UNIT-1
Introduction to medical parasitology

INTRODUCTION

- **Parasitology** is the study of parasites, their hosts, and the relationship between them.
- Medical parasitology is the subject which deals with the parasites that infect human being, the diseases caused by them, clinical feature and the response generated by human being against them.
- It's also concerned with the various methods of their diagnosis, treatment and finally their prevention & control.

Definitions of keys terms

- **A parasite:** a living organism that acquires some of its basic nutritional requirements through its intimate contact with another living organism.
- Parasites may be simple unicellular protozoa or complex multicellular metazoan.
- **Eukaryote:** a cell with a well“ defined chromosome in a membrane“ bound nucleus.
- **All parasitic organisms are eukaryotes**

- **Protozoa:** Unicellular organisms.
- **Metazoa:** multicellular organisms, e.g. worms and arthropods.
- **Vector:** a living carrier (e.g. an arthropod) that transports a pathogenic organism from an infected to a non-infected host.

Different Kind of Host

Host: the organism in or on which the parasite lives/ or An organism that harbors the parasite usually larger than the parasite.

- **Definitive host:** the organism in which the adult or sexually mature stage of the parasite lives.
- **Intermediate host:** the organism in which the parasite lives during a period of its development only.
- **Paratenic host:** Paratenic hosts are those that harbour the immature/infective stage of a parasite in an encapsulated form and helps in dissemination of the parasite to the definitive host.

- **Reservoir hosts** are hosts that harbour a parasite of another host without itself getting affected, but act a source of infection for the original host.
- **Carrier hosts** are hosts that have a residual population of the parasite and acts as a source of infection for the same type of host.
- **Transport hosts** are those that harbour the immature/infective stage of a parasite of another host and help in disseminating the parasite without any development in itself.

Types of Parasites

- **An endoparasite:** a parasite that lives within another living organisms.
- **An ectoparasite:** a parasite that lives on the external surface of another living organisms e.g. lice, ticks.
- **Temporary parasites:** These parasites spend only part of their lives as a parasite and another part as free-living organism. Examples are: ***Ascaris.***
- **permanent parasite**. A **parasite**, such as a fluke or an itch mite, that lives on its host until maturity or spends its entire life on its host.

- **Facultative parasites**:These organisms are normally free living and infect a host only by accident.
- **Accidental** or incidental **parasite**:These are **parasites** that establish themselves in or on a host in which they do not normally live. For example, it is common for nematodes, normally **parasitic** in insects, to live for a short time in the intestines of birds or for a dog flea to bite a human.
- **Obligatory parasites**: These parasites can only survive in a host and therefore go directly from one host to another. This may involve complex life cycles.
  Examples are: ***Taenia.***
- **Wondering or Aberrant parasites**: **Parasites** which cannot reach normal destination and infects a host where they cannot develop further are known as aberrant or **wandering parasites**.

The routes of transmission

- **Congenital transmission**: from mother to infant transmission.
- **Contact transmission: d**irect contact and Indirect contact.
- **Food transmission:** The infectious stage of parasite is contaminated food/the meat of the intermediate hosts containing infectious stage of parasites.
- **Water transmission**: Drink or contact the water contaminated the infectious stage of parasites.
- **Soil transmission**: contamination of the soil by feces containing the certain stage of parasites.
- **Arthropod transmission**: Vectors of certain parasitic diseases.

UNIT-2
General characteristics, morphology, classification of Protozoa and Helminthes

General characteristics, morphology, classification of Protozoa

- **Defination:** Protozoa are one-celled animals found worldwide in most habitats. Most species are free living, but all higher animals are infected with one or more species of protozoa. Infections range from asymptomatic to life threatening, depending on the species and strain of the parasite and the resistance of the host.

- **Structure:** Protozoa are microscopic unicellular eukaryotes that have a relatively complex internal structure and carry out complex metabolic activities. Some protozoa have structures for propulsion or other types of movement.

Characteristics of Protozoa

- There are about 50,000 known species of Phylum Protozoa.
- Protozoans exhibit mainly two forms of life; free-living (aquatic, freshwater, seawater) and parasitic (ectoparasites or endoparasites). They are also commensal in habitat.
- They are small, usually microscopic, not visualize without a microscope
- They are the simplest and primitive of all animals.
- They have a simple body organization. i.e. with a protoplasmic grade of organization.
- The body is unicellular (without tissue and organs).

- They have one or more nuclei which are monomorphic or dimorphic.
- Body naked or bounded by a pellicle, but in some forms may be covered with shells and often provided with an internal skeleton.
- They are solitary (existing alone/single) or colonial (individuals are alike and independent).
- Body shape variables may be spherical, oval, elongated or flattened.
- Body symmetry either none or bilateral or radial or spherical.
- Body form usually constant, varied in some, while changing with environment or age in many.

- Body protoplasm is differentiated into an outer ectoplasm and inner endoplasm.
- The single-cell body performs all the essential and vital activities, which characterize the animal body; hence only subcellular physiological division of labor.
- Locomotory organs are fingers like pseudopodia, whip-like flagella, hair-like cilia or none.
- Nutrition may be holozoic (animal-like), holophytic (plant-like), saprozoic or parasitic.
- Digestion occurs intracellularly which takes place inside the food vacuoles.
- Respiration occurs by diffusion through the general body surface.

Classification on basis of protozoan locomotion:

- **Mastigophora**
  - Move by one to many flagella.
  - Example: *Euglena*.
- **Sarcodina**
  - Locomotory organelles are pseudopodia.
  - The amoeboid form is predominant.
  - Some have a hard shell.
  - They generally do not form spores.
  - The formation of gametes and flagellated young ones are common.
  - Nutrition holozoic or saprozoic.

- **Sporozoa**
  - Locomotory organelles absent.
  - Spores usually present.
  - Exclusively endoparasites.
  - Cilia or flagella may be present in gametes.
  - Syngamy takes place after which many spores are formed.
  - The spores are simple and contain one to many sporozoites.
  - Sporozoites are the infective phase.
  - The nucleus is of the single type

- **Ciliate**
  - They possess cilia or compound ciliary structure as locomotory or food acquiring organelles.
  - There is the presence of an infraciliary system, composed of basal granules below the cell surface and interconnected by longitudinal fibrils.
  - Most ciliates possess a cell mouth or cytostome.
  - Anal aperture (cytopyge) permanent.
  - Two types of nuclei, one vegetative (macronucleus) and the other reproductive (micronucleus).
  - Sexual reproduction never involves the formation of free gametes.
  - One or more contractile vacuoles present even in marine and parasitic types.

Classification on basis of protozoan Pathogenicity:

- **Sarcomastigophora**
  - Locomotor organelles are pseudopodia or flagella.
  - The nucleus is of a single type (monomorphic).
  - There is no spore formation.
  - Syngamy occurs in reproduction.

- **Ciliophora**
  - They possess simple ciliary organelles for locomotion, infraciliature is subpeculiar.
  - They have two nuclei, a trophic macronucleus, and a reproductive micronucleus.
  - Binary fission is perkinetal.
  - Conjugation takes place with the fusion of nuclei, autogamy and cytogamy also occur.
  - There are never any free gametes.
  - Nutrition is mixotrophic or heterotrophic.
  - They usually have a cytostome.

General characteristics, morphology, classification of Helminthes

- Helminths are parasitic worms that feed on a living host to gain nourishment and protection, while causing poor nutrient absorption, weakness and disease in the host. These worms and larvae live in the small bowel and are referred to as intestinal parasites.
- All helminths are multicellular eukaryotic invertebrates with tube-like or flattened bodies exhibiting bilateral symmetry.
- The following are examples of helminths:
  - Giant roundworm
  - Threadworm
  - Pinworm
  - Roundworm Necator Americanus
  - Toxocara cati

Characteristics of helminths

- **HABITAT:** Endoparasites found in animals.
- **SYMMETRY:** Bilateral symmetry.
- **ORGANISATION LEVEL:** Organ level.
- **SKELETON:** Dorsoventrally flattened body called flatworms too.
- **REPRODUCTION:** Hermaphrodites
- **FERTILISATION:** Internal
- **DEVELOPMENT:** Indirect (Larvae formation)

The following groups of worms are classed as helminths:

- Nematodes or roundworms
- Trematodes, which includes flukes or flatworms
- Cestodes or tapeworms
- Monogenans, also members of the flatworm phylum

Characterstics of Nematodes or roundworms

The Nematodes present in the soil feed on the bacteria, fungi, and other nematodes, and play an important role in nutrient recycling.

- Their body is bilaterally symmetrical and triploblastic.
- They are cylindrical in shape.
- They exhibit tissue level organization.
- Their body has a cavity or pseudocoelom.
- The alimentary canal is distinct, with the mouth and the anus.
- They are sexually dimorphic.
- They are devoid of the circulatory system and respiratory system.

- They are free-living or parasitic.
- Parasitic nematodes cause diseases in the host.
- Fertilization is internal and reproduction is sexual.
- Their cuticle moults periodically.
- The epidermis is synctical and contains dorsal or ventral nerve cords.
- The body-wall muscles are longitudinal.
- They possess amoeboid sperm cells.
- They consist of chemosensory organs called aphids situated on the lips.

Characterstics of Trematodes

- **Trematodes** are flattened oval or worm-like animals, usually no more than a few centimetres in length, although species as small as 1 millimetre (0.039 in) are known.
- Their most distinctive external feature is the presence of two suckers, one close to the mouth, and the other on the underside of the animal.
- These are parasites. Definitive host is human and intermediate host is fresh water snail. Second intermediate host is fish or crab.
- Body form : Leaf shaped, unsegmented flatworm, called fluckes.
- Symmetry : Bilateral symmetry.
- Size : 1 mm to several centimeters in length.

- Germ layer : They are triploblastic animal. They consist of three germ layers, ectoderm, mesoderm and endoderm.
- Body cavity : Body cavity is absent. Space between body wall and alimentary canal is filled with parenchymatous connective tissues.
- Suckers : Sucker is strong muscular cup shaped structure. One sucker surround mouth called oral sucker. Other sucker is present on ventral surface of worm called ventral sucker.
- Alimentary canal : Incomplete alimentary canal. Mouth is present and anus is absent. Oesophagus bifurcate in front of ventral sucker. It is called blind intestinal caeca or crura. They may reunite to form single caecum.

General Characters of Cestodes:

- Cestodes have a head, called a scolex, which has suckers. These suckers are used to attach to a person's intestinal tract. Some cestodes also have hooks on their head as well.
- Although cestodes can be found in a person's digestive tract, ironically they don't have one themselves. They absorb nutrients through a skin-like covering instead.
- Another thing that cestodes don't have? A body cavity. Instead, their insides are filled with spongy cells that suspend their internal organs.
- Shape: Flat and tape-like and body is segmented.

- Sexes: Hermaphrodite
- Alimentary canal: Only sucker is present. No gut.
- Head end: Suckers with or without hooks.
- Body cavity: Absent
- Most cestodes require 2 hosts to complete their lifecycle. A definitive host and an intermediate host

Unit-3
Blood and Stool collection

Blood Specimen Collection and Processing

- The first step in acquiring a quality lab test result for any patient is the specimen collection procedure. The venipuncture procedure is complex, requiring both knowledge and skill to perform. Several essential steps are required for every successful collection procedure:

Venipuncture Procedure:

- A phlebotomist must have a professional, courteous, and understanding manner in all contact with all patients.
- The first step to the collection is to positively identify the patient by two forms of identification; ask the patient to state and spell his/her name and give you his/her birth date. Check these against the requisition (paper or electronic).

- Check the requisition form for requested tests, other patient information and any special draw requirements. Gather the tubes and supplies that you will need for the draw.
- Position the patient in a chair, or sitting or lying on a bed.
- Wash your hands.
- Select a suitable site for venipuncture, by placing the tourniquet 3 to 4 inches above the selected puncture site on the patient. See below for venipuncture site selection “notes.”
- Do not put the tourniquet on too tightly or leave it on the patient longer than 1 minute.
- Next, put on non-latex gloves, and palpate for a vein.

- When a vein is selected, cleanse the area in a circular motion, beginning at the site and working outward. Allow the area to air dry. After the area is cleansed, it should not be touched or palpated again. If you find it necessary to reevaluate the site by palpation, the area needs to be re-cleansed before the venipuncture is performed.
- Ask the patient to make a fist; avoid “pumping the fist.” Grasp the patient’s arm firmly using your thumb to draw the skin taut and anchor the vein. Swiftly insert the needle through the skin into the lumen of the vein. The needle should form a 15-30 degree angle with the arm surface. Avoid excess probing.
- When the last tube is filling, remove the tourniquet.

- Remove the needle from the patient's arm using a swift backward motion.
- Place gauze immediately on the puncture site. Apply and hold adequate pressure to avoid formation of a hematoma. After holding pressure for 1-2 minutes, tape a fresh piece of gauze or Band-Aid to the puncture site.
- Dispose of contaminated materials/supplies in designated containers.

Fingerstick Procedure:

- The best locations for fingersticks are the 3rd (middle) and 4th (ring) fingers of the non-dominant hand. Do not use the tip of the finger or the center of the finger.
- Avoid the side of the finger where there is less soft tissue, where vessels and nerves are located, and where the bone is closer to the surface. The 2nd (index) finger tends to have thicker, callused skin. The fifth finger tends to have less soft tissue overlying the bone. Avoid puncturing a finger that is cold or cyanotic, swollen, scarred, or covered with a rash.
- When a site is selected, put on gloves, and cleanse the selected puncture area.

- Massage the finger toward the selected site prior to the puncture.
- Using a sterile safety lancet, make a skin puncture just off the center of the finger pad. The puncture should be made perpendicular to the ridges of the fingerprint so that the drop of blood does not run down the ridges.
- Wipe away the first drop of blood, which tends to contain excess tissue fluid.
- Collect drops of blood into the collection tube/device by gentle pressure on the finger. Avoid excessive pressure or “milking” that may squeeze tissue fluid into the drop of blood.
- Cap, rotate and invert the collection device to mix the blood collected.
- Have the patient hold a small gauze pad over the puncture site for a few minutes to stop the bleeding.
- Dispose of contaminated materials/supplies in designated containers.
- Label all appropriate tubes at the patient bedside.

Heel prick Procedure (infants):

- Prewarming the infant's heel (42° C for 3 to 5 minutes) is important to increase the flow of blood for collection.
- Wash your hands, and put gloves on. Clean the site to be punctured with an alcohol sponge. Dry the cleaned area with a dry gauze pad.
- Hold the baby's foot firmly to avoid sudden movement.
- Using a sterile blood safety lancet, puncture the side of the heel in the appropriate regions shown above. Make the cut across the heel print lines so that a drop of blood can well up and not run down along the lines.

- Wipe away the first drop of blood with a piece of clean, dry cotton gauze. Since newborns do not often bleed immediately, use gentle pressure to produce a rounded drop of blood. Do not use excessive pressure because the blood may become diluted with tissue fluid.
- Fill the required microtainer(s) as needed.
- When finished, elevate the heel, place a piece of clean, dry cotton on the puncture site, and hold it in place until the bleeding has stopped. Apply tape or Band-Aid to area if needed.
- Be sure to dispose of the lancet in the appropriate sharps container. Dispose of contaminated materials in appropriate waste receptacles.
- Remove your gloves and wash your hands.

Labeling The Sample

All specimens must be received by the laboratory with a legible label containing at least two (2) unique identifiers.

- The specimen must be labeled with the patient's full name (preferably last name first, then first name last) and one of the following:
  - Geisinger medical record number (MRN) - for Geisinger locations, this is the required second identifier
  - Patient's full date of birth (must include the month, day, and year)
  - Unique requisition identifier/label

Blood Sample Handling and Processing:

Pre-centrifugation Handling - The first critical step in the lab testing process, after obtaining the sample, is the preparation of the blood samples. Specimen integrity can be maintained by following some basic handling processes:

- Fill tubes to the stated draw volume to ensure the proper blood-to-additive ratio. Allow the tubes to fill until the vacuum is exhausted and blood flow ceases.
- Tubes should be stored at 4-25°C (39-77°F).
- Tubes should not be used beyond the designated expiration date.

- Mix all gel barrier and additive tubes by gentle inversion 5 to10 times immediately after the draw. This assists in the clotting process. This also assures homogenous mixing of the additives with the blood in all types of additive tubes.
- Serum separator tubes should clot for a full 30 minutes in a vertical position prior to centrifugation. Short clotting times can result in fibrin formation, which may interfere with complete gel barrier formation.

Blood Sample Centrifugation – It is recommended that serum be physically separated from contact with cells as soon as possible, with a maximum time limit of 2 hours from the time of collection.

- Complete gel barrier formation (gel barrier tubes) is time, temperature and G-force dependent. The uniformity of the barrier is time dependent; an incomplete bar

- In general, for a horizontal, swing-bucket centrifuge, the recommended spin time is 10 minutes. For a fixed-angle centrifuge, the recommended spin time is 15 minutes.
- Tubes should remain closed at all times during the centrifugation process.
- Place the closed tubes in the centrifuge as a “balanced load” noting the following: Opposing tube holders must be identical and contain the same cushion or none at all.
  - Opposing tube holders must be empty or loaded with equally weighted samples (tubes of the same size and equal in fill).
  - If an odd number of samples is to be spun, fill a tube with water to match the weight of the unpaired sample and place it across from this sample.

Stool collection

- label a clean, screw-top container with your name, date of birth and the date
- place something in the toilet to catch the poo, such as a potty or an empty plastic food container, or spread clean newspaper or plastic wrap over the rim of the toilet
- make sure the poo doesn't touch the inside of the toilet
- use the spoon or spatula that comes with the container to collect the poo, then screw the lid shut

- if you've been given a container, aim to fill around a third of it – that's about the size of a walnut if you're using your own container
- put anything you used to collect the poo in a plastic bag, tie it up and put it the bin
- wash your hands thoroughly with soap and warm running water

- **TRANSPORT**

1. Specimens are acceptable for culture as long as the transport fluid has not turned yellow.
2. Do not refrigerate.
3. Transport ASAP to the laboratory.

Unit-4
concentration techniques

Introduction

- Fecal concentration has become a routine procedure as a part of the complete ova and parasite examination for parasites and allows the detection of small numbers of organisms that may be missed by using only a direct wet smear.
- Each stool specimen was examined by the following techniques.
  1. Macroscopic examination:
  The colour, consistency and the nature of the faeces were recorded. The stool specimens were examined for the presence of worms like Ascaris, Enterobius, proglottids of Taenia, adult Hookworm and Trichuris, either with the naked eye or with the aid of a hand lens.

- **2. Direct microscopic examination by using saline and iodine preparations:**
  On a 1mm thick microscopic slide, a small amount of stool sample was emulsified in 1-2 drops of saline or iodine solution. A cover slip was placed on it by taking care that the preparation was free of air bubbles and macroscopic debris.
- **3. The microscopic examination after the various concentration techniques:**
  - **Simple salt floatation:**
    - Briefly, about 1gm of faeces was emulsified with 3-4 ml of saturated salt solution in a 20ml conical glass test tube.
    - It was stirred well and more salt solution was added till the container was nearly full, with the stirring being continued.

- Any coarse matter which floated up was removed and the tube was placed on a levelled surface with a glass slide being placed over the top of the tube, which was in contact with the fluid.
- It was allowed to stand for 30 minutes. The slide was removed and observed for the presence of eggs/cysts.

- **Zinc sulphate centrifugal floatation:**
  - 1g of the stool specimen was emulsified in 10 parts of tap water and it was strained through a wire gauze.
  - The filtrate was collected in a Wassermann tube and centrifuged at 2,500 rmp. The supernatant was discarded and the sediment was re-suspended in water.
  - This step was repeated till the supernatant became clear. To the sediment, 3-4 ml of 33% Zinc sulphate solution was added, it was mixed well and it was filled with ZnSO4 solution, about half an inch of the rim.
  - Several loopfuls of the supernatant fluid were removed with a bacteriological loop and they were observed for parasites.

- **Formol-ether concentration:**

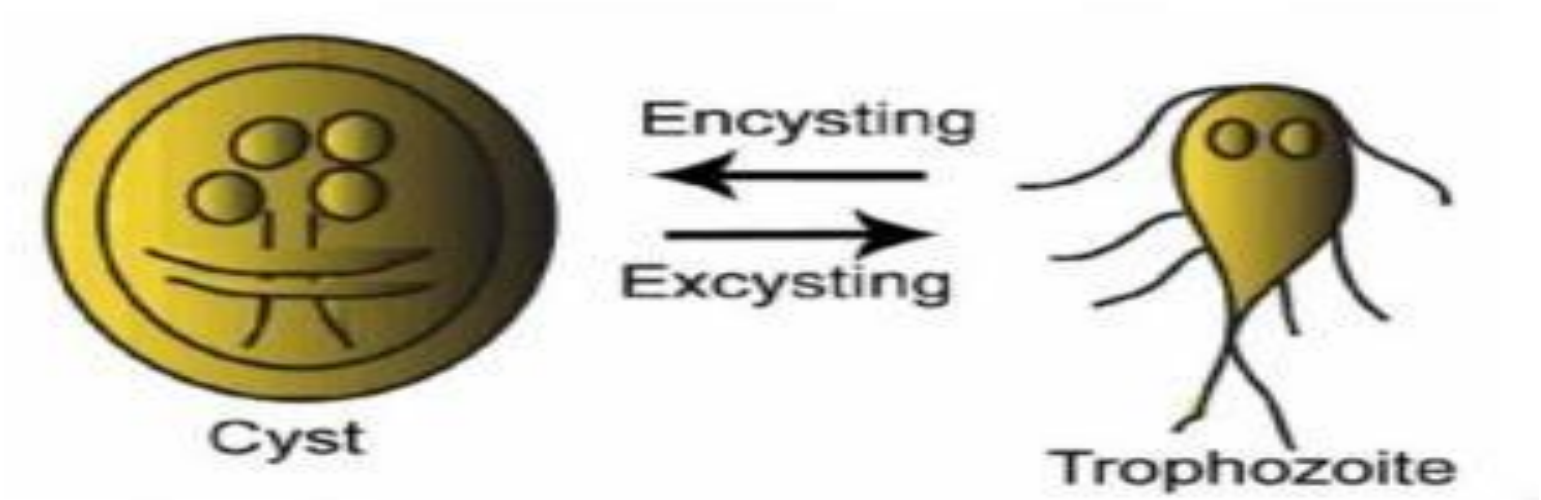
  - 1g of stool was emulsified in 7ml of 10% formol saline and it was kept for 10 minutes for fixation.
  - It was then strained through a wire gauze.
  - The filtrate was added to 3 ml of ether and it was centrifuged at 2000 rpm for 2 minutes.
  - It was allowed to settle.
  - The supernatant was removed and a wet mount was made of the deposit to look for parasites.

- **Formol-ether concentration which was modified by Allen and Ridely:**
  - It was a modification of the formol-ether method where the centrifugation was done at 3000 rpm for 60 seconds instead of 2000 rpm for 2 min.
  - The sediment was used for the parasitic examination.

Unit-5
Giardia and Entamoeba histolytica

Giardia lamblia:

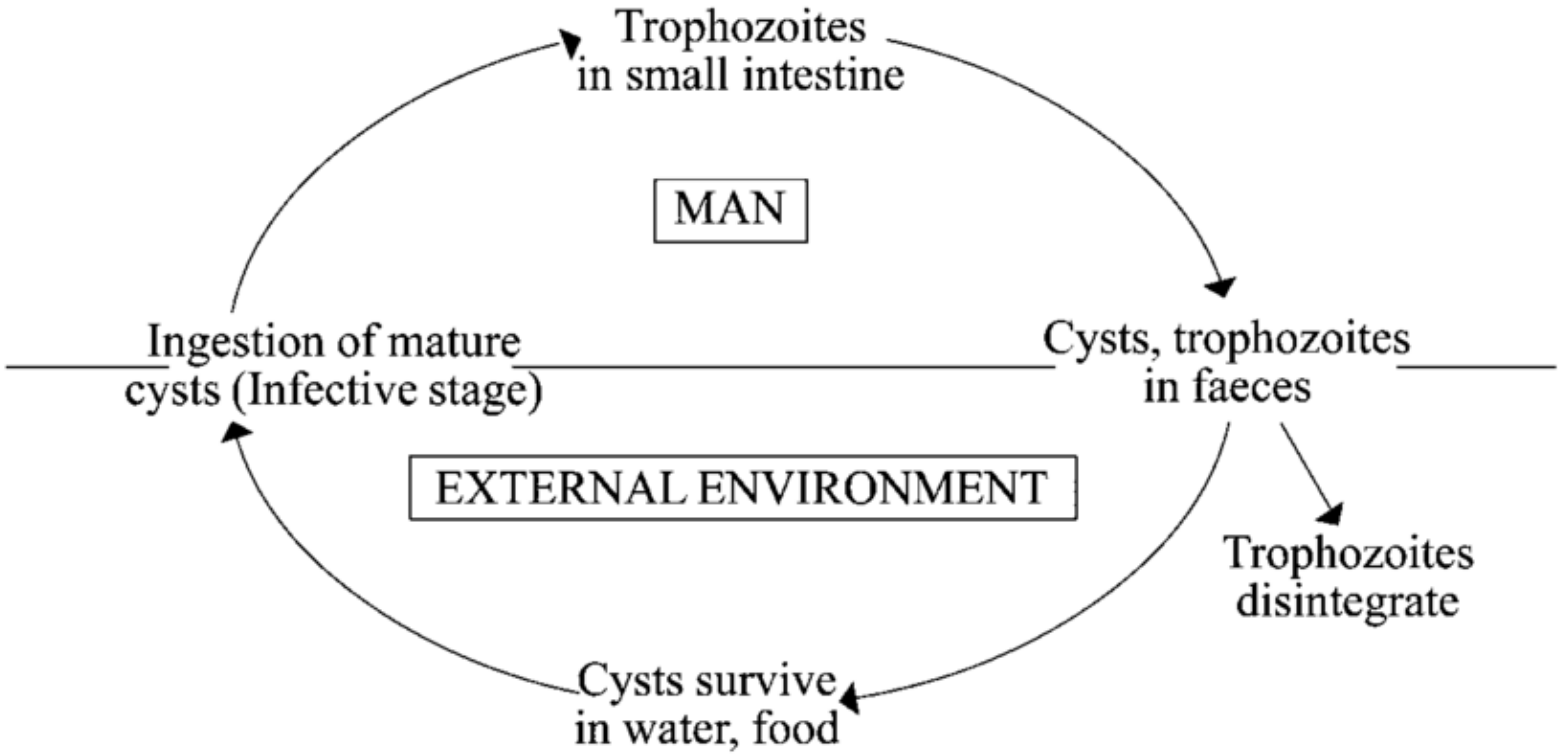
- **Geographical distribution**: worldwide; found in the soil, water or surfaces contaminated with feces of infected human/animal.
- Habitat: **Duodenum and upper part of jejunum** of human.



Morphology

- **Exists in two form**
  - **Cyst: Oval cyst** is thick walled with **four nuclei** and several internal fibers. **Each cyst gives rise to two trophozoites** during excystation in the intestinal tract.
  - **Trophozoite:** Pear-shaped with two nuclei, four pairs of flagella and a suction disk.
- Giardia is protected by an outer shell that allows it to survive outside the body for long periods of time and makes it tolerant to chlorine disinfection.

Life cycle of Giardia



- Giardia cysts are the infective stage of *G. intestinalis*.
- As few as 10 cysts can cause infection.
- These cysts are ingested by consuming contaminated food or water, or fecal-orally. They can survive outside the body for several months, and are also relatively resistant to chlorination, UV exposure and freezing.
- When cysts are ingested, the low pH of the stomach acid produces excystation, in which the activated flagella breaks through the cyst wall.
- This occurs in the small intestine, specifically the duodenum.
- Excystation releases trophozoites, with each cyst producing two trophozoites.

- Within the small intestine, the trophozoites reproduce asexually (longitudinal binary fission) and either float free or are attached to the mucosa of the lumen.
- Some trophozoites then encyst in the small intestine.
- Encystation occurs most likely as a result of exposure to bile salts and fatty acids, and a more alkaline environment.
- Both cysts and trophozoites are then passed in the feces, and are infectious immediately or shortly afterward.
- Person-to-person transmission is possible.
- Animals can also be infected with Giardia, and beavers have been associated with giardia outbreaks, although not definitively.

Laboratory Diagnosis:

Faecal specimen containing *Giardia lamblia* may have an offensive odour and are pale colored, fatty and float in water.

- **Ova and parasite (O+P) examination**
  - *Giardia* cysts can be **excreted intermittently,** so many cases (>50%) of giardiasis will be missed with a single O+P examination, resulting in under diagnosis.
  - Multiple stool collections (i.e., **three stool specimens collected on separate days**) increase test sensitivity
  - Use of concentration method increases sensitivity.

- Microscopically examination of freshly passed stools is used for the demonstration of Giardia trophozoite and cysts.
- **Fresh diarrhoeic specimen:** Try to find *Giardia lamblia* trophozoites. Generally difficult to detect as they attach themselves to the wall of the intestine. A Giemsa or Field's stained faecal smear should be examined if giardiasis is suspected but no trophozoites are detected in a wet mount of the faeces.
- **Formed faecal specimen**: Look for the *Giardia lamblia* cyst.

- **Fecal immunoassays** that are more sensitive and specific can be used:
  - An ELISA test that detects a Giardia cyst wall **antigen** in the stool can be used .

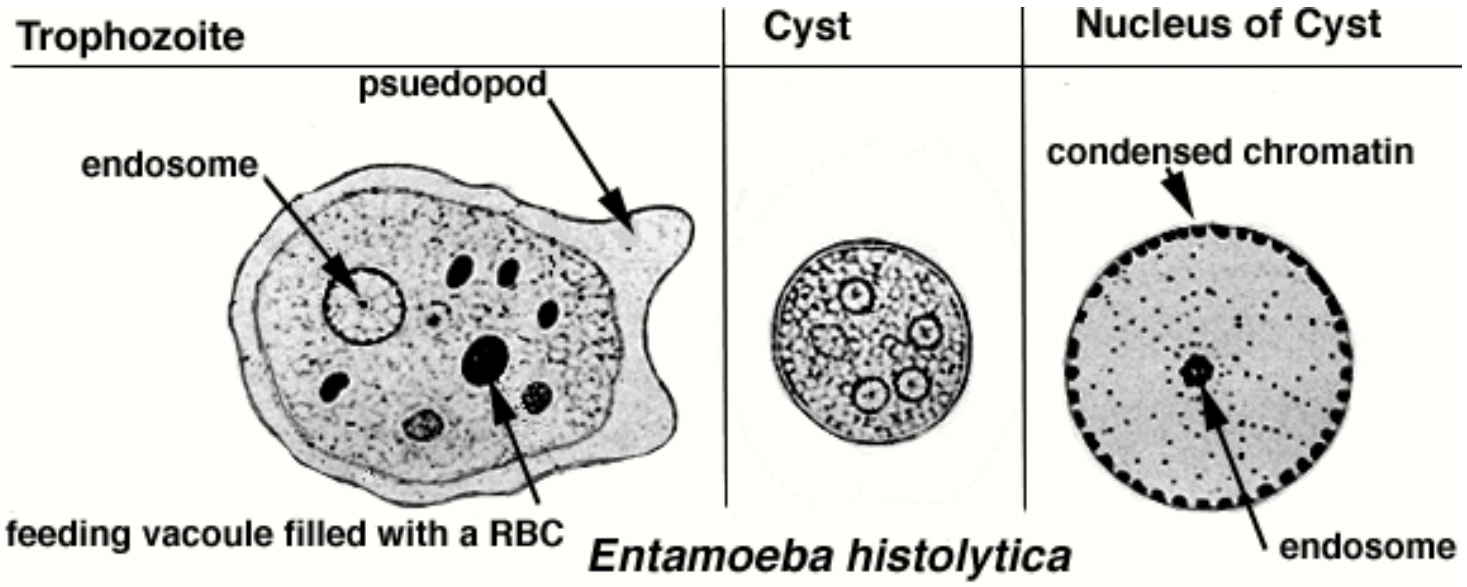
- **String test (Entero-Test):**
  - Swallowing a weighted piece of string until it reaches a duodenum.
  - The trophozoite adhere to the string and can be visualized after withdrawal of the string.
- **Polymerase Chain Reaction (PCR)** can be used to identify the subtypes of *Giardia lamblia*.

Entamoeba histolytica

- *Entamoeba histolytica* is a common protozoan parasite found in the large intestine of human. The parasite is responsible for amoebiasis and liver absceses. It is the third leading parasite cause of death in the developing countries.

Morphology:

- Parasite occurs in three stages; trophozoite, precyst and cyst



Trophozoite:

- It is the growing and feeding stage of parasite
- **Sape;** not fixed because of constantly changing position
- **Size:** ranging from 18-40 µm; average being 20-30 µm
- **Cytoplasm:** cytoplasm is divided into two portion; a clear transparent ectoplasm and a granular endoplasm. Ingested RBCs, tissue granules and food materials are also found in endoplasm
- **Nucleus:** It is single, spherical shape and size ranging from 4-6µ Nucleus contains central karyosome and fine peripheral chromatin.
- Trophozoites are actively motile with the help of pseudopodia.
- Trophozoites are anaerobic parasite, (present in large intestine)

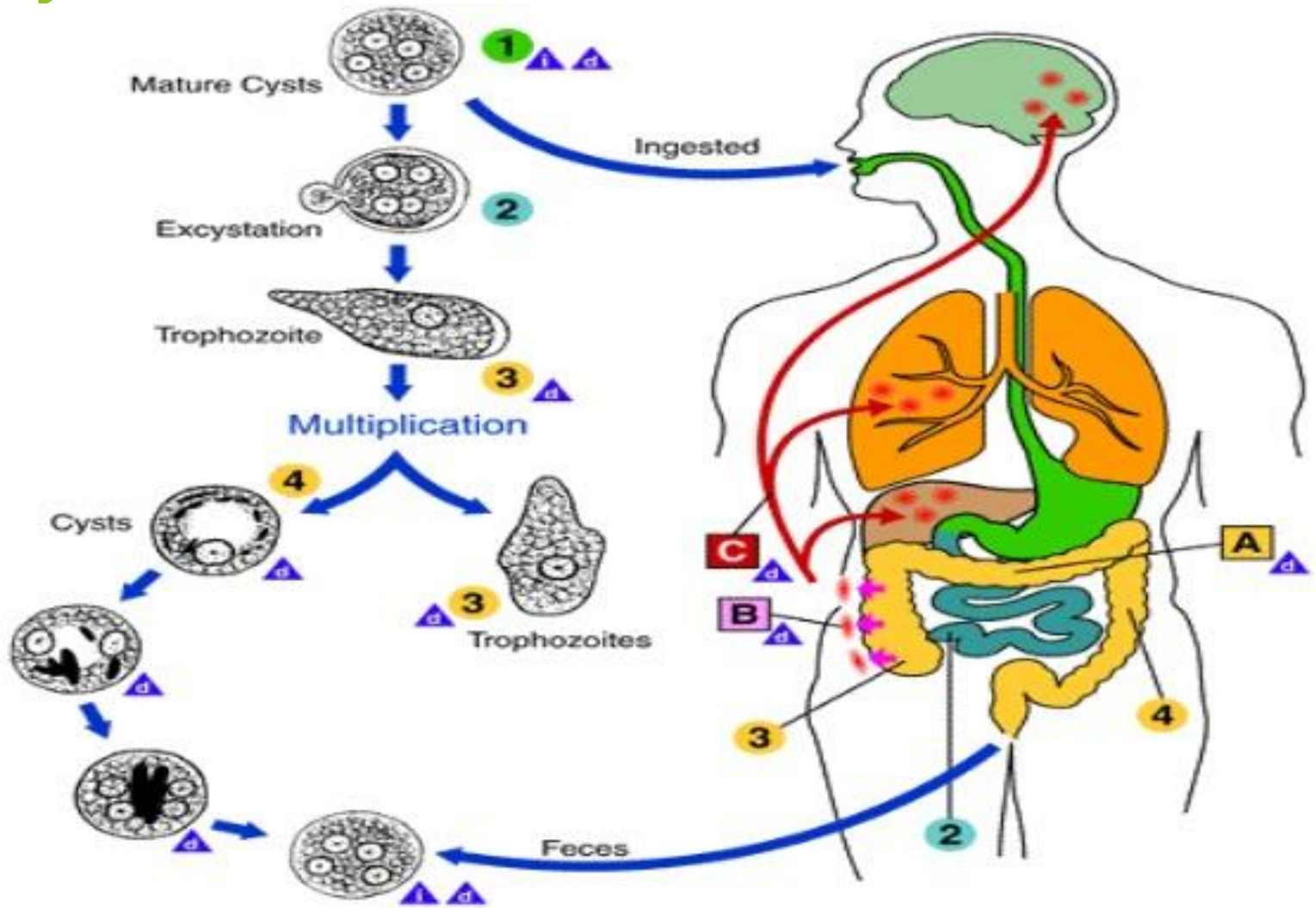
Pre cyst:

- It is the intermediate stage between trophozoite and cyst
- It is smaller in size; 10-20µ
- It is round or slightly ovoid with blunt pseudopodium projecting from periphery
- No RBC or food materials are found on its endoplasm.

Cyst

- It is the infective form of parasite.
- **Shape:** It is round or round or oval in shape
- **Size:** 12-15 µm in diameter
- It is surrounded by a highly retractile membrane called cyst wall. The cyst wall is resistant to digestion by gastric juice in human stomach
- **Nucleus:** A mature cyst is quadri-nucleated.
- **Cytoplasm:** Cytoplasm shows chromatid bars and glycogen masses but no RBCs or food particles.
- Mature cyst passed out in stool from infected patient and remained without fouther development in soil for few days.

Life cycle:



Life Cycle of Entamoeba histolytica

- Life cycle of *histolytica is* relatively simple and consists of infective cyst and invasive trophozoites stage.
- Life cycle completes in single host, ie human
- Human get infected with E. histolytica cyst from contaminated food and water. Infection can also acquired directly by ano-genital or oro-genital sexual contact.
- The mature Cyst is resistant to low pH of stomach, so remain unaffected by the gastric juices.
- The cyst wall is then lysed by intestinal trypsin and when the cyst reaches the caecum or lower part of illium excystation occurs. The neutral or alkaline environment as well as bile components favor excystation.

- Excystation of a cyst gives 8 trophozoites. Trophozoites are actively and carried to large intestine by peristalsis of small intestine. Trophozoites then gain maturity and divide by binary fission.
- The trophozoies adhere to mucus lining of intestine by lectin and secretes proteolytic enzymes which causes tissue destruction and necrosis. Parasite, when gain access to blood, migrates and causes extra-intestinal diseases.
- When the load of trophozoites increases, some of the trophozoites stop multiplying and revert to cyst form by the process of encystation.
- These cysts are released in faeces completing the life cycle.

Lab Diagnosis

- **Specimen:** stool, pus or liver abscesses, sputum and biopsy samples
  - **Stool macroscopy:** in amoebic dysentery stool is offensive, semi-solid, dark brown color and acidic in nature, mixed with blood, mucus and faecal materials.
  - **Microscopy**: Normal saline preparation of fresh faecal material revels trophozoites with RBCs in its cytoplasm and its amoebic motility.
  - **Stool Ag detection:** ELISA to detect 170KD lectin of *E. histolytica*
  - **Stool culture:** Robinson's medium and NH polyxenic culture medium are used to culture *E. histolytica*

- **Serology:** IHA, IFA etc are used to detect antibody in serum against *E. histolytica*
- **PCR:** It is sensitive test , used to differentiate *E. histolytica* with other Entamoeba species
- **Radiological finding:** X-rays, MRI, CT scan, ultrasonography etc for extra intestinal amoebiasis..
- **Blood test:** blood count, Liver function test, Kidney function test
- **Intradermal test**

Unit-6
Ancylostoma and Ascaris lumbricoides

Ascaris lumbricoides

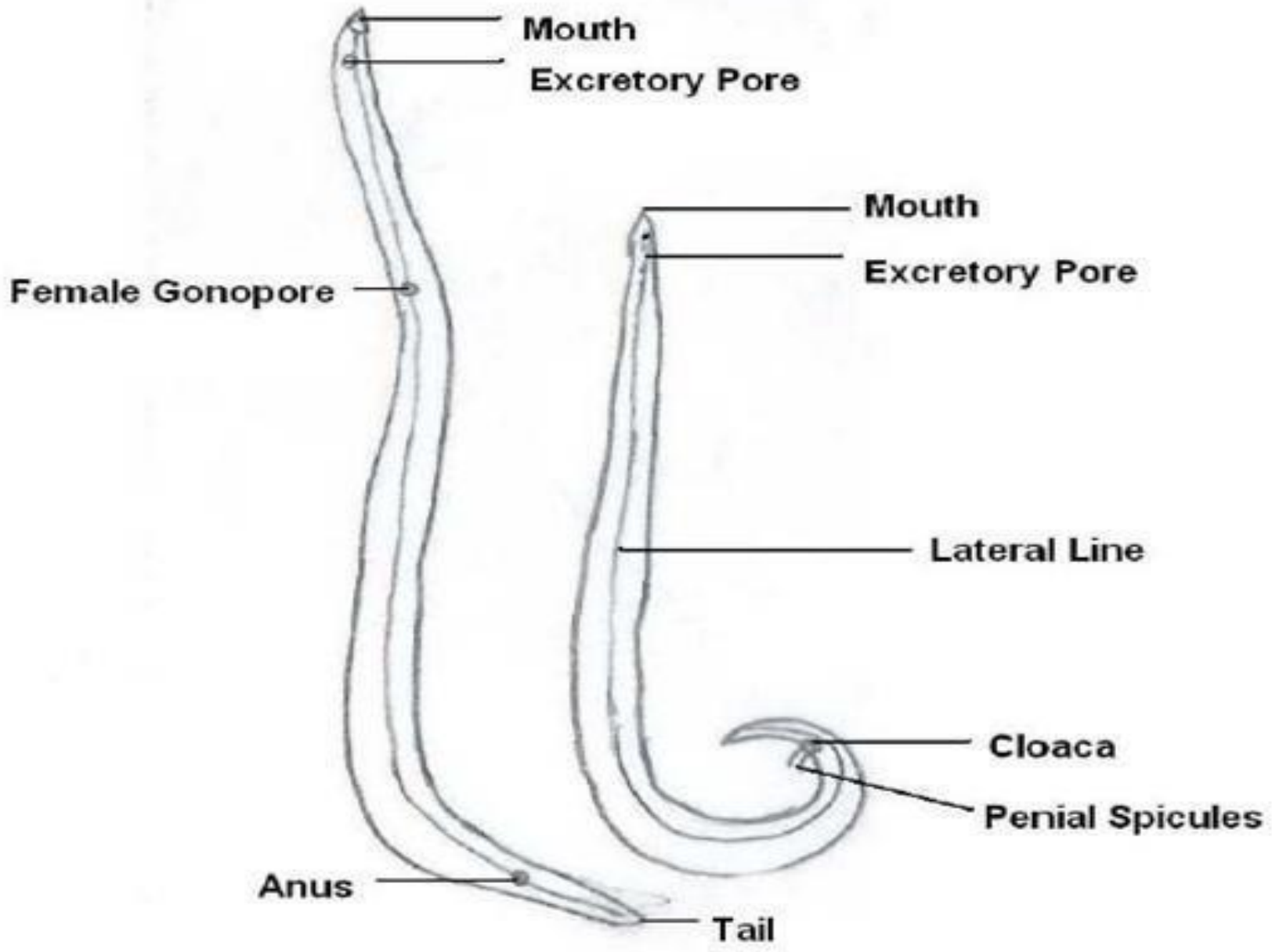
- Ascaris lumbricoides is an intestinal round worm. It is the largest intestinal nematode to infect Human. The adult worm lives in small intestine and grow to a length of more than 30 cm. Human is only the natural host and reservoir of infection.
- The round worm infection occurs worldwide. The number of infected persons is estimated to be more than 2 billion. The main epidemic region with prevalence rate of approx. 10-90% includes countries on South east Asia, Africa and latin America.

Morphology:

- **Adult:**

  The round worm resembles to earthworm. It is elongated tapering to both end, anterior being thinner than posterior. Freshly excreted worm is yellowish pink in color, which gradually changes to white.

- The worm is sexually diamorphic.
  - Adult male: 15-30 cm in length, 3-4 mm in diameter, tail curved
  - Adult female; 20-40 cm length, 2-6mm diameter, tail straight

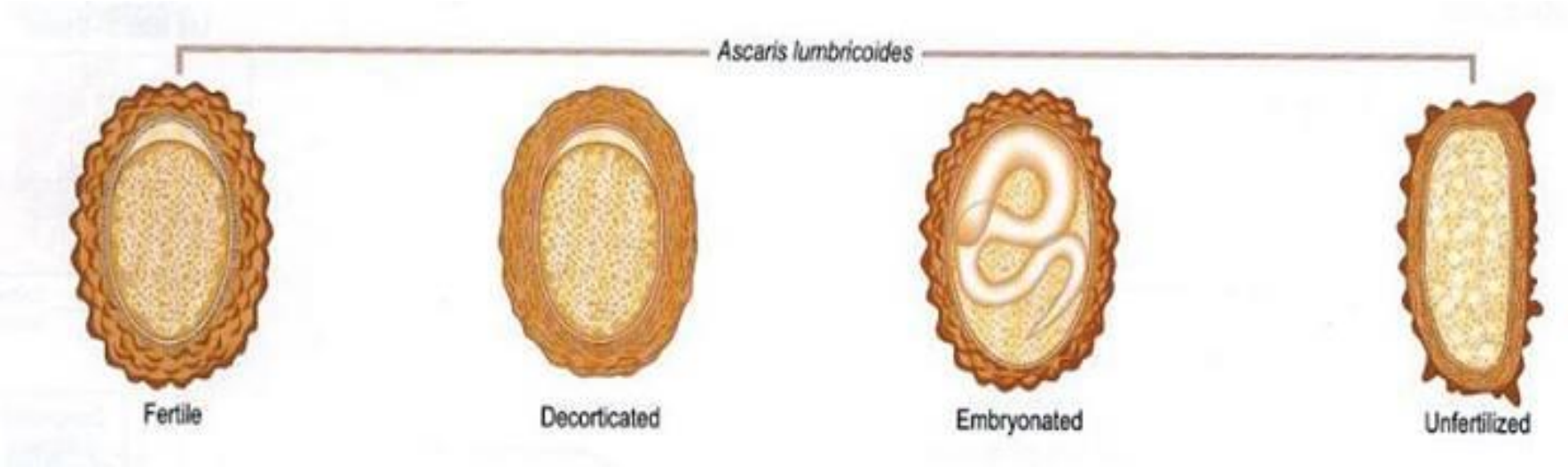

Mouth
Excretory Pore
Mouth
Excretory Pore
Female Gonopore
Lateral Line
Cloaca
Penial Spicules
Anus
Tail

- **Egg:**

  Ascaris egg is round or oval, 60*40 µm size, thick brown shell and have rough surface. It is the infective form of parasite.

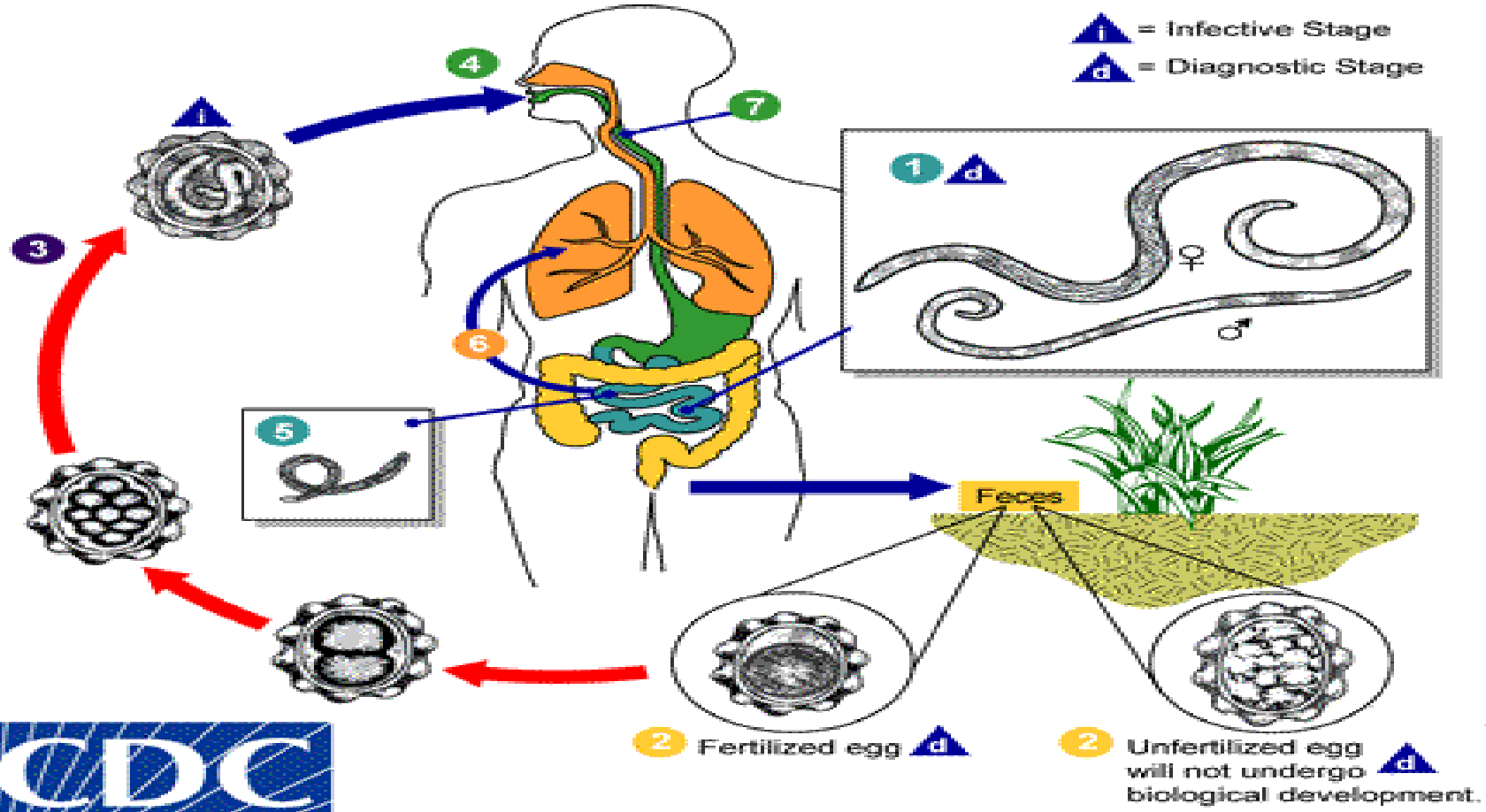
  i) Unfertilized egg; large, more elongated (38-55*78-105) µm

  ii) fertilized egg; ovoid (35-50*50-70)µm, golden brown color



Life cycle:

- The life cycle of Ascaris completes in single host. Human.
- Adult worm lives in small intestine



Stages in life cycle:

- **Stage I: Eggs in faeces**
  - Sexually mature female produces as many as 200,000 eggs per day, which are shed along with faeces in unembryonated form. They are non infective.
- **Stage II: Development in soil**
  - Embryonation occurs in soil as optimum temperature of 20-25C with sufficient moisture and O2
  - Infective larva develops within egg in about 3-6 weeks.

- **Stage III: Human infection and liberation of larvae**
  - Human get infection with ingestion of embryonated egg contaminated food and water
  - Within embryonated state inside egg, first stage larvae develops into second stage larvae. This second stage larvae is known as Rhabtitiform larvae
  - Second stage larve is stimulated to hatch out by the presence of alkaline pH in small intestine and solubilization of its outer layer by bile.

- **Stage IV: migration of larvae through lungs**
  - Hatched out larvae penetrates the intestinal wall and carried to liver through portal circulation
  - It then travels via blood to heart and to lungs by pulmonary circulation within 4-7 days of infection.
  - The larvae in lungs molds twice, enlarge and breaks into alveoli.

- **Stage V: Re-entry to stomach and small intestine**
  - From alveoli, the Larvae then pass up through bronchi and into trachea and then swallowed.
  - The larvae passes down the oesophagus to the stomach and reached into small intestine once again.
  - Small intestine is the normal habitat of *Ascaris* and it colonises here.
  - Within intestine parasite molds twice and mature into adult worm.
  - Sexual maturation occurs with 6-10 weeks and the mature female discharges its eggs in intestinal lumen and excreted along with faeces, continuing the life cycle.
  - The life span of parasite is 12-18 months

Lab diagnosis:

- **Specimen:** stool, sputum
- **Microscopy:** examination of stool by saline emulsion or concentration by floatation methods employed to unembryonated egg
- **X-ray**
- **Serodiagnosis:** Indirect haemagglutination test, Immuno-fluorescence assay
- **Ultrasonography** and CT scan
- **Other test:** blood count shown peripheral eosinophilia

Ancylostoma duodenale

- *Ancylostoma duodenale*, also known as the old hookworm is a common hookworm of human.
- It causes ancylostomiasis in humans, characterized by non-deficiency anemia and hypoalbuminemia.
- The adult worm lives in the small intestine of a man particularly in the jejunum, less often in the duodenum and rarely in the ileum.

Morphology:

- **Adult worm:**
  - It is small greyish white, cylindrical worm.
  - When freshly passed the worm has a reddish-brown color due to the ingested blood in intestinal tract.
  - The anterior end of the worm is bent slightly in the same direction of the body curve resembling a hook, hence they are called hookworms.
  - The large and conspicuous buccal capsule is lined with a hard substance

- There are five glands connected with the digestive system:
  - one of them called the esophageal gland, secretes a ferment which prevents the clotting of blood.

- The buccal capsule is provided with 6 teeth, 4-hook like on the ventral surface and had a pair of knob-like (triangular plates) on the dorsal surface.

- The sexes are easily differentiated by their size, the shape of the tail and position of the genital opening.

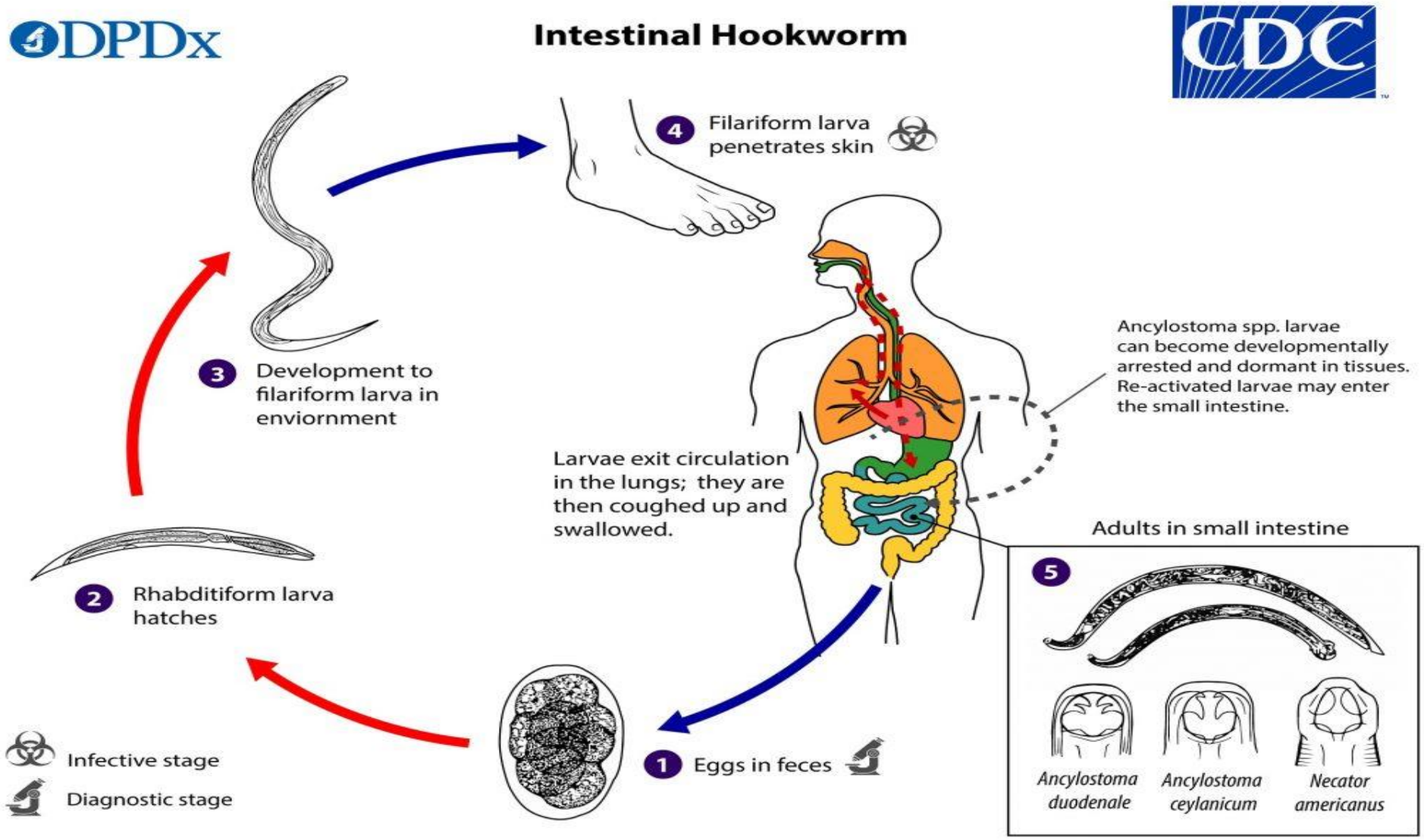
- **Male:**
  - shorter, measuring 8mm in length, the posterior end is expanded in an umbrella like fashion (copulatory bursa).
  - -Genital opening is present posteriorly and opens with the cloaca.
- **Female:**
  - longer than males, measuring 12.5 mm in length.
  - The posterior end is tapering and possess no bursa.
  - The genital pore is present at the junction of posterior and middle third of the body.
  - Owing to the position of the genital opening, the worm assumes the Y-shaped figure during copulation.

- Copulatory bursa in males is characteristic bell shaped used to catch and hold the female nematode during mating.
- – It is membranous, asymmetrical and consists of 13 fingers like rays in 3 lobes: 1 dorsal and 2 lateral.
- -Dorsal lobe consists 3 (1 single dorsal ray and 2 external dorsal rays: the two lateral lobes contain 1/3 pairs of lateral rays and 2 pairs of ventral rays).

Eggs:

- Eggs are oval in shape.
- 60 mm in length and 40 mm in breadth colorless.
- They are surrounded by a transparent hyaline shell-membrane.
- Eggs contain an un-segmented ovum usually with 4 blastomeres.
- A clear space is present between the egg shell and segmented ovum.
- These eggs floats in saturated solution of common salt.
- The eggs of *Necator* is slightly smaller than *Ancylostoma*.
- The female *Ancylostoma* produces 10,000-20,000 eggs per day whereas *N. americanus* produce less eggs i.e. 3000-6000 per day.

Life cycle:


DPDx
Intestinal Hookworm
CDC
4 Filariform larva penetrates skin
3 Development to filariform larva in enviornment
2 Rhabditiform larva hatches
1 Eggs in feces
Larvae exit circulation in the lungs; they are then coughed up and swallowed.
Ancylostoma spp. larvae can become developmentally arrested and dormant in tissues. Re-activated larvae may enter the small intestine.
Adults in small intestine
5
Ancylostoma duodenale
Ancylostoma ceylanicum
Necator americanus
Infective stage
Diagnostic stage

The following are the various stages of the life cycle:

- **Stage 1: Passage of eggs from the infected host**
  - The eggs containing segmented oval with 4 blastomeres, are passed out in the feces of human host.
- **Stage 2: Development in soil**
  - Eggs under the favorable conditions (damp, warm, well-oxygenated soil) hatch to rhabditiform larva (250mm in length) i.e. L1 larva within 48 hours.
  - The rhabditiform larva moults twice, on the 3rd and 5th day to develop into a filariform larva (500—600 mm in length), the infective stage of the parasite.
  - The time taken for development from eggs to filariform larva is on an average 8-10 days.

- **Stage 3: Entrance to new host**
  - The L3 larva cast of their sheath and gain entrance into the body by penetrating the skin, through the epidermis to dermis and subcutaneous tissue.
- **Stage4: Migration**
  - On reaching the subcutaneous tissue, the larva enters into the lymphatic small vessels.
  - They pass through the lymph-vascular system into the venom circulation and carried via the right heart into the pulmonary capillaries.
  - They break the lung capillaries and enter into the alveolar spaces.

- They then ascend upward the bronchial tree to trachea and larynx, crawl up over the epiglottis to the back of pharynx and ultimately swallowed.
- During migration or entering the esophagus, the larva undergoes a third moult to form fourth stage (L4 larva) equipped with a buccal capsule allowing adherence to the gut wall.

- **Stage 3: Entrance to new host**
  - The L3 larva cast of their sheath and gain entrance into the body by penetrating the skin, through the epidermis to dermis and subcutaneous tissue.

- **Stage4: Migration**
  - On reaching the subcutaneous tissue, the larva enters into the lymphatic small vessels.
  - They pass through the lymph-vascular system into the venom circulation and carried via the right heart into the pulmonary capillaries.
  - They break the lung capillaries and enter into the alveolar spaces.
  - They then ascend upward the bronchial tree to trachea and larynx, crawl up over the epiglottis to the back of pharynx and ultimately swallowed.
  - During migration or entering the esophagus, the larva undergoes a third moult to form fourth stage (L4 larva) equipped with a buccal capsule allowing adherence to the gut wall.

- **Stage5: Localization and laying of eggs**

  - The growing larva settle down in the small intestine, undergo a fourth moult to develop into an adolescent worm.
  - At this stage, the provisional toothless buccal capsule formed is cast off and definitive buccal capsule complete with teeth is formed.
  - During 4-5 weeks, the adults who remain attached to mucosa of the small intestine becomes sexually mature.
  - The fertilized females begin to lay eggs which are excreted out in the feces.
  - The cycle is thus repeated.
  - The interval between the time of skin infection and the first appearance of eggs in feces is about 6 weeks.

Laboratory diagnosis:

- **Specimen:**Stool, duodenal content obtained by the duodenal intubation (Ryles tube) may sometimes reveal either egg or the adult worms.
- **Stool culture:**
  - Harada- mori method of culture of stool is carried out to demonstrate L3 larva.
  - The eggs present in the stool are smeared in moist filter paper, after an incubation of 5-7 days at room temperature, L3 larva hatch out of eggs.

- **Imaging methods:**
  - On chest X-ray a patchy infiltrate may be demonstrated in the migratory phase of larva in the lungs.
- **Blood test:**
  - This is carried out to ascertain the nature of anemia and the presence of eosinophilia.
- **Occult blood test:**
  - occult blood in the stool gives a position reaction in case of hookworm infection
  - Charcot-Leyden crystals are often found in the stool.

- **Stool microscopy:**
  - A specific diagnosis of hookworm infection is based on the microscopic identification of eggs.
  - This is carried out by examination of a direct wet mount of the stool.
  - In case of light hook worm infection, detection of eggs can be done using concentration methods-formalin ether concentration technique or simple salt floatation technique.
  - Kato-Katz is a useful method for quantitation estimation of hook worm eggs present in the stool.
  - The intensity of the infection is determined by counting the no. of eggs in a measured volume of feces.

Unit-7
T solium, T saginata

Taenia solium; Pork tape worm

- *Taenia solium* commonly known as the pork tapeworm or the armed tapeworm.
- It is a flat-ribbon like tape worms that causes intestinal taeniasis.
- Adult worms are rarely pathogenic but the encysted larval stage (cysticercus cellulosae) of the worm caused a serious disease in human called **Cysticercosis.**
- **Habitat:** The adult worm inhabits the small intestine (upper jejunum) of human.

Morphology:

1. **Adult worm:**
   - Adult *Taenia solium* is a flattened ribbon like tapeworm that is white in color.
   - The adult worm measures about 2-3 meters in length.
   - The body of parasite can be divided into 3 parts:- Head (Scolex), neck and body (strobila)

   i. Scolex (Head):

   - It measures 1 mm in diameter, about the size of a pin head.
   - It is globular in shape and has 4 circular suckers.

- The head is provided with a rostellum armed with a double row of alternating large and small hooklets (130-180mm long).
- The presence of hooklets gave its name armed tape worm.

ii. Neck:

- The neck is short measuring 5-10 mm in length.

iii. Body (Strobila):

- The body or Strobila consists of segments or proglottids.
- The total number of proglottids are about 800-900.
- The proglottids may be immature, mature or gravid.
- The gravid segment measures 12 X 6 mm in diameter and looks grayish-black and transparent when fully developed.
- The worm is hermaphrodite and each segment containing both male and female reproductive organs.
- The common genital pore is marginal, thick-lipped and is situated near the middle of each segment alternating between the right and left side.

- Testes consists of 150-200 follicles.
- An ovary is two in number which has a third (accessory lobe).
- The ovary is situated in the posterior side of the segment.
- The gravid consists of a median longitudinal stem of uterus having 7-13 branches on each side of the segment.
- Uterus is completely filled with eggs and each gravid consist nearly 30,000-50,000 eggs.
- The vaginal opening is not guarded by a muscular sphincter.
- The gravid segment are expelled passively, in chains of 5 to 6 at a time and not singly.

2. Eggs:

- Eggs are similar to those of *Taenia saginata*.
- Each egg is round, brown in color, measures 40-50 µm in diameter
- Each egg consists of two shells.
- The outer shell is thin, transparent and represents the remnant of yolk mass.
- The inner shell, also known as embryophore is brown, thick walled and radially striated. It encloses the embryo.
- The embryo measures 14-20 µm in diameter with hooklets.
- Eggs do not float in saturated solution of common salt (NaCl).
- Eggs are infective to pigs as well as to humans.

3. Cysticercus cellulosae larvae

- Larvae is cysticercus cellulosae and is the Infective form of parasite.
- It is also known as Taenia cyst.
- The larval form develops in the muscle of pigs as well as various organs of the human.
- A mature cyst is an opalescent ellipsoidal body and measures 8-10 mm width by 15mm in length. It has a fluid filled milky white bladder like structure.

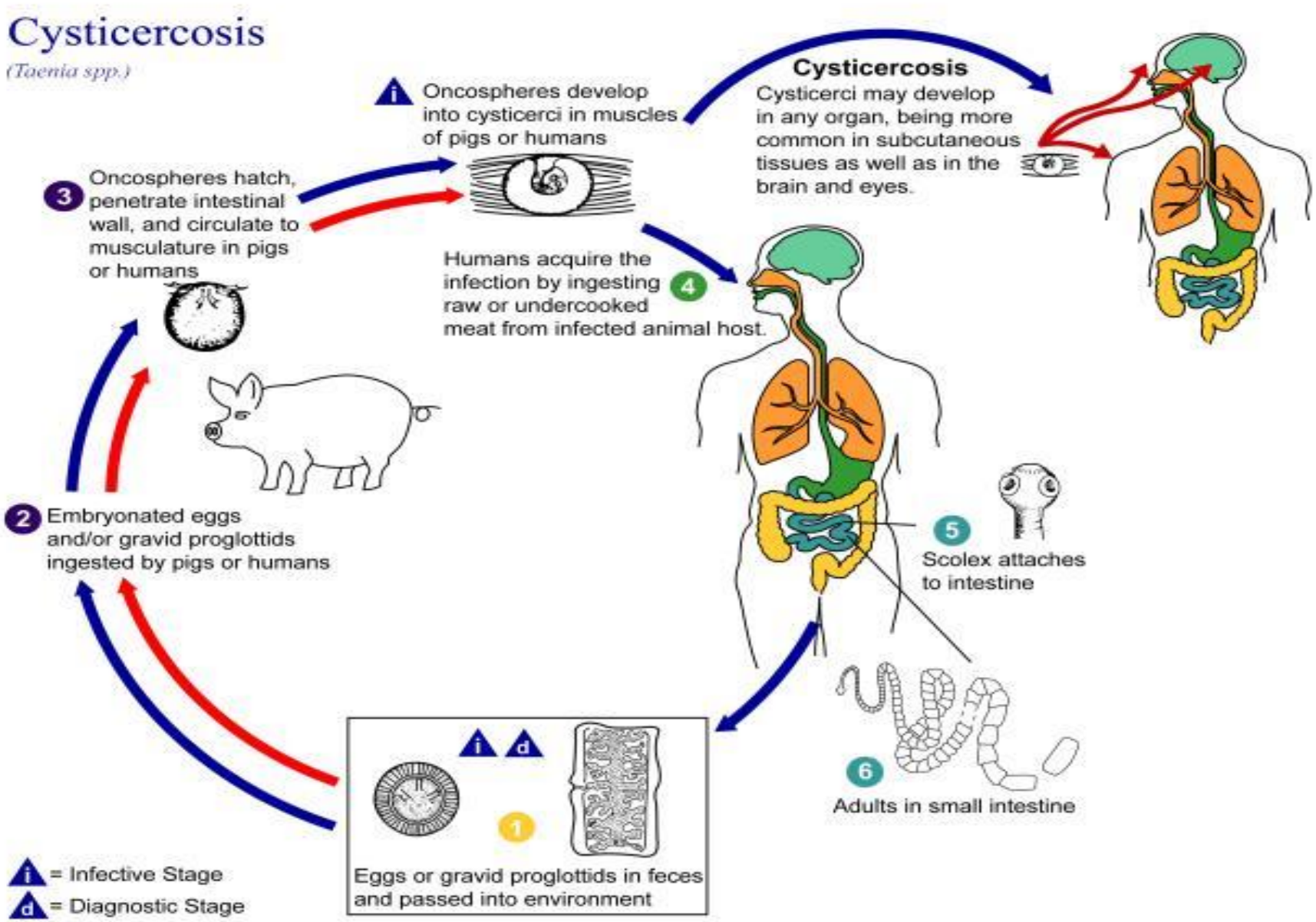
- The long axis of cyst lies parallel with the muscle fiber. The cyst is separated from the host tissue by a thin collagenous capsule. There is a dense milk white spot at the side, where the scolex with its hooks and suckers remain invaginated.
- The cavity of cyst is fill with a clear fluid rich in albumin and salts.
- The larvae can live for about 8 months in muscles of pig and can only develop into adults when ingested by man.

Life cycle of *Taenia solium*:

- The life cycle is completed in two hosts.
- Definitive host: Human
- Intermediate Hosts: Pig, occasionally human.
- Humans acquire infection by ingestion of inadequately or improperly cooked pork infected with cysticerci.
- Inside the alimentary canal of man the scolex on coming incontact with bile exvaginates and anchor to the gut wall with its hooks and suckers.
- The larvae develops into an adult worm by gradual strobilisation.
- The worm grows to sexual maturity in 2-3 months and start producing eggs which are then passes in the faeces along with the gravid segments.

- The pig gets infection by ingestion of eggs or gravid proglottids passed in human faeces.
- In the intestine of pig, the oncospheres hatch out of eggs.
- They attach to the intestinal mucosa by hooks, penetrate the gut-wall and gain entrance into the portal vessels or mesenteric lymphatic, finally reaching the systematic circulation.
- Usually they travel via the portal vein and successively reach the liver, right side of heart, lungs, left side of heart, brain or other tissue with high blood flow.
- The naked onchospheres are filtered out from the circulating blood into the muscular tissue where they ultimately settle down and undergo further development.
- They lose their hooklets, enlarge, and develops into a fluid-filled cyst within a period of 9-10 weeks.

- They remain viable for up to 8 weeks in muscle of pig during which they remain infective for human.
- The new host gets infection by ingestion of the infected meat of pig and the cycle is repeated.
- Occasionally humans get infection by eating food or drinking water contaminated with eggs.
- On ingestion, the onchospheres are released from the eggs in the intestine. These larva invade the intestinal mucosa and are then carried by the circulation to different tissue where they develops into cysts.
- In human most cysts are produced in the CNS, skeletal muscles, eye and subcutaneous tissue giving rise to a condition called **cysticercosis**.


Cysticercosis
(Taenia spp.)
i Oncospheres develop into cysticerci in muscles of pigs or humans
Cysticercosis
Cysticerci may develop in any organ, being more common in subcutaneous tissues as well as in the brain and eyes.
3 Oncospheres hatch, penetrate intestinal wall, and circulate to musculature in pigs or humans
Humans acquire the infection by ingesting 4 raw or undercooked meat from infected animal host.
2 Embryonated eggs and/or gravid proglottids ingested by pigs or humans
5 Scolex attaches to intestine
6 Adults in small intestine
i d
1
Eggs or gravid proglottids in feces and passed into environment
i = Infective Stage
d = Diagnostic Stage

Laboratory Diagnosis of *Taenia solium:*

- **Specimen:** Faeces, muscle tissue, blood, csf

1. Macroscopic examination:

- A naked examination of the specimen can be made for segment or proglottids.
- The whitish segment can easily be recognized against the dark yellow mass of the faeces.

2. Stool Microscopy:

Demonstration of eggs and less frequently proglottids and scolex in faeces is used as tool for diagnosis.

Eggs:

- Eggs are demonstrated by a thick faecal smear examination.
- The eggs shed irregularly, so 2-3 stool sample should be collected.
- Eggs can be seen in perianal area and can be detected by cellophane swab.
- Since eggs of T. soluim and T. saginata are similar, species diagnosis can be made by Acid-fast staining. Eggs of T. soluim are non-acid fast whereas T. saginata is acid fast.

Proglottids:

- The gravid proglottids are found in faeces or recovered from the under clothings.
- They are washed in clean water and placed between two sides.
- The sides are held by adhesive tape at each end and are examined by hand less for lateral branches.
- Demonstration can be facilitated by staining them with india ink, injected through the genital pores.

Observation:

- Taenia soluim: 7-12 lateral branches on each side of utetine stem
- T. saginata: 10-20 lateral branches on each side of utenine stem.

Scolex:

- T. soluim – bears a row of hooks
- T. saginata- lacks hooks.
- The scolex is not always recovered following the treatment and the method is hazardous.

Antigen detection:

- This is a very useful for screening the cases of intestinal taeniasis.
- Antigens capture ELISA polyclonal antisera raised against Taenia is employed to detect antigen in faeces.

Serodiagnosis:

- Serological tests are employed to detect anti-cysticercus antibodies in serum or CSF.
- ELISA (sensitivity 75%, specificity 85%). Antigen can be detected by ELISA using specific monoclonal antibodies.
- Enzyme-linked immunoelectro transfer blot (EIIB). Sensitivity 90 % specificity 50-70%.
- Detection of antigens in serum or CSF indicates recent or viable infection.

Histopathological diagnosis

- Diagnosis of Neurocycticercosis (NCC) is made by demonstrating cysticerci in the biopsy tissue obtained from brain during post mortem.
- Skeletal cysticercosis can be diagnosed by histological examination of biopsy.

Imaging method:

- X-ray of the soft tissue in arm and thigh, chest and neck may show dead, calcified or elongated cysts.
- X-ray of the skull may reveal cerebral calcification and intracranial lesions in the neurocysticercosis.
- CT scan is best method for detecting dead, calcified and multiple cysts is pathognomonic of neurocysticercosis.

T saginata is also known as beef worm and the morphology, life cycle and lab diagnosis is same as like Taenia solium

Unit-8
Malarial Parasite

- ***Plasmodium,*** the parasite responsible for human malaria is among the most researched genera of parasite in the world. Malarial infection in humans continues to grow in tropic and sub-tropic areas despite extensive studies on control measures.
- **There are four species of malaria that affect humans:**

  • *Plasmodium falciparum:* the commonest species in the hotter parts of the world and responsible for much sickness and even death.

  • *P. vivax:* the commonest species in the cooler parts of the tropics, the largest of the malaria parasites found in humans, and the cause of much illness.

  • *P. malariae:* a less common species but one that occurs throughout much of the world.

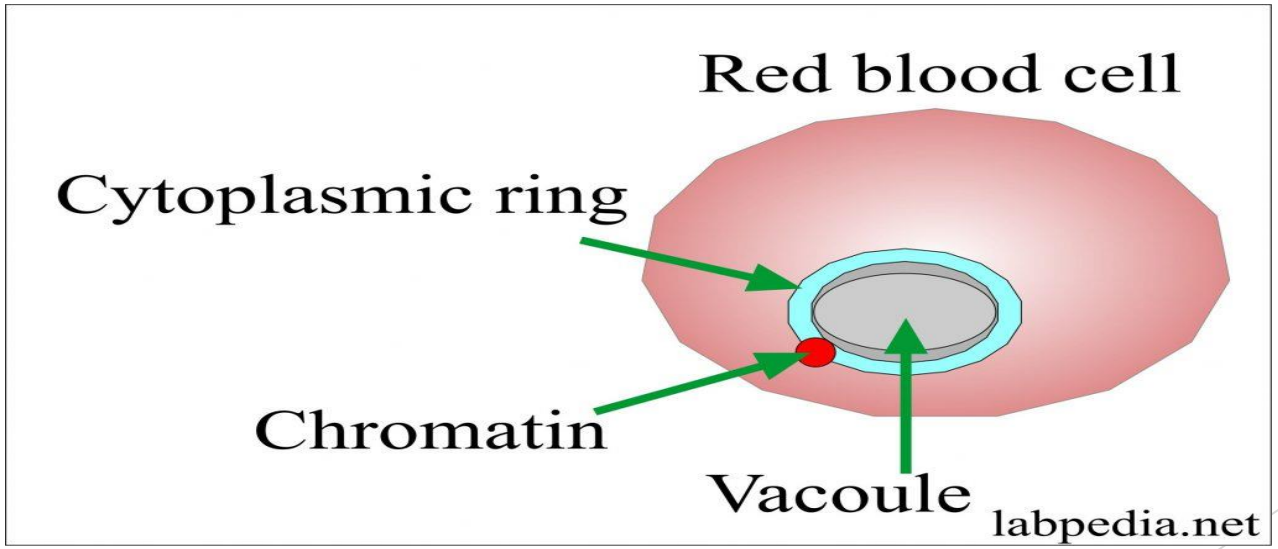
  • *P. ovale:* a relatively rare species but reported from time to time in many countries, especially in Africa; sometimes confused with *P. vivax*.

- Plasmodium falciparum is the most virulent species of Plasmodium in human. It causes malignant tertian or falciparum malaria. The name ‘falciparum’ is derived by Welch from ‘falx’ meaning sickle or crescent and ‘parere’ meaning to bring forth.

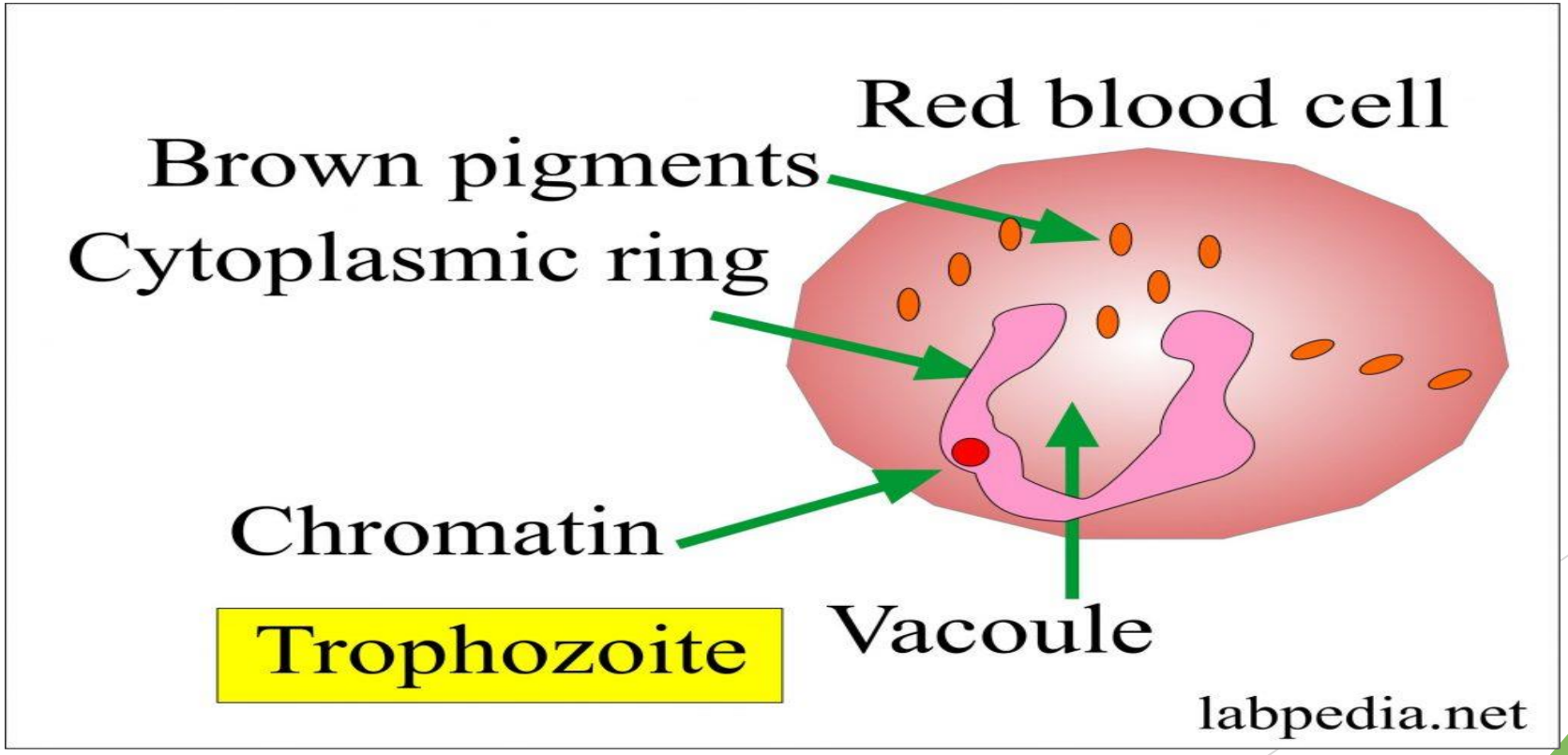
- **Habitat:**Various stages of malarial parasites are found inside the parenchymal cellsof liver and inside RBCs of Human.

Morphology:

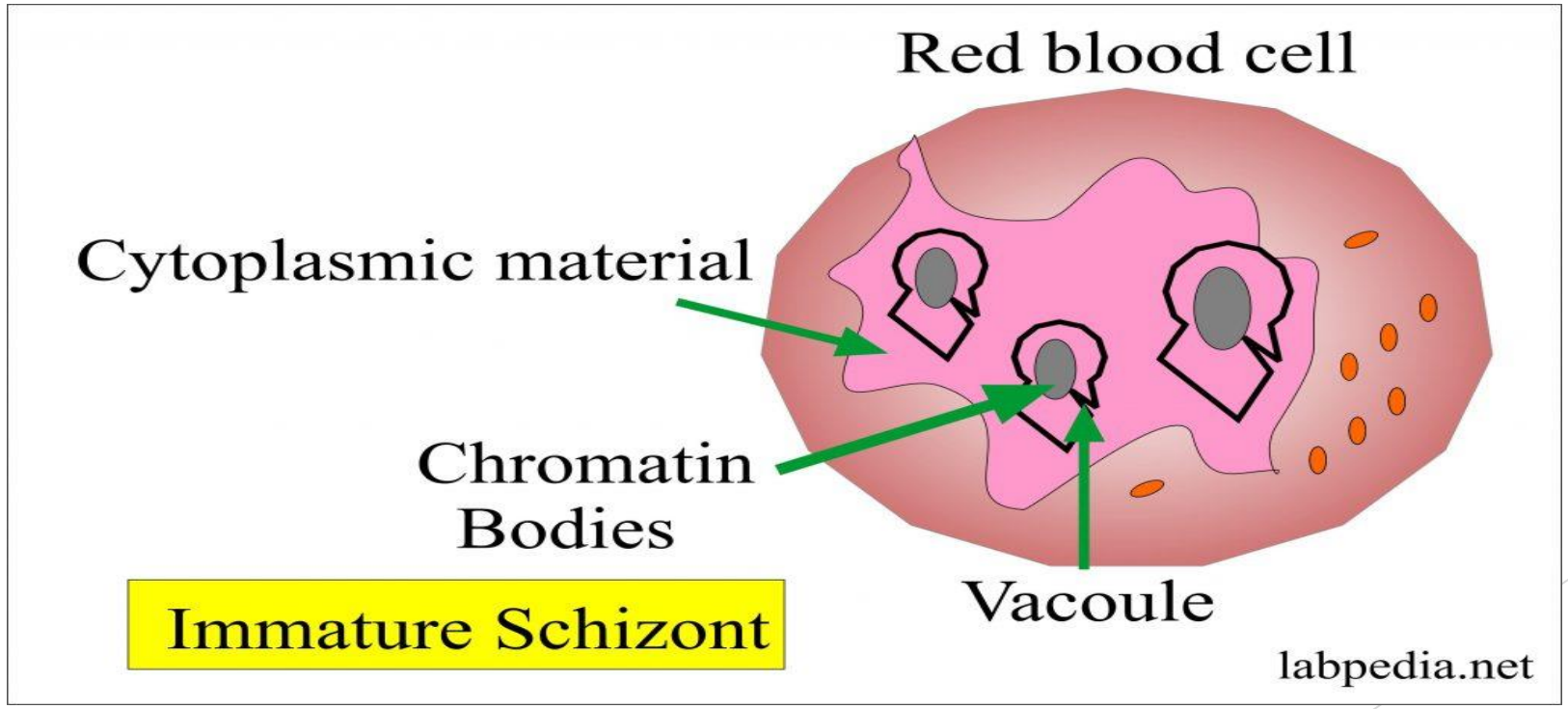
- **Ring form** is early trophozoites.
  - This is a ring-like malarial parasite following the invasion of RBCs.
  - Giemsa stain shows it as a blue stain cytoplasmic circle connected to red chromatin dot.
  - The space inside the ring is known as the vacuole.



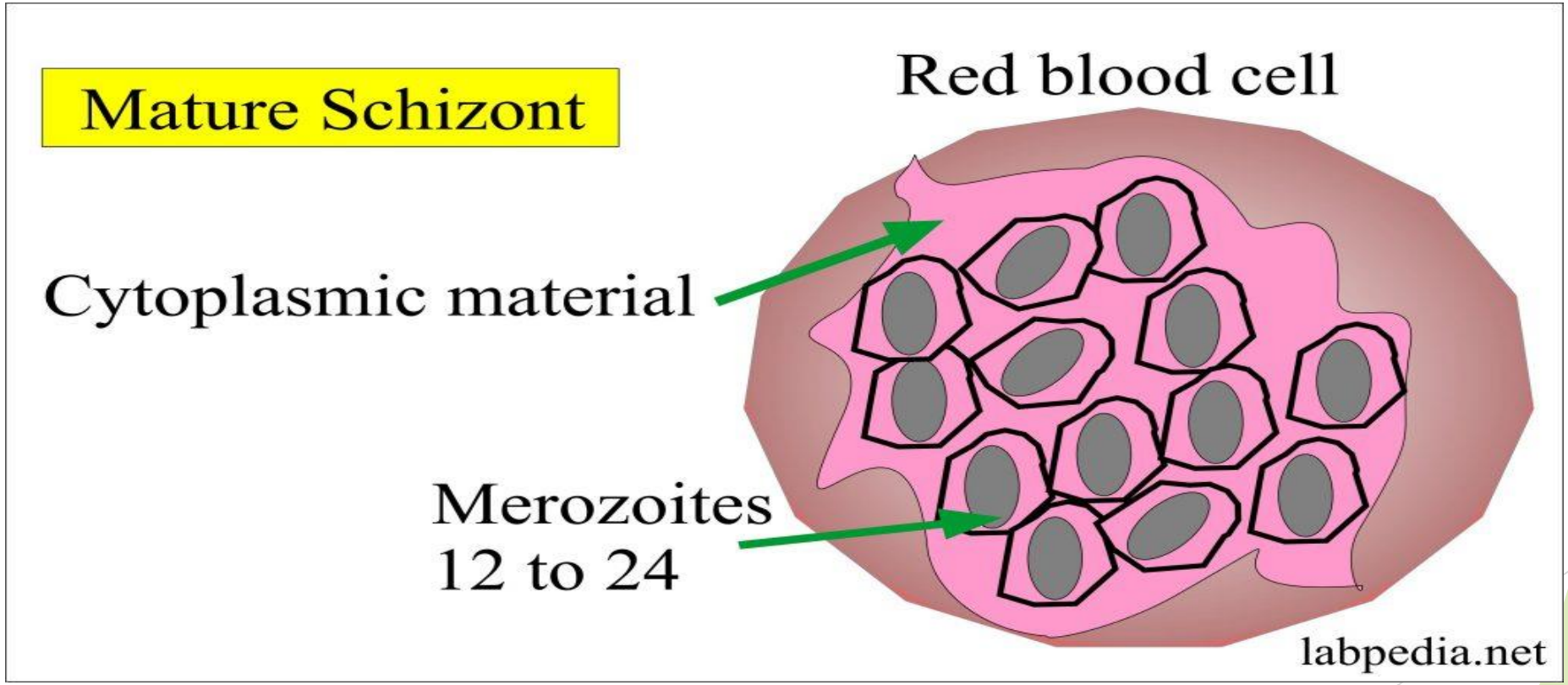
- **Trophozoites:** The shape varies according to the type of malarial parasite.
  - There are a cytoplasmic circle and the chromatin dot.
  - More space is taken by the developing trophozoites.
  - Pigments are brown in color.



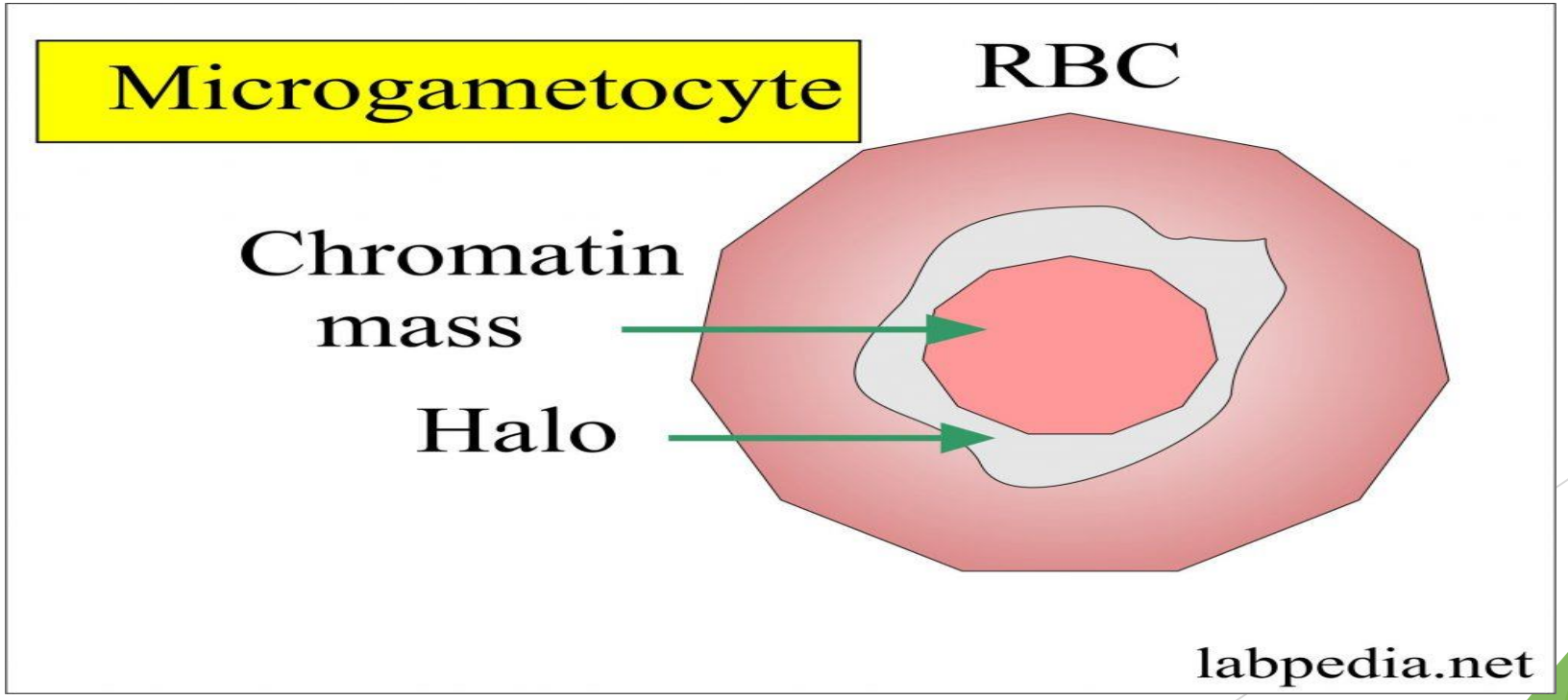
- **Immature schizonts:** There is active chromatin replication.
  - Visible cytoplasmic material surrounds the growing chromatin.
  - Pigments are often brown.
  - It occupies more space in the RBC.



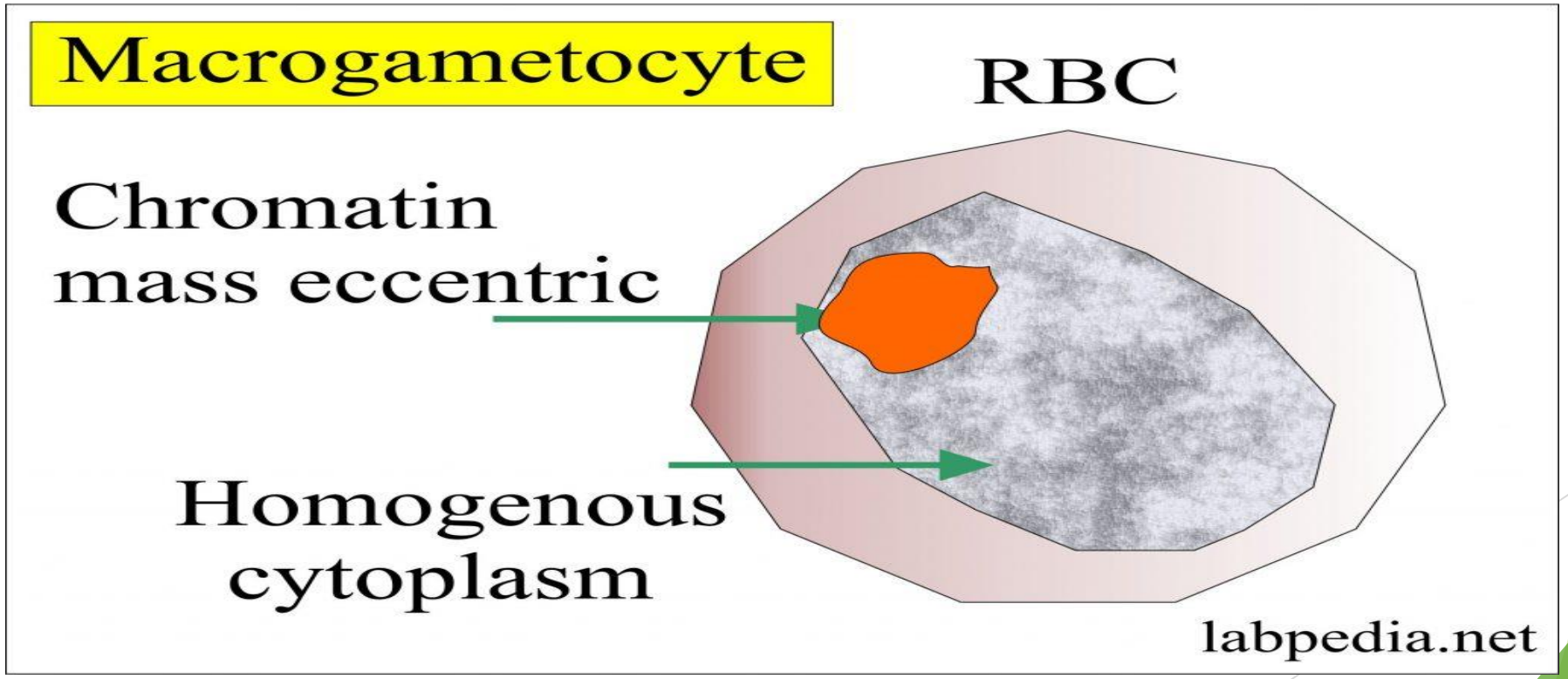
- **Mature Schizonts:** These are characterized by the presence of merozoites.



- **Microgametocytes:** In Plasmodium falciparum is crescent-shaped, in others is typical to a round shape.
  - There is large diffuse chromatin mass which stains pink to purple.
  - Chromatin mass is surrounded by colorless to pale halo.
  - Pigments are usually visible



- **Macrogametes:** These are round to oval in shape with the exception of P.falciparum which is the crescent shape.
  - Chromatin is surrounded partially or completely by the cytoplasm.
  - Pigments are also present



Lifecycle:

- **Asexual cycle:**

The majority of sporozoites migrate to the liver and invade hepatocytes.

- Initially, elongated sporozoite has transformed into a rounded form.
- This rounded form then matures within the hepatocyte to a schizont containing many merozoites.
- This cycle takes 5 to 16 days.
- Merozoites leave the liver and enter the blood, this is an asexual cycle.

- **The sexual cycle:**

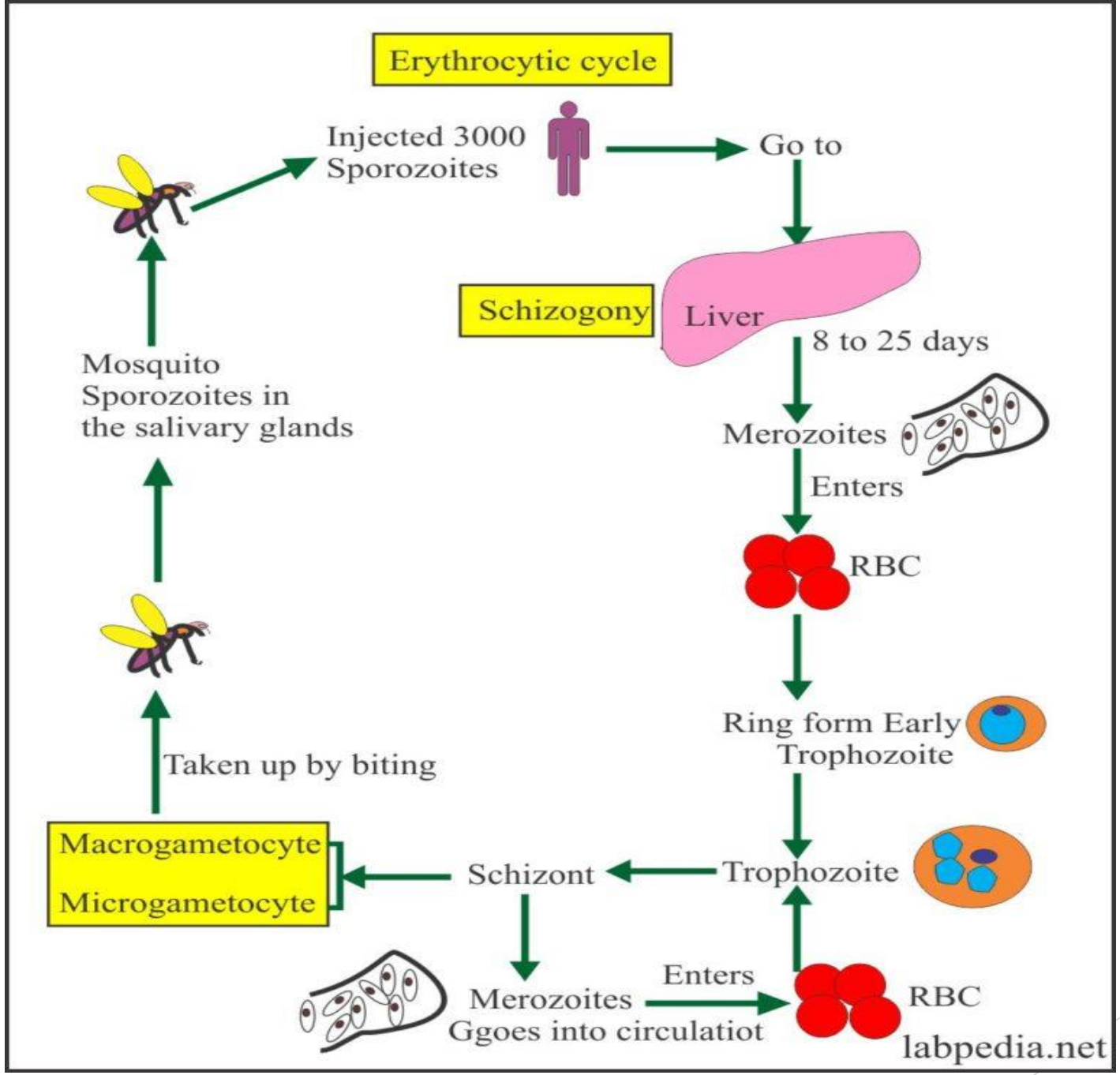
It starts when the mosquito sucks the blood from the patients.

- Micro and macrogametocytes in the stomach of mosquito combine and form zygote.
- This forms oocyst and sporozoites.
- Sporozoites are injected into humans containing merozoites.

RBCs Cycle (Erythrocytic Cycle) Asexual Cycle:

Now Merozoites enter the RBCs and start the **Asexual** cycle. produce more merozoites. This cycle repeats 1 to 3 days.

- This multiplication can result in thousands of parasite-infected cells in the host bloodstream.
- The patient may develop signs and symptoms of illness and complications of malaria.
- The complication of malaria parasite if not treated then it may last for months.
- Some of the merozoites transform into a sexual form called as male and female gametocytes.
- These gametocytes circulate in the blood are taken up by the biting mosquito.

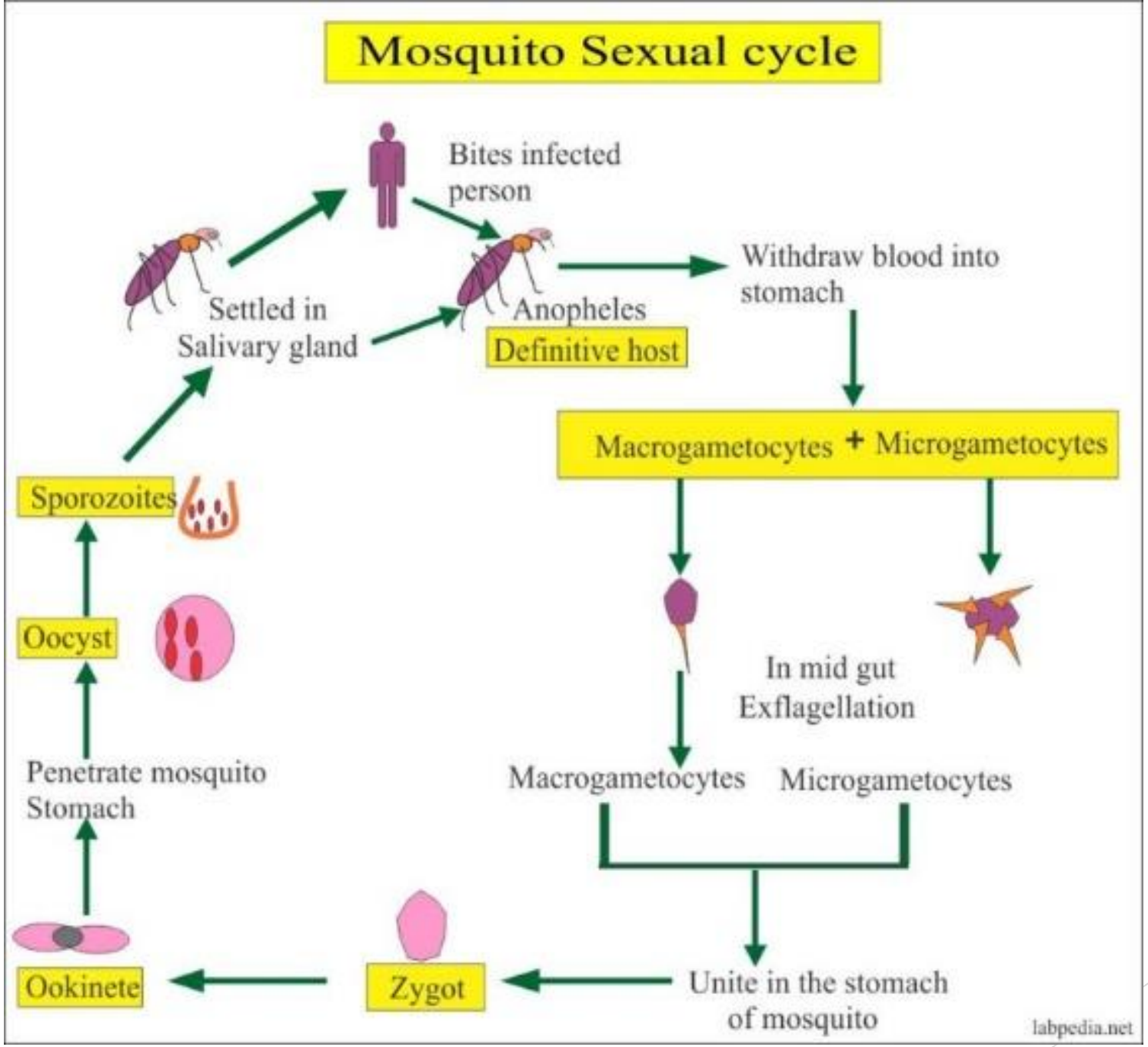


MP RBCs cycle

The Life Cycle In The Mosquito (Sexual Cycle):

When the mosquito bites the infected humans, then suck the blood and these gametocytes go into along the blood.In mosquito RBCs, burst and gametocytes are released These will get into more mature form **Gametes**.

- Male and female gametes fuse to form diploid zygotes.
- Zygotes develop into actively moving ookinetes that burrow into the mosquito midgut wall and form oocysts.
- Growth and division of each oocyst produce thousands of active haploid forms called sporozoites.
- After 8-15 days, the oocyst bursts, releasing sporozoites into the body cavity of the mosquito, from which they travel to and invade the mosquito salivary glands.
- Mosquito is ready to infect humans



MP sexual life cycle

Lab Diagnosis

- History of the patient in suspected areas.
- Blood smear:
  - Make a blood smear when the patient has a fever. Thin and Thick smears are made.
  - The thick smear is more helpful to find M.Parasites.
    - The thin smear is good to identify the type of malarial parasite.
  - Collect blood 6 to 8 hourly till 48 hours to declare negative for malaria.
  - Giemsa stain is the best choice.
-

- Serologic methods are based on immunochromatic techniques. Tests most often use a dipstick or cassette format and provide results in 2-15 minutes.
- Polymerase chain reaction (PCR): Parasite nucleic acids are detected using the PCR technique. This is more sensitive than smear microscopy. This is of limited value for the diagnosis of acutely ill patients because of the time needed for this procedure.

Unit-9
Virology

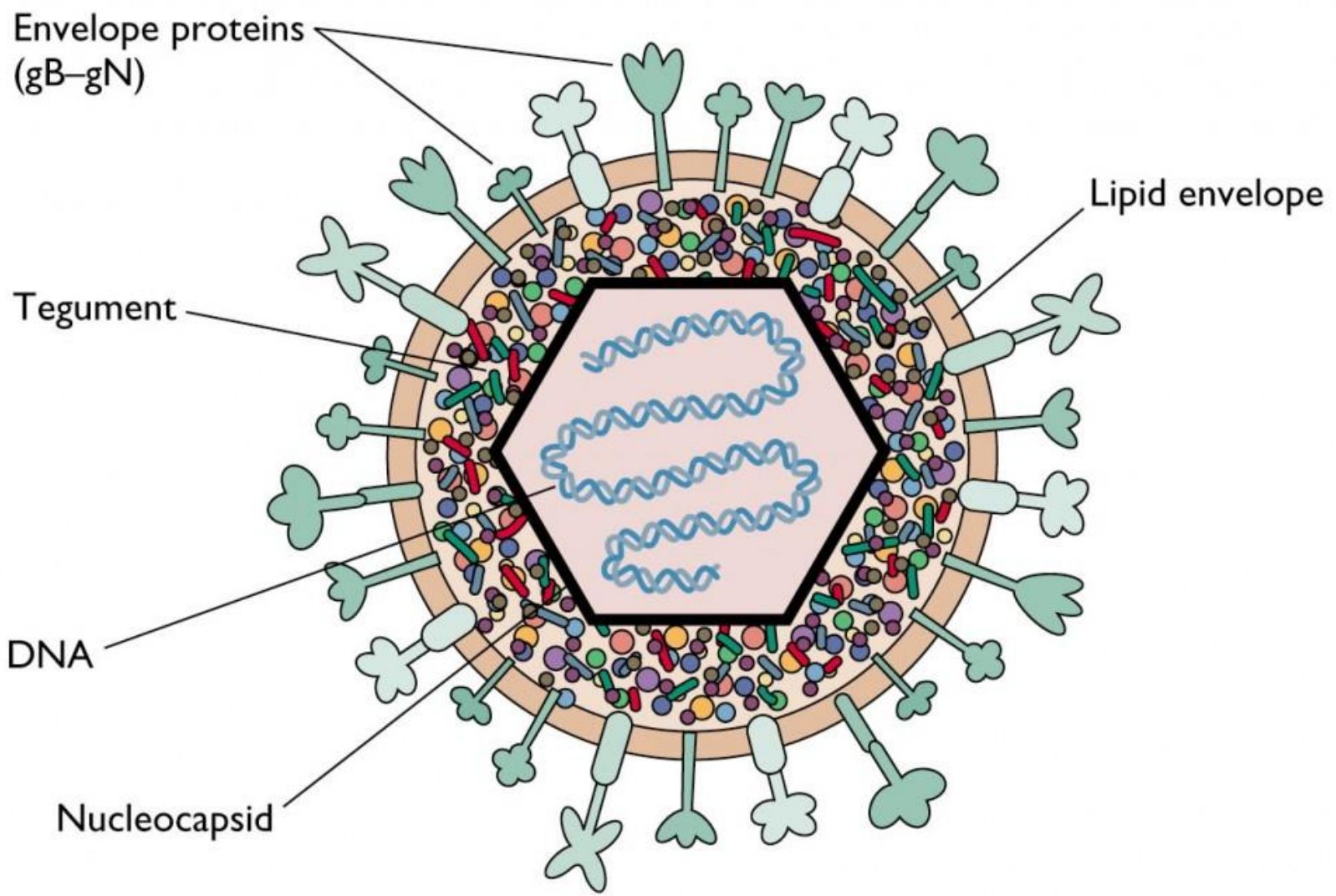
Virology

- virology is the branch of microbiology that deals with the study of viruses (as well as various virus-like particles), their characteristics, classification, as well as the relationship with their respective hosts.
- Compared to the other organisms in microbiology, viruses are very unique with different characteristics (with regards to multiplication, structure, etc) that set them apart.
- Viruses infect all major groups of organisms: vertebrates, invertebrates, plants, fungi, bacteria but some viruses have a broader host range than others; however, none can cross the eukaryotic/prokaryotic boundary.

Virus: Structure and Symmetry

- Virus are very small infectious agents with size ranging from 20-300nm in diameter.
- Viruses are non-cellular entities so they are also called as particles.
- Virus lacks their own independent metabolism and cannot replicate outside the host cell. So they are also called as obligate intracellular parasites.
- Virus that infects bacteria are called bacteriophage or simply phage. Animal virus infects animals and similarly plant virus infects plants.

Structure of virus



- A basic structure of virus is nucleic acid core (either DNA or RNA but not both) surrounded by protein coat.
- Central core of nucleic acid of a virus is called genome and the protein coat surrounding is called as capsid.
- In some virus, an envelope made up of glycoprotein and phospholipid bilayer is present outside the capsid.

The basic structural components of a virus are

1. Genome:

- Virus contains either DNA or RNA as genetic material but not both. Virus which contains DNA as genetic material are called DNA virus and those containing RNA are called RNA virus.
- Unlike other living cell where ds DNA is always a genetic material, a viral genome may consists of linear or circular ds DNA, single stranded DNA, ss linear RNA or ds linear RNA.
- Examples; Reo virus is a RNA virus which contains ds RNA genome. Parvovirus contains ss DNA, Papovavirus contains ds circular DNA as genetic materials.

2. Capsid:

- Capsid is the outer layer. Sometime it is refered as coat or shell.
- Capsid serves as impenetrable shell around the nucleic acid core.
- Capsid also helps to introduce viral genome into host cell during infection.
- The protein coat or capsid is made up of number of morphological similar sub units called capsomere. Each capsomere is further composed of protomere.
- Capsomere are arranged precisely and tightly together in a repetitive pattern to form complete capsid.
- The number of capsomere in a capsid varies from virus to virus.
- The complete complex of nucleic acid and protein coat of a virus particle is called as virus nucleo-capsid.
- Structure of capsid give the symmetry to the virus. Virus particle may be either cubicl or helical or binal or complex symmetry.

3. Envelope:

- Some virus contains envelope that surrounds nucleocapsid. The virus without envelope is called naked virus.
- The envelope is a bilayer of lipoprotein and glycoprotein.
- The envelope is acquired by the progeny virus from host cell during virus release by budding process.
- In some virus the glycoprotein projects out in the form of spike called peplomere. Some of the peplomers or glycoprotein spike such as Haemaglutinin and Neuraminidase which are involved in binding of virus to host cell.

4. Enzymes:

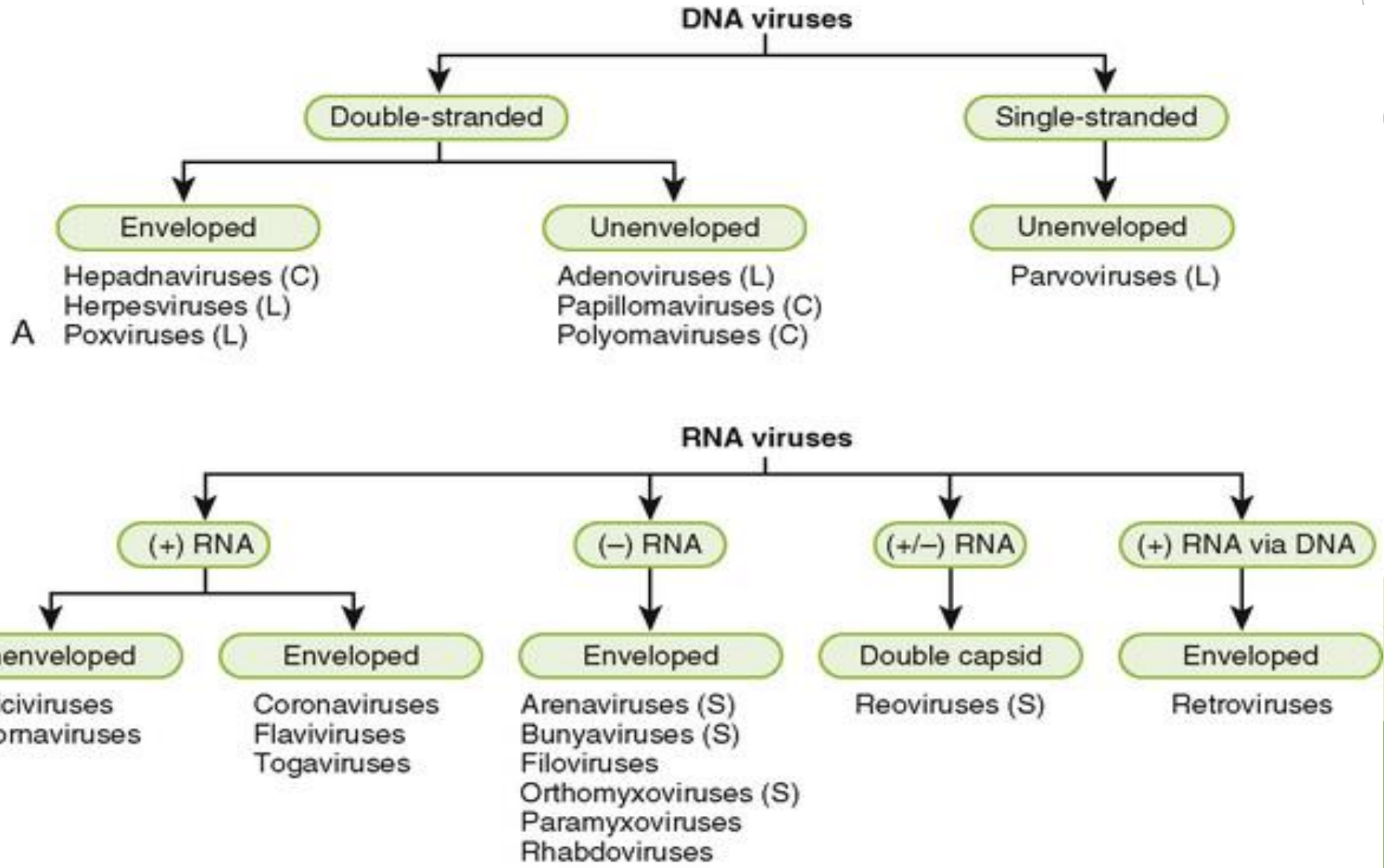
- Some virus contains enzymes which play central role during infection process. Eg. Some bacteriophage contains an enzyme lysozyme, which makes small hole in bacterial cell that allows viral nucleic acid to get in.
- Some virus contains their own nucleic acid polymerase which transcribe the viral genome into mRNA during replication process. Eg. Retro virus are RNA virus that replicates inside host cell as DNA intermediate. These virus possess an RNA dependent DNA polymerase called reverse transcriptase.

Some of the general characteristics of a virus include:

- Can only reproduce (through synthesis and assembly) in living cells
- Contain DNA/RNA or both in some cases
- Are not capable of sexual or asexual modes of reproduction
- Are not cells - They are acellular particles that lack normal cell organelles and cytoplasm
- Very small compared to other single-celled organisms
- They are parasites that fully depend on living cells for replication.
- They can pass through filters, through which bacteria cannot pass.
- They are ultra-microscopic and can only be visualized under electron microscope.

Classification of virus

Classification of virus on the basis of nucleic acid



1. DNA virus:

- viral genome is DNA

  i) Double stranded DNA virus: eg. Adenovirus, Herpesvirus

  ii) Single stranded DNA virus: eg. Parvovirus, φ174 virus

2. RNA virus:

- genome is RNA

  i) Double stranded RNA virus: eg. Reo virus

  ii) Single stranded RNA virus: these are further classified into two groups

- Positive sense RNA (+RNA): Polio virus, Hepatitis A
- Negative sense RNA (-RNA): Rabies virus, Influenza virus

Classification of virus on the basis of structure

1. Cubical virus: they are also known as icosahedral symmetry virus Eg. Reo virus, Picorna virus

2. Spiral virus: they are also known as helical symmetry virus Eg. Paramyxovirus, orthomyxovirus

3. Radial symmetry virus: eg.Bacteriophage

4. Complex virus: eg. Pox virus

Classification of virus on the basis of replication properties and site of replication

1. Replication and assembly in cytoplasm of host: Eg. All RNA virus replicate and assemble in cytoplasm of host cell except Influenza virus

2. Replication in nucleus and assembly in cytoplasm of host: Eg. Influenza virus, Pox virus

3. Replication and assembly in nucleus of host: All DNA viruses replicate and assemble in nucleus of host cell except Pox virus.

4. Virus replication through ds DNA intermediate: Eg. All DNA virus, Retro virus and some tumor causing RNA virus replicates through ds DNA as intermediates.

5. Virus replication through ss RNA intermediate: Eg. All RNA virus except Reo virus and tumor causing RNA viruses.

Classification of virus on the basis of host range:

1. Bacteriophage: Phage are virus infecting bacteria. Eg, λ phage, T2, T4, φ174, MV-11

2. Plant virus: Those virus that infects plants. Eg. TMV, cauliflower mosaic virus

3. Animal virus: Those virus that infects animals. Eg. Polio virus, Retro virus, Herpes virus, Adeno virus

4. Insect virus: Virus that infects insects. Eg. Baculovirus, Sacbrood virus, Entomopox virus, Granulosis virus

Classification of virus on the basis of mode of transmission:

1. Virus transmitted through respiratory route: Eg, Swine flu, Rhino virus

2. Virus transmitted through faeco-oral route: Eg. Hepatitis A virus, Polio virus, Rota virus

3. Virus transmitted through sexual contacts: Eg. Retro virus

4. Virus transmitted through blood transfusion: Eg. Hepatitis B virus, HIV

5. Zoonotic virus: virus transmitted through biting of infected animals; Eg. Rabies virus, Alpha virus, Flavi virus

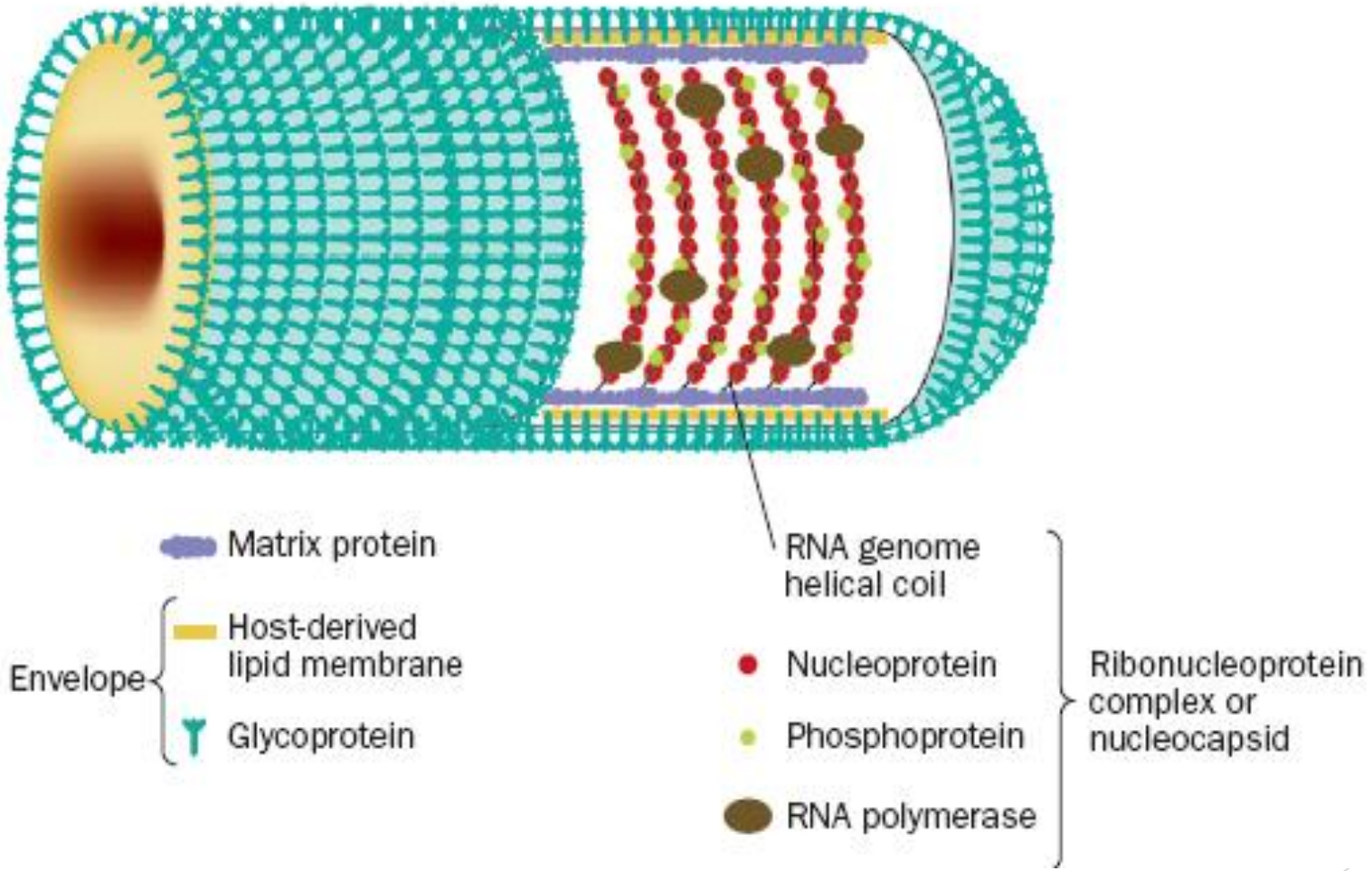
Unit-10
Medically important viruses

Pathogenicity, Lab diagnosis and prevention of – - Rabies

Structure of Rabies Virus

- The virus is enveloped, rod shaped particles measuring 75 × 180 nm.
- Mature virion appears either as bullet shaped particles with one rounded and one flattened end or as bacilliform particles.
- The particles are surrounded by a membranous lipid envelope.
- The particles have a buoyant density in CsCl of about 1.19 g/cm3.
- The virion outer surface is covered with protruding spikes which is 10nm long.
- The peplomers (spikes) are composed of trimers of the viral glycoprotein.
- Inside the envelope is a ribonucleocapsid which encloses single-stranded, negative-sense RNA genome (12 kb; molecular weight 4.6 *10ˆ6).

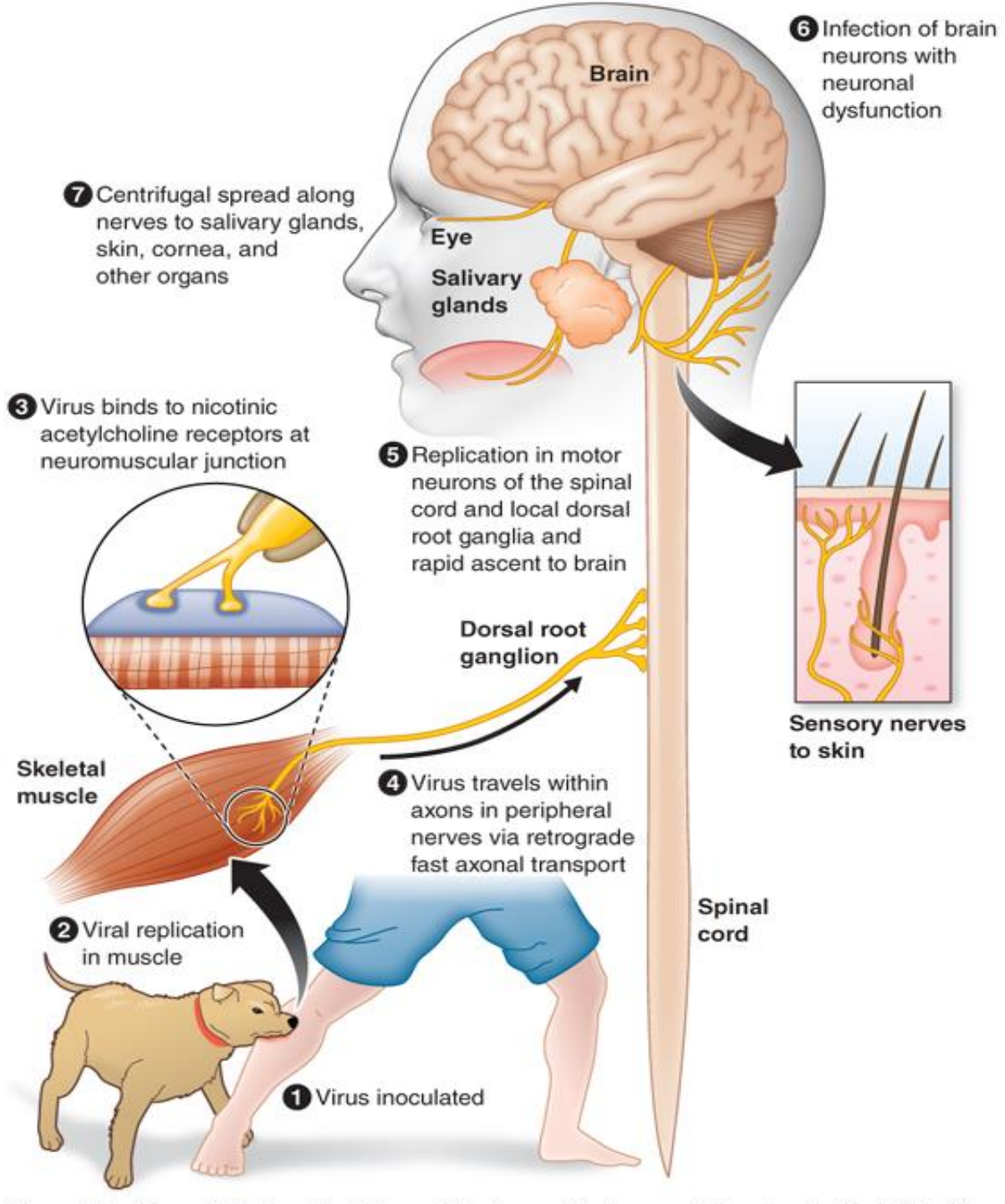
- The genome encode for five proteins designated as glycoprotein(G), nucleoprotein(N), phosphoprotein(P), matrix protein(M) and large polymerase protein(L).



Pathogenesis of Rabies Virus

- After inoculation of infectious saliva by bite, virus may persist and replicate in muscle tissue before progressing to the peripheral nervous tissue via neuromuscular junction.
- Neurotropsim is a main feature associated with viral replication residing exclusively to neurons.
- A significant interaction of G protein and acetylcholine receptor provide evidence of viral attachment.
- After peripheral nerve entry, virus moves centripetally within axons to the CNS via transportation by retrograde axonal flow.

- Incubation period is dependent on the distance between site of bite and CNS.
- Apart from this, it also depends on age of host, immune status of host, viral strain involved and amount of inoculation.
- In the CNS, the multiplication of the virus occurs in the grey matter and spreads in the endoneurium of Schwann cell.
- Virus spread may be facilitated by movement across cell to cell junctions.
- After period of multiplication, it disseminate into tissues and organs via efferent neurons.


6 Infection of brain neurons with neuronal dysfunction
Brain
7 Centrifugal spread along nerves to salivary glands, skin, cornea, and other organs
Eye
Salivary glands
3 Virus binds to nicotinic acetylcholine receptors at neuromuscular junction
5 Replication in motor neurons of the spinal cord and local dorsal root ganglia and rapid ascent to brain
Dorsal root ganglion
Sensory nerves to skin
Skeletal muscle
4 Virus travels within axons in peripheral nerves via retrograde fast axonal transport
Spinal cord
2 Viral replication in muscle
1 Virus inoculated

Diagnosis of Rabies Virus

Specimen: saliva, corneal biopsy, brain tissue, neck skin biopsy

Histopathology

- Detection of Negri bodies by Seller's staining technique which comprises use of basic fuchsin and methylene blue.
- Negri bodies are purplish pink, sharply demarcated, more or less spherical, and 2–10 μm in diameter, and they have a distinctive internal structure with basophilic granules in an eosinophilic matrix.

Antigen detection

- Tissues infected with rabies virus are currently identified most rapidly and accurately by means of immunofluorescence or immunoperoxidase staining using antirabies monoclonal antibodies.

Antibody detection

- Antibodies develop slowly in infected persons or animals during progression of the disease but promptly after vaccination with cell-derived vaccines.
- Serum antibodies to rabies can be detected by immunofluorescence or neutralization tests.

Virus isolation

- Available tissue is inoculated intracerebrally into suckling mice.
- Infection in mice results in encephalitis and death.
- The central nervous system of the inoculated animal is examined for Negri bodies and rabies antigen.
- However, virus isolation takes too long to be useful in making a decision about whether to give vaccine.

Molecular method

- Reverse transcription-polymerase chain reaction testing can be used to amplify parts of a rabies virus genome from fixed or unfixed brain tissue or saliva.
- Sequencing of amplified products using Nucleic Acid Sequence Based Amplification can allow identification of the infecting virus strain.

Prevention of Rabies Virus

- This include preexposure and post exposure prophylaxis.
- Preexposure prophylaxis is given to individuals who are at risk that include veterians, animal handlers, laboratory workers.
- Human diploid cell line vaccine (HDCV) is given at two doses at 4 weeks interval.
- Post exposure prophylaxis includes
- Animal examination for 10days for symptoms and are then killed.
- Wound management by surgical debrigement and cleaning of wound with soap and water and quaternary ammonium compounds.

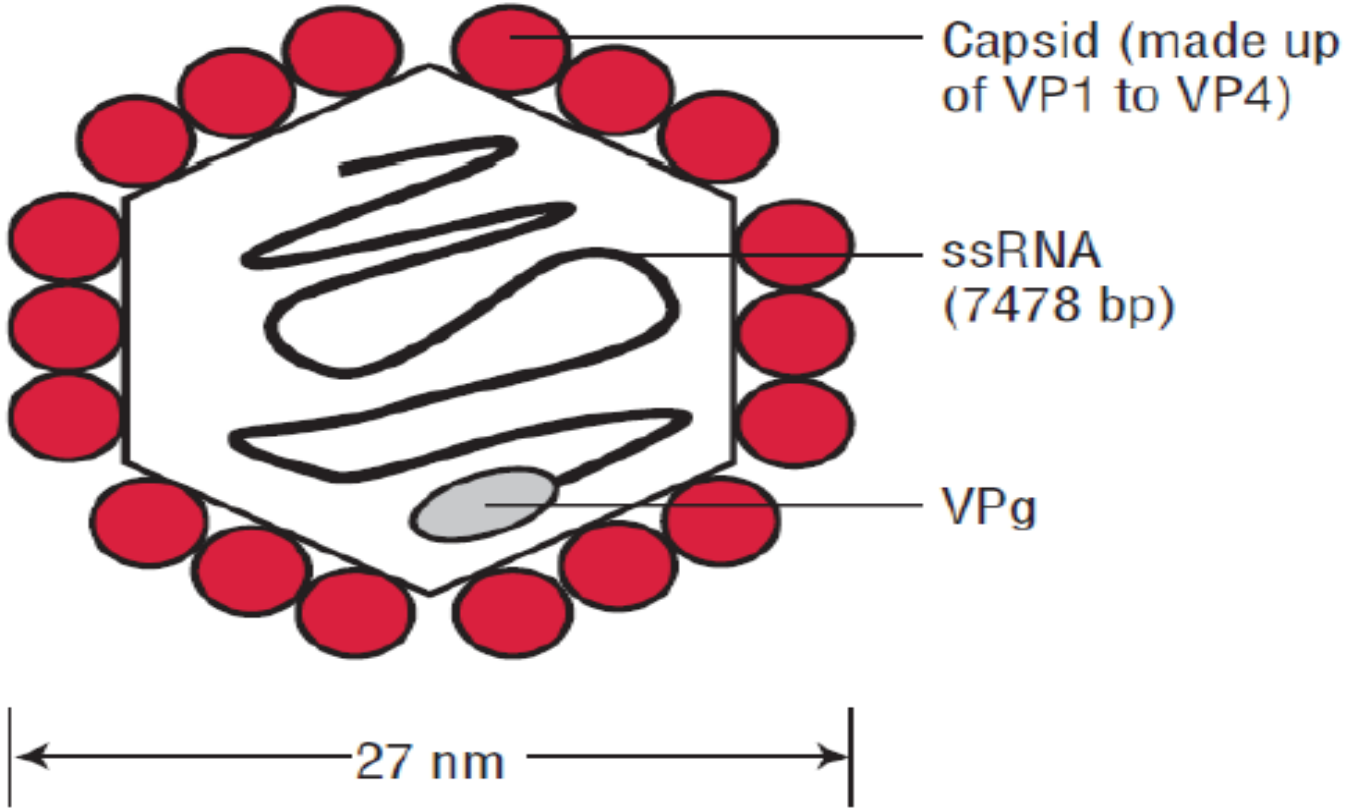
- Passive immunization using Human Rabies Immune globulin (HRIG) collected from immunized persons and infiltrated at site of wound or administered intramuscularly as soon as possible after rabies exposure.
- Active immunization with modern tissue culture vaccines consists of a series of four doses, all administered intramuscularly in the deltoid region, 1 mL each, over a 2-week period (days 0, 3, 7, and 14).
- For persons with immunosuppression, the recommended postexposure prophylaxis series includes five doses of vaccine administered on days 0, 3, 7, 14, and 30.
- Use of both active and passive immunization is strongly recommended for proper treatment.

Pathogenicity, Lab diagnosis and prevention of – - Polio

Structure of Polio Virus

- Poliovirus is a member of a family of viruses called the *Picornaviridae*.
- Virions are spherical in shape with a diameter of about 27nm.
- The particles are simple in that they are composed of a protein shell surrounding the naked RNA genome.
- The genome is monopartite, linear ssRNA(+) genome of 7.2-8.5 kb, polyadenylated, composed of a single ORF encoding a polyprotein.
- The capsids are composed of four structural proteins: VP1, VP2, VP3, and VP4.
- The basic building block of the picornavirus capsid is the protomer, which contains one copy each of VP1, VP2, VP3, and VP4.
- The shell is formed by VP1 to VP3, and VP4 lies on its inner surface.

- The virus particles lack a lipid envelope, and their infectivity is insensitive to organic solvents.



Pathogenesis of Polio Virus

- The mouth is the portal of entry for the virus, transmitted by fecal oral route on ingestion of contaminated water.
- Virus initially multiply in the oropharynx and gastrointestinal mucosa.
- The virus is regularly present in the throat and in the stools before the onset of illness.
- Virions are resistant to acidity of stomach and to lytic activities of the protease and other enzymes of the intestinal tract and bile.
- On entering the body, the virus infects and multiplies in the tonsils and Peyer's patch of ileum.
- The incubation period is 9-12 days.
- The virus then spreads to regional lymph nodes and enters the blood causing primary viremia.

- Antibodies to the virus appear early in the disease, usually before paralysis occurs.
- The antibodies are produced to prevent infection from spreading.
- On continued infection and multiplication of virus in the ReticuloEndothelial System (RES), it invades the blood stream causing secondary viremia.
- During this period of viremia, the poliovirus crosses the blood brain barrier and gain access to the brain.
- The virus shows tissue tropism by specifically combining with neural cells.
- The virus recognizes the receptor present on the anterior horn of spinal cord, dorsal root ganglia and motor neurons.
- The destruction of motor neurons leads to paralysis.
- The virus also infects brain stem causing bulbar poliomyelitis.

Laboratory Diagnosis of Polio Virus

Specimen: stool, rectal swab, throat swab, CSF (rare)

Microscopy

- Virus can be detected in stool specimens by direct electron microscopy or also by immune electron microscopy.
- Although virus is rarely demonstrated in CSF, microscopy of CSF demonstrates predominantly lymphocytic pleocytosis.

Virus isolation

- Virus may be recovered from pharyangeal aspirations and feces.
- Virus isolation from feces and throat swab is carried out by cultivation on monkey kidney, human amnion, HeLa cells, Hep-2, Buffalo green monkey (BGM), MRC-5 and other cell cultures.
- Cytopathogenic effects appear in 3–6 days.
- Cytopathic effects include cell retraction, increased refractivity, cytoplasmic granularity, and nuclear pyknosis.
- An isolated virus is identified and typed by neutralization with specific antiserum.

Serodiagnosis

- Demonstration of fourfold increase of antibody titer in the serum sample collected at the time of acute illness and time of convalescence.
- Neutralization test and complement fixation test is carried out to demonstrate antibodies presence.

Molecular diagnosis

- Virus can also be identified more rapidly by polymerase chain reaction (PCR) assays.

Prevention and Control of Polio Virus

- Provision of clean water, improved hygienic practices and sanitation are important for reducing the risk of transmission in endemic countries.
- Immunization is the cornerstone of polio eradication and both live-virus and killed-virus vaccines are available.
- Formalin-inactivated vaccine (Salk) is prepared from virus grown in monkey kidney cultures.
- Killed-virus vaccine induces humoral antibodies but does not induce local intestinal immunity so that virus is still able to multiply in the gut.
- Live attenuated vaccine (Sabin) is grown in primary monkey or human diploid cell cultures and delivered orally.

- The live polio vaccine infects, multiplies, and immunizes the host against virulent strains.
- The vaccine produces not only immunoglobulin M (IgM) and IgG antibodies in the blood but also secretory IgA antibodies in the intestine, enabling mucosal immunity.
- Both killed-virus and live-virus vaccines induce antibodies and protect the CNS from subsequent invasion by wild virus.
- Oral polio vaccine has been the vaccine used predominantly in the past in global campaigns and is still used in endemic areas.
- It has the advantages of inducing both humoral and intestinal immunity and of being cheap and easy to administer.

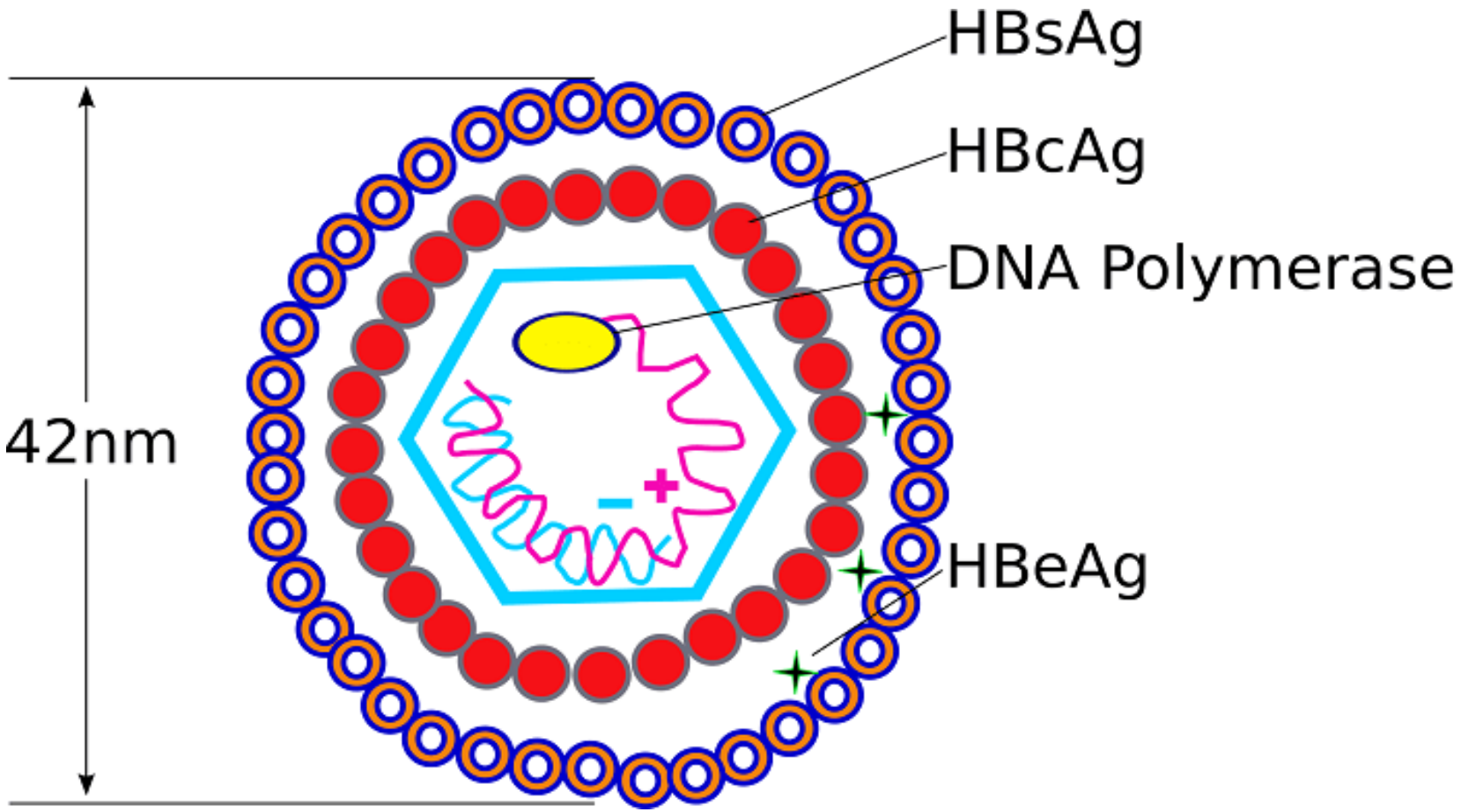
- However, the gut develops a far greater degree of resistance after administration of live-virus vaccine indicating it as a potential limiting factor of interference for oral vaccine.
- The disadvantage is the small risk of vaccine associated paralytic poliomyelitis (VAPP), which occurs in about 4 out of every 1,000,000 vaccinated children and unvaccinated contacts.
- Inactivated poliovirus vaccine is injected intramuscularly and does not carry any risk of VAPP.
- The disadvantage of inactivated vaccine is that it does not confer intestinal immunity and is not effective for outbreak control and is more expensive and requires better trained staff for deliverance.
- European countries have gradually shifted from OPV to IPV over the last decades and today all EU Member States use IPV in their childhood immunization programmes.

Pathogenicity, Lab diagnosis and prevention of – HBV (Hepatitis ‘B’ virus)

Hepatitis B virus:

- The hepatitis B virus The hepatitis B virus is a DNA virus belonging to the *Hepadnaviridae* family causing hepatitis B in humans.
- Hepatitis B is a viral infection that attacks the liver and can cause both acute and chronic disease.
- HBV is also called serum hepatitis.
- This is usually seen in young adults.

Structure of Hepatitis B Virus



- Contains icosahedral nucleocapsid, the core (27 nm) and outer envelope (4 nm)
- There is an outer shell (or envelope) composed of lipid and protein that is termed “surface antigen” or “HBsAg”.
- Inner protein shell that is referred to as the core particle or “HBcAg”, contains the viral DNA and enzymes used in viral replication (called “DNA polymerase”).
- HBeAg (hepatitis B e antigen) is the antigenic determinant that is closely associated with the nucleocapsid of HBV. It also circulates as a soluble protein in serum.

Pathogenesis of Hepatitis B

- Three mechanisms seem to be involved in liver cell injury during HBV infections.
- The first is an HLA class I restricted cytotoxic T-cell (CTL) response directed at HBcAg/HBeAg on HBV-infected hepatocytes.
- A second possible mechanism is a direct cytopathic effect of HBcAg expression in infected hepatocytes.
- A third possible mechanism is high-level expression and inefficient secretion of HBsAg.
- In the acute stage there are signs of inflammation in the portal tracts; the infiltrate is mainly lymphocytic.

- In the liver parenchyma, infected hepatocytes show ballooning and form acidophilic (Councilman) bodies as they die.
- In chronic hepatitis, damage extends out from the portal tracts, giving a piecemeal necrosis appearance. Some lobular inflammation is also seen. As the disease progresses, fibrosis and, eventually, cirrhosis develops.
- Chronic liver damage results from continuing, immune-mediated destruction of hepatocytes expressing viral antigens. In addition, autoimmune reactions may contribute to the damage as immune responses are induced to various liver-specific antigens.

Diagnosis of Hepatitis B

- The diagnosis of HBV infection and its associated disease is based on a constellation of clinical, biochemical, histological, and serologic findings.
- The laboratory can test for a wide range of HBV antigens and antibodies, using immnoassays based on enzyme reactivity (EIA) or chemiluminesence (CLIA) and ELISA.
- HBV DNA can be quantified in serum or plasma using real time polymerase chain reaction (PCR) assays.
- Focuses on the detection of the hepatitis B surface antigen HBsAg.

- Acute HBV infection is characterized by the presence of HBsAg and immunoglobulin M (IgM) antibody to the core antigen, HBcAg.
- During the initial phase of infection, patients are also seropositive for hepatitis B e antigen (HBeAg). HBeAg is usually a marker of high levels of replication of the virus. The presence of HBeAg indicates that the blood and body fluids of the infected individual are highly contagious.
- Chronic infection is characterized by the persistence of HBsAg for at least 6 months (with or without concurrent HBeAg). Persistence of HBsAg is the principal marker of risk for developing chronic liver disease and liver cancer (hepatocellular carcinoma) later in life.

Prevention and Control of Hepatitis B

- The hepatitis B vaccine is the mainstay of hepatitis B prevention.
- WHO recommends that all infants receive the hepatitis B vaccine as soon as possible after birth, preferably within 24 hours.
- Simple environmental procedures can limit the risk of infection to health care workers, laboratory personnel, and others. Examples of specific precautions include the following:
- Gloves should be used when handling all potentially infectious materials;
- protective garments should be worn and removed before leaving the work area.

- masks and eye protection should be worn whenever splashes or droplets from infectious material pose a risk;
- only disposable needles should be used;
- needles should be discarded directly into special containers without re-sheathing;
- work surfaces should be decontaminated using a bleach solution; and laboratory personnel should refrain from mouth pipetting, eating, drinking, and smoking in the work area.
- Metal objects and instruments can be disinfected by autoclaving or by exposure to ethylene oxide gas.

Unit-11
Virological Samples

Commonly used virological specimen are:

- **Throat swab :** Swab tonsillar area and posterior wall of pharynx. For swabing cotton tiped sterile swab is used. After swabing place it in a 3-4 ml (VTM and send it lab immediately)
- **Respiratory aspirates :** A plastic disposable suction aspirator is used. In this aspirator, respiratory secretion are collected. Fine gauge rubber catheter helps in collection of respiratory secretions. In babies it is passed through nose. In adult it is passed through pharynx.

- **Nasal washings :** In still about 5 ml saline into each nostril. Then collect it into screw capped bottle.Alternatively a cotton swab may be used. It should be left in nostril for 15-20 sec. Rotated firmly to collect newly formed secretions.
- **Mucous membrane swab** : Swab the mouth (buccal cavity), lips or genital area. Then put it into VTM.
- **Conjunctiva Swab** : Roll dry cotton swab(preferably sterile) gently along lower conjunctiva surface. Store in VTM.
- **Vesicle fluid** : Tuberculin syringe and 15 ml gauge needle are used to aspirate several vesicle. If possible, choose fresh, plump vesicle. Store vesicle fluid in sterile container.

- **Cerebrospinal, pericardial and pleural fluid.** Place about 1 ml of fluid in dry, sterile container.
- **Saliva, urine, stool :** These specimens should be collected in sterile container, Rectal swab may be used if stool can not be obtained.
- **Tissue :** Collected tissue is placed in sterile container containing 3-4 ml VTM. It must be stored at 4 degree C.
- **Blood :** Collect 10 ml blood in sterile container containing heparin, EDTA or citrate. These chemical act as anticoagulants. For serology, collect blood in plain sterile tube.

TRANSPORT AND STORAGE OF SAMPLE

- Some specimens are sent by postage or courier. We must follow all rules and regulations of postal department.
- Inside lab, specimen should be kept in safety cabinet. It must be handled inside safety cabinet.
- Every specimen containing isolated virus, should be treated as urgent. It should be sent to virus laboratory without delay as soon as possible.
- Specimen must be placed in transport medium. This virus transport medium should be kept at 4 degree C. It should not be stored at O degree C or in ice box or in deep freezer.

- During transportation and storage of specimen, all precaution must be taken. Virus in specimen must be inactive form. Factors like dehydration, heat, freezing, sudden change in pH, oxidising agent and U.V light may inactivate/ destroy virus.
- Sterile, leak proof containers should be used for transportation of specimen, especially aspirates, fluids and tissues.