الشركة القابضة
لمياه الشرب والصرف الصحى

برنامج المسار الوظيفي للعاملين بقطاع مياه الشرب والصرف الصحي

دليل المتدرب

التحاليل البيولوجية مجهريات (ديدان و ووحيدات الخلية و هائمات حيوانية مائية) للمياه

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1. TABLE OF CONTENTS

List of Tables:

List of Figures:

Biology and Parasitology

1. INTRODUCTION

1.1. Biology

Biology is the study of living organisms, which divided into many specialized fields that cover their morphology, physiology, anatomy, behavior, origin, and distribution.

1.1.1. Microbiology

Microbiology is the science which studying only on the very small living organisms whose microscopic size or less.

1.1.2. Living Organisms and Different Types of Cells

Living organism is anything exerts at least one symptom (criterion) of life, ex. Nutrition, respiration, growth, execration, and (or) reproduction, etc…, the living organism may consists of either organelles (in case of primitive organisms) or organs (in case of higher organisms), which integrate together to perform all vital processes.

The **cell** is the building unit of any living organism, which consists of at least one cell or more. Based on the cell structure and maturation, organisms can be classified into acellular (viruses), prokaryotic (bacteria), or eukaryotic (algae, protozoa, and higher animals and plants).

Acellular organism (Non-cellular) is an organism that lacks a true cellular structure for at least part of its life cycle. This kind of organisms considered to be semi-living organisms, as they exert symptoms of life only within their host cells. Viruses are the only example of this group.

Prokaryotic cell is any cell that lacks a membrane-bound nucleus, mitochondria, or any other membrane-bound organelle (Fig. 1). Prokaryotes (prokaryotic organisms) are divided into two domains, Archaea and Bacteria.

Eukaryotic cell is any cell has a nucleus and other organelles enclosed within membranes. Eukaryotes (Eukaryotic organisms) belong to the domain Eukaryota, and can be single-celled or multicellular (Fig. 1).

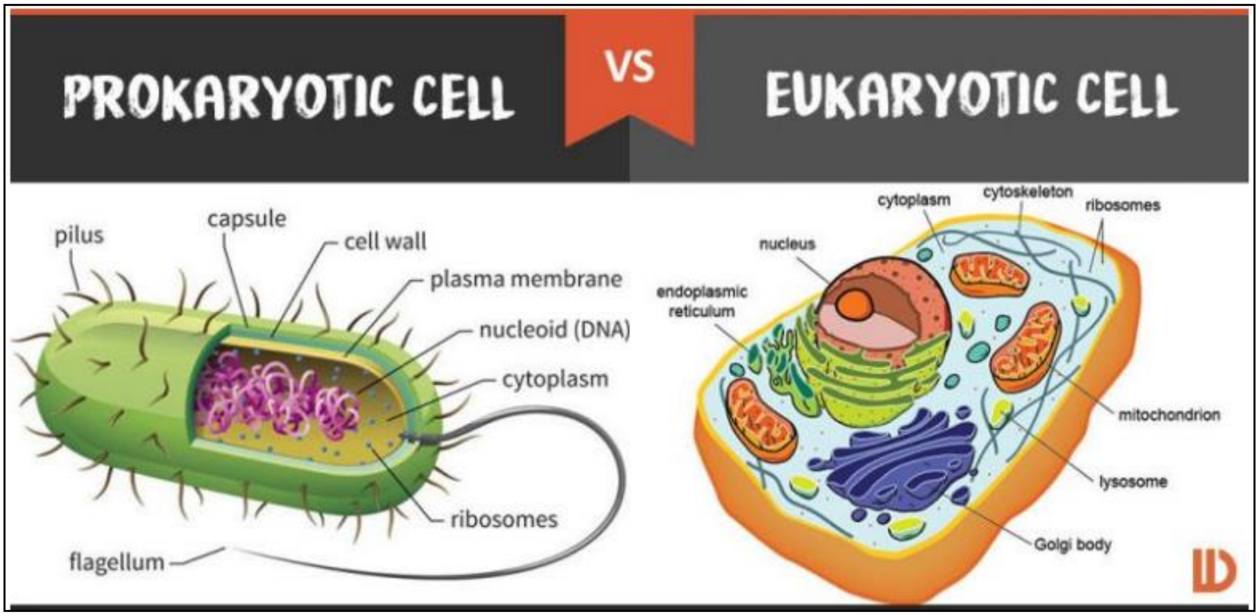


Figure 1: Differences between the structure of prokaryotic and eukaryotic cell.

1.2. Free Living Organism:

An organism that is not directly dependent on another organism for survival.

1.3. Parasitology

Parasitology is the study of parasites, their hosts, and the relationship between them. As a biological discipline, the scope of parasitology is not determined by the organism or environment in question, but by their way of life. This means it forms a synthesis of other disciplines, and depends on techniques from fields such as cell biology, bioinformatics, biochemistry, molecular biology, immunology, genetics, evolution and ecology.

1.3.1. Parasite

Parasitism is a non-mutual relationship between species, where one species, the parasite, benefits at the expense of the other, the host.

1.3.2. Host cell

Host is an organism that harbors (receive and host) a parasitic, a mutualistic, or [3] a commensalism guest (symbiont), typically providing nourishment and shelter.

Examples include animals playing host to parasitic worms (e.g. nematodes), cells harboring pathogenic (disease-causing) viruses, a bean plant hosting mutualistic (helpful) nitrogen-fixing bacteria.

1.3.3. Pathogenicity

Pathogenicity refers to the ability of an organism to cause disease (i.e. harm the host). This ability represents a genetic component of the pathogen and the overt damage done to the host is a property of the host-pathogen interactions.

1.3.4. Routes of Transmission

In medicine, public health, and biology, transmission is the passing of a pathogen causing communicable disease from an infected host individual or group to a particular individual or group, regardless of whether the other individual was previously infected.

1.3.5. Classification of the routes of transmission

They can be classified according to the type of contact between the patient (the infected individual) and the susceptible individual (potential patient).

Table 1: Different ways of disease transmissions

Type of transmission	Sub type	Description	Example
One to One Contact between the infected patient and the potential patient	Direct	Direct physical contact (body surface to body surface) between infected or colonized individual and susceptible host.	Common cold; sexually transmitted diseases
	Indirect	Infectious agent deposited onto an object or surface (fomite) and survives long enough to transfer to another person who subsequently touches the object.	RSV; Norwalk; rhinovirus; perhaps influenza.
	Droplet	Contact, but transmission is through the air. Droplets are relatively large (>5 µm) and projected up to about one meter.	Sneezing; coughing, or (in health care) during suctioning
No contact between the patient and the potential patient	Airborne	Transmission via aerosols (airborne particles <5µm) that contain organisms in droplet nuclei or in dusts. Can be spread via ventilation systems.	TB; varicella; measles; chickenpox; smallpox
	Vehicle (Food & Waterborne)	A single contaminated source spreads the infection (or poison) to multiple hosts. This can be a common source or a point source.	Food-borne outbreak from infected batch of food or from contaminated certain water resource
	Vector	Transmission by insect or animal vectors.	Mosquitoes and malaria.

1.4. Taxonomy of Living Organisms

Biological classification (taxonomy) aims to simplify and order the immense diversity of life into coherent units called taxa that have widely accepted names and whose members share important properties. There are many attempts to classify living organisms starting by Linnaeus in 1735. Table (2) summarizes the main historical attempts of classification.

Table 2: Different examples on attempts of scientists to classify living organisms

<table>
<tr><td>Name</td><td>Linnaeus</td><td>Haeckel</td><td>Chatton</td><td>Copeland</td><td>Whittaker</td><td>Woese et al.</td><td>Cavalier-Smith</td></tr>
<tr><td>Year</td><td>1735</td><td>1866</td><td>1925</td><td>1938</td><td>1969</td><td>1990</td><td>1998</td></tr>
<tr><td>Number of Kingdoms</td><td>2 kingdoms</td><td>3 kingdoms</td><td>2 empires</td><td>4 kingdoms</td><td>5 Kingdoms</td><td>3 domains</td><td>5 kingdoms</td></tr>
<tr><td rowspan="6">Classification</td><td rowspan="3">Not treated</td><td rowspan="3">Protista</td><td rowspan="2">Prokaryota</td><td rowspan="2">Monera</td><td rowspan="2">Monera</td><td>Bacteria</td><td rowspan="2">Bacteria</td></tr>
<tr><td>Archaea</td></tr>
<tr><td rowspan="4">Eukaryota</td><td>Protoctista</td><td>Protista</td><td rowspan="4">Eukaryota</td><td>Protista</td></tr>
<tr><td rowspan="2">Vegetabilia</td><td rowspan="2">Plantae</td><td rowspan="2">Plantae</td><td>Plantae</td><td>Plantae</td></tr>
<tr><td>Fungi</td><td>Fungi</td></tr>
<tr><td>Animalia</td><td>Animalia</td><td>Animalia</td><td>Animalia</td><td>Animalia</td></tr>
</table>

Currently, the most common and accepted classification adopts <u>five</u> kingdoms (fig. 2) in which all living things are divided into: **Bacteria** Kingdom, **Protista** Kingdom, **Fungi** Kingdom, **Plant** Kingdom, and **Animal** Kingdom.

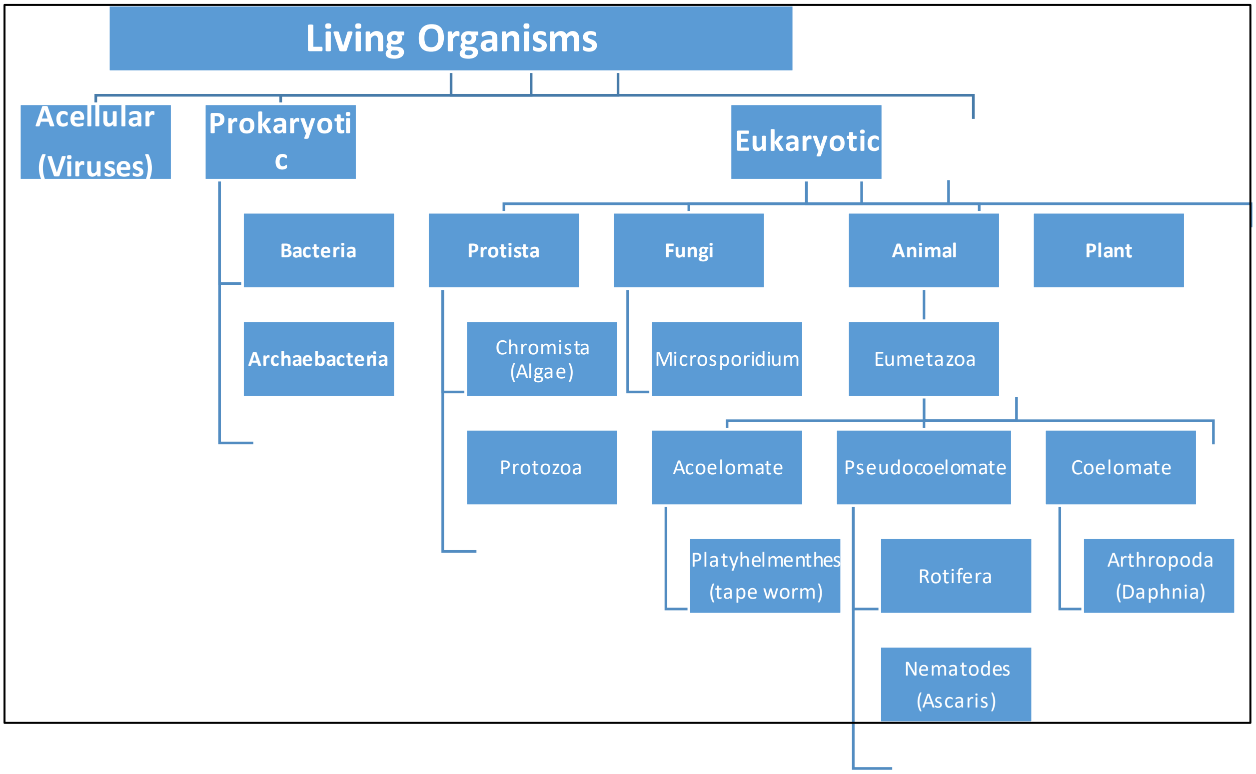


Figure 2: Classification of living organisms, showing the main microorganisms and eumetazoa groups.

1.4.1. Bacteria Kingdom (Monera)

The Monera or bacteria Kingdom consists of organisms that are made up of one cell (unicellular). These unicellular organisms are made of a very simple cell (Fig. 1) that often **lacks many cell parts, such as a nucleus and other organelles such as mitochondria (i.e. Prokaryote).** Bacteria and Archaebacteria are the two types of this kingdom.

1.4.2. Protista Kingdom (Algae and Protozoa)

Protists are similar to Monera in that they are **unicellular**. Protists are a bit more complex because they **contain a nucleus (i.e. Eukaryote)**. They do not undergo tissue formation through the process of embryological layering and they are either **autotrophic (make their own food i.e. algae)** or **heterotrophic (don't make their own food i.e. protozoa)**.

1.4.3. Fungi Kingdom

Fungi have their own kingdom because there is no other organism like them. They were once thought to be plants but they differ from plants in one major way. Fungi cannot make their own food (**heterotrophic**). Mushrooms are a type of fungi.

1.4.4. Plant Kingdom

All plants are a part of the Plant Kingdom. They all share the common characteristic of being able to make their own food using water and sunlight (**autotrophic**). Because they only require a few simple requirements, plants can grow almost anywhere. Plants include trees, grass, and flowers.

1.4.5. Animal Kingdom

Organisms in the Animal Kingdom are **multicellular** and able to form tissue. They rely on other organisms for food (**heterotrophic**). This kingdom is by far the largest (size wise) of all the kingdoms.

2. PROTOZOA

2.1. General Characteristics of Protozoa

2.1.1. Shape, size, and cell structure of Protozoa

Protozoa are unicellular, eukaryotic microorganisms. Protozoal cells have **no cell walls** and therefore can assume an infinite variety of shapes. Some genera have cells surrounded by hard shells, while the cells of other genera are enclosed only in a cell membrane.vMost protozoa have a single nucleus, but some have both a macronucleus and one or more micronuclei. Contractile vacuoles may be present in protozoa to remove excess water, and food vacuoles are often observed. Size of protozoa ranges from microscopic size to large one which could be seen by naked eye.

2.1.2. Nutrition of Protozoa

They are all heterotrophic, either parasitic, predators of other unicellular organisms like algae, or saprophytic free living obtains large food particles by phagocytosis. However, some protozoa are free living and opportunistic parasitic. The food particle is ingested into a food vacuole. Lysosomal enzymes then digest the nutrients in the particle, and the products of digestion are distributed throughout the cell. They usually lack the capability for photosynthesis, although the genus Euglena is renowned for motility as well as photosynthesis (and is therefore considered both an alga and a protozoan).

2.1.3. Reproduction of Protozoa

Although most protozoa reproduce by asexual methods (binary fission), sexual reproduction has been observed in several species.

2.1.4. Habitat of Protozoa

Protozoa are located in most moist habitats. Free-living species inhabit freshwater and marine environments, and terrestrial species inhabit decaying organic matter. Some species are parasites of plants and animals.

Most protozoal species are aerobic, but some anaerobic species have been found in the human intestine and animal rumen.

2.1.5. Locomotion of Protozoa

Many protozoal species move independently by one of three types of locomotor organelles: flagella, cilia, and pseudopodia. How a protozoan moves is an important consideration in assigning it to a group.

2.1.6. Life Cycle of Protozoa

Many protozoa alternate between a free-living vegetative form known as a **trophozoite** and a resting form called a **cyst**. The protozoal cyst is somewhat analogous to the bacterial spore, since it resists harsh conditions in the environment. Many protozoal parasites are taken into the body in the cyst form.

2.1.7. Importance of protozoa

1. Protozoa play an important role as zooplankton, the free-floating aquatic organisms of the oceans. There, they are found at the bases of many food chains, and they participate in many food webs.
2. Many protozoa are parasitic on human, animals, or plants, so, they are significantly important from health and economic prospection.
3. Protozoa has an important role in decreasing (consuming) the organic load in both surface water and wastewater during its treatment process in wastewater plants. So, it has a crucial role to maintain and restore the quality of water resources.
4. The relative abundance and diversity of protozoa are used as indicators of organic and toxic pollution.

2.2. Classification of Protozoa

Historically, the Protozoa were classified as "unicellular animals", as distinct from the Protophyta, single-celled photosynthetic organisms (algae) which were considered primitive plants. Both groups were commonly given the rank of phylum, under the kingdom Protista. In older systems of classification, the phylum Protozoa was commonly divided into several sub-groups, reflecting the means of locomotion. Classification schemes differed, but throughout much of the 20th century the major groups of Protozoa included:

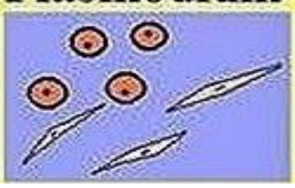
Phylum	Common Name	Locomotion	Examples
Sarcodina	sarcodines	**pseudopodia**	**Amoeba** 
Ciliophora	ciliates	**cilia**	**Paramecium** 
Sarco-mastigophora (Zoomastigina)	zooflagellates	**flagella**	**Trypanosma**  Giardia
Apicomplexa (Sporozoa)	sporozoans	**none in adult form**	**Plasmodium** 

Figure 3: Main phyla of protozoa

with the emergence of molecular phylogenetics and tools enabling researchers to directly compare the DNA of different organisms, it became evident that, of the main sub-groups of Protozoa, only the ciliates (Ciliophora) formed a natural group, or monophyletic clade (that is, a distinct lineage of organisms sharing common ancestry). The other classes or subphyla of Protozoa were all polyphyletic groups made up of organisms that, despite similarities of appearance or way of life, were not necessarily closely related to one another. In the system of eukaryote classification currently endorsed by the International Society of

Protistologists, members of the old phylum Protozoa have been distributed among a variety of supergroups.

2.2.1. Sarcodina (Amoeba)

In older classification systems, most amoebas were placed in the class or subphylum Sarcodina, a grouping of single-celled organisms that possess pseudopods or move by protoplasmic flow. However, molecular phylogenetic studies have shown that Sarcodina is not a monophyletic group whose members share common descent. Consequently, amoeboid organisms are no longer classified together in one group.

The best known amoeboid protists are the "giant amoebae" *Chaos carolinense* and *Amoeba proteus*, both of which have been widely cultivated and studied in classrooms and laboratories. Other well-known species include the so-called "brain-eating amoeba" *Naegleria fowleri*, the intestinal parasite *Entamoeba histolytica*, which causes amoebic dysentery, and the multicellular "social amoeba" or slime mould

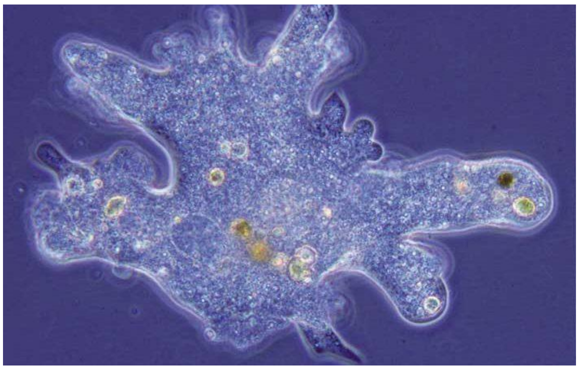


Fig 4: *Chaos carolinense*

Fig 5: *Amoeba proteus*

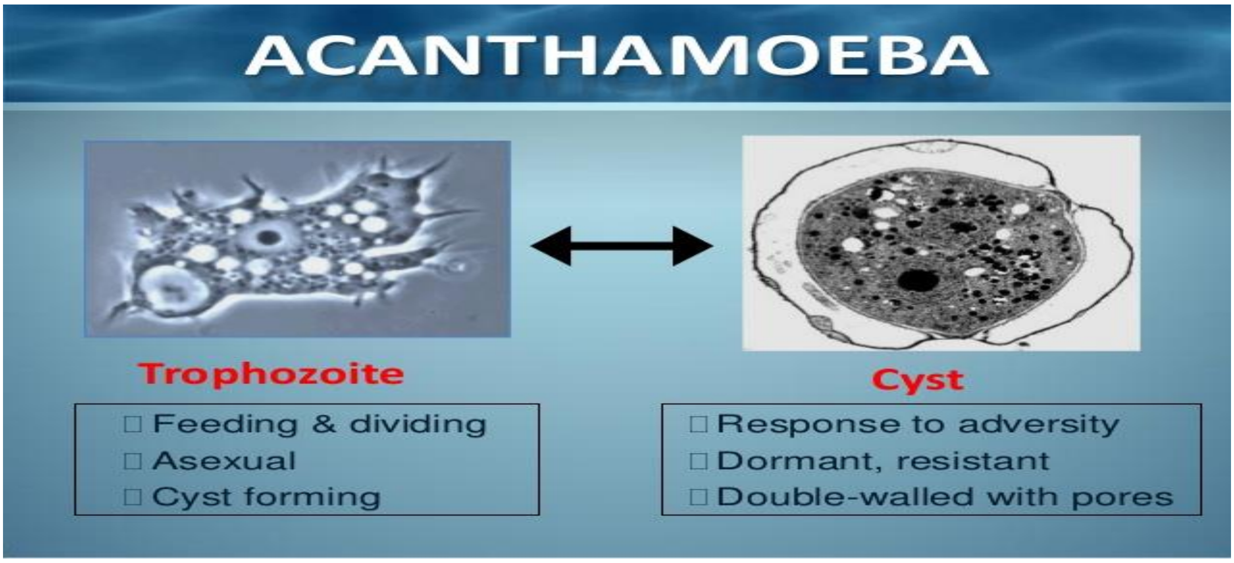


Figure 6: Trophozoite and cyst of Acanthamoeba

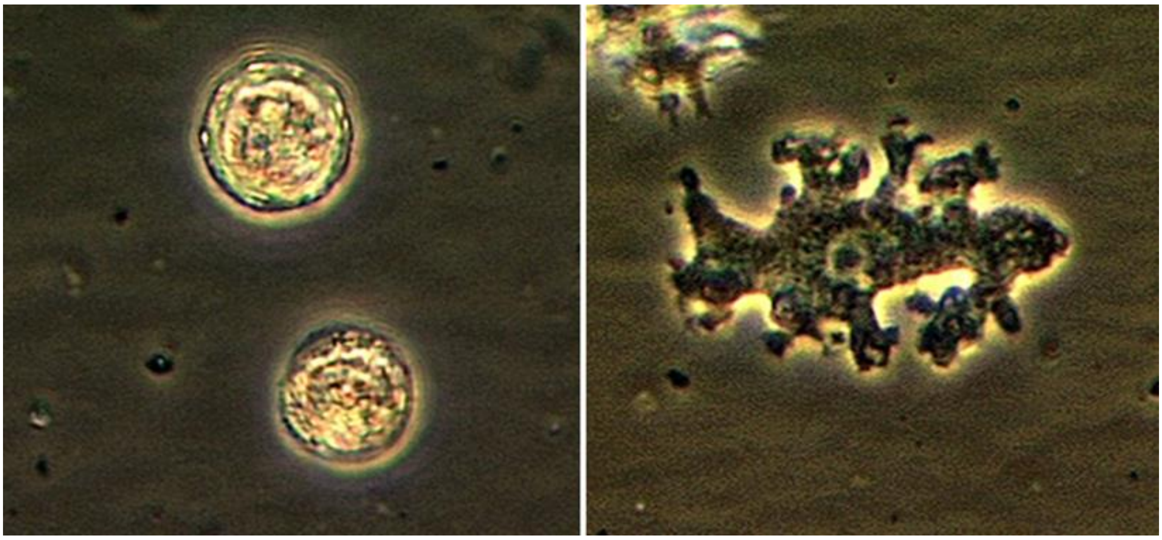


Figure 7: Cyst and trophozoite of *Balamuthia*.

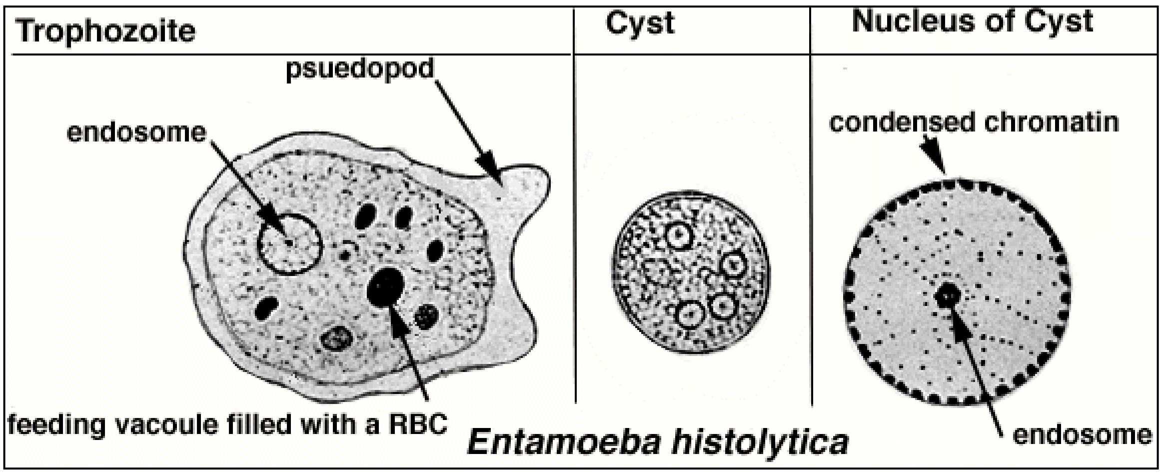


Figure8: Trophozoite and cyst of Entamoeba Histolytica

2.2.1.1. Shape and Size of Amoeba

Size is mostly ranges between 400 and 600 microns.Amoebas are classified based on the morphology and internal structure of their pseudopods as follow:

Species (e.g., amoeba) exhibit bulbous pseudopods with a tubular mid-section and rounded ends. Other species (e.g., Euglypha and Gromia) have pseudopods which appear thin and thread-like.

Foraminifera produce slender pseudopods that branch and merge with one another to form net-like structures.

Others are characterized by rigid, needle-like pseudopods with a complex network of microtubules.

2.2.1.2. Habitat of Amoeba

It could be found in:

Freshwater, salt water, or in wet soil, typically on decaying vegetation matter.

Also found in animals (including people).

2.2.1.1.Locomotion of Amoeba

It moves by extending finger-like protrusions of their cells called pseudopodia. It is slow-moving and possesses a cytoplasm that "flows" against a thin, flexible cell membrane. This criterion is used to engulf or capture substances and bring them into the cell (endocytosis).

2.2.1.2.Nutrition of Amoeba

It obtains its food by endocytosis; which could be **phagocytosis** (eat) and **pinocytosis** (drink). You can see several engulfed food particles as circles within each amoebae cell above.

2.2.1.3.Reproduction of Amoeba

It is asexual by binary fission.

2.2.1.4.Life Cycle of Some infective Amoeba

There are 2 forms (stages) of *Acanthamoeba* life cycle (Fig. 10), stated as following:

Active feeding stage: during this stage the trophozoites are actively dividing by feeding on bacteria, yeast and algae.

Dormant cyst stage: Cysts form once there is a change in the environment of the trophozoites – e.g. nutrient deprivation or changes in temperature. The cysts are resistant to chlorination and antibiotics.

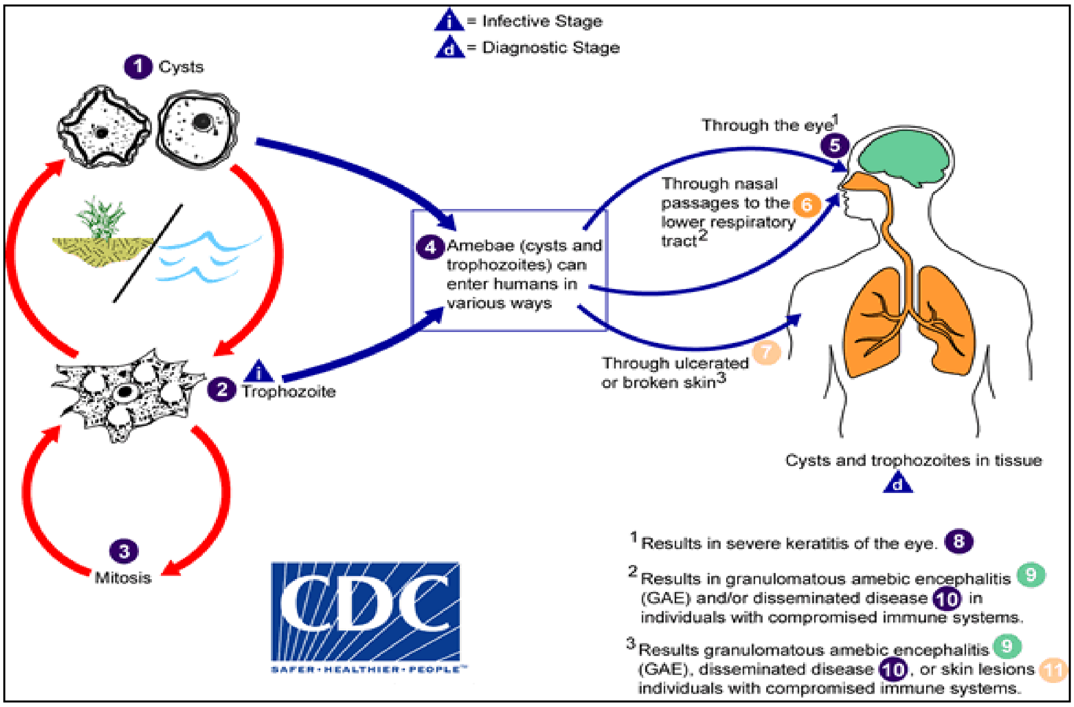


Figure 9: Life cycle of Acanthamoeba showing routes of infection

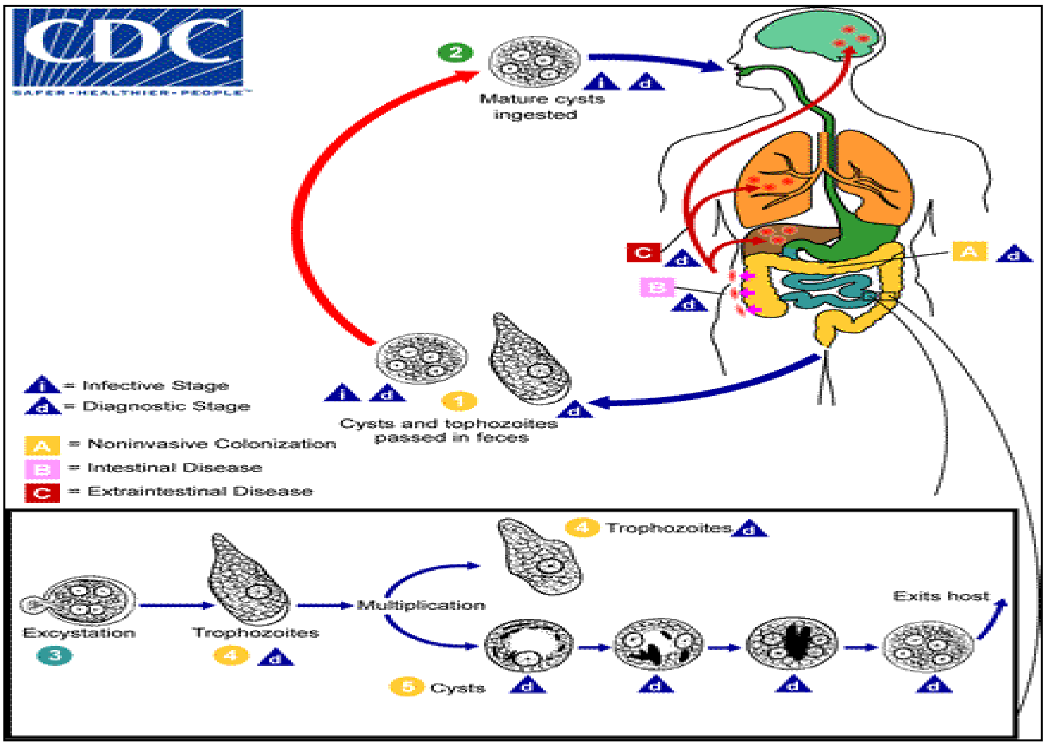


Figure 3: Life cycle of Entamoeba histolytica showing sites of infections inside human body and the route of infection.

2.2.1.5. Pathogenicity of Amoeba

The following table (3) shows the most common pathogenic organisms belong to Sarcodina

Table 3: Diseases caused by some organisms of Sarcodina (Amoeba), and the way of infections

S	Genus of the pathogenic Sarcodina	Disease(s) caused by the pathogen	Way(s) of transmission
1	*Acanthamoeba spp.*	**Keratitis**: is a rare but serious infection of the eye that can result in permanent visual impairment or blindness.	Eye contacts with contaminated water or equipment (contact lenses)
		Granulomatous Amebic Encephalitis (GAE): A serious infection of the brain and spinal cord that typically occurs in persons with a compromised immune system. 3. The infection could be disseminated in the body.	It could penetrate the skin or inhaled to lungs, and then transferred to the brain through blood stream. The infection may affect other parts of the body
2	*Balamuthia mandrillaris*	Skin infections 2. Sinus infections 3. Granulomatous Amebic Encephalitis (GAE)	Disease can begin with a skin wound and can then progress to the brain and other organs
3	*Sappinia pedata*	Rare GAE	
4	*Entamoeba histolytica*	GIT infection, it burrows in the large intestine wall causing bloody diarrhea, colitis, and tissue destruction.	Ingesting cyst through water or food, or by direct contact.
5	*Naegleria fowleri*	Naegleriasis: (also known as primary amoebic meningoencephalitis) is an infection of the brain, rare but fetal disease.	Contact to poorly chlorinated swimming pool through inhaling the organism.

2.2.1.6. Importance and significance of Amoeba

1. Because of the ease with which they may be obtained and kept in the lab, they are common objects of study, both as representative protozoa and to demonstrate cell structure and function.
2. Many genera have health hazards, like Granulomatous Amebic Encephalitis (GAE), keratitis, colitis, and bloody diarrhea.
3. Existence of any living amoeba in drinking water means incompliance of water criteria with the stated Egyptian and international guidelines.

2.2.2. Flagellates (Zoomastigophora)

2.2.2.1. Examples of flagellates

Giardia lamblia (Fig. 11 & 12), *Trypanosoma brucei* (Fig. 13&14), *Trichomonas vaginalis* (Fig. 15), *Euglena gracilis* (Fig. 16).

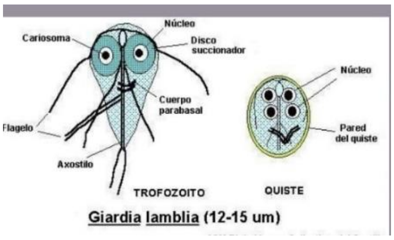


Figure 4: Trophozoite and cyst of Giardia.

Figure 12: Trophozoite of Giardia by electron microscope.

Figure 5: Trypanosoma by electron microscope

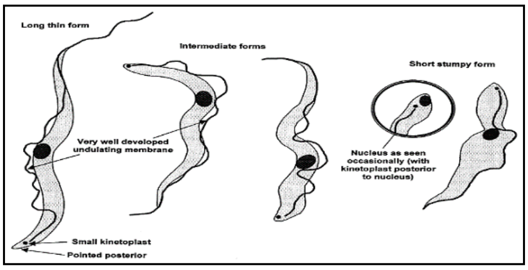


Figure 6: Trypanosoma

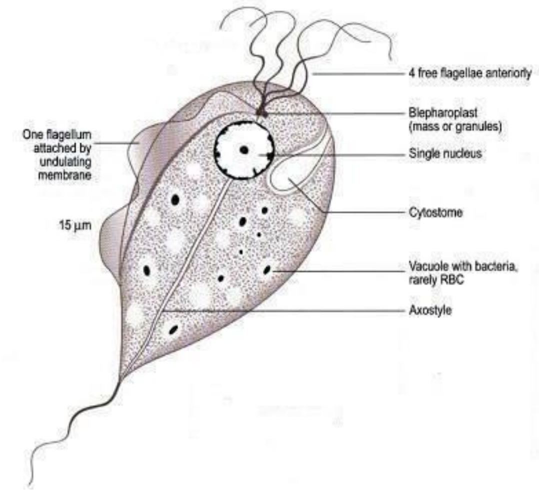


Figure 7: Diagrammatic structure of trophozoite of Trichomonas.

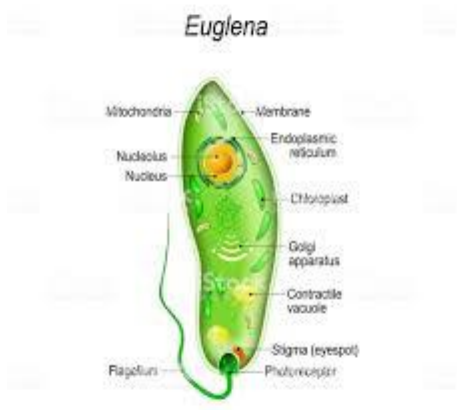


Figure 8: Euglena

2.2.2.2.Shape and Size of flagellates

Flagellates are eukaryotic single-celled with one or more flagella, which are whip-like organelles often used for propulsion. Flagellates range in size from 5-20 µm.

2.2.2.3.Habitat of flagellates

Most are free-living organisms, however, a number are parasitic or pathogenic for animals and humans.

2.2.2.4.Locomotion of flagellates

They move using their flagella for propulsion.

2.2.2.5.Nutrition of flagellates

Many flagellates are autotrophic (fix their own energy from inorganic sources) as well as heterotrophic (depend upon energy and carbon fixed by some other organism).

2.2.2.6.Reproduction of flagellates

They multiply by binary fission and some species possess cyst stages.

2.2.2.7.Life Cycle of flagellates

2.2.2.7.1. **Life cycle of Giardia**

The cycle is composed of two stages: an actively multiplying **trophozoite** and a resistant **cyst**. Cysts survive in food and water. When ingested, the cyst passes through the stomach, where the acid environment triggers excystation, which usually takes place in the duodenum. The trophozoites attach to the duodenal or proximal jejunal mucosa, probably via contraction of the ventral disk, and replicate by repeated binary fission. Cyst formation takes place as the trophozoites move through the colon (Fig. 17).

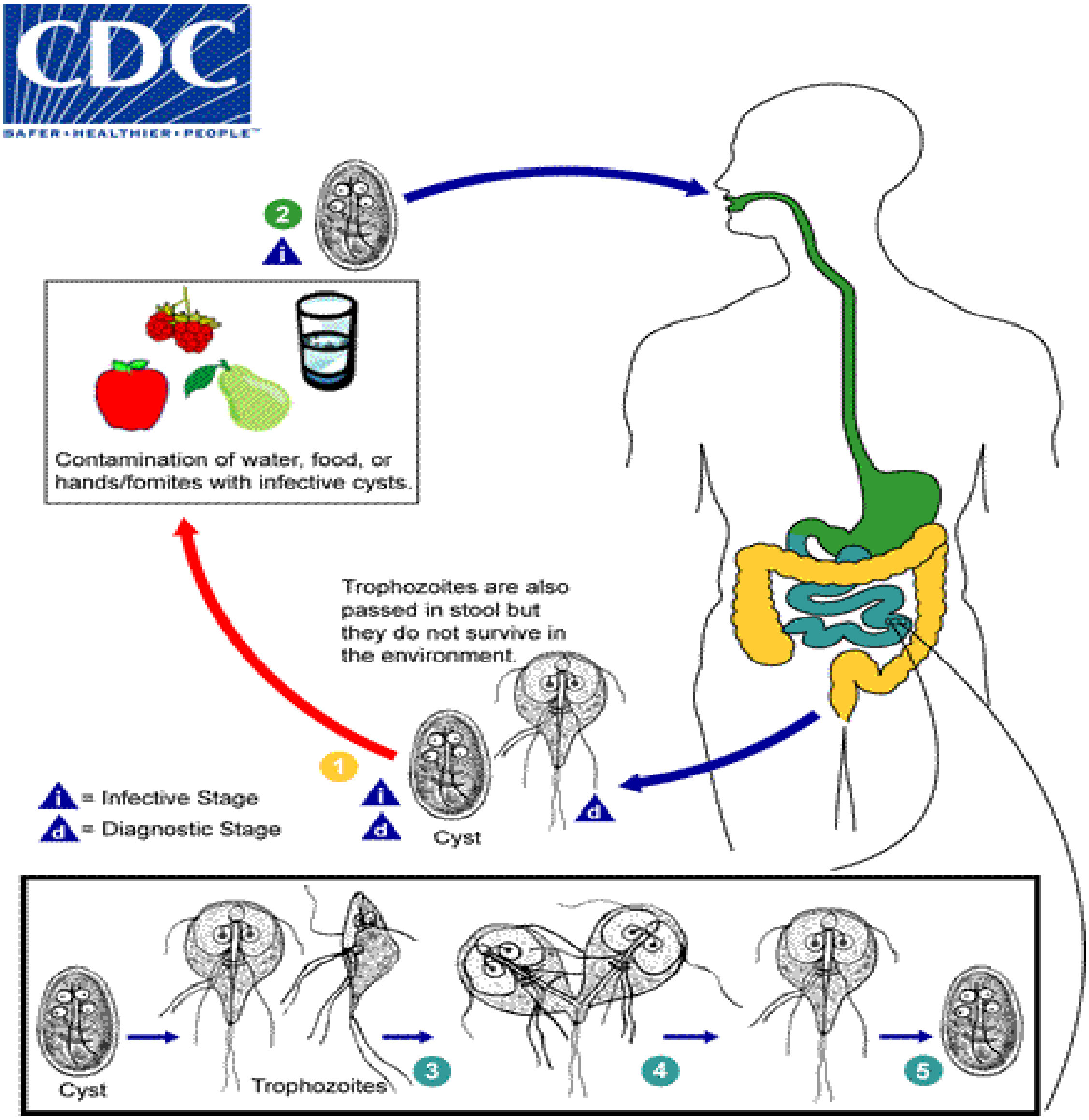


Figure 9: Life cycle of Giardia showing the route of transmission.

2.2.2.8.Pathogenicity of flagellates

Table 4: Some examples of the pathogenic flagellate protozoa are listed, showing routes of transmission.

S	Genus of the pathogenic Sarcodina	Disease(s) caused by the pathogen	Way(s) of transmission
1	Giardia lamblia	**Giardiasis**: is an infection of the small intestine, the main symptoms are: diarrhea, gas, fatty or foul-smelling stools (they may float), and stomach or abdominal cramping.	Ingesting the **cyst** through the contaminated food or water.

2.2.3. Ciliophora (Ciliates)

2.2.3.1.Classes, shapes and Examples of Ciliates

All ciliates are free living with only one exception (*Balantidium coli*), which is parasitic for human and some animals. Free living ciliates could be classified into three groups based on the distribution of cilia around the cell, and their way to move and feed, and they can be listed as following:

2.2.3.1.1. **Free-Swimming Ciliates**

- Free-swimming ciliates such as *Litonotus* (Fig. 18) and Paramecium possess ciliate on all surfaces of the body and usually can be found suspended or swimming freely in the bulk solution.
- Free-swimmers swim faster than flagellates so they are more efficient to obtain food.
- Free-swimmers are usually found when the environment is highly oxygenated with no large flocs have been formed so that it is easier to swim around.
- If free-swimming ciliates are present as the dominant protozoan group, this could indicate a wastewater environment that is not yet stabilized and a sludge that is intermediate in health.

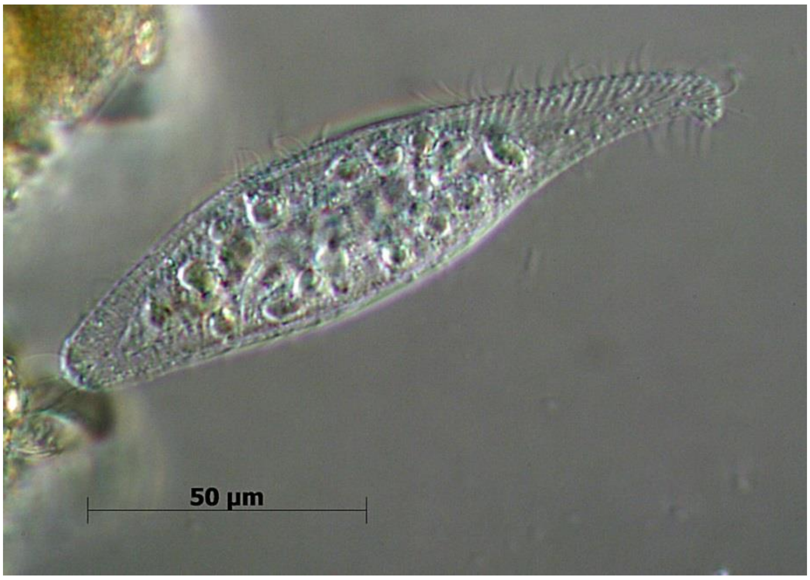


Figure 10: Litonotus as an example for free swimming ciliates

2.2.3.1.2. **Crawling Ciliates**

- Crawling ciliates, such as *Aspidisca* (Fig. 19) possess ciliate only on the ventral or belly surface where the mouth opening is located.
- The beating of the cilia gives the appearance that it is crawling as it moves across the surface of floc particles.
- Some of the cilia are modified to form "spikes" that help to anchor the organism to the floc particle.
- Crawling ciliates are found in large numbers when the bacterial population, relatively large flocs, and dissolved oxygen concentration of the wastewater treatment process are high and the wastewater environment is stable.

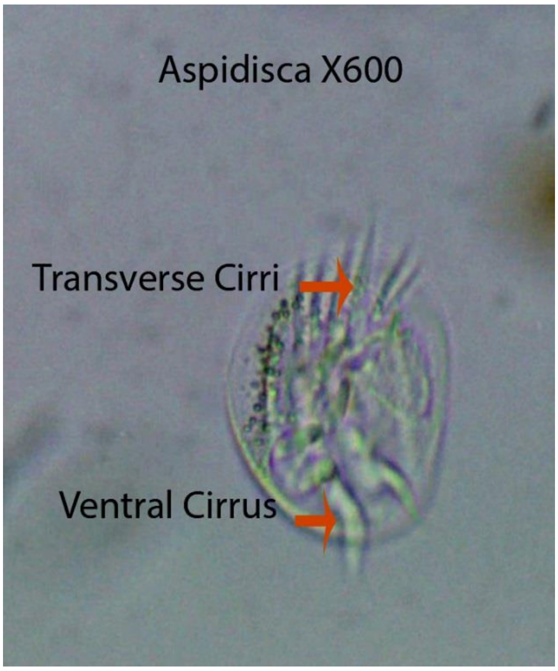


Figure 19: Aspidisca as an example of crawling ciliates.

2.2.3.1.3. **Stalked Ciliate**

- Stalked ciliates, such as *Carchesium* (fig. 20) have cilia around the mouth opening only and are used to attach to floc particles and to withdraw bacterial cells to their mouths (the most efficient among ciliates).
- Stalked ciliates have an enlarged anterior portion or "head" and a slender posterior portion or "stalk".

Figure 11: Carchesium as an example of stalked ciliates.

2.2.3.2.Pathogenicity of Ciliates and Life Cycle

Balantidium is the only ciliated protozoan known to infect humans. **Balantidiasis** is acquired by humans via the feco-oral route from the normal host (fig. 21), the pig, where it is asymptomatic. **Contaminated water is the most common mechanism of transmission**.

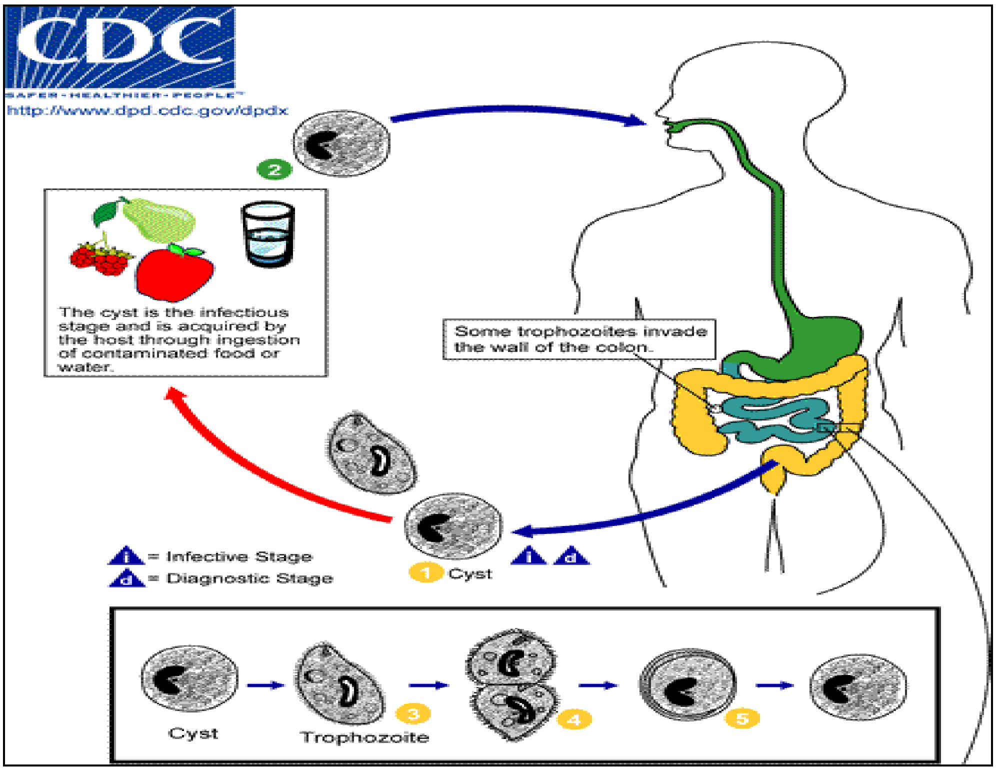


Figure 12: Life cycle of Balantidium coli; the only parasite of ciliates

2.2.3.3.Importance and significance

1. They are usually an indicator of good quality sludge and typically found in young to medium age sludge.
2. They are important because they work with the bacteria in the activated sludge process by feeding on them and helping to clarify the effluent.

2.2.4. Sporozoan (Apicomplexa)

2.2.4.1.General Characteristics and Examples

- They are a large phylum of obligatory parasitic organisms. Most of them possess a unique form of organelle that comprises a type of plastid called an **apicoplast**, which enables the apicomplexa to penetrate host cell.
- The Sporozoa (Apicomplexa) are **unicellular** and **spore-forming**.
- Examples of sporozoa are: Malaria (*Plasmodium*) and Cryptosporidiosis (*Cryptosporidium parvum*).

2.2.4.2.Cryptosporidium parvum

Association with Water

Oocysts of *Cryptosporidium spp* are widely distributed in water (about 87% of raw water samples and 27% of drinking water samples). *Cryptosporidium spp* have the ability to resist and overcome both chlorination and filtration processes.

The highly transmissible nature of Cryptosporidium spp. underscores the risk for acquiring cryptosporidium infection in areas of high endemicity, especially by travelers from regions where the rate of exposure to the parasite is lower.

Life Cycle of Cryptosporidium

- As shown in figure (22), Following ingestion of oocyst (and possibly inhalation) by a suitable host the number 3, excystation the letter A occurs.
- The sporozoites are released and parasitize epithelial cells (The letter B, The letter C) of the gastrointestinal tract or other tissues such as the respiratory tract.
- In these cells, the parasites undergo **asexual multiplication** (schizogony or merogony) (The letter D, The letter E, The letter F) **and then sexual multiplication** (gametogony) producing microgamonts (male) the letter G and macrogamonts (female) the letter H.

- Upon fertilization of the macrogamonts by the microgametes (The letter I), oocysts (The letter J, The letter K) develop that sporulate in the infected host.
- Two different types of oocysts are produced, the thick-walled, which is commonly, excreted from the host the letter J, and the thin-walled oocyst the letter K, which is primarily involved in autoinfection.
- Oocysts are infective upon excretion, thus permitting direct and immediate fecal-oral transmission.

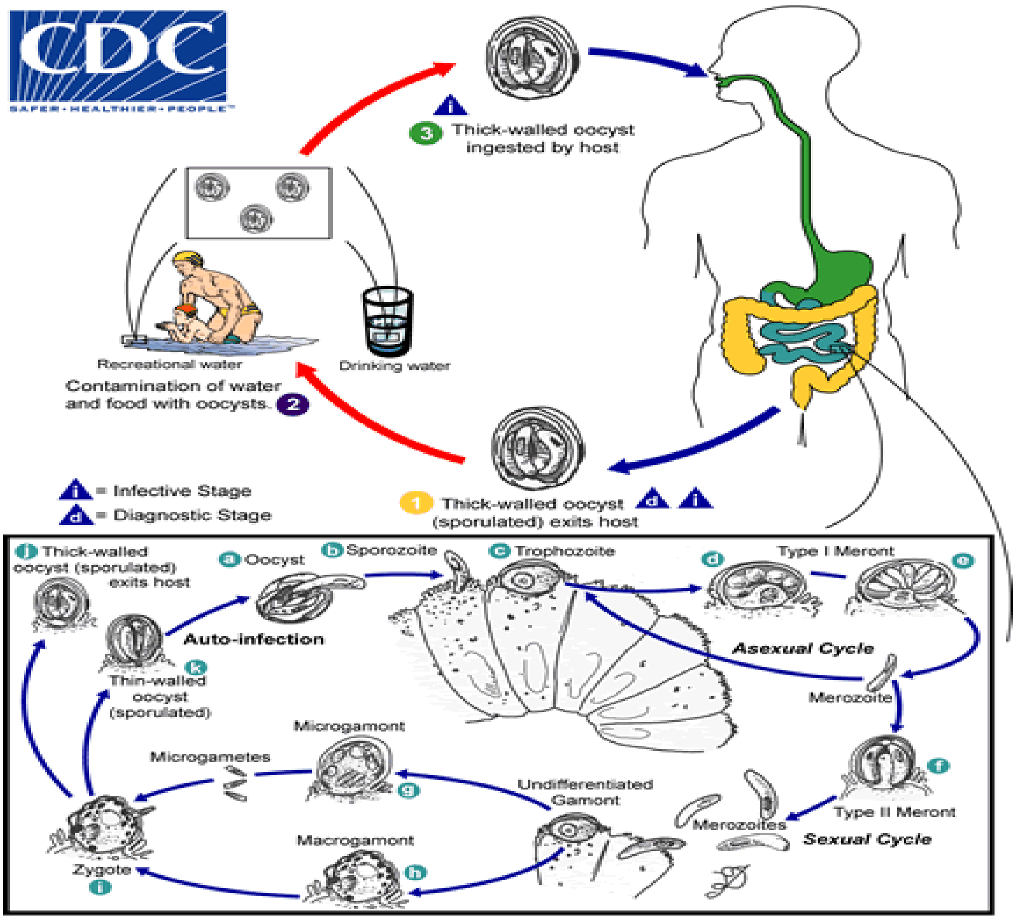


Figure 13: ***Cryptosporidium*** **Life Cycle**

2.2.4.3.Cyclospora cayetanensis

2.2.4.3.1. General Background

Cyclospora cayetanensis is a protozoan that causes disease in humans, especially to adult foreigners visiting regions where the species is endemic and acquiring the infection; consequently, *C. cayetanensis* is a cause of **"traveler's diarrhea".**

There is a significant difference between *Cyclospora cayetanensis* and *Cryptosporidium*, in that *Cryptosporidium* is able to infect **directly** new individual after its spores being introduced to environment through feces, while in case of *Cyclospora cayetanensis*, its cyst introduced not infective to the environment, and require further **maturation** before being infective.

2.2.4.3.2. Routes of Transmission

Food and water contaminated with sporulated oocyst, which was maturated in the environment after being introduced by feces to the environment.

2.2.4.3.3. Infective Stage under Microscope

Sporulated oocyst ingested in food or water (fig. 23)

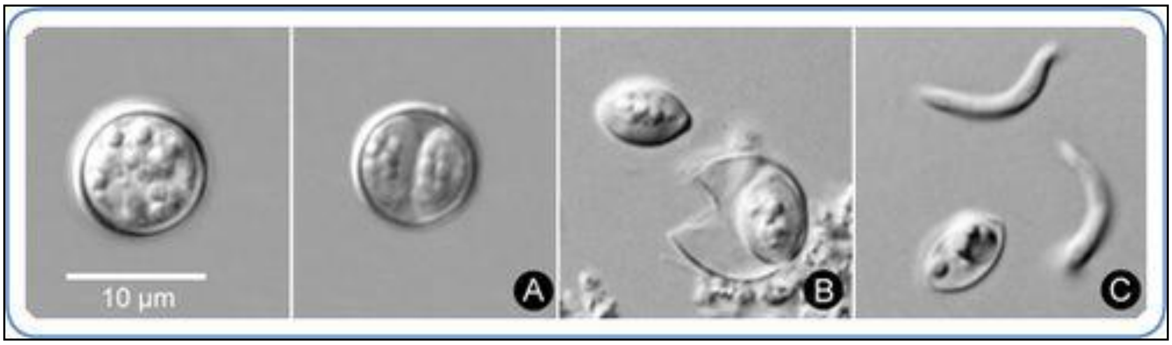


Figure 14: Sporulation and Rupturing of Cyclospora cayetanensis oocyst

2.2.4.3.4. Pathogenicity

It causes travellers' diarrhea.

2.2.4.3.5. Life Cycle

- As seen in figure (23), When freshly passed in stools, the oocyst is not infective (number 1) (thus, direct fecal-oral transmission cannot occur; this differentiates *Cyclospora* from another important coccidian parasite, *Cryptosporidium*).

- In the environment (number 2), sporulation occurs after days or weeks at temperatures between 22°C to 32°C, resulting in division of the sporont into two sporocysts.
- Each sporocyst containing two elongate sporozoites (number 3).
- Fresh food and water can serve as vehicles for transmission (number 4) and the sporulated oocysts are ingested (in contaminated food or water) (number 5).
- The oocysts elaborate (excyst) in the gastrointestinal tract, freeing the sporozoites which invade the epithelial cells of the small intestine (number 6).
- Inside the cells they undergo asexual multiplication and sexual development to mature into oocysts, which will be shed in stools (number 7).

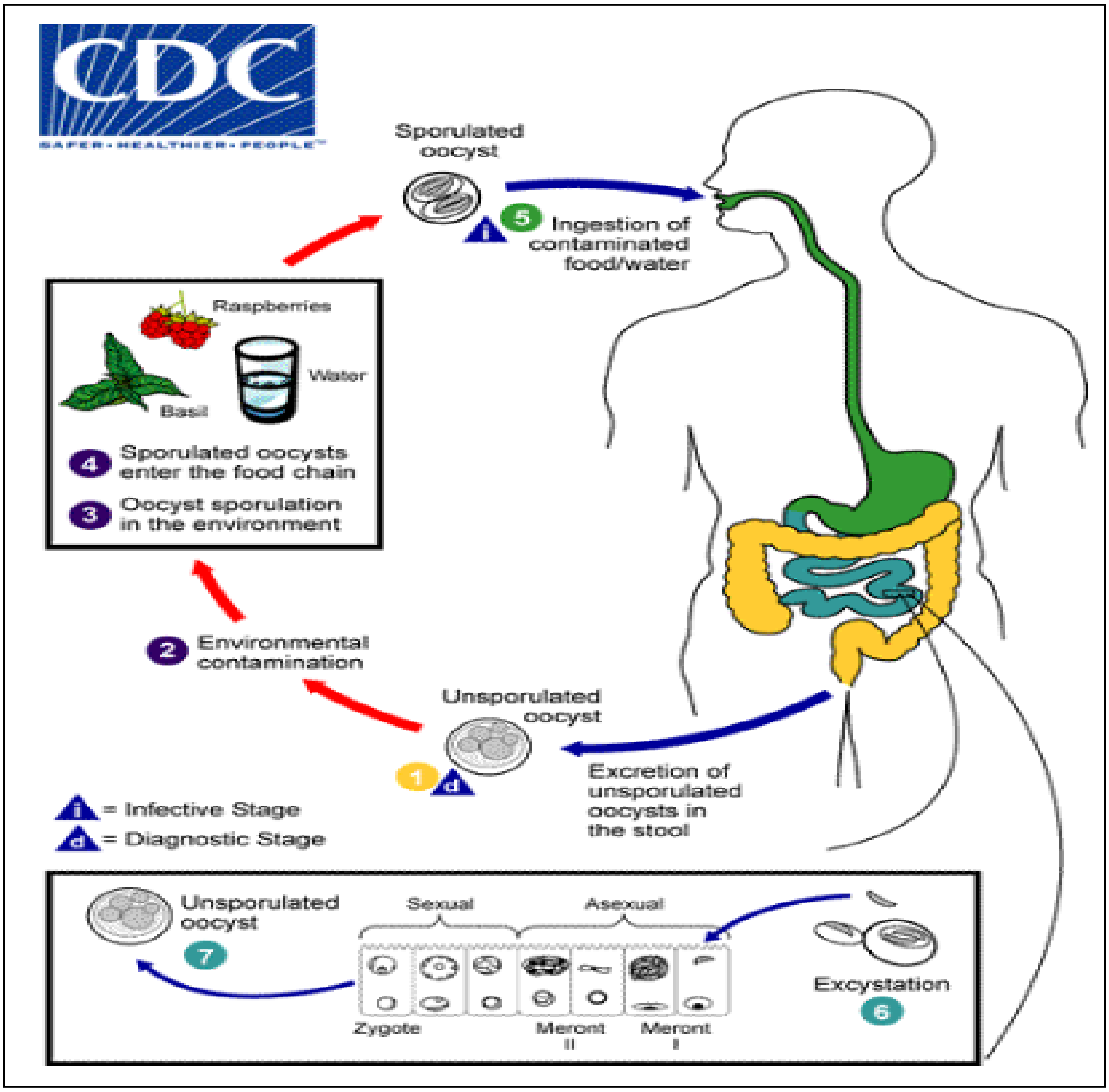


Figure 15: Life Cycle of *Cyclospora cayetanensis*

3. WORMS AND ZOOPLANKTON (ANIMAL KINGDOM)

3.1. Zooplankton

Zooplankton are heterotrophic (sometimes detritivorous) plankton. Plankton are organisms drifting in oceans, seas, and bodies of fresh water. Individual zooplankton are usually microscopic, but some (such as jellyfish) are larger and visible to the naked eye.

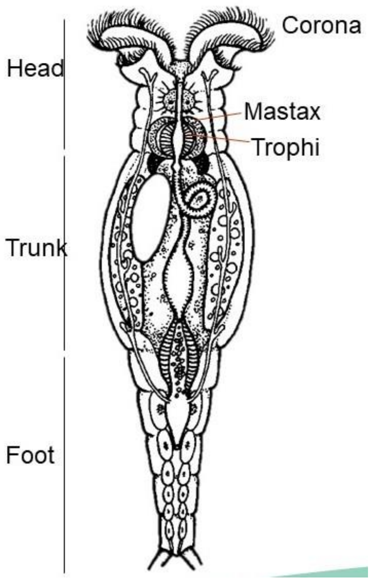
Zooplankton can also act as a disease reservoir. Crustacean zooplankton have been found to house the bacterium *Vibrio cholerae*, which causes cholera, by allowing the cholera vibrios to attach to their chitinous exoskeletons. This symbiotic relationship enhances the bacterium's ability to survive in an aquatic environment, as the exoskeleton provides the bacterium with carbon and nitrogen.

Most zooplankton belong to three major groups: rotifers, Cladocera, and Copepoda

3.1.2. Rotifera: -

Mostly littoral, sessile, but some are completely planktonic. May be dominant zooplankton in some lakes. Omnivorous, small (<12 µm).

3.1.2.1. Charcters of Rotifera:



- Bilaterally symmetrical.
- Body has more than two cell layers, tissues and organs.
- Body cavity is a pseudocoelom[second body cavity].
- Body possesses a through gut with an anus.
- Body covered in an external layer of chitin called a lorica.
- Has a nervous system with a brain and paired nerves.
- Has no circulatory or respiratory organs.
- Reproduction mostly parthenogenetic [an asexual form of reproduction found in females where growth and development of embryos or seeds occurs without fertilization by a male.] , otherwise sexual and gonochoristic[reproduction with two distinct sexes].
- Feed on bacteria, and protists, or are parasitic.
- All live in aquatic environments either free swimming or attached.
- 3 general regions: head, trunk, foot
- Ciliary **corona** for feeding, locomotion
- Complete digestive tract

- **mastax** = muscular pharynx
- **trophus** = grind food

3.1.2.2.Reproduction :

Sexual : complex life cycle with different types of eggs usually dioecious but in some groups, males are absent (parthenogenesis)

Parthenogenesis: unisexual reproduction where females produce offspring from unfertilized eggs (virgin birth).

Reproduction during most of growing season by diploid female parthenogenesis Diploid eggs produced via mitosis. Develop into amictic females Continues for generations during good conditions (Fig. 24 and 25).

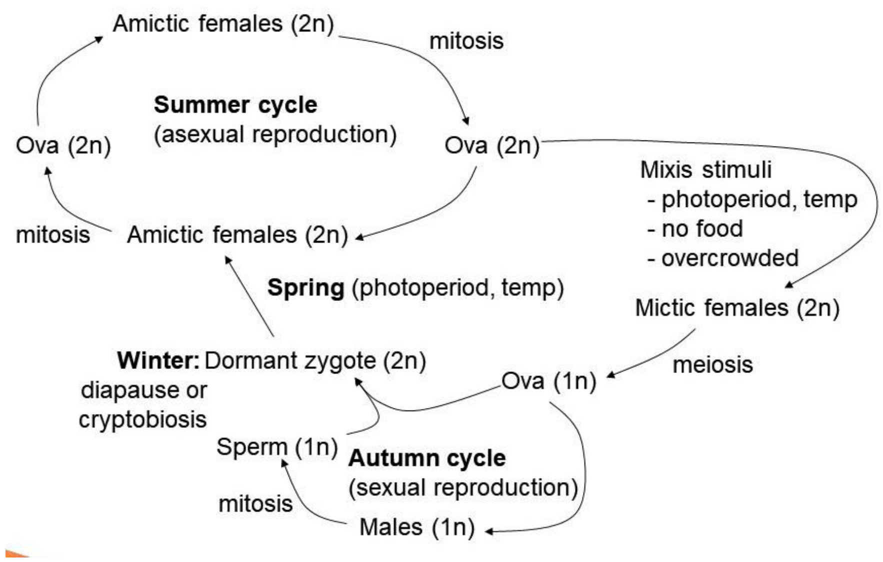


Figure 16: Life Cycle of Rotifera

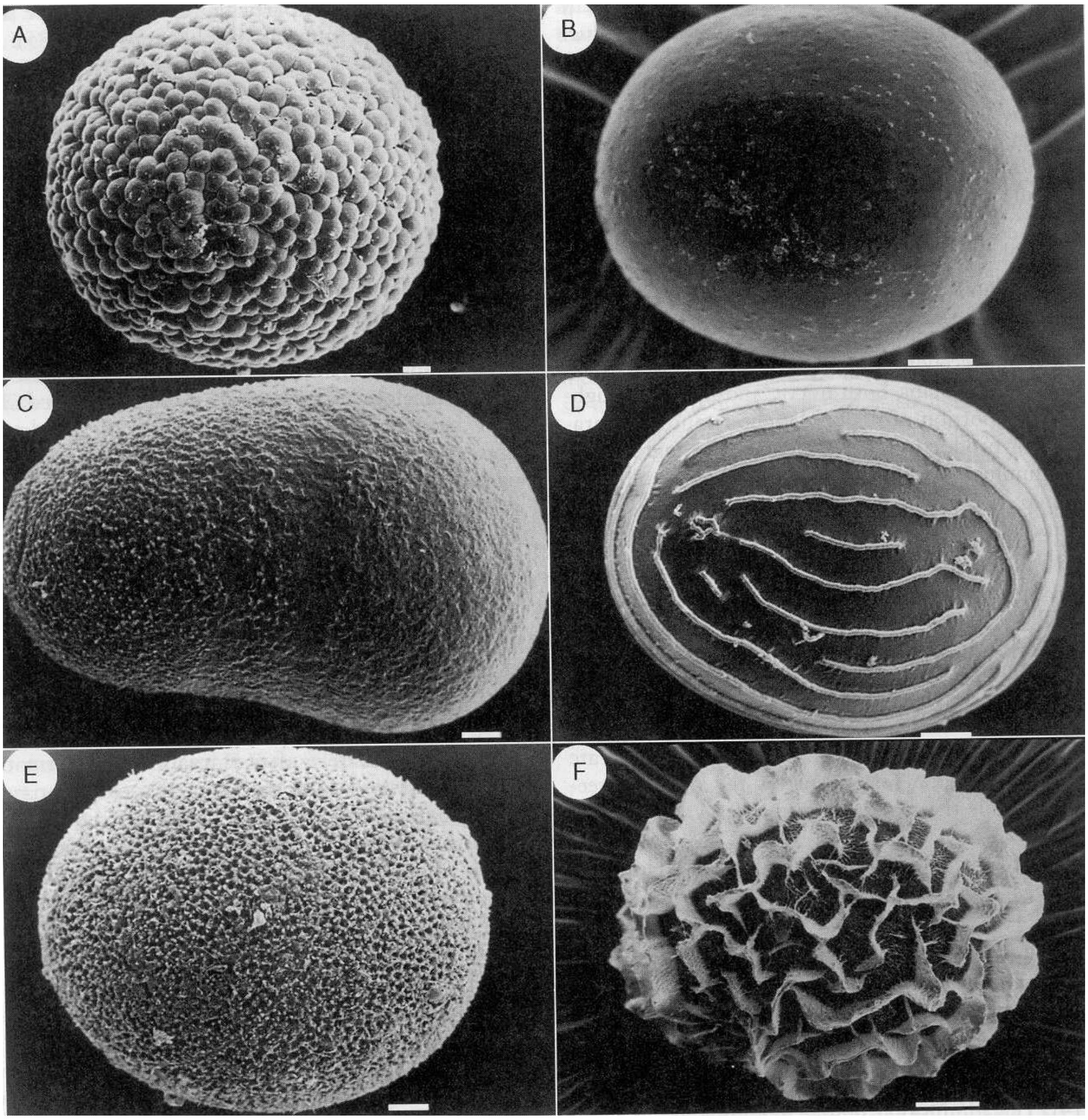


Figure 17: Resting Egg of Rotifera

3.1.2.3.Rotifer Population Dynamics

Different species exhibit different population peaks. Some in early summer, others in winter/early spring, others multiple times in summer

3.1.2.4.Rotifer Cyclomorphosis Fig 26

- Seasonal polymorphism
- Elongation, enlargement or reduction, production of spines
- Reduce sinking rate in warmer water
- Cope with larger prey
- Better resist predation
- Spines prevent ingestion.
- Formation of spines induced by organic substance (kairomone) produced by predator

Figure 18 Rotifer Cyclomorphosis

3.1.3. Cladocera (ex. Daphnia):

Small crustaceans (0.2-3.0 mm) with head, and body covered by bivalve carapace. and is divided into segments, although this division is not visible. The head is fused, and is generally bent down towards the body with a visible notch separating the two. In most species, the rest of the body is covered by a carapace, with a ventral gap in which the five or six pairs of legs lie. The most prominent features are the compound eyes, the second antennae, and a pair of abdominal setae. In many species, the carapace is translucent or nearly so and as a result they make excellent subjects for the microscope as one can observe the beating heart. They swim by using large 2nd antennae. Filter phytoplankton, detritus for food (some are predators). Size of phytoplankton ingested proportional to body size. The rate of filter feeding increases with size and temperature. Selective filtering by cladocerans can remove big “chunks” of the phytoplankton, and alter phytoplankton succession. (Fig 27).

Figure 19 Daphnia magna

3.1.3.1.Cladocera Reproduction

Reproduction similar to that of rotifers. Parthenogenesis by diploid females throughout most of the growing season. The Process Continues until interrupted by unfavorable conditions. When temperature reductions, drying, reduced photoperiod, crowding (competition for food), decrease in food size/quality. Some eggs develop into diploid males. Females produce haploid eggsm ate with males. Then fertilized eggs overwinter in thickened brood pouch (ephippium). Ephippia can withstand severe conditions. It can be transported by birds to other waters. It hatch under favorable conditions into parthenogenetic females Similar to those of rotifers. Some overwinter as adults, others as resting eggs (ephippia). Increased food and temperature enhance production (Fig 28).

Figure 28 ephippia

3.1.3.2.Diurnal Vertical Migrations

Most migrate to surface at dusk, downward at dawn (light intensity the stimulus) Movements may be >50 m and rapid (20 m/hr).

Reasons for migration:

1) avoid visual feeding fish in epilimnion by coming up to feed on phytoplankton after dark
2) improve food utilization - filter faster in warmer water, assimilate better in cooler water

3.1.3.3.Cladoceran Cyclomorphosis

Cyclomorphosis of Daphnia made an extension of head to form helmet and increase of caudal spine length. That caused by increased temperature, turbulence, photoperiod, food etc… (Fig 29). Advantage of allowing for continued growth of transparent peripheral structures without enlarging central portion of body visible to fish and reduces of predation.

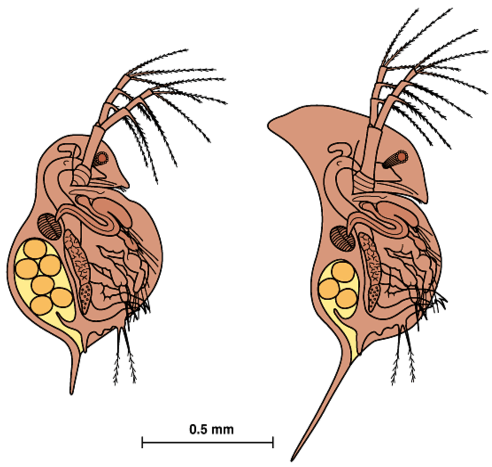


Figure 29 Cladoceran Cyclomorphosis

3.1.4. Copepoda :

Copepods are a group of small crustaceans found in nearly every freshwater and saltwater habitat. Some species are planktonic (drifting in sea waters), some are benthic (living on the ocean floor), and some continental species may live in limnoterrestrial habitats and other wet terrestrial places, such as swamps, under leaf fall in wet forests, bogs, springs, ephemeral ponds, and puddles, damp moss, or water-filled recesses (phytotelmata) of plants such as bromeliads and pitcher plants. Many live underground in marine and freshwater caves, sinkholes, or stream beds. Copepods are sometimes used as biodiversity indicators.

As with other crustaceans, copepods have a larval form. For copepods, the egg hatches into a nauplius form, with a head and a tail but no true thorax or abdomen. The larva molts several times until it resembles the adult and then, after more molts, achieves adult development. The nauplius form is so different from the adult form that it was once thought to be a separate species.

- Several different groups based on differences in body structure 2 major groups: cyclopoids and calanoids. Cyclopoids with short 1st antennae (Fig 30) and Calanoids with long 1st antennae (Fig 31).

Fig 30

Fig 31

3.1.4.1. Copepod Reproduction:-

Eggs hatch into nauplius larvae with 3 pairs of legs (Fig 32). Nauplius grow and molt several times to become copepodite. It grow and molt more before becoming adult. It take longer period of time from egg to adult than in rotifers, cladocerans may have resting eggs (overwinter). Predation by cyclopoid copepods may kill up to 30% of nauplii or copepodites (of own or other species). This predation may result in vertical, seasonal separation of similar species.

Figure 32 Nauplius

3.1.4.2.Copepoda Cyclomorphosis

No cyclomorphosis

3.1.5. Zooplankton Generalities:

Increased population with higher temps and decreased with increased food availability. Highest assimilation when feeding on prime food - right type, size

Lower when feeding on bacteria on vice versa lowest when feeding on detritus. It is Avoide of shores. Productivity correlated with phytoplankton production. Mode of filter-feeders have higher productivity than predators. Many zooplankton abundant in littoral areas. Associated with macrophytes, sediments . It abundance related to plant surface area, algal/detrital abundance. Abundance generally highest in spring and fall, lowest in mid-summer. It is Low corresponded with heavy predation by insect larvae, small fish.

3.2. Worms: -

They are many different distantly related animals that typically have a long cylindrical tube-like body and no limbs. Worms vary in size from microscopic to over 1 metre in length for marine polychaete worms (bristle worms), 6.7 metres for the African giant earthworm, *Microchaetus rappi*, and 58 metres for the marine nemertean worm, *Lineus longissimus*. Various types of worm occupy a small variety of parasitic niches, living inside the bodies of other animals. Free-living worm species do not live on land, but instead, live in marine or freshwater environments, or underground by burrowing. In biology, "worm" refers to an obsolete taxon, *vermes*, used by Carolus Linnaeus and Jean-Baptiste Lamarck for all non-arthropod invertebrate animals, now seen to be paraphyletic. Worms may also be called helminths, particularly in medical terminology when referring to parasitic worms, especially the Nematoda (roundworms) and Cestoda (tapeworms) which reside in the intestines of their host. When an animal or human is said to "have worms", it means that it is infested with parasitic worms, typically roundworms or tapeworms. Lungworm is also a common parasitic worm found in various animal species such as fish and cats.

3.2.1. Informal grouping:

Worms can be divided into several groups, but are still technically decomposers. The first of these, Platyhelminthes, includes the flatworms, tapeworms, and flukes. They have a flat, ribbon- or leaf-shaped body with a pair of eyes at the front. Some are parasites.

The second group contains the threadworms, roundworms, and hookworms. This phylum is called Nematoda. Threadworms may be microscopic, such as the vinegar eelworm, or more than 1 metre long. They are found in damp earth, moss, decaying substances, fresh water, or salt water. Some roundworms are also parasites. The Guinea worm, for example, gets under the skin of the feet and legs of people living in tropical countries.

The third group consists of the segmented worms, with bodies divided into segments, or rings. This phylum is called Annelida. Among these are the earthworms and the bristle worms of the sea.

Familiar worms include the earthworms, members of phylum Annelida. Other invertebrate groups may be called worms, especially colloquially. In particular, many unrelated insect larvae are called "worms", such as the railroad worm, woodworm, glowworm, bloodworm, inchworm, mealworm, silkworm, and woolly bear worm.

3.2.2. Platyhelminthes:

Platyhelminthes (flat worm) are bilaterally symmetrical animals: their left and right sides are mirror images of each other; this also implies they have distinct top and bottom surfaces and distinct head and tail ends. Like other bilaterians, they have three main cell layers (endoderm, mesoderm, and ectoderm). Unlike other bilaterians, Platyhelminthes have no internal body cavity, so are described as acoelomates.

They also lack specialized circulatory and respiratory organs (unlike Nematodes and Rotifera); both of these facts are defining features when classifying a flatworm's anatomy. Their bodies are soft and unsegmented.

3.2.2.1.Examples of pathogenic Platyhelminthes

Cestoda (tapeworm): Infects human through ingesting the undercooked pork or beef (ex. *Taenia*) (fig. 33).

Trematodes (Flukes): It includes two groups of parasitic flatworms, known as flukes. They are internal parasites of molluscs and vertebrates. Most trematodes have a complex life cycle with at least two hosts. The primary host, where the flukes sexually reproduce, is a vertebrate. The intermediate host, in which asexual reproduction occurs, is usually a snail. (ex. *Fasciola sp.* And *Schistosoma sp.*).

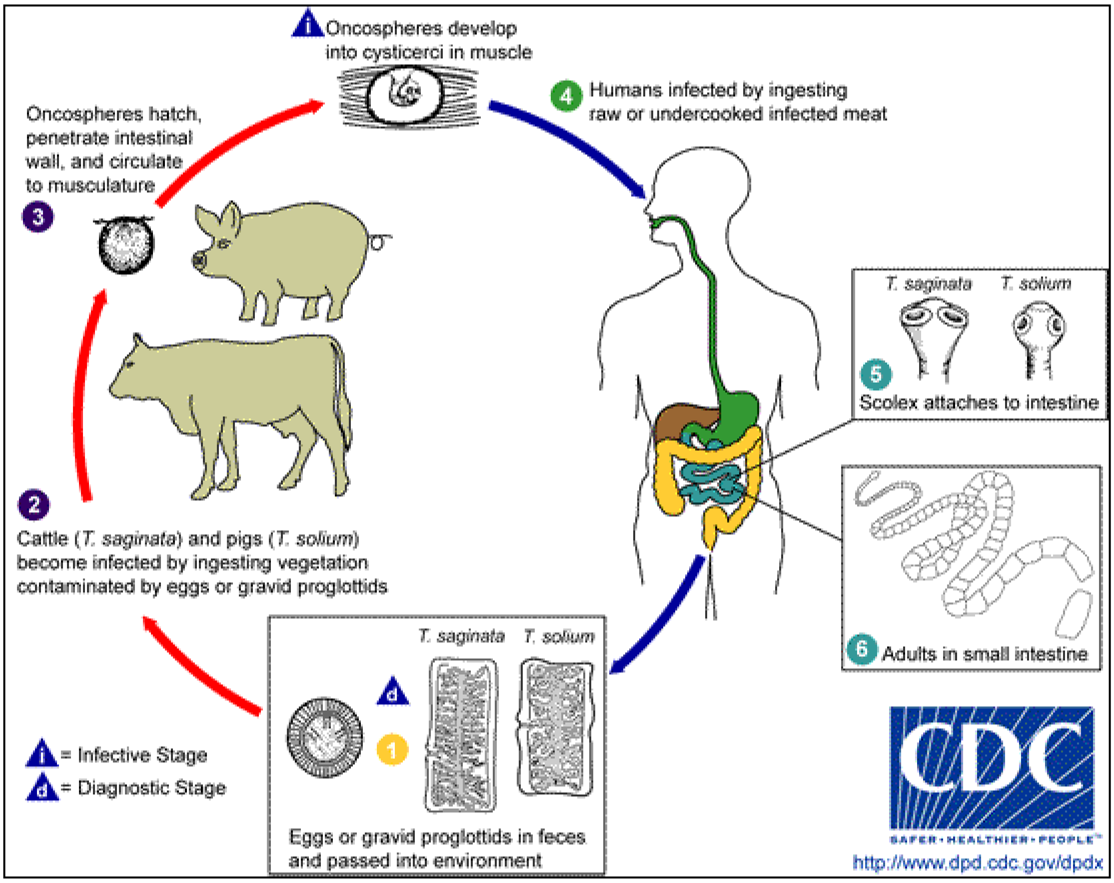


Figure 33 : Life cycle of the eucestode *Taenia*: Inset 5 shows the scolex, which has four *Taenia solium*, a disk with hooks on the end. Inset 6 shows the tapeworm's whole body, in which the scolex is the tiny, round tip in the top left corner, and a mature proglottid has just detached.

3.2.3. Nematoda:

The nematodes are quite species diverse (about 10,000 species) and the many parasitic forms have a significant impact on humans.Most nematodes are under 5cm and many are microscopic. However, some parasitic forms can be over a meter in length.

Nematodes use their pseudocoelom as a hydrostatic skeleton. A pseudocoelom is a fluid-filled body cavity in which mesoderm lines only the outer edge of the developing blastocoel. No peritoneal lining develops.

The body has a thick cuticle (made primarily of collagen) secreted by the underlying epidermis, which resists the high hydrostatic pressure exerted by the fluid in the pseudocoelom.

Beneath the epidermis is a layer of longitudinal muscles. Muscles in nematodes are not arranged in antagonistic pairs, the antagonistic role is played by the cuticle. Contraction of a longitudinal muscle on one side is transmitted through the hydrostatic skeleton and stretches the cuticle on the opposite side of the body. When the muscle relaxes, the cuticle contracts and the body returns to its resting position.

Nematodes have a complete gut with a mouth, muscular pharynx, intestine, rectum, and anus. Most nematodes are dioecious and males are smaller than females.

Fertilization is internal and juveniles go through several developmental stages, each time molting or shedding their cuticle.

3.2.3.1.Free-living nematodes:

The slender, tapered body of nematodes equips them to live in interstitial spaces. Most free-living nematodes are less than 2.5mm in length and often are microscopic. The largest soil dwelling nematodes may be 7mm long and the largest marine forms a whopping 5cm. Most free-living nematodes are carnivorous. However, some feed on algae and fungi and some are detritivores. Others feed on plants, especially the roots. Many root feeding nematodes are major agricultural pests. These species pierce root cells and suck out their contents. Nematodes are estimated to destroy 12% of the world's cash crops annually.

3.2.3.2.Parasitic nematodes:

There are a great many species of parasitic nematodes and they attack virtually all groups of animals and plants. Parasitic forms include ascarids, hookworms, Guinea worms, trichina worms, pinworms, and filarial worms.

3.2.3.3.*Ascaris lumbricoides*:

Ascaris lumbricoides is the largest intestinal roundworm and is the most common helminths infection of humans worldwide. Infestation of **ascariasis** can cause morbidity by:

(1) Compromising nutritional status, (2) affecting cognitive processes, (3) inducing tissue reactions such as granuloma to larval stages, and (4) by causing intestinal obstruction, which can be fatal.

Shape and Size of *Ascaris*: (fig. 34)

- Adult: cylindrical shape, creamy white or pinkish in color
- Male: average 15–30 cm and is more slender than female
- Female: average 20–35 cm in length

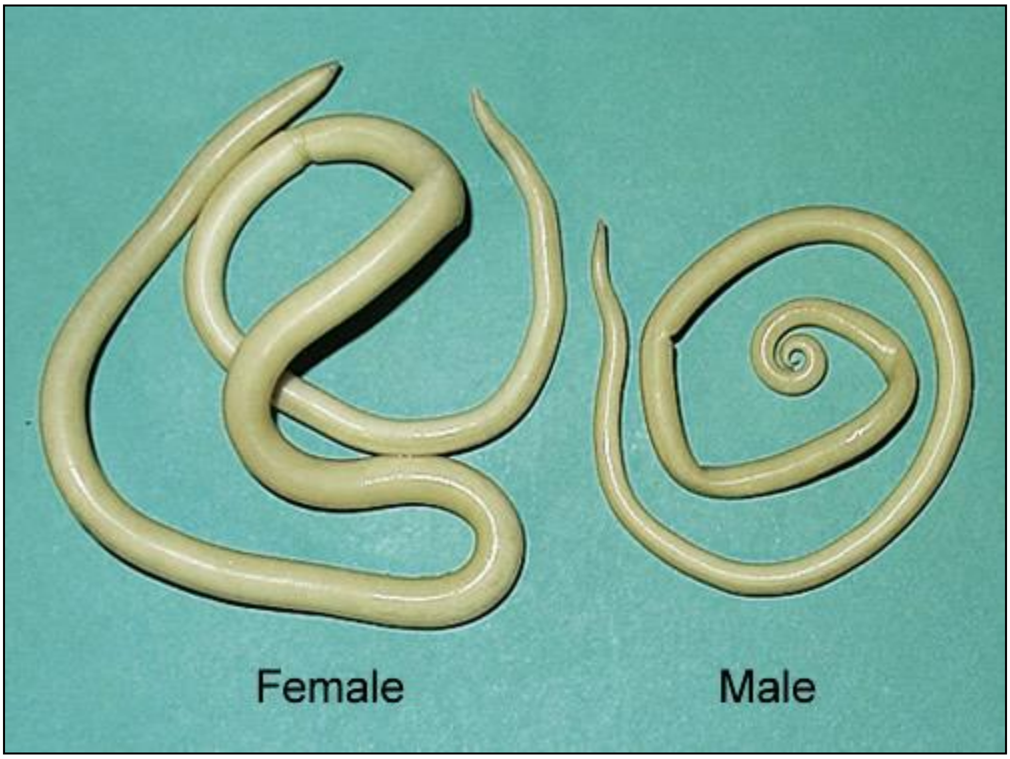


Figure 34: Adult male and female *Ascaris*

<u>Life Cycle of *Ascaris*:</u> (fig. 35)

Adult worms (number 1) live in the lumen of the small intestine. A female may produce approximately 200,000 eggs per day, which are passed with the feces (number 2). Unfertilized eggs may be ingested but are not infective. Fertile eggs embryonate and become infective after 18 days to several weeks (number 3). After infective eggs are swallowed (number 4). The larvae hatch (number 5), invade the intestinal mucosa, and are carried via the portal, then systemic circulation to the lungs (number 6). The larvae mature further in the lungs (10 to 14 days), penetrate the alveolar walls, ascend the bronchial tree to the throat, and are swallowed (number 7). Upon reaching the small intestine, they develop into adult worms (number 1). Between 2 and 3 months are required from ingestion of the infective eggs to oviposit by the adult female. Adult worms can live 1 to 2 years.

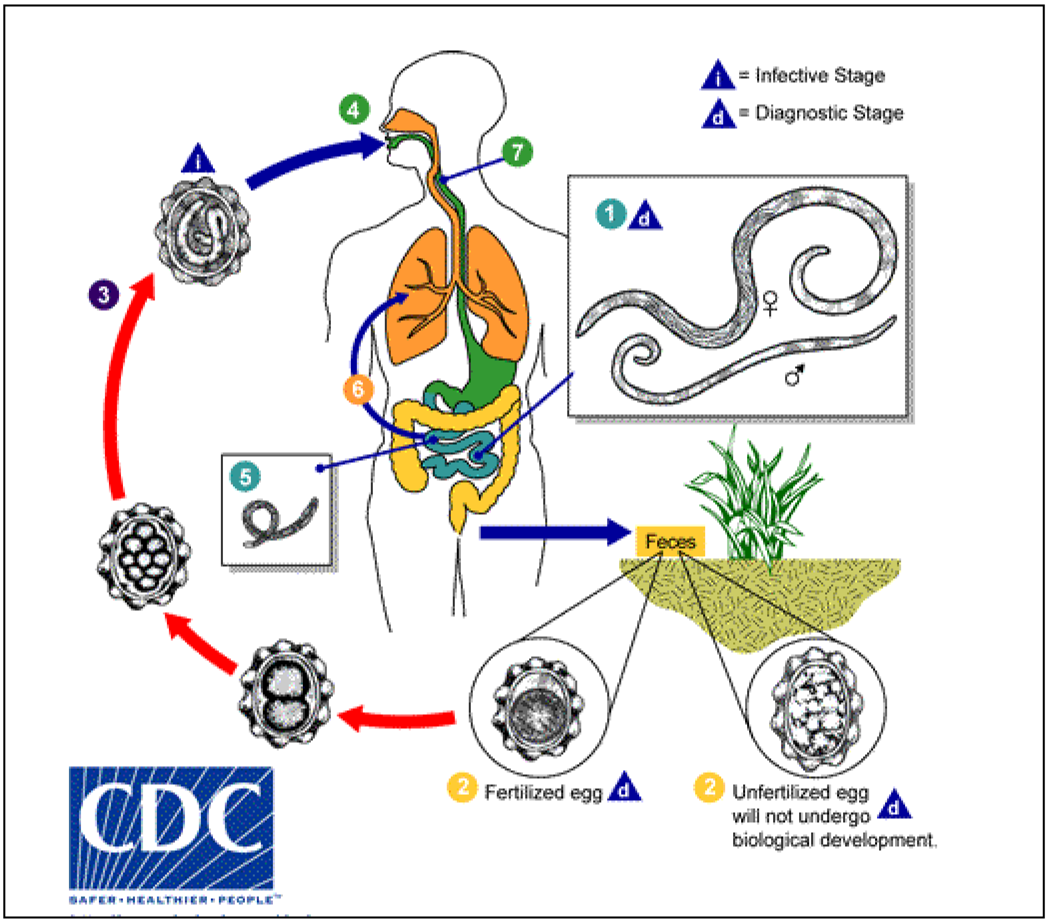


Figure 35: Ascaris lumbricoides life cycle

3.2.3.4. Hookworms:

At least two species of hookworms infect man, *Necator americanus* and *Ancylostoma duodenale*. They live in small intestine.

3.1.2.1.1. Morphology:

Adults: They look like an odd piece thread and are about 1cm. They are white or light pinkish when living. The male's posterior end is expanded to form a copulatory bursa (Fig 36).

3. **Eggs**: 60×40 μm in size, oval in shape, shell is thin and colorless. Content is 2-8cells (Fig 37).

Figure 36: Adults of Hookworm

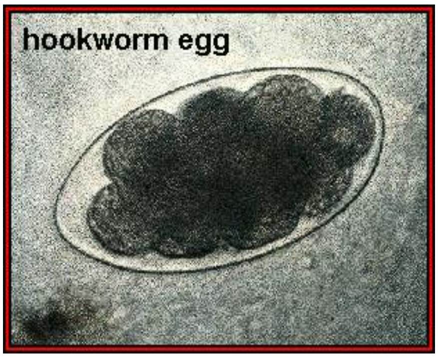


Figure 37: Egg of Hookworm

	A. duodenale	*N. americanus*
Size	Larger	smaller
Shape	single curve, looks like C	double curves, looks like S
Mouth	2 pairs of ventral teeth (Fig 36)	1peir of ventral cutting plates (Fig 37)
Copulatory Bursa	circle in shape (Fig 38)	oval in shape (Fig 39)

Copulatory spicule	1pair with separate endings	1pair of which unite to form a terminal hooklet
caudal spine	Present	absent
vulva position	post-equatorial	pre-equatorial

Figure 38: ***A. duodenale***

Figure 39: ***N. americanus***

Figure 40: ***A. duodenale***

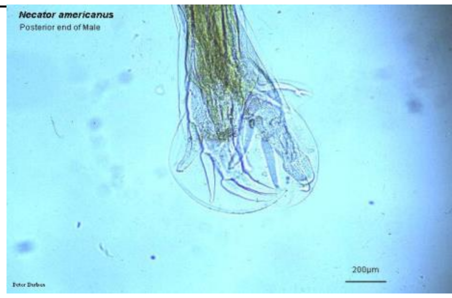


Figure 41: ***N. americanus***

Shape, Size, and Structure of *Ancylostoma duodenale*:

Ancylostoma duodenale (also known as the hookworm), is small cylindrical worm, greyish-white in color worm (fig. 42 and 43). Males are 8 mm to 11 mm long, while female are 10 mm to 13 mm long.

Figure 42: Adult of *Ancylostoma duodenale*

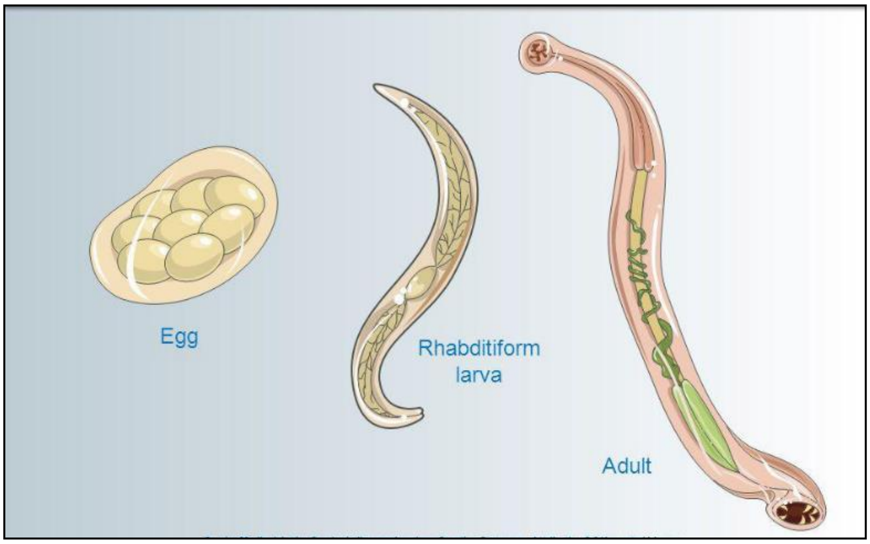


Figure 43: Egg, larva (infective phase), and adult of *Ancylostoma duodenale*

Habitat of *Ancylostoma duodenale*:

It lives in the small intestine of hosts such as humans, cats and dogs, where it is able to mate and mature.

Impact of *Ancylostoma duodenale* on Health:

- A light infection: causes abdominal pain, and loss of appetite.
- Heavy infection: causes severe protein deficiency or iron deficiency anemia. Protein deficiency may lead to dry skin, edema and potbelly, while iron deficiency anemia might result in mental dullness and heart failure.

Life Cycle of *Ancylostoma duodenale*: (fig. 44)

- After a rhabditiform "infective" larva penetrates the intact skin – most commonly through the feet – the larva enters the blood circulation.
- It is then carried to the lungs, breaks into alveoli, ascends the bronchi and trachea and is coughed up and swallowed back into the small intestine where it matures.
- The larva later matures into an adult in the small intestine and female worms can lay 25,000 eggs per day.
- The eggs are released into the feces and reside on soil, when deposited on warm, moist soil a larva rapidly develops in the egg and hatches after 1 to 2 days.
- This rhabditiform larva becomes a skin penetrating infective larva within 5–10 days to begin a new cycle of infection.

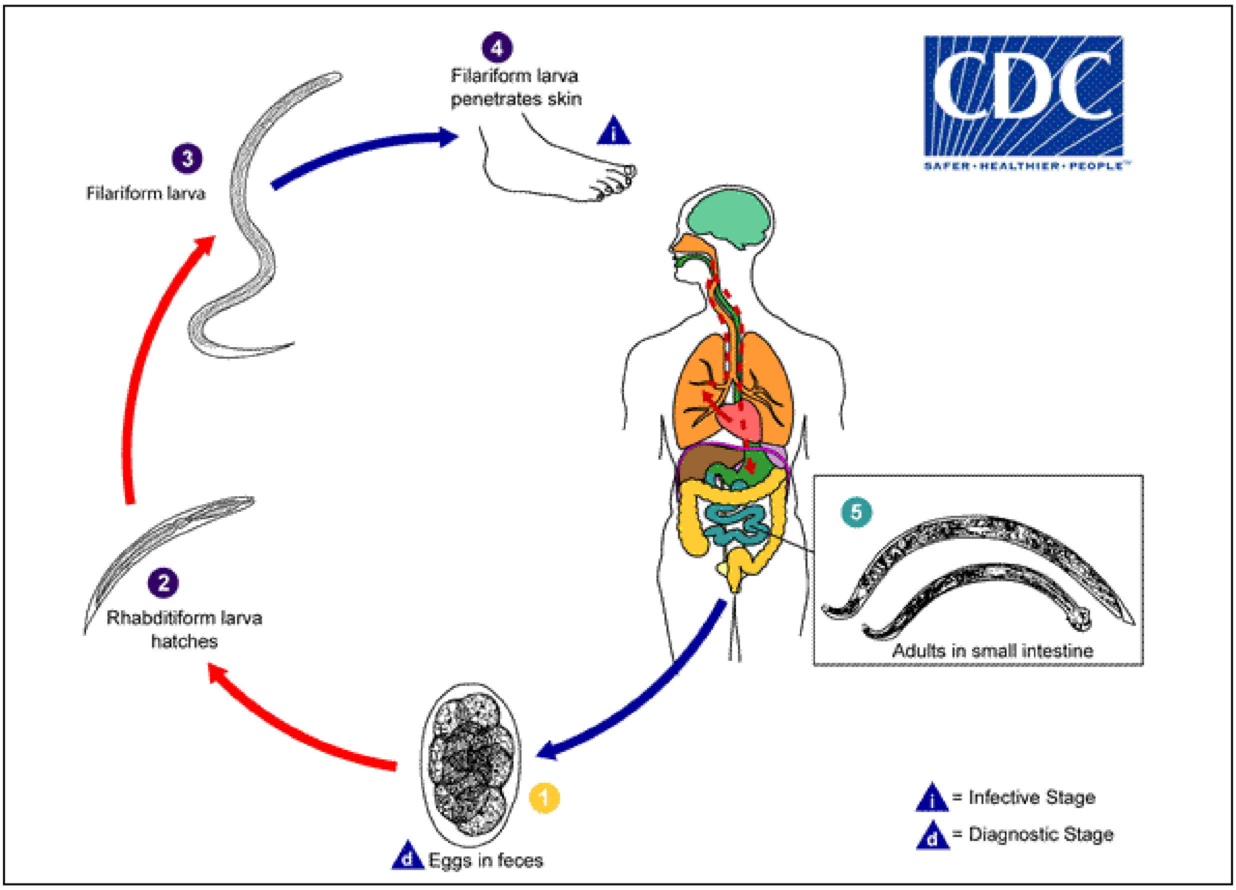


Figure 44: Life Cycle of *Ancylostoma duodenale*

4. DETECTION METHODS

Microscopic Zooplanktons, Worms and Protozoa can be detected by several methods according to Organisms:

Direct filtration- used for detect Most Protozoa, Microscopic Zooplankton and Microscopic worms (Adult and eggs). It is most common used method in Water Laboratory in Egypt.

Culture Method – used for detect Free Living Amoeba (*Acanthamoeba* and *Neglaria*). It needs special requirement for safety of Lab and Biologist. Method applied in Reference laboratory of Water and Some of water companies. (See Appendix)

Immuno Magnetic assay – Detection of *Giardia* and *Cryptosporidium*. It need special equipment and chemicals. This method applied only in Reference Laboratory for Water. (See Appendix).

4.1. The Optical Microscope

The optical microscope, often referred to as the light microscope, is a type of microscope that commonly uses visible light and a system of lenses to magnify images of small objects.

4.1.1. Types

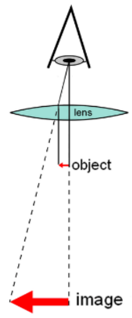
There are two basic types of optical microscopes: simple microscopes and compound microscopes. A simple microscope is one which uses a single lens for magnification, such as a magnifying glass. A compound microscope uses several lenses to enhance the magnification of an object. The vast majority of modern research microscopes are compound microscopes while some cheaper commercial digital microscopes are simple single lens microscopes. Compound microscopes can be further divided into a variety of other types of microscopes which differ in their optical configurations, cost, and intended purposes.

4.1.1.1. Regular microscope

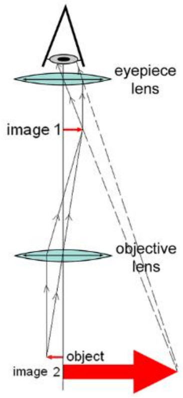
A regular microscope uses a lens or set of lenses to enlarge an object through angular magnification alone, giving the viewer an erect enlarged virtual image. The use of a single convex lens or groups of lenses are found in simple magnification devices such as the magnifying glass, loupes, and eyepieces for telescopes and microscopes.

4.1.1.2.Compound microscope

A compound microscope uses a lens close to the object being viewed to collect light (called the objective lens) which focuses a real image of the object inside the microscope.



That image is then magnified by a second lens or group of lenses (called the eyepiece) that gives the viewer an enlarged inverted virtual image of the object. The use of a compound objective/eyepiece combination allows for much higher magnification. Common compound microscopes often feature exchangeable objective lenses, allowing the user to quickly adjust the magnification. A compound microscope also enables more advanced illumination setups, such as phase contrast.



4.1.1.3.Fluorescence microscopy

Modern biological microscopy depends heavily on the development of fluorescent probes for specific structures within a cell. In contrast to normal transilluminated light microscopy, in fluorescence microscopy the sample is illuminated through the objective lens with a narrow set of wavelengths of light. This light interacts with fluorophores in the sample which then emit light of a longer wavelength. It is this emitted light which makes up the image.

4.1.1.4.Components

All modern optical microscopes designed for viewing samples by transmitted light share the same basic components of the light path. In addition, the vast majority of microscopes have the same 'structural' components (numbered below according to the image on the right):

Eyepiece (ocular lens) (1)

Objective turret, revolver, or revolving nose piece (to hold multiple objective lenses) (2)

Objective lenses (3)

Focus knobs (to move the stage)

Coarse adjustment (4)

Fine adjustment (5)

Stage (to hold the specimen) (6)

Light source (a light or a mirror) (7)

Diaphragm and condenser (8)

Mechanical stage (9)

4.1.1.4.1. Eyepiece (ocular lens)

The eyepiece, or ocular lens, is a cylinder containing two or more lenses; its function is to bring the image into focus for the eye. The eyepiece is inserted into the top end of the body tube. Eyepieces are interchangeable and many different eyepieces can be inserted with different degrees of magnification. Typical magnification values for eyepieces include 5X, 10X (the most common), 15X and 20X.

4.1.1.4.2. Objective turret (revolver or revolving nose piece)

Objective turret, revolver, or revolving nose piece is the part that holds the set of objective lenses. It allows the user to switch between objective lenses.

4.1.1.4.3. Objective

At the lower end of a typical compound optical microscope, there are one or more objective lenses that collect light from the sample. The objective is usually in a cylinder housing containing a glass single or multi-element compound lens. Typically there will be around three objective lenses screwed into a circular nose piece which may be rotated to select the required objective lens. These arrangements are designed to be parfocal, which means that when one changes from one lens to another on a microscope, the sample stays in focus. Microscope objectives are characterized by two parameters, namely, magnification and numerical aperture. The former typically ranges from 5X to 100× while the latter ranges from 0.14 to 0.7, corresponding to focal lengths of about 40 to 2 mm, respectively. Objective lenses with higher magnifications normally have a higher numerical aperture and a shorter depth of field in the resulting image. Some high performance objective lenses may require matched eyepieces to deliver the best optical performance.

4.1.1.4.4. Oil immersion objective

Some microscopes make use of oil-immersion objectives or water-immersion objectives for greater resolution at high magnification. An oil immersion lens usually has a magnification of 40 to 100×.

4.1.1.4.5. Focus knobs

Adjustment knobs move the stage up and down with separate adjustment for coarse and fine focusing. The same controls enable the microscope to adjust to specimens of different thickness. In older designs of microscopes, the focus adjustment wheels move the microscope tube up or down relative to the stand and had a fixed stage.

4.1.1.4.6. **Frame**

he whole of the optical assembly is traditionally attached to a rigid arm, which in turn is attached to a robust U-shaped foot to provide the necessary rigidity. The arm angle may be adjustable to allow the viewing angle to be adjusted.

4.1.1.4.7. **Stage**

The stage is a platform below the objective which supports the specimen being viewed. In the center of the stage is a hole through which light passes to illuminate the specimen. The stage usually has arms to hold slides (rectangular glass plates with typical dimensions of 25×75 mm, on which the specimen is mounted).

At magnifications higher than 100× moving a slide by hand is not practical. A mechanical stage, typical of medium and higher priced microscopes, allows tiny movements of the slide via control knobs that reposition the sample/slide as desired. If a microscope did not originally have a mechanical stage it may be possible to add one.

All stages move up and down for focus. With a mechanical stage slides move on two horizontal axes for positioning the specimen to examine specimen details.

4.1.1.4.8. **Light source**

Many sources of light can be used. At its simplest, daylight is directed via a mirror. Most microscopes, however, have their own adjustable and controllable light source – often a halogen lamp, although illumination using LEDs and lasers are becoming a more common provision. Köhler illumination is often provided on more expensive instruments.

4.1.1.4.9. **Condenser**

The condenser is a lens designed to focus light from the illumination source onto the sample. The condenser may also include other features, such as a diaphragm and/or filters, to manage the quality and intensity of the illumination. For illumination techniques like dark field, phase contrast and differential interference contrast microscopy additional optical components must be precisely aligned in the light path.

4.1.1.4.10. **Magnification**

The actual power or magnification of a compound optical microscope is the product of the powers of the ocular (eyepiece) and the objective lens. The maximum normal magnifications of the ocular and objective are 10× and 100× respectively, giving a final magnification of 1,000×.

4.2. Direct Filtration – Concentration and Staining Method

4.2.1. Scope

The direct method is suitable for the detection of Protozoa, Microscopic Zooplankton and Microscopic worms (Adult and eggs) in drinking, raw or ground water samples.

Equipment

- 47 mm stainless steel filtration manifold.
- Centrifuge
- 47 mm filter membranes, no greater than 0.8µm pore size.
- Vacuum pump
- Petri dishes 47 mm
- Centrifuge tubes.
- Microscope
- Glass slide

4.2.2. Chemical and Reagents

- Distilled water
- Autoclaved distilled water
- Methanol
- Ethyl alcohol 95%
- Glacial acetic acid
- Ethyl alcohol 100%
- Xylene
- Chromotrope 2R
- Fast green
- phosphotungstic acid

4.2.3. Procedure

4.2.3.1.Filtration – Concentration – Examination Procedures

1. Water samples take in container (1 L at least)

2. Samples should be transport and stored at (5 ± 3) °C unless they are to be analyzed immediately.
3. Samples should be analyzed within 24 h of collection.
4. Place the membrane filter into the housing and clamp on the upper part.
5. Pump the water sample through the filter.
6. Rinse the container with 1 L of filtered distilled water and pump the washings through the filter after sample filtration to confirm there were no parasites on the surface of the water tank of the filtration unit.
7. Remove the filter from the filter housing and place into a suitable clean Petri dish.
8. Add suitable amount of distilled water in the Petri dish and gently scrap the surface of the filter for 1 min.
9. Decant the washings into a 10 ml centrifuge tube refer to Working instruction for centrifuge.
10. Repeat the wash procedure with further distilled water and add this to the centrifuge tube.
11. Centrifuge the tube at 2000 rpm for 15 min.
12. Genteelly and carefully aspirate off the supernatant leaving the pellet. If no pellet is visible, extra care shall be taken to avoid aspirating oocysts and cysts during this step.
13. Spread the obtained pellet on the glass slide and examine by light microscope to detect parasites, fresh water Nematode and Rotifera.

4.2.4. Equipment Cleaning

Wash all equipment that is reused thoroughly in water containing detergent and then rinse in distilled water to remove any oocysts and cysts that may be attached to the equipment. Wash equipment used for positive control procedures separately (if possible in a separate area) from equipment used for the analysis of samples.

4.2.5. Results and Possible Microscopic Observations

4.2.5.1.Egg of Some Worms

They could be seen under the microscope as in figures (45, 46, 47, 48, and 49).

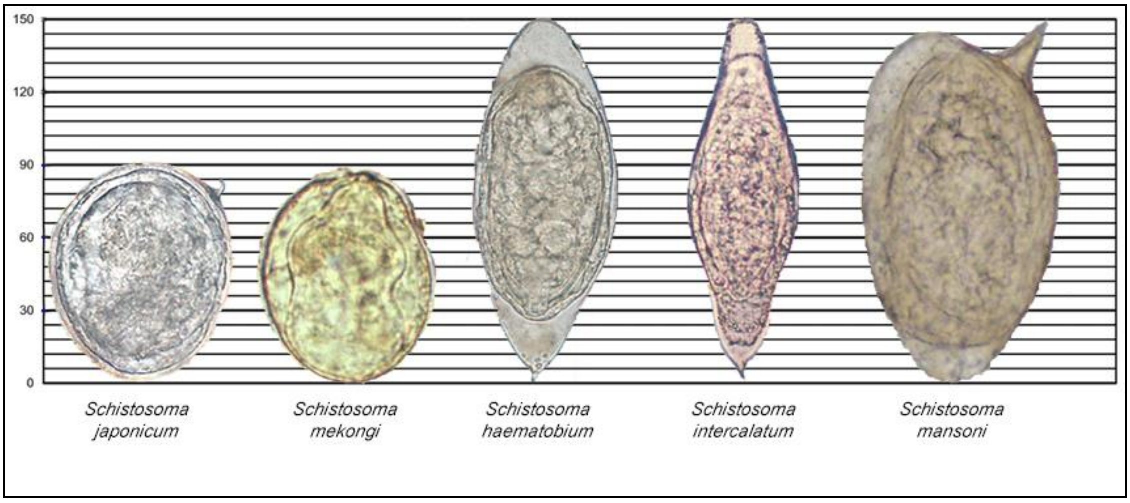


Figure 45: Some worm eggs images under microscope

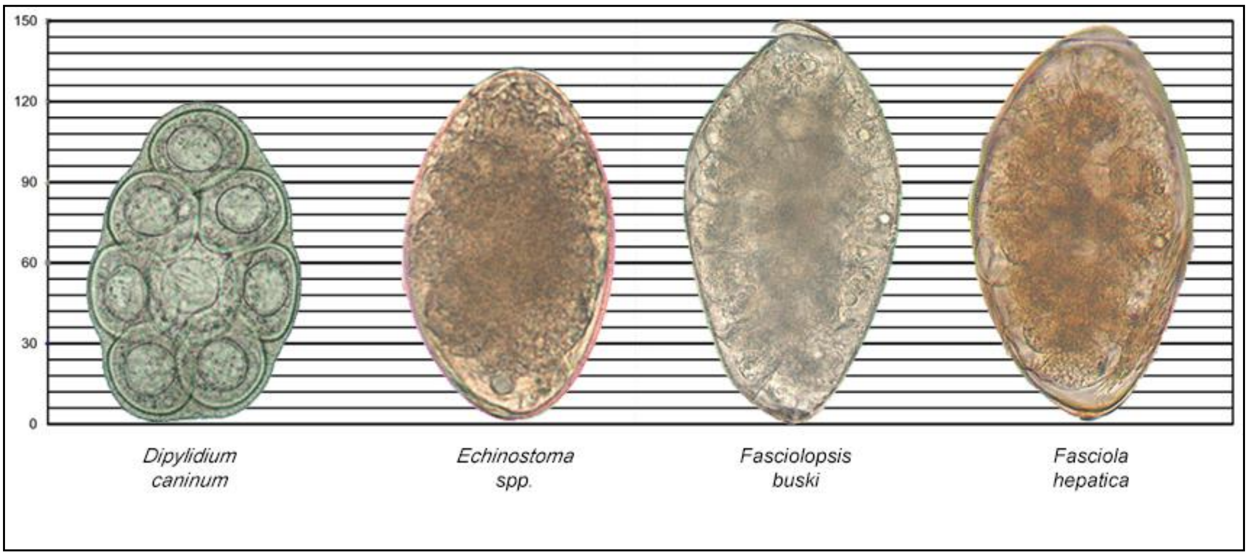


Figure 46: Some worm eggs images under microscope

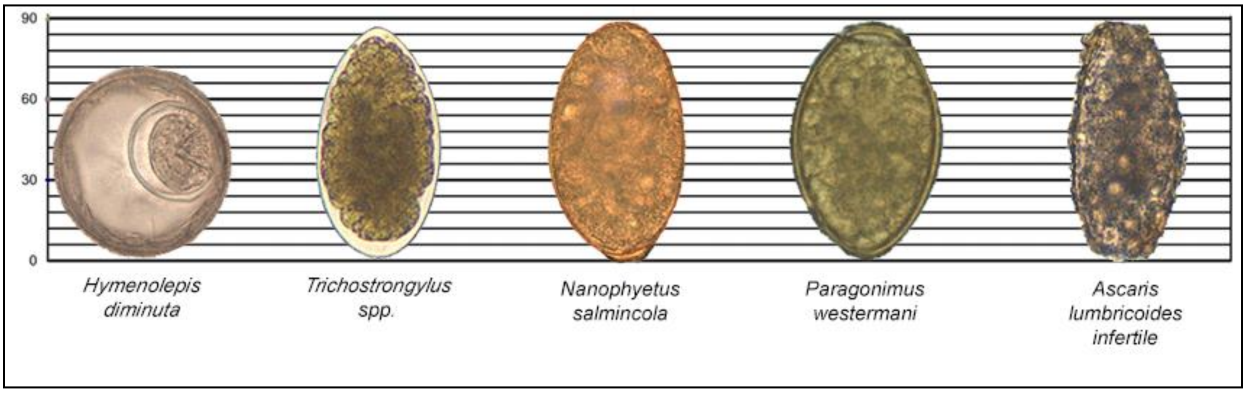


Figure 47: Some worm eggs images under microscope

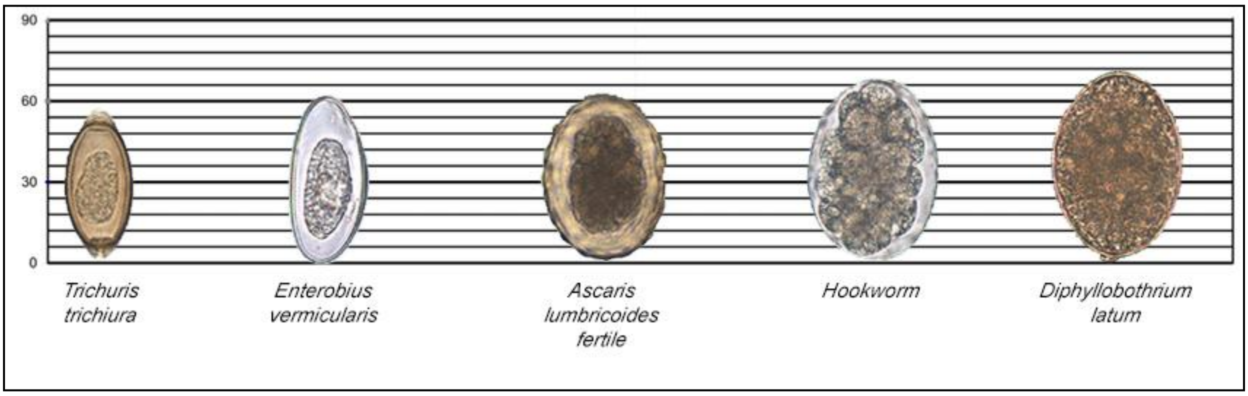


Figure 48: Some worm eggs images under microscope

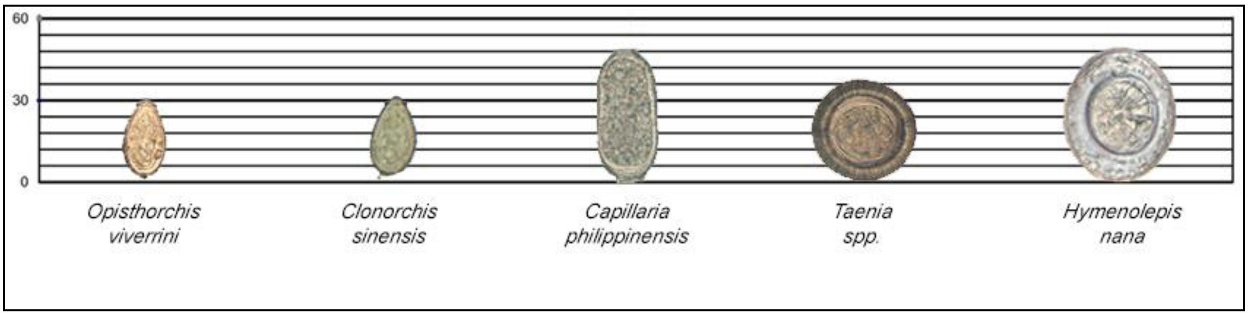


Figure 49: Some worm eggs images under microscope

للاقتراحات والشكاوى قم بمسح الصورة (QR)

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