Chapter- 1

Introduction

1. INTRODUCTION

1.1. Protozoan Parasitic Diseases: General Concept

Protozoa are considered to be the simplest organisms in the animal kingdom. They are all single-celled and considered being a subkingdom of the kingdom Protista, although in the classical system they were placed in the kingdom Animalia. Some protozoa are more closely related to animals, others to plants, and still others are relatively unique. Although it is not appropriate to group them together into a single taxonomic category, the research tools used to study any unicellular organism are usually the same, and the field of protozoology has been created to carry out this research. Protozoans are found in all moist habitats, but we know little about their specific geographic distribution. Because of their small size, production of resistant cysts, and ease of distribution from one place to another, many species appear to be cosmopolitan and may be collected in similar microhabitats worldwide [1]. Other species may have relatively narrow limits to their distribution. More than 50,000 species have been described, most of which are free-living organisms; protozoa are found in almost every possible habitat. The fossil record in the form of shells in sedimentary rocks shows that protozoa were present in the Pre-Cambrian era. Antonvan Leeuwenhoek was the first person to see protozoa, using microscopes he constructed with simple lenses. Between 1674 and 1716, he described, in addition to free-living protozoa, several parasitic species from animals, and *Giardia lamblia* from his own stools. Virtually all humans have protozoa living in or on their body at some time, and many persons are infected with one or more species throughout their life. Some species are considered commensally, i.e., normally not harmful, whereas others are pathogens and usually produce disease. Protozoan diseases range from very mild to life- threatening. Individuals whose defenses are able to control but not eliminate a parasitic infection become carriers and constitute a source of infection for others. In geographic areas of high prevalence, well-tolerated infections are often not treated to eradicate the parasite because eradication would lower the individual's immunity to the parasite and result in a high likelihood of re-infection [2- 4].

Many protozoan infections that are mild in normal individuals can be life- threatening in immuno-suppressed patients, particularly patients with acquired immune deficiency syndrome (AIDS). Evidence suggests that many healthy persons harbor low numbers of

Pneumocystis carinii in their lungs. However, this parasite produces a frequently fatal pneumonia in immuno-suppressed patients such as those with AIDS. *Toxoplasma gondii,* a very common protozoan parasite, usually causes a rather mild initial illness followed by a long-lasting latent infection. AIDS patients, however, can develop fatal toxoplasmic encephalitis. *Cryptosporidium* was described in the 19th century, but widespread human infection has only recently been recognized. *Cryptosporidium* is another protozoan that can produce serious complications in patients with AIDS. Microsporidiosis in humans was reported in only a few instances prior to the appearance of AIDS. It has now become a more common infection in AIDS patients. As more thorough studies of patients with AIDS are made, it is likely that other rare or unusual protozoan infections will be diagnosed [5].

1.2. Structure [6,7]

Most parasitic protozoa in humans are less than 50μm in size. The smallest (mainly intracellular forms) are 1 to 10 μm long, but *Balantidium coli* may measure 150 μm. Protozoa are unicellular eukaryotes. As in all eukaryotes, the nucleus is enclosed in a membrane. In protozoa other than ciliates, the nucleus is vesicular, with scattered chromatin giving a diffuse appearance to the nucleus; all nuclei in the individual organism appear alike. One type of vesicular nucleus contains a more or less central body, called an endosome or karyosome. The endosome lacks DNA in the parasitic amebas and trypanosomes. In the phylum Apicomplexa, on the other hand, the vesicular nucleus has one or more nucleoli that contain DNA. The ciliates have both a micronucleus and macronucleus, which appear quite homogeneous in composition.

The organelles of protozoa have functions similar to the organs of higher animals. The plasma membrane enclosing the cytoplasm also covers the projecting locomotors structures such as pseudopodia, cilia, and flagella. The outer surface layer of some protozoa, termed a pellicle, is sufficiently rigid to maintain a distinctive shape, as in the trypanosomes and *Giardia*. However, these organisms can readily twist and bend when moving through their environment. In most protozoa the cytoplasm is differentiated into ectoplasm (the outer, transparent layer) and endoplasm (the inner layer containing organelles); the structure of the cytoplasm is most easily seen in species with projecting pseudopodia, such as the amebas. Some protozoa have a cytosome or cell "mouth" for

ingesting fluids or solid particles. Contractile vacuoles for osmoregulation occur in some, such as *Naegleria* and *Balantidium*. Many protozoa have sub-pellicular microtubules; in the Apicomplexa, which have no external organelles for locomotion, these provide a means for slow movement. The trichomonads and trypanosomes have a distinctive undulating membrane between the body wall and a flagellum. Many other structures occur in parasitic protozoa, including the Golgi apparatus, mitochondria, lysosomes, food vacuoles, conoids in the Apicomplexa, and other specialized structures. Electron microscopy is essential to visualize the details of protozoal structure. From the point of view of functional and physiologic complexity, a protozoan is more like an animal than like a single cell.

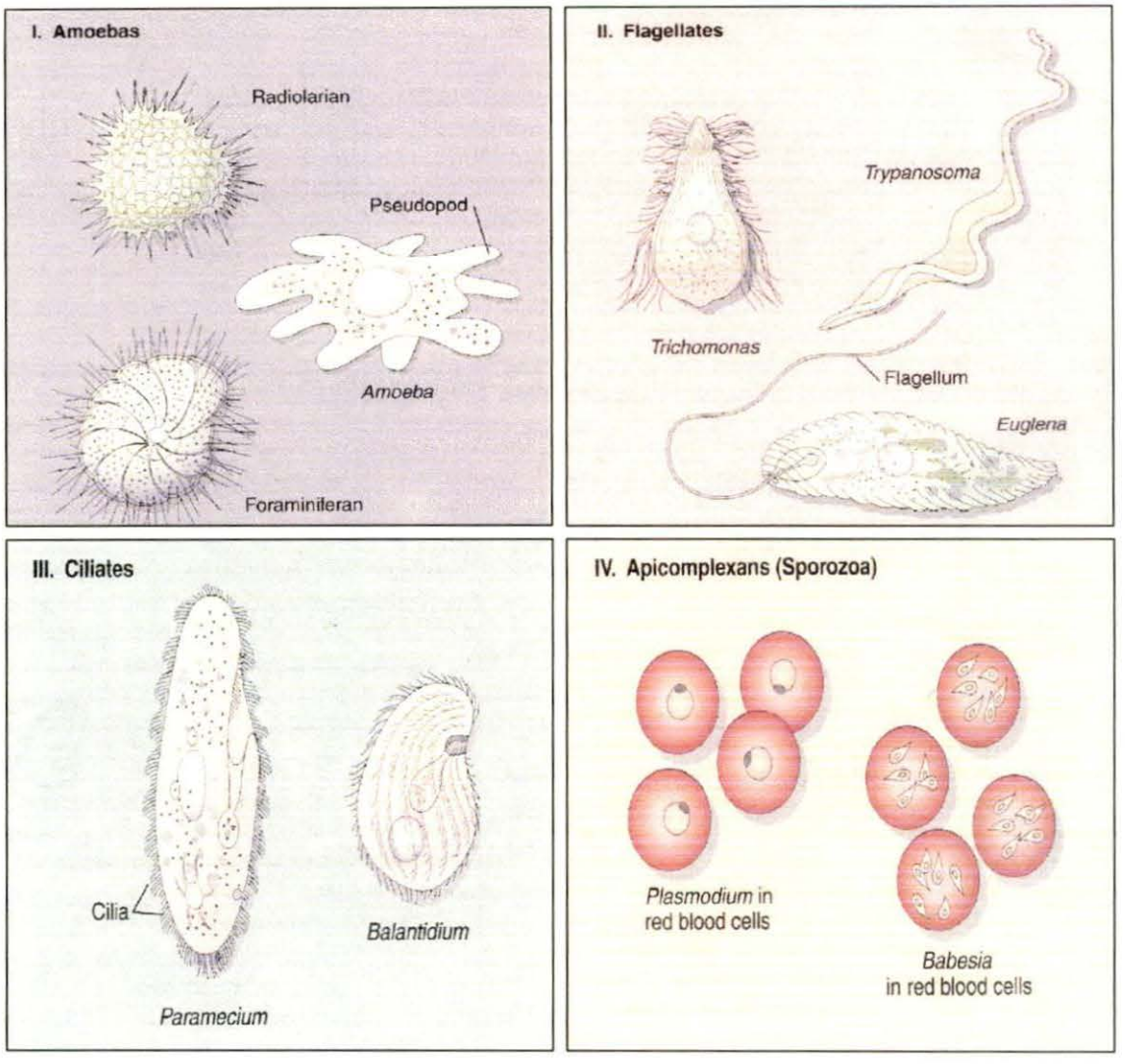

Fig. 1. Fine structure of different protozoan parasite

1.3. Characteristics of Protozoa

- Largest organisms included in microbial world.
- Except a few, they lack chlorophyll or other photosynthetic pigments.
- Though unicellular, but able to perform all functions characteristic of multi-cellular organisms.
- Mostly live in water, damp soil or mud, in drainage ditches or puddles, in ponds or ocean.
- A great diversity in form.
- Cells enclosed by a membrane, which in some cases surrounded by a pellicle containing chitin like material for rigidity. Cell wall, however, is absent. True nucleus, flagella, cilia are present. Freshwater forms take in water by osmosis and eliminate it via organelles called contractile vacuoles.
- Nutrition by ingestion i.e. engulfs food particles by phagocytosis or through special organs of ingestion. A membrane encloses the particle, forming a food vacuole. The latter commonly joins with a submicroscopic organelle, lysosome that contains digestive enzymes.
- Generally heterotrophic, saprobic, some pathogenic. The feeding form is commonly known as the trophozite.
- Aerobic in Whittaker's system, they along with certain algae are placed in the kingdom Protista.

1.4. Classification

In 1985 the Society of Protozoologists published a taxonomic scheme that distributed the Protozoa into six phyla. Two of these phyla the Sarcomastigophora and the Apicomplexa contain the most important species causing human disease. This scheme is based on morphology as revealed by light, electron, and scanning microscopy. *Dientamoeba fragilis*, for example, had been thought to be an ameba and placed in the family Entamoebidae. However, internal structures seen by electron microscopy showed that it is properly placed in the order Trichomonadida of flagellate protozoa. In some instances,

organisms that appear identical under the microscope have been assigned different species names on the basis of such criteria as geographic distribution and clinical manifestations; a good example is the genus *Leishmania*, for which subspecies names are often used. Biochemical methods have been employed on strains and species to determine isoenzyme patterns or to identify relevant nucleotide sequences in RNA, DNA, or both. Extensive studies have been made on the kinetoplast, a unique mitochondrion found in the hemoflagellates and other members of the order Kinetoplastida. The DNA associated with this organelle is of great interest. Cloning is widely used in taxonomic studies, for example to study differences in virulence or disease manifestations in isolates of a single species obtained from different hosts or geographic regions. Antibodies (particularly monoclonal antibodies) to known species or to specific antigens from a species are being employed to identify unknown isolates. Eventually, molecular taxonomy may prove to be a more reliable basis than morphology for protozoan taxonomy, but the microscope is still the most practical tool for identifying a protozoan parasite.

Table: 1.1. Classification of parasitic protozoa and associated diseases

Phylum	Subphylum	Representative genera	Major diseases produced in human beings
Sarcomastigophora (With flagella, Pseudopodia, or both)	Mastigophora	Leishmania	Visceral, cutaneous and mucocutaneous infection
		Trypanosoma	Sleeping sickness Chagas' disease
		Giardia	Diarrhea
		Trichomonas	vaginitis
	Sarcodina (Pseudopodia)	Entamoeba	Dysentery, liver abcess
		Dientamoeba	Colitis
		Naegleria and Acanthamoeba	CNS and corneal ulcer
		Babesia	Babesiosis
Apicomplexa (Apical complex)		Plasmodium	Malaria
		Isospora	Diarrhoea
		Sarcocystis	Diarrhoea
		Cryptosporidium	Diarrhoea
		Toxoplasma	Toxoplasmosis
Microspora		Enterocytozoon	Diarrhoea
Ciliophora (with cillia)		Balantidium	Dysentery

1.5. Life cycle

During its life cycle, a protozoan generally passes through several stages that differ in structure and activity. Trophozoite (Greek for "animal that feeds") is a general term for the active, feeding, multiplying stage of most protozoa. In parasitic species this is the stage usually associated with pathogenesis. In the hemoflagellates the terms amastigote, promastigote, epimastigote, and trypomastigote designate trophozoite stages that differ in the absence or presence of a flagellum and in the position of the kinetoplast associated with the flagellum. A variety of terms are employed for stages in the Apicomplexa, such as tachyzoite and bradyzoite for *Toxoplasma gondii*. Other stages in the complex asexual and sexual life cycles seen in this phylum are the merozoite (the form resulting from fission of a multinucleate schizont) and sexual stages such as gametocytes and gametes. Some protozoa form cysts that contain one or more infective forms. Multiplication occurs in the cysts of some species so that excystation releases more than one organism. For example, when the trophozoite of *Entamoeba histolytica* first forms a cyst, it has a single nucleus. As the cyst matures nuclear division produces four nuclei and during excystation four uninucleate metacystic amebas appear. Similarly, a freshly encysted *Giardia lamblia* has the same number of internal structures (organelles) as the trophozoite. However, as the cyst matures the organelles double and two trophozoites are formed. Cysts passed in stools have a protective wall, enabling the parasite to survive in the outside environment for a period ranging from days to a year, depending on the species and environmental conditions. Cysts formed in tissues do not usually have a heavy protective wall and rely upon carnivorism for transmission. Oocysts are stages resulting from sexual reproduction in the Apicomplexa. Some apicomplexan oocysts are passed in the feces of the host, but the oocysts of *Plasmodium*, the agent of malaria, develop in the body cavity of the mosquito vector.

1.6. Reproduction

Reproduction in the protozoa may be asexual, as in the amebas and flagellates that infect humans, or both asexual and sexual, as in the apicomplexa of medical importance. The most common type of asexual multiplication is binary fission, in which the organelles are duplicated and the protozoan then divides into two complete organisms. Division is longitudinal in the flagellates and transverse in the ciliates; amebas have no apparent

anterior-posterior axis. Endodyogeny is a form of asexual division seen in *Toxoplasma* and some related organisms. Two daughter cells form within the parent cell, which then ruptures, releasing the smaller progeny, which grow to full size before repeating the process. In schizogony, a common form of asexual division in the Apicomplexa, the nucleus divides a number of times, and then the cytoplasm divides into smaller uninucleate merozoites. In *Plasmodium, Toxoplasma*, and other apicomplexans, the sexual cycle involves the production of gametes (gamogony), fertilization to form the zygote, encystation of the zygote to form an oocyst, and the formation of infective sporozoites (sporogony) within the oocyst.

Some protozoa have complex life cycles requiring two different host species; others require only a single host to complete the life cycle. A single infective protozoan entering a susceptible host has the potential to produce an immense population. However, reproduction is limited by events such as death of the host or by the host's defense mechanisms, which may either eliminate the parasite or balance parasite reproduction to yield a chronic infection. For example: leishmania, plasmodium species.

Most free-living protozoa reproduce by cell division (exchange of genetic material is a separate process and is not involved in reproduction in protozoa). The relative importance for population growth of biotic versus chemical-physical components of the environment is difficult to ascertain from the existing survey data.

1.7. Nutrition

The nutrition of all protozoa is holozoic; that is, they require organic materials, which may be particulate or in solution. Amebas engulf particulate food or droplets through a sort of temporary mouth, perform digestion and absorption in a food vacuole, and eject the waste substances. Many protozoa have a permanent mouth, the cytosome or micropore, through which ingested food passes to become enclosed in food vacuoles. Pinocytosis is a method of ingesting nutrient materials whereby fluid is drawn through small, temporary openings in the body wall. The ingested material becomes enclosed within a membrane to form a food vacuole.

Protozoa are found living actively in nutrient-poor to organically rich waters and in fresh water varying between 0- 50°C . Nonetheless, it appears that rates of population growth increase when food is not constrained and temperature is increased [8-10]. Comparisons

of oxygen consumption in various taxonomic groups show wide variation [11], with some aerobic forms able to function at extremely low oxygen tensions and to thereby avoid competition and predation. Many parasitic and a few free-living species are obligatory anaerobes (grow without atmospheric oxygen). Of the free-living forms, the best known is the plagiopylid ciliates that live in the anaerobic sulfide-rich sediments of marine wetlands. The importance of plagiopylids in recycling nutrients to aerobic zones of wetlands is potentially great [12].

Protozoa have metabolic pathways similar to those of higher animals and require the same types of organic and inorganic compounds. In recent years, significant advances have been made in devising chemically defined media for the in vitro cultivation of parasitic protozoa. The resulting organisms are free of various substances that are present in organisms grown in complex media or isolated from a host and which can interfere with immunologic or biochemical studies. Research on the metabolism of parasites is of immediate interest because pathways that are essential for the parasite but not the host are potential targets for antiprotozoal compounds that would block that pathway but be safe for humans. Many antiprotozoal drugs were used empirically long before their mechanism of action was known. The sulfa drugs, which block folate synthesis in malaria parasites, are one example.

The rapid multiplication rate of many parasites increases the chances for mutation; hence, changes in virulence, drug susceptibility, and other characteristics may take place. Chloroquine resistances in *Plasmodium falciparum* and arsenic resistance in *Trypanosoma rhodesiense* are two examples.

Competition for nutrients is not usually an important factor in pathogenesis because the amounts utilized by parasitic protozoa are relatively small. Some parasites that inhabit the small intestine can significantly interfere with digestion and absorption and affect the nutritional status of the host; *Giardia* and *Cryptosporidium* are examples. The destruction of the host's cells and tissues as a result of the parasites' metabolic activities increases the host's nutritional needs. This may be a major factor in the outcome of an infection in a malnourished individual. Finally, extracellular or intracellular parasites that destroy cells while feeding can lead to organ dysfunction and serious or life-threatening consequences.

1.8. Protozoa: As parasites

Protozoa generally exist in two basic forms: the active, growing form called the "trophozoite;" and the dormant, resistant form called the "cyst." The trophozoite form proliferates tissues, causing damage that result in clinical diseases. The cyst is able to survive in an external environment and is usually the form that is transmitted from host to host. Some protozoa go through an intermediate stage in blood-sucking insects.

The four groups of protozoa that are mainly responsible for human disease include the following: sarcodina, ciliophora, mastigophora and sporozoa. All grouped according to their form of locomotion.

- **Sarcodina**, commonly known as amoebas, move by extending a section of their cytoplasm (called a pseudopodium or false foot) in one direction, causing the remainder to follow. They are usually found in marine and fresh water. Members include eight species (see Endoparasites). Three are parasitic to humans, with one causing more of a problem than the others. (*Entamoeba histolytica* causes the disease amebiasis.)
- **Ciliophora or Ciliates**, move by using the many fine cilia that beat in rhythmic patterns to propel the organism. Members include and Paramecium, but only one species causes disease in humans; and that is of a dysentery nature. *Balantidium coli* are a large oval-shaped cell that is the largest intestinal protozoa found in humans. Increasingly, it is showing up in the human intestinal tract, where it can invade and destroy the intestinal lining. Its normal habitat is the intestinal tract of hogs, but it can also be found in marine and fresh water worldwide, causing the disease known as balantidiasis. The life cycle is similar to that of the amoeba *E. histolytica* and has been associated with chronic fatigue syndrome.
- **Mastigophora** is a subphylum of protozoa that has one or more whip like flagella that propel the organism like swimmers. They are commonly known as Flagellates and are normally found in fresh water. Two relatively mild diseases, trichomoniasis and giardiasis, are produced from them, as well as the more serious diseases of trypanosomiasis and leishmaniasis.

- **Sporozoa** (singular sporozoon) is a class of parasitic protozoa that include Plasmodium and Toxoplasma. These two are commonly known as the parasites, found in vectors responsible for malaria and toxoplasmosis. They have both a sexual and asexual phase. They mainly target the epithelial cells of the intestinal tract, but can also be found in the liver and other organs

Protozoa are infamous for their role in causing disease, and parasitic species are among the best-known protozoa. Nevertheless, our knowledge has large gaps, especially of normally free-living protozoa that may become pathogenic in immuno-compromised individuals. For example, microsporidia comprise a unique group of obligate, intracellular parasitic protozoa. Microsporidia are amazingly diverse organisms with more than 700 species and 80 genera that are capable of infecting a variety of plant, animal, and even other protist hosts. They are found worldwide and have the ability to thrive in many ecological conditions. Until the past few years, their ubiquity did not cause a threat to human health, and few systematists worked to describe and classify the species. Since 1985, however, physicians have documented an unusual rise in worldwide infections in AIDS patients caused by four different genera of microsporidia (*Encephalitozoon*, *Nosema*, *Pleistophora*, and *Enterocytozoon).* According to the Centers for Disease Control in the United States, difficulties in identifying microsporidian species are impeding diagnosis and effective treatment of AIDS patients. There are over 50,000 species of protozoa, of which a fifth are parasitic, some 10,000 species. They infect vertebrates and invertebrates and some are even parasitic in plants. Parasitic protozoa are, in general, small, have short generation times, high rates of reproduction and a tendency to induce immunity to reinfection in those hosts that survive. Structurally a protozoan is equivalent to a single eukaryotic cell. Among the unique features in protozoa are the mega- and micronucleus found in Ciliates and the *kinetoplast*, a DNA containing structure in the mitochondrion of kinetoplastid flagellates. Parasitic protozoa are in no way simple or degenerate and adaptations to parasitism frequently include complex life cycles and specialized ways of entering and maintaining themselves in their hosts. It is, therefore, surprisingly humans and their domesticated animals should act as hosts to protozoa, but the diseases thus caused are out of all proportion to the number of species involved. The protozoa that infect humans range from forms that are never pathogenic to those that cause malaria, sleeping sickness, Chagas' disease and leishmaniasis, now

regarded as being among the major diseases of tropical countries, and which together threaten over one quarter of the population of the world [13].

Table: 1. 2. List of protozoan diseases in human

Disease	Causative agent	Motion by	Transmission
Amoebiasis	*Entamoeba histolytica* (Sarcodina)	Pseudopodia	Water, Food
Giardiasis	*Giardia lambila* (Mastigophora)	Flagella	Water, Contact
Trichomoniasis	*Trichomonas vaginalis* (Mastigophora)	Flagella	Sexual,Contact
African Sleeping Sickness	*Trypanosoma brucei* (Mastigophora)	Flagella	Tsetse fly (Glossina)
American Sleeping Sickness	*Trypanosoma cruzi* (Mastigophora)	Flagella	Triatomid bug (Triatoma)
Leishmaniasis (Kala - azar)	*Leishmania donovani* (Mastigophora)	Flagella	Sandfly (Phlemotomus)
Balantidiasis	*Balantidium coli* (Ciliophora)	Cilia	Food, Water
Toxoplasmosis	*Toxoplasma gondii* (Sporozoa)	NA	Cats, Food
Malaria	Plasmodium Spp. (Sporozoa)	NA	Mosquito (Anopheles)
Babesiosis	*Babesia microti* (Sporozoa)	NA	Tick (Ixodes)