known only from Southeast Asia. The only species found in Africa is *Triatoma rubrofasciata*; it is found throughout the tropics, presumably spread worldwide via sheeps.

The New World triatomine species occur from just south of the Great Lakes region of the United States to southern Argentina, with all but a few species concentrated in subtropical and tropical regions. All triatomines have the potential to transmit *Trypanosoma cruzi,* the etiologic agent of Chagas disease. Of the 119 described triatomine species, about half have been shown to be vectors, and about a dozen of these are considered vectors of major epidemiological importance.

Table 1: Major Triatomine Vectors of *Trypanosoma cruzi* and Their Geographic Distribution

Species	Geographic range
Rhodnius prolixus	Southern Mexico, Guatemala, El Salvador, Honduras, Nicaragua, Costa Rica, Colombia, Venezuela
Triatoma infestans	Peru, Bolivia, Brazil (from Mato Grosso across to northeastern Goiás and Paraiba, south to Rio Grande do Sul), Paraguay, Argentina, Uruguay, and

	Chile
Triatoma dimidiata	Mexico south to Ecuador and Peru
Triatoma pallidipennis	Mexico
Triatoma phyllosoma	Mexico
Rhodnius pallescens	Panama, Colombia
Triatoma maculata	Colombia, Venezuela, Netherlands Antilles, Guyana, Suriname
Triatoma brasiliensis	northeastern Brazil
Panstrongylus herreri	northern Peru
Panstrongylus megistus	Brazil (especially coastal), Paraguay, Argentina, Uruguay
Triatoma guasayana	Bolivia, Paraguay, Argentina
Triatoma sordida	Bolivia. Brazil. Paraguay. Uruguay, Argentina

Morphology

- Most Reduviidae are medium to large bugs and often show elongate or ovoid body shapes.
- Some of the most distinctive characteristics of the assassin bugs are the neck-like shape of the head behind the eyes and the labium, which is short, strongly curved, and inflexible.
- Other relevant body structures include the membrane of hemelytra, usually with two or three elongated cells; the presence of a fossula spongiosa at the apex of the fore and

mid tibiae in many taxa; and the presence of Brindley's glands between the metathorax and the first abdominal segment.

- The female genitalia have a lateral spermathecae; males with the eighth abdominal segment telescoped largely into the seventh segment and usually with symmetrical genitalia.
- Holometabolous metamorphosis.
- Abdomen lacks cerci.
- Triatomines are relatively large bugs, up to 34 mm in length.
- They usually have wings, which are held in a concavity on top of the abdomen.
- The head is narrow, and large eyes are located midway or far back on the sides of the head. Two ocelli may be present behind the eyes.
- The antennae are slender and in four segments. The apparently three-segmented labial tube folds backward at rest into a groove between the forelegs. The bugs can make a squeaking sound by rubbing the labium against ridges in the groove (stridulation).
- The color pattern varies, with an overall black or piceous color and spotted patterns of yellow, brown, orange, or red

Fig- Adult female kissing bug of the species *Triatoma rubida*, the most abundant triatomine species in southern Arizona.

Biology

The various reduyiids characteristically frequent different sites; for example, some species are normally found on the ground, some in trees, and some in human dwellings. The eggs, numbering from a few dozen to a thousand depending on species, are deposited in the normal habitat of the adult. There are usually five nymphal instars. Triatomines do not seem

to be very choosy about their food sources; whatever vertebrate is available in their habitat is apparently acceptable. Triatomines that inhabit human dwellings feed on humans, dogs, cats, and rats; other species depend more on wild animals.

Research on *trypanosoma cruzi*, as well as on xenodiagnosis, demands a supply of lab-reared bugs, and much effort has gone into the development of rearing techniques, especially feeding stimuli. Temperature seems to be a stimulus for *Triatoma infestans*; in experimental feeders mammalian body temperatures evoked a feeding response, although crop filling did not depend on blood temperature. Evidently *Triatoma infestans* can detect objects that are near mammalian body temperatures and orient toward them when seeking food. *Triatoma infestans* is also somewhat particular in what it eats, being partial to citrated over heparinized blood (sodium citrate and heparin are both anticoagulants), but mouse odor added to the feeder does not make the blood more appetizing.

Life cycle

Mating in triatomines can involve a fairly complex set of behaviors. In one laboratory study involving *Triatoma mazzottii*, nine steps were identified, including vigilance on the part of the male, female advancement, gyrations, copulation, and separation, all happening in about 10 minutes. Only about 1 in 10 of the matings was completed, however, due to a combination of nonreceptiveness on the part of females and indifference on the part of males.

Egg laying follows a circadian rhythm in *Rhodnius prolixus*, and lab-reared populations can be made to lay more or less synchronously using light-dark cycles. The restricted timing of egg laying persists when the insects are transferred to total darkness, suggesting environmental control of population level ovulation and oviposition. Both egg laying and feeding in *Rhodnius prolixus* are likely under hormonal control; serotonin is evidently secreted from tissues associated with the abdominal nerves and builds up in the hemolymph during feeding. Blood meals are essential to egg production, but adults may lay eggs without feeding.

Life cycle of assassin bugs

- Kissing bugs go through incomplete metamorphosis, meaning they have an egg, nymph, and adult stage. They have 5-8 nymphs or instar stages. At each stage, they will take at least one blood meal before molting to the next instar. Blood meal hosts may include humans, dogs, wildlife, chickens, and more. Right after feeding, some species of kissing bugs will defecate (poop), which is how the parasite *Trypanosoma cruzi* is transmitted.
- Time needed between life stages will vary based on temperature and host availability. Once they reach adulthood, female kissing bugs will mate, feed, and lay eggs. She will mate with several different individuals and can lay hundreds of eggs in her lifetime.
- Adults can lay up to 200-300 eggs in rafts (bundles) of 30–60.

- **Eggs:** Eggs are barrel-shaped and laid upright in clusters or rows on leaves or stems. Eggs hatch within 2 weeks.
- **Nymphs:** They look similar to adults but don't have wings and most common in tree crops. Also found in broadleaf crops such as cotton, pulses, canola and sunflower. Nymphs Feeds on many different insects, but prefers soft-bodied prey such as caterpillars and small bugs like green mirids. Wingless nymphs pass through 5 growth stages before reaching adulthood.
- **Adults:** Adults may live for 6–10 or even upto 24 months. Adults are 10–30mm long and have distinct, elongated heads with raised eyes, long curved proboscises, abdomens with a slight waist. They have long, slender back legs and front legs are enlarged to grab prey.

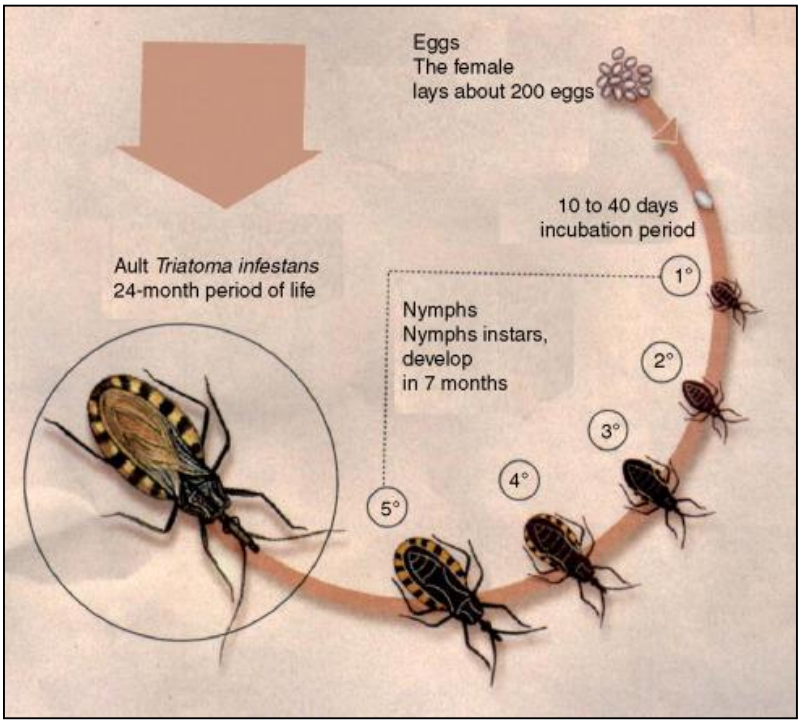

Fig- Life cycle of Assassin bugs (*Triatoma infestans).*

Transmission

T. cruzi can be transmitted by various triatomine bugs in the genera *Triatoma*, *Panstrongylus*, and *Rhodnius*. The primary vectors for human infection are the species of triatomine bugs that inhabit human dwellings, namely *Triatoma infestans*, *Rhodnius prolixus*, *Triatoma dimidiata* and *Panstrongylus megistus.* These insects are known by a number of local names, including vinchuca in Argentina, Bolivia, Chile and Paraguay, barbeiro (the barber) in Brazil, pito in Colombia, chinche in Central America, and chipo in Venezuela.

Bugs live in mud, thatch or huts. They hide in walls or roof during day and come out during night.

↓

The bugs tend to feed at night, preferring moist surfaces near the eyes or mouth. A triatomine bug can become infected with *T. cruzi* when it feeds on an infected host. *T. cruzi* replicates in the insect's intestinal tract and is shed in the bug's feces. When an infected triatomine feeds, it pierces the skin and takes in a blood meal, defecating at the same time to make room for the new meal. The bite is typically painless, but causes itching. Scratching at the bite introduces the *T. cruzi*-laden feces into the bite wound, initiating infection.

↓

Once entered inside body the parasites multiply and spread and cause Chagas disease.

In addition to classical vector spread, Chagas disease can be transmitted through consumption of food or drink contaminated with triatomine insects or their feces. Since heating or drying kills the parasites, drinks and especially fruit juices are the most frequent source of infection.

Pathogenesis

- **Reduviidae Bugs as Vectors: -**
  - Assassin bugs are large blood-sucking insects with sharp beaks, inflict an extremely painful bite and inject a lethal nerve poison that liquefies tissues.
  - There are about 2,500 known species of reduviid, or assassin bugs. Characteristically, a short, three-jointed proboscis protrudes from the tip of the head, and the insects feed primarily on body fluids of other insects although some attack humans and other animals. Members of three genera, *Triatoma*, *Panstrongylus*, *and Rhodnius*, are the major vectors for **Chagas' disease**. While several dozen species belonging to these genera occur in various countries of North and South America, relatively few serve as significant vectors for Chagas' disease. Most species are arboreal and feed on the blood of wild animals, and their importance lies in their role in maintaining the infection in sylvatic reservoirs. Species that commonly inhabit or occasionally intrude into human dwellings are the major transmitters to humans. Such insects, unlike transient mosquitoes, actually invade the homes and establish stable colonies in cracks and crevices of walls and in thatched roofs.
  - The adults emerge at night for a blood meal once or twice a week. Their bites are painful, often resulting in **itchy swellings** from toxins injected during feedings.

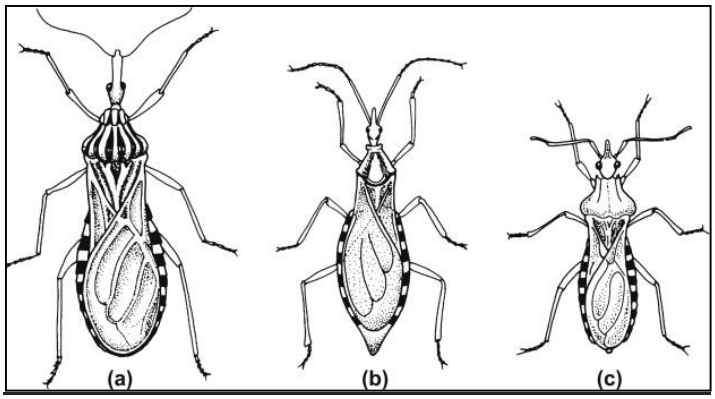

Fig- Some bloodsucking reduviids. (a) *Panstrongylus*. (b) *Triatoma*. (c) *Rhodnius*.

Chagas disease can cause -

1. **Acute Phase**
    - **Early Symptoms**: This phase may last for a few weeks to a few months. Symptoms can include fever, malaise, rash, and swelling at the site of entry. In some cases, a noticeable swelling called a **chagoma** may appear at the site of the bite, or a **Romaña's sign** (swelling of the eyelid) if the parasite enters through the eye.
    - **Parasitemia**: The parasite rapidly multiplies in the bloodstream, leading to a high level of parasites in the blood. This phase is often characterized by significant immune system activation.
2. **Immune Response**
    - **Immune Reaction**: The host's immune system responds to the infection with both innate and adaptive immune responses. However, *T. cruzi* has evolved mechanisms to evade the immune system, leading to chronic infection.
3. **Chronic Phase**
    - **Latency**: If untreated, the acute phase can progress to a chronic phase, which may remain asymptomatic for years or even decades.
    - **Chronic Symptoms**: About 20-30% of individuals will develop chronic complications, including:
        - **Cardiac**: Chronic Chagas cardiomyopathy, which can cause heart failure, arrhythmias, and other cardiac issues.
        - **Gastrointestinal**: Megaesophagus and megacolon, leading to difficulties with swallowing, digestion, and bowel movements.

4. **Chronic Inflammation and Tissue Damage**
    - **Autoimmunity**: Chronic inflammation can lead to tissue damage and autoimmunity, where the immune system attacks the body's own tissues, particularly in the heart and digestive tract.
    - **Fibrosis**: Prolonged inflammation can lead to fibrosis, which impairs organ function.

Control

- **Treatment:**
    - Reducing the effects of a bite from a member of the Reduviidae family, which includes the notorious kissing bugs, involves a few key steps:
        1. **Clean the Area**: Wash the bite area with soap and water to reduce the risk of infection.
        2. **Apply Antiseptic**: Use an antiseptic solution to prevent any potential infection.
        3. **Use Anti-itch Cream**: Apply an over-the-counter hydrocortisone cream or an antihistamine cream to alleviate itching and swelling.
        4. **Take Oral Antihistamines**: If itching is severe, oral antihistamines like diphenhydramine (Benadryl) may help reduce allergic reactions and itching.
        5. **Apply a Cold Compress**: A cold compress can help reduce swelling and provide relief from itching.
        6. **Pain Relief**: Over the counter pain relievers such as ibuprofen or acetaminophen can be used to alleviate pain and discomfort.
        7. **Avoid Scratching**: Scratching can lead to infection or worsen the irritation.
        8. **Monitor for Infection**: Watch for signs of infection, such as increased redness, warmth, swelling, or pus. If these occur, or if you experience severe symptoms like difficulty breathing, seek medical attention promptly.
    - If the bite is from a kissing bug and if it is concerned about Chagas disease, which these bugs can transmit, consult a healthcare provider. Early diagnosis and treatments are crucial for managing this disease.
- **Prevention:**
    - The number of triatomines in a house increases with the number of people living there, but the triatomine population can be reduced by reducing the number of hiding places for the bugs that is, by improving construction and altering nearby

environments. For example, removal of stacked fire wood from near houses and replacement of dirt floors with concrete, eliminates *Triatoma dimidiata* infestation.

- Reducing the number of other food sources for the bugs around the dwelling, such as dogs, birds, and rats, also is of value in controlling triatomines.
- As with bedbugs, residual insecticides around the potential hiding places are effective in control, and paint with insecticide (**chlorpyrifos**) has been used with some success for control of triatomes, especially on wood interiors. However, nutritional state affects the susceptibility of *T. infestans* to insecticide.
- Preventing an assassin bug infestation in the home requires sealing off possible points of entry. Make sure to seal any cracks or crevices in exterior walls with a silicone-based caulk, replace weather stripping, install door sweeps and repair any torn screens in doors or windows.
- **Precocene II**, a natural product extracted from the plant *Ageratum* sp., shows promise as a fumigant against triatomines. It is cytotoxic to the corpora allata, preventing production of juvenile hormone. Precocene blocks oogenesis in adult females and causes immatures to molt precociously to sterile adults. Triatomine bugs occur in the United States from New England to California. Similarly *T. cruzi* has been found from coast to coast in wild mammals, including wood rats, raccoons, opossums, and skunks. Several cases of human infection were diagnosed in Arizona.
- The most effective professional products for conenose bug control include wettable powder or microencapsulated formulations of pyrethroid insecticides such as cypermethrin, lambda-cyhalothrin, deltamethrin, or cyfluthrin.
- **Insect control:** In areas where Reduviidae bugs are common, take measures to reduce exposure, such as using insect screens, bed nets, and insect repellents.
- **Avoid Rubbing the bite:** Do not rub or scratch the bite site, as this can increase the risk of introducing *T. cruzi* from the bug's feces into your body.

Different genus of Reduviidae bugs: -

Scientists has contributed an annotated taxonomic study on the Reduviidae. Of the many genera, Triatoma, Rhodnius, Panstrongylus, Melanolestes, and Rasahus are the most important.

- **Genus *Triatoma*:** Several species of *Triatoma* are naturally infected with the hemoflagellate *Trypanosoma cruzi*. Among these, *Triatoma sanguisuga* (the "**Mexican bedbug**"), found in the United States and Central America, is one of the most common vectors.

- This bug measures 18-20 mm in length and is characterized by a flattened body that is dark brown and splattered with reddish orange or pinkish areas on the abdomen, on the tips and bases of the hemelytra, and along the lateral and anterior margin of the pronotum.
- Similarly, *Triatoma protracta*, which is widely distributed along the Pacific Coast of North America, is naturally infected with *Trypanosoma cruzi. T. protracta* is also known to be naturally infected in Arizona and Texas. Both of these species are vicious biters of various mammals, including humans. Their bites result in very painful and itchy swellings due to a toxin injected during feeding.
- In Hawaii, *Triatoma rubrofasciata* produces similar symptoms, and the anaphylactic reactions to the bites result in intense itching welts.
- Other species of *Triatoma* known to be naturally infected with *T. cruzi* are *T. infestans* in southern Brazil, Uruguay, Chile, Paraguay, Argentina, and southern Bolivia: *T. dimidiata* in Mexico, Panama, Guatemala, and San Salvador, *T. hegneri*, *T. rubida*, *T. barberi*, and *T. gerstaeckeri* in Texas; and *T. rubida* and *T. recurva* in Arizona. There are more than 10 million infected persons in South and Central America today. A few cases have been reported from Arizona. Since the *Trypanosoma cruzi* population in the blood of infected humans is small and difficult to detect, the diagnostic technique originated by Brumpt, known as xenodiagnosis, is widely used. This technique involves allowing uninfected Triatoma to bite the individual and examining the digestive tract of the bug for flagellates after a period of incubation.

- **Genus *Rhodnius*:** *Rhodnius prolixus* and *R. pallescens* are two representatives of the genus that reportedly are naturally infected with *Trypanosoma cruzi*. The former ranges from Brazil north to Colombia and is found in San Salvador and Mexico, whereas the latter is found in Panama. While working with *R. prolixus* in 1912, Brumpt demonstrated that *T. cruzi* is not transmitted through the bite of the bug. Rather, the infective form of the flagellate passes out in the vector's feces and is mechanically deposited via contaminated hands on the extremely susceptible mucous membranes of the nose, eyes, or mouth, or is rubbed into skin perforations. *Rhodnius brumpti* and *R. domesticus* also are found in Brazil.

Fig- *Rhodnius prolixus.*

- **Genus *Panstrongylus*:** This genus contains several species, including *P. megistus* and *P. rufotuberculatus*, which are suitable vectors for *Trypanosoma cruzi*. *Panstrongylus megistus*, which is widely distributed in Brazil, the Guianas, and Paraguay, is a nocturnal hematophagous species, hiding by day in cracks and crevices in houses. The adults measuring 30-32 mm in length are black with red markings on the prothorax, wings, and abdomen. *Panstrongylus rubrofasciata*, another bloodfeeder, is also widely distributed, being found in the Orient, Ethiopia, Central America, the West Indies, and Florida. The bites of neither *P. megistus* nor *P. rubrofasciata* result in such a severe reaction as those of *Triatoma* sp.

Fig- *Panstrongylus megistus.*

- **Genus *Melanolestes*:** This genus includes *M picipes* and *M. abdominalis*. *M. picipes* is black and is found under stones, logs, and masses throughout North America. It bites humans, and the host's reaction to its toxins is severe. *M. abdominalis* has habits similar to those of *M. picipes* and is also widely distributed throughout North America. Members of *Melanolestes* are known to serve as vectors for microorganisms.

Fig- *Melanolestes picipes.*

- **Genus *Rasahus*:** *Rasahus biguttatus* and *R. thoracicus*, commonly referred to as the **corsair bugs**, are severe biters and cause damaging inflammations. Neither of these serves as a vector. *Rasahus biguttatus* is found in the southern United States, the West Indies, and South America, whereas *R. thoracicus* is found in the western United States and Mexico.

Fig- *Rasahus biguttatus.*

Probable questions:

1. Discuss the epidemiology of reduviid bug.
2. Comment on Major Triatomine Vectors of *Trypanosoma cruzi* and Their Geographic Distribution.
3. Describe the morphology of *Triatoma rubida*
4. Describe the life cycle of Assassin bugs (*Triatoma infestans) with diagram*
5. Elastrate the transmission procedure of *Trypanosoma cruzi*.
6. Write down the pathogenecity of Reduviidae Bugs as Vectors.
7. Write down the symptoms of Chagas disease.
8. Which treatments can be applied when a person is bitten by a bug of the Reduviidae family.
9. State the prevention method of triatomine population.
10. Write short notes on *Rasahus biguttatus.*

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Unit XIII

Structure, biology and control of non-biting dipterans

Objective: In this unit we will discuss about Structure, biology and control of non-biting dipterans.

Introduction

The family of Chironomidae is a group of Diptera insects belonging to the suborder of Nematocera, commonly called “non-biting midges” in the adult stage and “bloodworms” in the larval stage. The Chironomidae are often the most abundant group of macroinvertebrates, in number of species and individuals, encountered in all aquatic environments of freshwater, brackish, terrestrial and even the sea. Likewise, Chironomidae occur in all the continents. The Chironomidae family is divided into 11 sub-families that have different ecological statues. Despite the wealth of data on Chironomidae in the Holarctic region, other parts of the world are poorly studied and few guides to identifying Chironomidae have been produced. This chapter includes a theoretical synthesis on the Chironomidae, it deals with the Biology (life cycle and description of different stages), description of all subfamilies and the ecology of this important family of Diptera.

Fig: Non biting midges

- The Chironomidae family is a group of Diptera insects belonging to the suborder of Nematocera. Members of this family are commonly called “non-biting midges” in the adult stage and “bloodworms” in the larval stage.

- The Chironomidae are often the most abundant group of macroinvertebrates, in number of species and individuals, found in all freshwater aquatic environments. They are widely distributed and live in both lentic and lotic ecosystems. Indeed, the Chironomidae are among the few insects living in the sea and the ocean. Likewise, they occur in all continents where they have been found alive at heights of 5600 m on the glaciers of the Himalayas and in the depths of the lakes. Several qualitative observations showed that larvae of terrestrial Chironomidae are able to colonize the vegetation above the soil surface on heathlands.
- Chironomidae are holometabolous insects, their larvae, pupae and adults form an integral part of the trophic chain serving as food for other invertebrates, fish, birds and amphibians. The larval and pupal stages are generally subservient to aquatic habitats while the adults are aerial and often collected at more or less distances from their emergence habitats.

Morphology

- Chironomidae are Diptera belonging to the morphological group of the Culiciforma, so their general appearance is that of a mosquito. They are Nematoceran and as such, they are characterized by long antennae (more or less as long as the head). Their mouthparts are much regressed and the atrophy of the mandibles in the adult stage does not allow them to bite.
- Chironomidae undergo during their life cycle four morphologically very different stages which, while having a general appearance identical from one subfamily to another, present anatomical variations which constitute essential bases of their systematics.

Structure

Eggs

- The egg of Chironomidae, like all insects, is of the centrolecithic type, rich in yolk which constitutes a central mass of nutrient reserves. The cytoplasm containing several nuclei is peripheral.
- The eggshell has, from the inside to the outside, the yolk envelope and the chorion separated by a protective waxy layer. In general, the chorion of eggs of Chironomidae is not very thick and contains protrusions and has a micropyle. However, it may be smooth in other species such as *Tanytarsus barbitarsis* or thick providing some protection against desiccation in eggs of Telmatogetoninae.
- In general, all Chironomidae laid their eggs in the form of gelatinous masses in contact with water. However, members of the Telmatogetoninae subfamily are an exception since their eggs are laid individually without a gelatinous matrix.

Number of eggs

- Often, the egg masses of Chironomidae contain approximately 20 to 30 eggs. This number can increase to over 3000 in large species. In fact, the largest number of eggs laid was recorded in *Chironomus tentans* with 3300 eggs in a single mass. However, there may also be intraspecific variations.

Shape and size of the eggs

- The shape of the eggs in Chironomidae is usually elliptical or kidney-shaped. Likewise, the eggs can also be deltoids in some Telmatogetoninae (*TelmatogeIton japonicus*) and some Orthocladiinae such as *Orthocladius* sp. and *Eukiefferiella claripennis*.
- Egg sizes vary greatly between species. Indeed, the smallest eggs are those of *Corynoneura* and *Thienemanniella* whose size is around 170 µm long and 70 µm wide, while *Tanypus punctipennis*, a large Tanypodinae, lays eggs 612 µm long and 135 µm wide. Generally, in Chironomidae the ratio: length/width is 2.5 to 3.

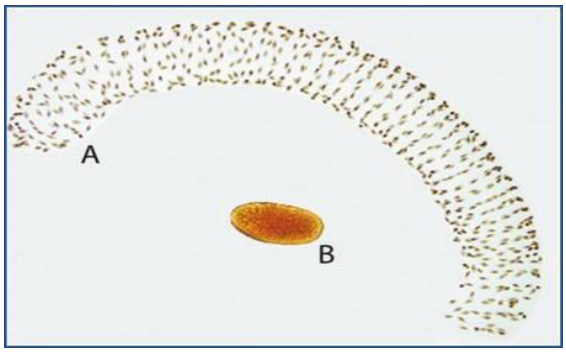

Fig*: Chironomus striatipennis.* (A): Egg mass; (B): Egg

Embryonic development

- The duration of embryonic development is largely influenced by environmental factors especially temperature. In fact, *Thiennemanniella vittata* eggs hatch in a minimum of 4 days at 20°C, 6 days at 15°C, 13 days at 10°C and 31 days at 5°C.

The larvae

- The Chironomidae undergo four larval stages but all morphological and taxonomic observations have been made on the last stage. The majority of structures appear in the early larval stages but many characters of the final stage, especially the shapes and ratios, do not apply to the early stages and do not allow good differentiation.
- The larvae of Chironomidae have a well-individualized, developed, exposed, complete, and non-retractile head capsule and a narrow, elongated segmented body that lacks thoracic legs.

Fig: Larva of non biting midges

The pupa

- The pupal stage of Chironomidae is very short compared to the larval stage, its duration is from a few hours to several days.
- The characters of the pupae of Chironomidae are best seen on their exuviae, which are very useful tools for species determination.
- The pupae of Chironomidae are comma shaped with a swollen cephalothorax and a dorsoventrally flattened abdomen. Their length varies from just under 3 to 18 mm. Their coloration usually follows that of the larva.

The adult (the imago)

- The body of the adult of Chironomidae consists of three parts:

Head

- The head: globular, it carries:
- The antennae: long and exhibit sexual dimorphism since they are fluffy in males and moniliform in females. The antenna of the adult Chironomidae consists of a narrow escape, a globose pedicel and a number (often 11–14) of flagellomeres. The number of antenna segments and their shape depend on the species.
- The eyes are very large and kidney-shaped.
- The mouthparts are very reduced.

Thorax

- Thorax generally well developed, it has three parts of equal importance: pronotum, mesonotum and metanotum. The thorax bears the wings and the legs.

Abdomen

- Abdomen composed of 10 segments, the seven anterior segments are flattened dorsoventrally. The female's abdomen is shorter and more swollen than that of the male. The dorsal part has coloring or ornamentation is often useful for identification. The last abdominal segments form the genitalias. The tergite IX has a posteromedial extension forming the anal point. Among the most distinctive characters of male genitalias are: basal gonocoxites and apical or subapical gonostyles. The gonocoxites of Chironomidae support a varying number of appendages called: volsellae and they are named according to their relative positions (middle, inferior and superior). Likewise, there may be other lobes associated with the aedeagus and the penis.

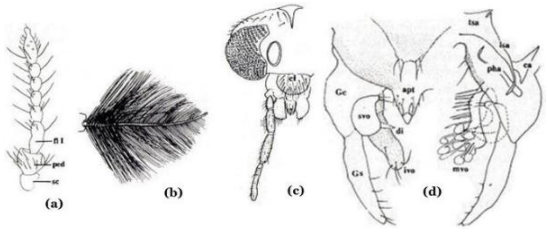

Fig: Morphology of the adult of non biting midges: (a): antenna of the female; (b) the male antenna; (c): the head; (d): male genitalia.

Life cycle

Chironomidae are Holometabolous insects, their development cycle comprises four morphologically very different states which, while having a general appearance identical from one subfamily to another, present anatomical variations which constitute one of the essential bases of systematics.

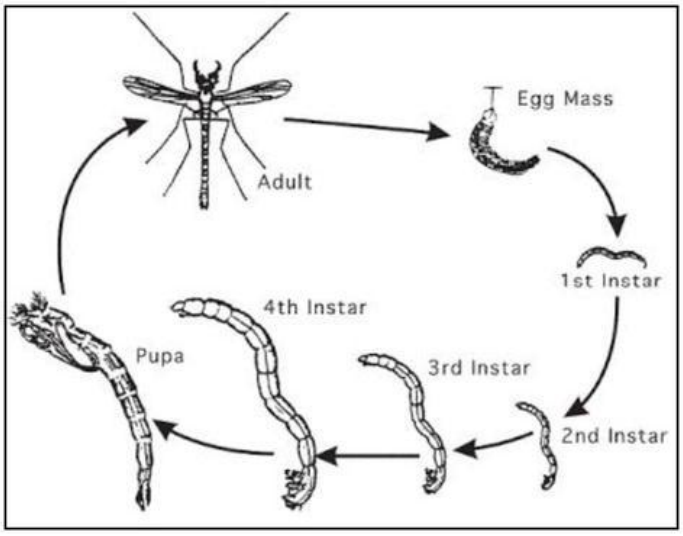

Fig- Life cycle of non biting midges

- The life cycle of Chironomidae begins with the deposition of eggs in water. These are gathered in gelatinous masses or deposited individually. The eggs may be free or attached to an object. They hatch after a more or less long period releasing the larvae. The latter undergo a reduced number of moults and go through four larval stages. The larvae can be free, sedentary or live inside a tube that they build with the substrate and salivary secretions.
- The larval stage is followed by that of the pupa. This can swim freely or, in tubicolous species, can remain partially included in the larval tube. At maturity, the pupa reach the surface of the water with the air produced in the intercuticular space of the adult. The adult emerges from the surface of the water in a very short period of time. Imaginal life is short. It does not take long for the adults to mate and lay the eggs.

Biology of the larvae

Feeding

- During the first hours of their post-embryonic life, the young larvae feed on the mucilaginous substance of the egg mass, then they pierce the envelope to become planktonic and become microdetritivores ingesting small organic particles.
- From the second instar, when the larvae are looking for a support, they feed on the detrital film of the bottom, ingesting both dead and living algae as well as inorganic particles.
- Based upon the feeding mode, larvae can be grouped in six categories: collectors (gatherers and filterers), shredders, scrapers, and predators (engulfers and piercers). They feed on a great variety of resources including coarse particulated organic matter fragmentation, periphyton, algae, microorganisms and dissolved organic matter.

Locomotion

- Larvae display three modes of motility: swimming, crawling and whole-body respiratory undulation. Swimming and respiratory undulation involve the use of metachronal waves of body bending which travel in a head-to-tail direction. Whereas swimming is produced by side-to-side flexures of the whole body, respiratory undulation employs a sinusoidal wave.

Biology of the pupae

- The pupal stage links two active stages in the life of insects: the larval and imaginal stages. While the pupal stage of most insects is immobile, the pupae of most Chironomidae are active for a very large part of their existence. In the majority of Chironomidae the pupae move to accomplish three main functions: moulting from

the larval cuticle, providing oxygen for respiration, and moving to the surface for emergence of the adult.

- Moulting from the larval cuticle
- The pupal cephalothorax is formed in the thoracic segments of the larva, and its large volume exercise a pressure on the dorsal suture. Undulations of the abdomen engage the points on the pupal tegument with the larval tegument, leading the pupa forward into the larval thorax. This extra pressure causes the rupture of the suture to push the back of the larval head to the first abdominal tergum, continuous undulations easily release the pupa outward .

Locomotion

- The pupa displays two swimming modes, somersaulting and eel-like whole-body undulation. The former being principally a brief, escape manoeuvre, the latter being a faster form of locomotion employed to deliver the pupa to the surface prior to adult emergence.
- The pupae of the majority of chironomidae swim freely, and they are susceptible to flotation and are provided with structures allowing them to adapt to this way of life. Indeed, these pupae have horns with a large plastron to allow them to float. Likewise, in some chironomidae (Pentaneurini) the anal macrosetae are covered with a sticky gelatinous material forming a natatory fringe that allows them to adhere to the substrate and with sudden flexion of the abdomen the pupae move to the surface. Tubicole species only leave their tubes to hatch on the surface of the water, and their anal fringes are therefore not used for swimming.

Biology of the adults

- The adults of Chironomidae are aerial. Swarm flight has long been discussed in several studies. Indeed, dense columnar swarms of Chironomidae are often observed, they extend from the tops of trees, the roofs of houses or around lakes.
- There is a close relationship between the size of the swarm and the number of matings. Indeed, the formation of flight in swarms allows a high rate of mating especially if the population density is low. Thus, the denser swarms attract more females.
- Most Chironomidae lay their eggs on or near water. The egg laying sites are very varied depending on the species. Indeed, egg masses of Chironomidae have been observed in lakes, rivers, streams, rice fields, sea and vegetation in the case of terrestrial species.
- In general, the eggs are laid on the surface of the water, it can be carried by the wind and the current and travel long distances before the eggs hatch, which

contributes significantly to ensure the dissemination of the species despite increased risk of destruction.

Physical, Mechanical and Cultural Control

Nutrient reduction: Dense larval populations usually occur in nutrient rich habitats. Fertilizer run-off from residential lawns and garden, golf courses and agricultural fields are sometimes responsible for the development of nuisance-level populations of midges. Community awareness and education about proper use of fertilizers can reduce excess run-off into lakes, ponds and streams and can help reduce midge populations. In some instances, it may be helpful to have your pond evaluated and get recommendations for nutrient management.

Winter draw down: Exposing bottom muds by draining ponds and lakes during winter months will kill over wintering midge larvae, reducing the size of the adult population emerging in the following spring. Understandably, this method may not be economically or logistically practical for many bodies of water.

Diversion of adults: Many lakes and reservoirs that experience nuisance populations of midges have homes, businesses and marinas constructed along the shore lines. After emergence, midge adults are attracted to shoreline lights. High intensity white light has been found to be highly attractive to adults. Keep window blinds closed and porch lights turned off during heavy emergence periods to reduce the number of adults attracted to these areas. Strategically placed high intensity white lights may divert midges away from populated areas.

Electrocutor traps: Electrocutor traps will attract and kill large numbers of midge adults. However, a single trap in a yard is unlikely to kill a sufficient number of midge adults to appreciably reduce nuisance populations. In addition, during heavy adult activity, the trap may malfunction as a result of becoming clogged with midge body fragments.

Lighting Modification: If you live near a lake side community or near a pond, you might try getting advice from your local government or electrical utility or a lighting consultant concerning the type of outdoor public lighting in your neighborhood. Some research has shown that LED lights are less attractive to midges and other night-flying insects as compared to metal halide and fluorescent lighting. There may be a situation where you would use brighter lights in an non-occupied area to attract them away from houses or where people are active outdoors. With residential structures, reducing exterior night-time lighting can help (as long as it doesn't compromise safety concerns). Close window shades to limit light shining through windows. Use subdued landscape type lighting if you wish. Avoid using floodlights except when needed.

Biological

- A large variety of aquatic organisms feed on midge larvae including dragonfly naiads (nymphs), predaceous diving beetles and a variety of fish species. Where the diversity of predaceous animals is high, the density of midge larvae is usually held below nuisance population levels. Shallow, organically rich lakes and heavily polluted habitats such as sewage waste lagoons are inhabited by fewer predaceous species compared to bodies of water that receive less nutrient-rich input.
- Predatory fish: Chironomid midges are a major component of the diet of many fish species. In particular, bottom-feeding fishes, such as catfish and carp, consume large numbers of midge larvae. However, the feeding habits of these fish has generally not been shown to reduce adult midge populations below nuisance levels adjacent to habitats with large larval populations.

Insecticidal Control

- Larvicides: Larvicides are insecticides that target the midge larvae. In fact, most of the common midge larvicides products are effective strictly against the larvae. For example, the insect growth regulator (IGR) S-methoprene (or Strike®) is registered for use in municipal wastewater treatment facilities to control midges and filter flies. IGRs are only effective against immature insects. The biological larvicide, *Bacillus thuringiensis* var. *israelensis* (Bti) is sold under several brand names and contains a protein toxin that affects midge and mosquito larvae as well as the larvae of a few other Diptera such as fungus gnats. Bti is only toxic after it is consumed by the midge larvae. Consequently, high organic content in water presents a competing food source for the midges and can reduce the pesticide's effectiveness against midges.
- In order for the treatment to be effective, you need to time your applications properly. Accordingly, dredge samples of bottom mud should be collected, sieved, and the chironomid larvae recovered and counted. Chemical treatments should be made when the number of larvae exceeds 100 per 6-inch square bottom sample. This treatment threshold is completely arbitrary and based on insecticide treatments made for the control of midge larvae in Florida and California. Without monitoring a midge population for one season, the relationship between numbers of immature midges found in the bottom mud and the subsequent numbers of nuisance adults cannot be established.
- Research has shown midge populations are often highest in the shallower edges of ponds. Treating a 20-foot wide band along the shoreline can be as effective (and less expensive) than trying to treat the entire pond.
- Adulticides: Many insecticides that are registered for control of the adult midges are the same products used for mosquitoes. These products are listed in the North Carolina Agricultural Chemicals Manual. Adulticides can be applied in the air as

ultra low volume sprays but are more commonly applied as diluted sprays to wall surfaces or vegetation where midge adults rest.

Fig: Sampling for midges in lake sediment

Fig: Spraying critical areas outside the house can help reduce adult midge populations.

Probable questions:

1. Write short notes about family of Chironomidae.
2. Describe the morphology of non-biting dipterans.
3. Describe the egg structure of family of Chironomidae.
4. Describe the Embryonic development of family of Chironomidae.
5. Describe the imago structure family of Chironomidae.
6. Describe the life cycle family of Chironomidae with diagram.
7. Write down the Biology of the larvae family of Chironomidae.
8. Write down the Biology of the pupa family of Chironomidae.

9. Write down the Biology of the adult family of Chironomidae.
10. Discuss the insecticidal Control of non-biting dipterans.
11. Discuss the biological Control of non-biting dipterans.
12. Discuss the physical Control of non-biting dipterans.
13. Discuss about the control techniques of non-biting dipterans.

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Unit XIV

Structure, biology & control of lice

Objective: In this unit we will learn about the structure, biology and control of lice.

Structure

The head louse is an obligate ectoparasite of humans. Head lice are wingless insects that spend their entire lives on the human scalp and feed exclusively on human blood. Humans are the only known hosts of this specific parasite, while chimpanzees and bonobos host a closely related species. Other species of lice infest most orders of mammals and all orders of birds. Lice differ from other hematophagic ectoparasites such as fleas in spending their entire lifecycle on a host. Head lice cannot fly, and their short, stumpy legs render them incapable of jumping, or even walking efficiently on flat surfaces. The non-disease-carrying head louse differs from the related disease-carrying body louse (*Pediculus humanus humanus*) in preferring to attach eggs to scalp hair rather than to clothing. The two subspecies are morphologically almost identical, but do not normally interbreed. From genetic studies, they are thought to have diverged as subspecies about 30,000-110,000 years ago, when many humans began to wear a significant amount of clothing. However, the degree of separation is contentious as they can produce fertile offspring in the laboratory. A much more distantly related species of hair-clinging louse, the pubic or crab louse, also infests humans. It is morphologically different from the other two species and is much closer in appearance to the lice which infest other primates Louse infestation of the body is known as pediculosis.

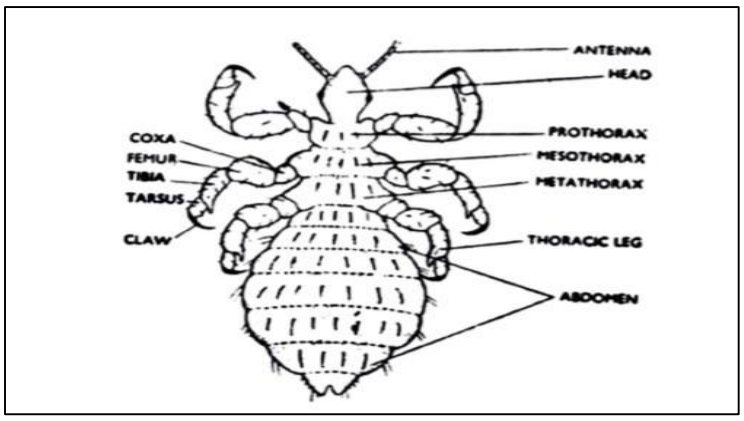

Fig- Structure of lice

I. Adult

Like other insects of the suborder Anoplura, adult head lice are small (2.5-3 mm long), dorsoventrally flattened and wingless. The thoracic segments are fused, but otherwise distinct from the head and abdomen, the latter being composed of seven visible segments.

Head lice are grey in general, but their precise color varies according to the environment in which they were raised. After feeding, consumed blood causes the louse body to take on a reddish color.

- Head: One pair of antennae, each with five segments, protrudes from the insect's head. Head lice also have one pair of eyes. Eyes are present in all species within the Pediculidae family, but are reduced or absent in most other members of the Anoplura suborder. Like other members of the Anoplura, head louse mouthparts are highly adapted for piercing the skin and sucking blood. These mouth parts are retracted into the insect's head except during feeding.
- Thorax: Six legs project from the fused segments of the thorax. As is typical in the Anoplura, these legs are short and terminate with a single claw and opposing "thumb". Between its claw and thumb, the louse grasps the hair of its host. With their short legs and large claws, lice are well adapted to clinging to the hair of their host. These adaptations leave them incapable of jumping, or even walking efficiently on flat surfaces. Lice can climb up strands of hair very quickly, allowing them to move quickly and reach another host.
- **Sex Differences**
  In male lice, the front two legs are slightly larger than the other four. This specialized pair of legs are used for holding the female during copulation. Males are slightly smaller than females and are characterized by a pointed end of the abdomen and a well-developed genital apparatus visible inside the abdomen. Females are characterized by two gonopods in the shape of a W at the end of their abdomens.

II. Eggs and Nits

Like most insects, head lice are oviparous. Females lay about three or four eggs per day. Louse eggs (also known as nits), are attached near the base of a host hair shaft. Eggs are usually laid on the base of the hair, 3-5 mm off the scalp surface. In warm climates, and especially the tropics, eggs may be laid 6 inches (15 cm) or more down the hair shaft.

To attach an egg, the adult female secretes a glue from her reproductive organ. This glue quickly hardens into a "nit sheath" that covers the hair shaft and large parts of the egg except for the operculum, a cap through which the embryo breathes. The glue was previously thought to be chitin-based, but more recent studies have shown it to be made of proteins similar to hair keratin.

Each egg is oval-shaped and about 0.8 mm in length. They are bright, transparent and tan to coffee-colored so long as they contain an embryo, but appear white after hatching. Head lice hatch typically six to nine days after oviposition. After hatching, the louse nymph leaves behind its egg shell, still attached to the hair shaft. The empty egg shell

remains in place until physically removed by abrasion or the host, or until it slowly disintegrates, which may take six or more months.

III. Development and Nymphs

Head lice, like other insects of the order Phthiraptera, are hemimetabolous. Newly hatched nymphs will moult three times before reaching the sexually mature adult stage. Thus, mobile head lice populations may contain eggs, nits, three nymphal instars, and the adults (male and female) (imago). Metamorphosis during head louse development is subtle. The only visible differences between different instars and the adult, other than size, is the relative length of the abdomen, which increases with each molt, as well as the existence of reproductive organs in the adults.

Aside from reproduction, nymph behavior is similar to the adult. Like adults, nymphs feed also only on human blood (hematophagia), and cannot survive long away from a host. Outside their hosts lice cannot survive more than 24 hrs. The time required for head lice to complete their nymph development to the imago lasts for 12-15 days.

Nymph mortality in captivity is about 38%, especially within the first two days of life. In the wild, mortality may instead be highest in the third instar. Nymph hazards are numerous. Failure to completely hatch from the egg is invariably fatal. Death during molting can also occur, although it is reportedly uncommon. During feeding, the nymph gut can rupture, dispersing the host's blood throughout the insect body. This results in death within a day or two. Whether the high mortality recorded under experimental conditions is representative of conditions in the wild is unclear.

- ## Reproduction and Lifespan:

Head lice reproduce sexually, and copulation is necessary for the female to produce fertile eggs. Parthenogenesis, the production of viable offspring by virgin females Pairing can begin within the first 10 hours of adult life. After 24 hours, adult lice copulate frequently, with mating occurring during any period of the night or day. Mating attachment frequently lasts more than an hour. Young males can successfully pair with older females, and vice versa.

A single young female confined with six or more males will die in a few days, having laid very few eggs. Similarly, death of a virgin female was reported after admitting a male to her confinement. The female laid only one egg after mating, and her entire body was tinged with red-a condition attributed to rupture of the alimentary canal during the sexual act. Old females frequently die following, if not during, copulation. During its lifespan of 4 weeks a female louse lays 50-150 eggs. Eggs hatch within 6-9 days, each nymphal stage last for 4-5 days and accordingly the period from egg to adults lasts for 18-24 days. Adult lice live for an additional 3-4 weeks.

Head lice infestation:

Head lice infestation, also known as *pediculosis capitis*, is the infection of the head hair and scalp by the head louse (*Pediculus humanus capitis*). Itching from lice bites is common. During a person's first infection, the itch may not develop for up to six weeks. If a person is infected again, symptoms may begin much more quickly. The itch may cause problems with sleeping. Generally, however, it is not a serious condition. While head lice appear to spread some other diseases in Africa, they do not appear to do so in Europe or North America.

Head lice are spread by direct contact with the hair of someone who is infected. The cause of head lice infestations in children is not related to cleanliness. Other animals, such as cats and dogs, do not play a role in transmission. Head lice feed only on human blood and are only able to survive on human head hair. When adults, they are about 2 to 3 mm long. When not attached to a human, they are unable to live beyond three days. Humans can also become infected with two other lice - the body louse and the crab louse. To make the diagnosis, live lice must be found. Using a comb can help with detection. Empty eggshells (known as nits) are not sufficient for the diagnosis.

Life cycle

There are three basic stages of head lice from inception to adulthood: the nit stage, the nymph stage, and the adult stage. Each stage is different, and the time it takes to complete each stage varies. Here is everything to know about each stage of the life cycle of lice.

i. The Nit Stage:

The life cycle of lice begins with the nit stage. Nits, also referred to as lice eggs, are the eggs laid by adult female lice (typically near the base of the hair shafts, close to the scalp). Nits are small, oval-shaped structures that are usually yellowish or whitish in color. They attach themselves firmly to the hair shafts using a glue-like substance, which ensures their stability and protection. Although they are often mistaken for dandruff or hair debris, lice eggs are more difficult to remove as they are firmly attached.

It takes approximately 8 to 9 days for nits to hatch. However, the hatching period can vary depending on factors such as temperature and humidity. Identifying the lice egg stage early on is crucial for preventing the further spread of lice infestation.

ii. The Nymph Stage:

Once the nits hatch, they release nymphs, which are the immature forms of lice. Nymphs closely resemble adult lice but are smaller in size. They go through three nymph stages: the first nymph, the second nymph, and the third nymph.

During each nymph stage, the nymph molts, shedding its exoskeleton to accommodate its growing body. This molting process occurs approximately every 7 to 10 days, but once

again, environmental factors can influence the duration. After each molt, the nymph emerges with a new exoskeleton, gradually increasing in size.

iii. The Adult Stage:

After completing the third nymph stage, the lice reach adulthood. Adult lice are fully developed and capable of reproduction. They are approximately the size of a sesame seed and have six legs equipped with claws. These claws allow them to grip onto hair strands and move around swiftly. Female adult lice have the ability to lay eggs, perpetuating the life cycle. They can lay up to six eggs, or nits, per day. The lifespan of an adult louse is typically around 30 days, during which they feed on human blood multiple times a day to survive and reproduce.

It is important to note that lice are obligate human parasites, meaning they rely on human blood for their survival. They cannot survive for more than a couple of days away from the human scalp, as they require the warmth and moisture provided by their host.

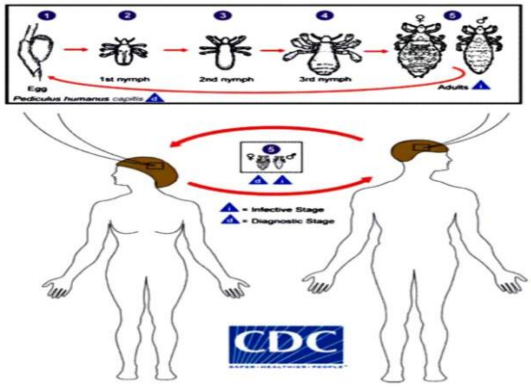

Fig- Life cycle of lice

Transmission

Head lice spreads through direct contact of the head of an infested person with the head of a non-infested person. The presence of live lice indicates an active infestation while the presence of nits indicates a past or currently inactive infection with the potential to become active. Head lice do not leap or spring as a means to transfer to their hosts; instead, they move by crawling. Transmission by indirect contact (e.g. sharing bedding, clothing, headwear, the same comb) is much less common. The cause of head lice infestations is not related to cleanliness. Neither hair length nor how often the hair is brushed affects the risk of infection. Pets are not vectors for head lice.

Other lice that infest humans are the body louse and the crab louse (aka pubic lice). The claws of these three species are adapted to attach to specific hair diameters. Pubic lice are most often spread by sexual contact with an infested person. Body lice can be found on clothing and they are not known to burrow into the skin.

Feeding Behaviour:

All stages except eggs are blood-feeders and bite the skin four to five times daily to feed. They inject saliva which contains an anticoagulant and suck blood. The digested blood is excreted as dark red frass. Although any part of the scalp may be colonized, lice favor the nape of the neck and the area behind the ears, where the eggs are usually laid. Head lice are repelled by light and move towards shadows or dark-coloured objects in their vicinity.

Distribution:

About 6-12 million people, mainly children, are treated annually for head lice in the United States alone. In the UK, it is estimated that two thirds of children will experience at least one case of head lice before leaving primary school. High levels of louse infestations have also been reported from all over the world, including Australia, Denmark, France, Ireland, Israel, and Sweden.

Diagnosis

The condition is diagnosed by finding live lice and unhatched eggs in the hair. Finding empty eggs is not enough. Dandruff, lint, sand, hair casts, and dried hairspray, can be mistaken for eggs and nits. This is made easier by using a magnifying glass or running a comb through the child's wet hair, the latter of which is the most assured method of diagnosis and can be used to monitor treatment. In questionable cases, a child can be referred to a health professional. However, head lice infestation is commonly over diagnosed, with extinct infestations being mistaken for active ones. Infestations are only considered extinct if nits are more than 0.25 inches away from the scalp and nymphs and adult lice are absent. As a result, lice-killing treatments are more often used on non-infested than infested children. The use of a louse comb is the most effective way to detect living lice. With both methods, special attention should be paid to the area near the ears and the nape of the neck. The use of a magnifying glass to examine the material collected between the teeth of the comb could prevent misdiagnosis.

The presence of nits alone, however, is not an accurate indicator of an active head louse infestation. Generally, white nits are empty egg casings, while brown nits may still contain viable louse larva. One way of determining the nit is to squeeze it between two fingernails; it gives a characteristic snapping pop sound as the egg bursts. Children with nits on their hair have a 35-40% chance of also being infested with living lice and eggs.

If lice are detected, the entire family needs to be checked (especially children up to the age of 13 years) with a louse comb, and only those who are infested with living lice should be treated. As long as no living lice are detected, the child should be considered negative for head louse infestation. Accordingly, a child should be treated with a pediculicide only when living lice are detected on their hair (not because they have louse eggs/nits on their hair and not because the scalp is itchy).

Control

Controlling lice involves a combination of physical removal, chemical treatments, and preventive measures. Here's a comprehensive approach:

1.Physical Removal-Combing:

Use a fine-toothed lice comb to remove lice and nits (lice eggs) from the hair. This should be done on wet hair for better results. Manual Removal:Handpick lice and nits, especially in hard-to-reach areas, and dispose of them properly.

2.Chemical Treatments:

- Over-the-Counter (OTC) Medications: These include shampoos, lotions, and creams containing permethrin, pyrethrin, or other insecticides. Follow the instructions carefully.
- Prescription Treatments: If OTC products fail, a healthcare provider may prescribe stronger treatments like ivermectin or spinosad.

3. Home and Environmental Cleaning:

- Wash Bedding and Clothing: Use hot water (at least 130F or 54C) to wash all clothing, bedding, and personal items used by the infested person within the last 48 hours.
- Vacuuming: Vacuum carpets, upholstery, and any surfaces where hair may have fallen.
- Sealing Non-Washable Items: Place non-washable items (e.g., stuffed animals) in a sealed plastic bag for at least two weeks to kill lice.

Prevention

Examination of the child's head at regular intervals using a louse comb allows the diagnosis of louse infestation at an early stage. Early diagnosis makes treatment easier and reduces the possibility of infesting others. In times and areas when louse infestations are common, weekly examinations of children, especially those 4-15 years old, carried out by their parents, will aid control. Additional examinations are necessary if the child came in contact with infested individuals, if the child frequently scratches their head, or if nits suddenly appear on the child's hair.

Clothes, towels, bedding, combs, and brushes, which came in contact with the infested individual, can be disinfected either by leaving them outside for at least two days or by washing them at 60 C (140 F) for 30 minutes. 28] This is because adult lice can survive only one to two days without a blood meal and are highly dependent on human body warmth.

Treatment:

Possible treatments include:

i. combing the hair frequently with a fine tooth comb or shaving the head completely. A number of topical medications are also effective, including malathion, ivermectin, and dimethicone. Dimethicone, which is a silicone oil, is often preferred due to the low risk of side effects.

ii. Pyrethroids such as permethrin have been commonly used; however, they have become less effective due to increasing pesticide resistance. There is little evidence for alternative medicines.

iii. Head-lice infestations are common, especially in children. In Europe, they infect between 1 and 20% of different groups of people. In the United States, between 6 and 12 million children are infected a year. They occur more often in girls than boys. It has been suggested that historically, head lice infection was beneficial, as they protected against the more dangerous body louse. Infestations may cause stigmatization of the infected individual.

iv. Dimethicone is between 70 and 97% effective with a low rate of side effects, and thus is seen as the preferred treatment. It works by physical means and there is no evidence of pesticide resistance. Ivermectin is around 80% effective, but can cause local skin irritation. Malathion has an effectiveness around 90%, but there's the possibility of toxicity. Pyrethroids such as permethrin, while commonly used, have lower rates of effectiveness due to the resistance among lice. Effectiveness varies from 10 to 80%, depending on the population studied. Medications within a lotion appear to work better than those within a shampoo. Benzyl alcohol appears effective but it is unclear if it is better than standard treatments. Abametapir was approved for medical use in the United States in July 2020.

v. Resistance to several commonly used treatments is increasing worldwide, with patterns of resistance varying by region. Head lice have demonstrated resistance to permethrin, malathion, phenothrin, and carbaryl in several countries around the world. A previous method used to delay resistance included utilizing a rotating list of recommended insecticides by health authorities. The mosaic model is the current recommendation, in which it is advised to use one product for a treatment course, followed by a different insecticide from another substance class if the first treatment fails.

vi. Tea tree oil has been promoted as a treatment for head lice; however, there is no clear evidence of its effectiveness. A 2012 review of head lice treatment recommended against the use of tea tree oil for children because it could cause skin irritation or allergic reactions, because of contraindications, and because of a lack of knowledge about the oil's safety and effectiveness. Other home remedies, such as putting vinegar, isopropyl alcohol, olive oil, mayonnaise, or melted butter

under a shower cap, have been disproven. The CDC states that swimming has no effect on drowning lice, and can decrease the effectiveness of some treatments.

Probable questions:

1. Write down the structure of head lice with diagram.
2. What is obligate Ectoparasites?
3. How can you identify the male lice and the female lice?
4. Write notes on nits of lice.
5. How the nymph stage of lice transforms to adult?
6. State the symptoms of head lice infestation in host body.
7. Describe the life cycle of head lice in host body.
8. What do lice and nits (eggs) look like?
9. How are lice spread?
10. How are head lice treated?
11. What is the treatment for head lice?
12. What can be done to prevent the spread of head lice?
13. What to if head lice are identified in a childcare setting

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The study materials of this book have been collected from books, various e- books, journals and other e-sources.