NATIONAL OPEN UNIVERSITY OF NIGERIA

FACULTY OF SCIENCES

COURSE CODE: BIO 310

COURSE TITLE: PROTOZOOLOGY

Course Code & Course Title:	BIO 310: Protozoology
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Reviewed: 2022

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Published by National Open University of Nigeria

Printed 2017

Reviewed 2022

Course Guide

Introduction

BIO 310: Protozoology is a one-semester, 2 credit- hour course in the Department of Biological Sciences. It is a 300 level, second semester undergraduate course offered to students admitted in the school of science and technology, school of education that are offering Biology or related programmes. This course is designed primarily for students in Biology disciplines to expose them to the beneficial and economic important of parasite surrounding our environment and also give them a basic background on how the parasite interact within our environment, may be through our food we eat. Also students should be able to get acquainted with genera and species of protozoans that constitutes intestinal and urogenital protozoans. Parasites are organism that obtains food and shelter from another organism and derives all benefits from this association. The scope of this course also encompasses the features of different parasites. One of the parasite are termed obligate when it can live only in a host; while the other one is classified as facultative when it can live both in a host as well as in free form. In this course we will also look at parasites that live inside the body which are termed endo-parasites whereas those that exist on the body surface are called ecto-parasites. Parasites that cause harm to the host are pathogenic parasites while those that benefit from the host without causing it any harm are known as commensals. At the end of this course, student should be able to have a clear knowledge of a parasite, the different types of parasites and also have a good knowledge of protozoans that are considered to be primarily of importance in animals but could also infect man (zoonotic importance).

The course guide tells you briefly what the course is all about, what course materials you will be using and how you can work your way through these materials. It gives you some guidance on your Tutor- Marked Assignments.

There is/are Self-Assessment Exercise(s) within the body of a unit and/or at the end of each unit. The exercise(s) is/are an overview of the unit to help you assess yourself at the end of every unit.

Course Competencies

This course is to provide a generalized survey of the protozoa parasite with reference to their external features, life cycle, ecological adaptation, symptom, pathology and their treatment and control.

Course Objectives

In addition to the course competencies, the course sets an overall objective which must be achieved. In addition to the course objectives, each of the units has its own specific

objectives. You are advised to read properly the specific objectives for each unit at the beginning of that unit. This will help you to ensure that you achieve the objectives.
As you go through each unit, you should from time to time go back to these objectives to ascertain the level at which you have progressed.

By the time you have finished going through this course, you should be able to:

- Have a clear knowledge of what parasites are and the different types of parasites
- Have a clear understanding of the phylum apicomplexa, its characteristics and replication
- Be clearly introduced to protozoans, its structure and functions.
- Have a good understanding of blood and tissue protozoans of major biological significance
- Be able to differentiate between the various protozoans using their morpholo gical and life cycle characteristics
- Be able to differentiate between the various species within the genera.
- Be acquinted with genera and species of protozoans that constitutes intestinal and urogenital protozoans.
- Know their epidemiology, morphological characteristics and life cycle.
- Have a good knowledge of protozoans that are considered to be primarily of importance in animals but could also infect man (zoonotic importance).
- Know protozoans parasitizing other organs aside from blood and intestines

Working through this Course

In this course, you will be advised to devote your time in reading through the material. You would be required to do all that has been stipulated in the course: study the course units, read the recommended reference textbooks and do all the unit(s) self- assessment exercise (s) and at some points, you are required to submit your assignment (TMAs) for assessment purpose. You should therefore avail yourself of the opportunity of being present during the tutorial sessions so that you would be able to compare knowledge with your colleagues.

Study Units

This course is divided into 4 modules with a total of twenty units which are divided as follows:

MODULE 1: GENERAL INTRODUCTION OF PROTOZOAN

Unit 1: Features/Relationship, roles and Reproduction of Protozoan

Unit 2: Ecology, Physiology and Biochemistry of Protozoan

Unit 3: Taxonomy of Protozoan

Unit4: Phylum Apicomplexa

Unit 5: Structure and Function of Protozoan

MODULE 2: BLOOD AND TISSUE PROTOZOA

Unit 1: Trypanosome

Unit 2: Leishmania

Unit 3: Plasmodium

Unit4: Babesia

Unit 5: Toxoplasma

MODULE 3: LUMINAL PROTOZOA

Unit 1: Amebae

Unit 2: Giardia

Unit 3: Trichomoniasis

Unit 4: *Blastocystis hominis*

Unit 5: *Dientamoeba fragilis*

MODULE 4: OTHER INTESTINAL/FREE LIVING PROTOZOA

Unit 1: *Balantidium coli*

Unit 2: *Cryptosporidium parvum*

Unit 3: *Isospora belli*

Unit 4: *Acanthamoeba* sp.

Unit 5: *Naegleria fowler*

References and Further Readings

You would be required to do all that has been stipulated in the course: study the course units and read the recommended reference textbooks in each unit of the course material.

Presentation Schedule

Presentation schedule for this course will be uploaded on the online course page.

Assessment

You are required to submit your assignment (TMAs) for assessment purpose.

How to get the Most from the Course

The course comes with a list of recommended textbooks. These textbooks are supplement to the course materials so that you can avail yourself of reading further. Therefore, it is advisable you acquire some of these textbooks and read them to broaden your scope of understanding.

Online Facilitation

Online facilitation for this course will hold once in a week for the period of eight weeks. The time and day for the online facilitation will be one hour between 5-6pm, every Tuesdays in a week for the period of eight weeks.

Course Information

Course Code: BIO 310
Course Title: Protozoology
Credit Unit: Two (2)
Course Status: Compulsory
Course Blub: This course is designed primarily for students in Biology disciplines to expose them to the beneficial and economic important of parasite surrounding our environment and also give them a basic background on how the parasite interract within our environment, Finally, the students should be acquainted with genera and species of protozoans that constitutes intestinal and urogenital protozoans.

Semester: Second Semester
Course Duration: 13 weeks
Required Hours for Study: 65 hours

Ice Breaker

I am Dr. Andem, Andem Bassey, a Senior Lecturer in the University of Calabar and external facilitator in National Open University. I facilitate and coordinate courses online such as BIO 405 and BIO 310, supervise project, coordinate seminar, coordinate field trip, moderate examination questions, review courses and mark exam scripts for National Open University. The links below are my research ID URL: https://scholar.google.com/citations, https://unical-ng.academia.edu/ANDEMANDEM,
https://www.researchgate.net/profile/Andem_Andem4/publications,
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Module 1: *General Introduction of Protozoans*

Module Structure

In this module we will discuss about the general introduction of protozoans with the following units:

Unit 1: Features/Relationship, roles and Reproduction of Protozoan

Unit 2: Ecology, Physiology and Biochemistry of Protozoan

Unit 3: Taxonomy of Protozoan

Unit4: Phylum Apicomplexa

Unit 5: Structure and Function of Protozoan

Glossary
End of the module Questions

Unit 1: Features/Relationship, roles and Reproduction of Protozoan

Unit Structure

1.1 Introduction
1.2 Intended Learning Outcomes (ILOs)
1.3 Morphology of Protozoa
1.4 Symbiotic Association/Relationship
1.5 Reproduction in Protozoa
1.6 Summary
1.7 References/Further Readings/Web Sources
1.8 Possible Answers to SAEs

1.1 Introduction

Deciphering the Greek roots results in defining protozoa as 'first' (proto) 'animals' (zoa). Although molecular phylogenetic studies indicate that protozoa are among the earliest branching eukaryotes. Such a definition does not provide much descriptive information. Protozoa are not easily defined because they are diverse and are often only distantly related to each other. “Due to the extreme diversity of the protozoa the only feature common to all protozoa is that they are unicellular eukaryotic micro-organisms. Protozoa possess typical eukaryotic organelles and in general exhibit the typical features of other eukaryotic cells. For example, a membrane bound nucleus containing the chromosomes is found in all protozoan species. However, in many protozoan species some of the organelles may be absent, or are morphologically or functionally different from those found in other eukaryotes. In addition, many of the protozoa have organelles that are unique to a particular group of protozoa.
A parasite is an organism that obtains food and shelter from another organism and derives all benefits from this association. The parasite is termed obligate when it can live only in a host; it isclassified as facultative when it can live both in a host as well as in free form. Parasites that live inside the body are termed endo-parasites whereas those that exist on the body surface are called ecto-parasites. Parasites that cause harm to the host are pathogenic parasites while those that benefit from the host without causing it any harm are known as commensals.

1.2 Intended Learning Outcomes (ILOs)

By the end of this lecture unit, students should be able to:

- have a good understanding on the feature, relationship, roles and reproduction of protozoan

1.3 Morphology of Protozoa

Protozoa exhibit a wide variety of morphologies. There is no one shape or morphology which would include a majority of the protozoa.

Shapes range from the amorphous and ever-changing forms of ameba to relatively rigid forms dictated in part by highly ordered cytoskeletons or secreted walls or shells.

Several protozoan species express photosynthetic or other pigments and thus are colored.

Many protozoan species exhibit complex life cycles with multiple stages. Sometimes the different life cycle stages are so dissimilar that they have been mistaken for completely different species.

Protozoa--except for a few colonial forms--are unicellular, or single-celled, organisms; although, some argue that they are actually 'acellular'. Thus, the vast majority of protozoa are microscopic. However, they do exhibit an incredibly large range of sizes. Extant species range in size from <1 µm (10^{-6} meter) to several mm.

Protozoa are found in moist environments virtually everywhere. As a group, the protozoa are extremely adaptable. Individual species, though, generally have specific niches. Like all other organisms, protozoa must be able to acquire and metabolize nutrients from their environment (i.e., heterotrophic).

Many protozoa simply absorb solutes (i.e., osmotrophy) from their media, while some are scavengers that ingest solid material (i.e., phagotrophy).

Predatory protozoa either actively hunt down or passively ambush other organisms (typically bacteria or other protozoa).

Some protozoa are photosynthetic and can capture the energy of the sun and convert it to usable chemical energy (i.e., autotrophic or phototrophic).

Many protozoa are not restricted to a single feeding mechanism and can utilize combinations of the above (i.e., mixotrophic).

Protozoa can also be viewed as free-living or symbiotic. Generally free-living organisms are found in the soil or aqueous environments, whereas symbionts live in close association with another organism. Symbiosis implies a physiological dependency of one organism on another organism and not just a close physical association between two organisms. Generally this dependency is in the form of nutrition. Different forms of symbiosis can be distinguished which reflect the nature of the association between the two organisms.

1.4 Symbiotic Association/Relationship

Commensalism: Is an interaction that is beneficial to one organism but has no effect on the other organism? For example, many protozoa live in the alimentary canal of another organism without harming it. These commensals are often scavengers or predators that exploit the abundance of nutrients or bacterial fauna provided by the host organism.

Mutualism: Is a special form of commensalism in which both organisms derive some benefit and have become dependent on each other. The classic example of mutualism is the protozoan *Trichonympha* found in the gut of

termites. *Trichonympha*, with the assistance of a symbiotic bacteria, digests the wood particles (i.e. cellulose) ingested by the termite.

Parasitism: Is a relationship in which one organism (the parasite) benefits at the expense of the other organism (the host)? Generally this host expense implies that the parasite takes in macromolecules from the host and releases others into the host. In some instances the parasitism will be overtly harmful to the host and referred to as being pathogenic.

Self-Assessment Exercise 1

1. ***Define Commensalism?***
2. ***Define mutualism?***

1.5 Reproduction in Protozoa

Protozoa, like all other organisms, reproduce. The most common form of reproduction in protozoa is asexual binary fission. In other words, a single organism will divide into two equal organisms. A slight modification of this binary fission, called budding, is when one of the newly formed cells is smaller than the other.

Typically the larger cell is called the mother and the smaller is the daughter. Some protozoa will form an intracellular bud and essentially give birth. Another variation of binary fission is a multiple fission or segmentation. In this situation, several rounds of nuclear replication occur without cytokinesis. This multinucleated cell will then form multiple progeny simultaneously. Many protozoa exhibit sexual reproduction in addition to the asexual forms of reproduction. This sexual reproduction can involve the production and fusion of gametes in processes similar to higher organisms. The Ciliophora undergo a conjugation in which opposite mating types will pair and directly exchange genetic material (i.e., DNA). Sometimes sexual reproduction is an obligatory step in the life cycle, whereas in other cases the organism can reproduce asexually with an occasional round of sexual reproduction.

What is a Parasite?

Self-Assessment Exercise 2

1. ***Highlight about four (4) characteristic of Protozoa?***
2. ***What is the different between Osmotrophy and Phagotrophy?***

1.6 Summary

In this unit, you have learnt about the general features of protozoa, Symbiotic Association/Relationship of protozoa and Reproduction in Protozoa. Protozoa can also be viewed as free-living or symbiotic. Generally free-living organisms are found in the soil or aqueous environments, whereas symbionts live in close association with another organism. Symbiosis implies a physiological dependency of one organism on another organism and not just a close physical association between two organisms.

1.7 References/Further Readings/Web Sources

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https://www.youtube.com/watch?v=cZrf6RAspvY

1.8 Possible Answers to SAEs

Answers to SAEs 1

1. *Commensalism is an interaction that is beneficial to one organism but has no effect on the other organism? For example, many protozoa live in the alimentary canal of another organism without harming it. These commensals are often scavengers or predators that exploit the abundance of nutrients or bacterial fauna provided by the host organism.*
2. *Mutualism is a special form of commensalism in which both organisms derive some benefit and have become dependent on each other. The classic example of mutualism is the protozoan Trichonympha found in the gut of termites. Trichonympha, with the assistance of a symbiotic bacteria, digests the wood particles (i.e. cellulose) ingested by the termite*.

Answers to SAEs 2

1. *Many protozoan species exhibit complex life cycles with multiple stages. Sometimes the different life cycle stages are so dissimilar that they have been mistaken for completely different species. Protozoa--except for a few colonial forms--are unicellular, or single-celled, organisms; although, some argue that they are actually 'acellular'. Thus, the vast majority of protozoa are microscopic. However, they do exhibit an incredibly large range of sizes. Extant species range in size from <1 μm (10^{-6} meter) to several mm. Protozoa are found in moist environments virtually everywhere. As a group, the protozoa are extremely adaptable. Individual species, though, generally have specific niches. Like all other organisms, protozoa must be able to acquire and metabolize nutrients from their environment (i.e., heterotrophic). Some protozoa are photosynthetic and can capture the energy of the sun and convert it to usable chemical energy (i.e., autotrophic or phototrophic).*
2. *Osmotrophy: protozoa that simply absorb solutes from their media, while Phagotrophy: some protozoan that are scavengers that ingest solid material.*

Unit 2: Ecology, Physiology and Biochemistry of Protozoa

Unit Structure

2.1 Introduction

Free-living protozoa are found in almost all ecosystems that contain, at least some of the time, free water. They have a critical role in the mobilization of nutrients in natural ecosystems. Their role is best conceived within the context of the microbial food web in which they include the most important bacterivores. In part, they facilitate the transfer of bacterial and algal production to successive trophic levels, but also they solubilize the nutrients within microbial biomass, allowing stimulation of microbial growth. As consumers, protozoa prey upon unicellular or filamentous algae, bacteria, microfungi, and micro-carrion. In the context of older ecological models of the micro- and meiofauna, protozoa may be a food source for macroinvertebrates.

2.2 Intended Learning Outcomes (ILOs)

By the end of this lecture unit, students should be able to:

- have a good understanding on the ecology, physiology and biochemistry of protozoan

2.3 Ecology of Protozoan

Protozoans play important roles in the fertility of soils. By grazing on soil bacteria, they regulate bacterial populations and maintain them in a state of physiological youth, i.e., in

the active growing phase. This enhances the rates at which bacteria decompose dead organic matter. Protozoans also excrete nitrogen and phosphorus, in the form of ammonium and orthophosphate, as products of their metabolism, and studies have shown that the presence of protozoans in soils enhances plant growth. Protozoans play important roles in wastewater treatment processes, in both activated sludge and slow percolating filter plants. In both processes, after solid wastes are removed from the sewage, the remaining liquid is mixed with the final sludge product, aerated, and oxidized by aerobic microorganisms to consume the organic wastes suspended in the fluid. In the activated sludge process, aerobic ciliates consume aerobic bacteria, which have flocculated (formed loose aggregates, making them easily separated from liquid). In the percolating filter process, substrates are steeped in microorganisms, such as fungi, algae, and bacteria, which provide food for oxidizing protozoans. In the final stages of both processes, solids settle out of the cleaned effluent in the settlement tank. Treatment plants with no ciliates and only small numbers of amoebae and flagellates produce turbid effluents containing high levels of bacteria and suspended solids. Good-quality, clean effluents are produced in the presence of large ciliated protozoan communities because they graze voraciously on dispersed bacteria and because they have the ability to flocculate suspended particulate matter and bacteria.

Protozoans probably play a similar role in polluted natural ecosystems. Indeed, there is evidence that they, by feeding on oil-degrading bacteria, increase bacterial growth in much the same way that they enhance rates of decomposition in soils, thereby speeding up the breakdown of oil spillages. Some radiolarians and foraminiferans harbour symbiotic algae that provide their protozoan hosts with a portion of the products of photosynthesis. The protozoans reciprocate by providing shelter and carbon and essential phytonutrients. “Many ciliates contain endosymbiotic algae, and one species, *Mesodinium rubrum*, has formed such a successful relationship with its red-pigmented algal symbiont that it has lost the ability to feed and relies entirely on symbiosis for its livelihood. *Mesodinium* often forms dense red blooms, or red tides, when it reaches high densities in water. Among the ciliates with endosymbionts, *Mesodinium* is the only completely photosynthetic species. Other ciliates achieve photosynthesis in another way”. Although they do not have symbiotic algae, they consume plantlike flagellates, sequester the organelles that contain the plant pigments, and use them for photosynthesis. These organelles are known as plastids. Because the isolated plastids eventually age and die, they must be replaced continuously. The impact of protozoan grazing on phytoplankton can be considerable. It has been estimated that at least half of the phytoplankton production in marine waters is consumed by protozoans. Like the soil protozoans, these planktonic protozoans excrete nitrogen and phosphorus at high rates. The protozoans are a fundamental component in recycling essential nutrients (nitrogen and phosphorus) to the phytoplankton. The protozoan cell carries out all of the processes including feeding, growth, reproduction, excretion, and movement necessary to sustain and propagate life. The cell is enclosed in a membrane called the plasma membrane”. Like all membranous structures in the eukaryotic cell, the plasma membrane is composed of mostly lipid and some protein molecules. The plasma membrane is a barrier between the cell cytoplasm and the outside liquid environment. Some substances,

such as oxygen, readily pass through the membrane by diffusion (passive transport), while others must be transported across at the expense of energy (active transport). Cilia and flagella arising from the cell are also sheathed in the cell membrane; this is in contrast to bacterial flagella, which are not surrounded by a membrane. The cell also has internal membranes, which are not as thick as the plasma membrane. Among these are the endoplasmic reticulum, whose membranes separate compartments of the cell, thereby allowing different conditions to be maintained in various parts e. g. separation of deleteriously reactive substances. Enzymes are arranged on the surface of the endoplasmic reticulum; one such enzyme system catalyzes the activity of the ribosomes during protein synthesis. The Golgi apparatus is a cluster of flattened vesicles, or cisternae, associated with the endoplasmic reticulum. The vesicles are involved in membrane maturation and the formation and storage of the products of cell synthesis, as in the formation of scales on the surface coat of some flagellates, for example. The scales are formed within the Golgi and are transported by the vesicles to the plasma membrane, where they are incorporated onto the surface of the cell. The Golgi apparatus is poorly evident in most ciliates and absent from some amoebae. All protozoans possess at least one nucleus, and many species are multinucleate.

Self-Assessment Exercise 1

1. ***Briefly explain the role of protozoan in the fertility of soil?***
2. ***Briefly explain the* impact of protozoan grazing on phytoplankton?**

2.4 Physiology and Biochemistry of Protozoan

The genetic material DNA (deoxyribonucleic acid) is contained within the chromosomes of the nucleus. Each nucleus is bounded by two unit membranes possessing pores that permit the passage of molecules between the cytoplasm and the nucleoplasm. Most ciliates have two types of nuclei: micronuclei and macronuclei. The macronucleus is the somatic, or non-reproductive, nucleus. It is large and it is polyploid, meaning that it contains more than two sets of chromosomes (the condition of two sets of chromosomes is described as diploid). In contrast, the micronucleus is germinal (responsible for transfer of genetic information during sexual reproduction) and diploid. The macronucleus can be quite variable in shape, resembling in some species a string of beads or a horseshoe. It directs the normal functioning of the cell and usually disintegrates during sexual reproduction, to be re-formed from the products of micronuclear division after the sexual phase is completed. Protozoans have transitory food or digestive vacuoles. The number of these membrane-bound cell organelles depends on the feeding habits of the organism. Some species may have many, whereas others may contain only one or two at any one time. In ciliates the food vacuoles form at the base of the cytopharynx, whereas in species without a cell “mouth,” or cytostome, the

vacuoles form near the cell membrane at the site where food is ingested. Within the cell, structural proteins of various types form the cytoskeleton (cell skeleton) and the locomotory appendages. They include microfilaments formed of a contractile protein also found in the muscles of animals (actin) and cylindrical microtubules formed from filaments of the protein tubulin. Microtubules are particularly important in the structural formation and functioning of cilia and flagella. Filopodia of certain rhizarian species are supported by microtubules.

Organisms acquire organic material from their environments and convert this material into energy or their own substance (i.e., biomolecules). Cells are made of distinct classes of biomolecules with specific functions. These macromolecules are synthesized from small molecular weight precursors or building blocks. These molecular precursors are components of interconnected metabolic pathways. The malaria parasite exhibits a rapid growth and multiplication rate during many stages of its life cycle. This necessitates that the parasites, like all other organisms, acquire nutrients and metabolize these various biological molecules in order to survive and reproduce. Obviously, the parasite's metabolism will be intertwined with that of the host's because of the intimate relationship between the host and parasite. These host-parasite interactions are further complicated by the complex life cycle of the parasite involving vertebrate and invertebrate hosts as well as different locations within each of these hosts. A better understanding of the parasite's metabolism may lead to the development of novel therapeutic strategies which exploit the uniqueness of the parasite. The malaria parasite, like all organisms, must acquire nutrients from the environment and convert these nutrients to other molecules or energy (i.e., catabolism). These other molecules and the energy are then used to maintain the homeostasis of the parasite, and in the growth and reproduction of the parasite (i.e., anabolism). Both anabolic and catabolic processes are catalyzed by enzymes. Growing and reproducing organisms require high levels of macromolecules and other biochemical for the maintenance of cellular structure and function. The malaria parasite needs to acquire these biochemical and precursors from the host. The unique life cycle and resulting microenvironments of the parasite has led to the evolution of metabolic pathways which differ from the human host. It may be possible to exploit these unique pathways and enzymes in the design of therapeutic strategies. For example, many anti-malarials are known to affect the food vacuole which is a special organelle for the digestion of host of host hemoglobin.

Outline at least two roles of protozoa in wastewater treatment processes.

Self-Assessment Exercise 2

1. ***In protozoa, differentiate catabolism and anabolism?***
2. ***Define Biomolecules in protozoan?***

2.5 Summary

In this unit, you have learnt about the ecology, physiology and biochemistry of protozoa. In this way they are different from bacteria which donot have a nucleus and whose single chromosome is coiled like a skein of wool in the cytoplasm. This primitive arrangement, found only in bacteria, rickettsia and certain algae, is called prokaryotic and such organisms may be regarded as neither animal nor plant, but as a separate kingdom of prokaryotic organisms, the Monera.

2.6 References/Further Readings/Web Sources

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2.7 Possible Answers to SAEs

Answers to SAEs 1

1. *By grazing on soil bacteria, they regulate bacterial populations and maintain them in a state of physiological youth, i.e., in the active growing phase. This enhances the rates at which bacteria decompose dead organic matter. Protozoans also excrete nitrogen and phosphorus, in the form of ammonium and orthophosphate, as products of their metabolism, and studies have shown that the presence of protozoans in soils enhances plant growth*.
2. *The impact of protozoan grazing on phytoplankton can be considerable. It has been estimated that at least half of the phytoplankton production in marine waters is consumed by protozoans. Like the soil protozoans, these planktonic protozoans excrete nitrogen and phosphorus at high rates. The protozoans are a fundamental component in recycling essential nutrients (nitrogen and phosphorus) to the phytoplankton. The protozoan cell carries out all of the processes including feeding, growth, reproduction, excretion, and movement necessary to sustain and propagate life.*

Answers to SAEs 2

1. ***Catabolism*** *in protozoa parasite, the malaria parasite, like all organisms, must acquire nutrients from the environment and convert these nutrients to other molecules or energy.* ***Anabolism*** *in protozoa parasite, these other molecules and the energy are then used to maintain the homeostasis of the parasite, and in the growth and reproduction of the parasite.*
2. *Biomolecules in protozoan is define as the organisms acquire organic material from their environments and convert this material into energy or their own substance.*

Unit 3: Taxonomy of Protozoa

Unit Structure

3.1 Introduction

Taxonomy is the science of naming and classifying organisms. In addition to assigning hierarchical taxonomic classifications, systematics also attempts to place organisms into groups reflect evolutionary relationships or phylogenies. However, taxonomic criteria are often arbitrary and taxonomy is always changing to reflect new discoveries and interpretations. Furthermore, utilitarian features, such as type of disease, host range and geographical distribution, are frequently used in the systematics of pathogenic micro-organisms. This is especially true for protozoan taxonomy. In addition, there is some debate on the overall philosophy in the classification of protozoa and the relationships between many protozoan groups are not known.

3.2 Intended Learning Outcomes (ILOs)

By the end of this lecture unit, students should be able to:

- Have a good understanding on the taxonomy of protozoa.

3.3 Historical Systematic of Protozoa

Historically protozoa were divided into four major groups: the ameba, the flagellates, the ciliates, and the sporozoa. The distinguishing features between the groups were based on motility (i.e., ameboid, flagella and cilia). The sporozoa were a heterogeneous group that produced spores during one stage of their life cycles and exhibited ‘gliding’ motility. However, such a classification scheme is quite arbitrary and does not necessarily reflect true evolutionary relationships between organisms. One problem with using motility as taxonomic criteria is that many protozoa utilize different types of motility during different stages of their life cycles. For example, *Naegleria* exists in an ameba form when food is plentiful and transforms into a flagellate when food is absent. In general, the amebas are a heterogeneous group and are all probably derived from flagellates. Among these four original protozoan groups only the ciliates are still considered a valid taxon.

Beginning the 1960's the electron microscope was used to identify Ultrastructural features which could serve as criteria for grouping protozoa. In many cases morphology leads to a classification which places organisms into monophyletic groups. Monophyletic means that all of the organisms in that group are probably derived from a common ancestor. For example, many of the protozoa formerly called sporozoa possess subcellular structures, collectively known as the apical organelles, and now form a monophyletic group called **Apicomplexa**. However, subcellular structures and metabolic pathways can be lost in some lineages and placing those descendants can be problamatic.

During the 1980's and continuing until present time molecular techniques are being applied to taxonomy. Possible evolutionary histories and relationships can be derived by

comparing DNA or protein sequences. Molecular sequence data has confirmed phylogenies based on other criteria, settled some debates, and led to a few surprises. For example, molecular data confirms that the apicomplexa are monophyletic, and furthermore, indicates that they are related to the ciliates and dinoflagellates. These three groups are now combined into a larger monophyletic group called alveolata. This relationship had been previously suspected and the name is in reference to morphological structures known as alveolar sacs. But the use of single genes can be an unreliable means of determining evolutionary relationships, especially among distantly related organisms, and molecular data should be interpreted with caution. Since the mid-1980's classification of protists has been in a state of flux and afflicted with some philosophical controversies. On one hand, there is some argument for retaining elements of the Bütschlian scheme based on motility due to its familiarity and simplicity. However, these schemes are often in conflict with phylogenetic data. In addition, the hierarchies and ranks of the traditional Linnaean systematics (i.e., phylum, class, order, family, genus and species) do not always fit well with micro-organisms, and it is often difficult to decide which hierarchical level is most appropriate for any particular protozoan group.

Previously many taxonomic schemes have defined five kingdoms of life: prokaryotes (bacteria), protists, plants, fungi, and animals. In these schemes the protozoa are part of the Protista along with unicellular algae, diatoms, oomycetes and slime molds. However, there has always been dissatisfaction with the protists group. This is in part due to protista being defined in part by negative criterion. In other words, organisms that do not fit in the other four kingdoms are defaulted into the protista. In addition, some protists are phylogenetically more closely related to the other three eukaryotic kingdoms than to other protists, and thus the protists are clearly polyphyletic.

Cavalier-Smith proposes five eukaryotic kingdoms consisting of the basal, and thus paraphyletic, kingdom Protozoa and four derived kingdoms: Animalia, Fungi, Plantae, and Chromista **(Figure 3.1**). Some of the former Protista are now included in the derived kingdoms that they are most closely related to resulting in the kingdom Protozoa becoming monophyletic. However, the alveolata, which include many traditional protozoa such as the apicomplexa, ciliates, and dinoflagellates, form a clade with the chromista, but are nonetheless placed in the protozoan kingdom. Thus the chromista are not holophyletic. Moving the alveolates into a new kingdom called Chromalveolata would solve this problem. However, this would probably result in controversy and confusion since many of the alveolates have been long considered to be protozoa.

Self-Assessment Exercise 1

1. ***What is the difference between Sporozoa, apical organelles and apicomplexa?***
2. ***Which Scientists proposed the five eukaryotic kingdoms consisting of the basal, and thus paraphyletic, kingdom Protozoa and four derived kingdoms?***

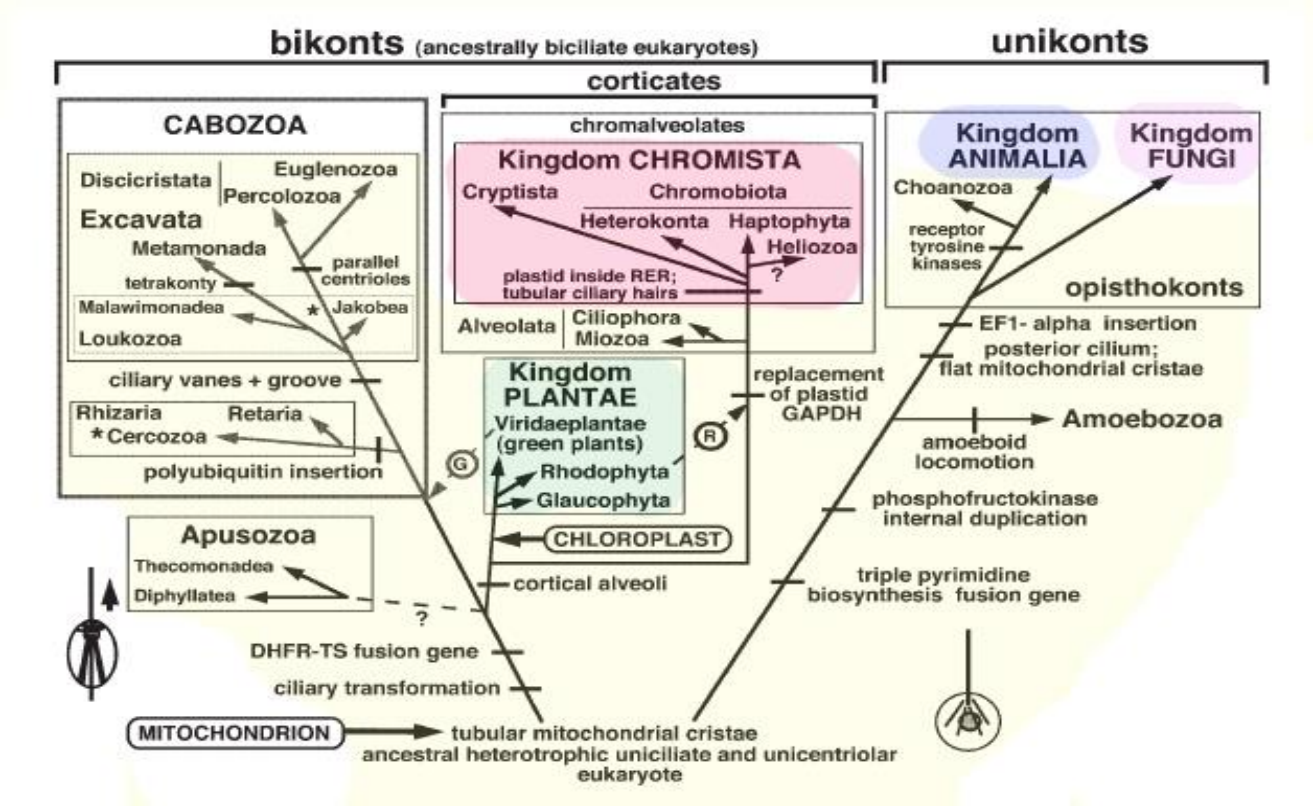

Figure 3.1 Classification of eukaryotes into 5 kingdoms: protozoa (yellow), plants (green), chromista (pink), animals (blue) and fungi (lavender). Modified from Calvaleir-Smith (2003), *European Journal of Protistol*. 39:338-348.

What is Taxonomy or Systematics?

Self-Assessment Exercise 2

1. ***What is monophyletic?***
2. ***Outline the five eukaryotic kingdoms consisting of the basal proposes by Cavalier-Smith***

3.4 Summary

In this unit, you have learnt about the taxonomy of protozoa. The classification of protozoa is further complicated by the original definition of protozoa being unicellular heterotrophs. It is now recognized that protozoa (or protists) can utilize multiple nutritional strategies and cannot be regarded as simply either plant-like (autotroph) or animal-like (heterotroph). Thus, the term protozoa cannot be regarded as a true taxonomic group. Despite the fact that the word protozoa per se are no longer a proper taxonomic name, it is still a useful and functional term.

3.5 References/Further Readings/Web Sources

Wilson, R. J. M. (2002). Progress with parasite plastids. *Journal of Molecular Biology*, 319:257-274.

Kuvardina, O. N., B. S. Leander, V. V. Aleshin, A. P. Myl'nikov, P. J. Keeling and T. G. Simdyanov (2002) The phylogeny of colpodellids (Alveolata) using small subunit rRNA gene sequeneces suggest they are the free-living sister group to Apicomplexans. *Journal of Eukaryotic Microbiology*, 49: 498-504.

Wilson, R. J. M. (2002) Progress with parasite plastids. *Journal of Molecular Biology*, 319: 257-274.

Moore, R. B., M. Obornik, J. Janouskovec, T. Chrudimsky, M. Vancova, D. H. Green, S. W. Wright, N. W. Davies, C. J. S. Bolch, K. Heimann, J. Slapeta, O. Hoegh-Guldberg, J. M. Logsdon and D. A. Carter (2008). A photosynthetic alveolate closely related to apicomplexan parasites. *Nature*, 451: 959-963.

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https://www.youtube.com/watch?v=fE3W1p7tMAk

https://www.youtube.com/watch?v=cZrf6RAspvY

3.6 Possible Answers to SAEs

Answers to SAEs 1

1. *Many of the protozoa formerly called sporozoa possess subcellular structures, collectively known as the apical organelles, and now form a monophyletic group called Apicomplexa.*
2. *Cavalier-Smith proposes five eukaryotic kingdoms consisting of the basal, and thus paraphyletic, kingdom Protozoa and four derived kingdoms: Animalia, Fungi, Plantae, and Chromista*.

Answers to SAEs 2

1. *Monophyletic means that all of the organisms in that group are probably derived from a common ancestor. For example, many of the protozoa formerly called sporozoa possess subcellular structures, collectively known as the apical organelles, and now form a monophyletic group called Apicomplexa*

2. *Kingdom Protozoa and four derived kingdoms such as Animalia, Fungi, Plantae, and Chromista*

Unit 4: The Phylum Apicomplexa

Unit Structure

4.1 Introduction

The Phylum Apicomplexa encompasses over 5,000 species of unicellular parasitic protozoa, many of which cause serious disease in humans and animals. For example, *Plasmodium* parasites are transmitted by mosquitoes, infect red blood cells and cause malaria, a disease with devastating impact on children in sub-Saharan Africa, Southeast Asia and South America. Other Apicomplexan parasites of medical importance include *Cryptosporidium* and *Toxoplasma*, which infect a large percentage of the population worldwide, and can cause debilitating and potentially fatal illness in immunocompromised individuals. *Toxoplasma* is a leading cause of congenital neurological birth defects; transmitted by consumption of undercooked meat or ingestion of material contaminated with cat feces (thisis why pregnant women are advised not to empty the kitty litter box!).

4. 2 Intended Learning Outcomes (ILOs)

By the end of this lecture unit, students should be able to:

- Have a good understanding about the phylum apicomplexa

4.3 General characteristics of Apicomplexa

Although they cause very different diseases, all apicomplexan parasites originated from a common ancestor and consequently share many characteristics:

- Apicomplexans are characterised by an apical complex, being a special organelle that appears as a conical structure on the tapered

(apical) end of the cell.

- Specialized secretory organelles, termed rhoptries and micronemes, that deploy their cargo in a coordinated fashion during parasite attachment to the host cell, invasion, establishment of the intracellular "parasitophorous vacuole" within which they reside and replicate, and modulation of the host cell.
- A plastid organelle, known as the "apicoplast" (apicomplexan plastid), acquired through "secondary endosymbiosis", in which an ancestral parasite ate a eukaryotic alga, and retained the algal plastid.
- All are obligate intracellular parasites, they must invade andreplicate within host cells in order to survive.
- They exhibit a complex life cycle, differentiating (developing) through various forms, often in different host species/tissues in the course of infection. In particular, sexual and asexual reproduction often occurs in different species (such as mosquitoes and humans for *Plasmodiu*m).
- Apicomplexan parasites contain all of the organelles one might expect in a eukaryote, including the nucleus, endoplasmic reticulum, Golgi apparatus, and mitochondrion.

Self-Assessment Exercise 1

1. ***A plastid organelle, known as?***
2. ***Apicomplexa complex life cycles that are characterised by three distinct processes namely?***

4.4 Life Cycle of Apicomplexa

The apicomplexa have complex life cycles that are characterised by three distinct processes: sporogony, merogony and gametogony (**Figure 4.4**). Although most apicomplexa exhibit this overall general life cycle the details can vary between species. Furthermore, the terminology used to describe these various life cycle stages vary between the species. The life cycle consists of both asexually reproducing forms and sexual stages. In monoxenous species (parasites whose development is restricted to a single species), all three of these processes will be carried out in a single host and often in a single cell type or tissue. Whereas, in heteroxenous species (whose development involves several host species), the various processes will be carried out in different hosts and generally involve different tissues. Sporogony occurs immediately after a sexual phase and consists of an asexual reproduction that culminates in the production of sporozoites. Sporozoites are an invasive form that will invade cells and develop into forms that undergo another asexual replication known as merogony. Merogony and the resulting merozoites are known by many different names depending of the species. In contrast to

sporogony, in which there is generally only one round of replication, quite often there are multiple rounds of merogony. In other words, the merozoites, which are also invasive forms, can reinvade cells and initiate another round of merogony. Sometimes these multiple rounds of merogony will involve a switch in the host organism or a switch in the type of cell invaded by the parasite resulting in distinct stages of merogony". As an alternative to asexual replication, merozoites can develop into gametes through a process variously called gametogony, gamogony or gametogenesis. As in other types of sexual reproduction, the gametes fuse to form a zygote, which will undergo sporogony (**Figure 4.4**).

4.5 How apicomplexan parasites replicate

Once the parasite has invaded a host cell, its survival is critically linked to replication. The replicative process occurs entirely within a specialized intracellular vacuole termed the parasitophorous vacuole. As the parasite divides, the host cell swells and eventually bursts (a lytic infection), causing disease through direct tissue damage. Similarly, in malaria, *Plasmodium* parasites lyse (Lysis: a breakdown of a cell caused by damage to its plasma or outer membrane) infected red blood cells, causing anemia (other factors may also exascerbate anemia in severe is ease). *Toxoplasma* damages multiple organs and systems, but is particularly noted for its ability to quickly destroy brain and foetal tissues.

Apicomplexan parasites replicate via an unusual process in which daughter parasites are assembled within a single mother cell. In the asexual "tachyzoite" stages of *Toxoplasma* infection, two daughter cells emerge from each mother (endodyogeny). In *Plasmodium* "merozoites" up to 16 or more daughters form within a single mother cell (schizogony). Assembly of daughter cells within the mother offers several advantages, such as the ability to eliminate indigestible waste products (such as the toxic heme left over after digestion of heamoglobin by Plasmodium) by simply leaving them behind. These parasites don't need lysosomes. Assembly of the cytoskeleton from the top (apical end) down also ensures that polarity is preserved, as required for invasion of the host cell. Replication by endodyogeny or schizogony also poses challenges: a complex process is required to ensure that each daughter inherits a complete set of organelles.

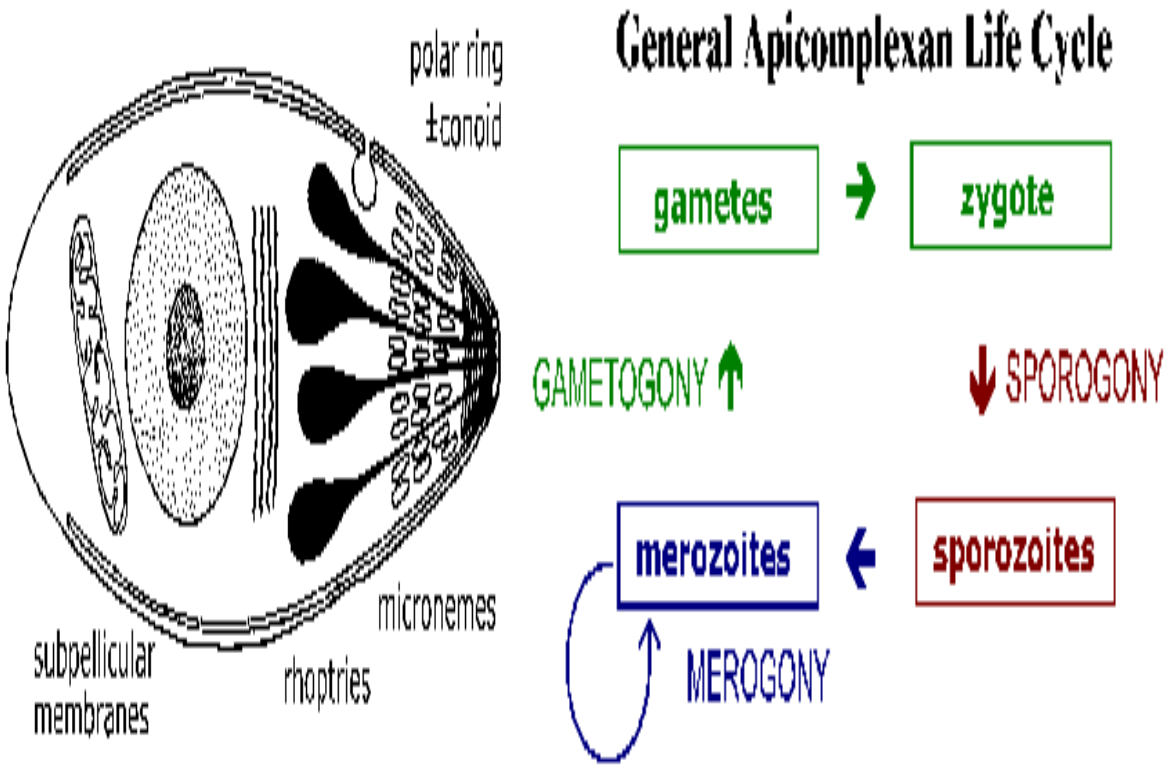

Figure 4.4: General Apicomplexan Structure and Life Cycle. Invasive and/or motile forms of apicomplexa exhibit distinctive Ultrastructural features, which can be seen with the electron microscope. At the very apical end is a ring of microtubules known as the polar ring
How do Apicomplexan parasite replicate.

Self-Assessment Exercise 2

1. ***Outline atleast four general characteristics of Apicomplexa?***
2. ***Briefly explain how apicomplexan parasites replicate in the host cells?***

4.6 Summary

In this unit, you have learnt about the phylum apicomplexa, general characteristics, life cycle of apicomplexa and how apicomplexan parasites replicate. Other Apicomplexan parasites of medical importance include *Cryptosporidium* and *Toxoplasma*, which infect a large percentage of the population worldwide, and can cause debilitating and potentially fatal illness in immunocompromised individuals. *Toxoplasma* is a leading cause of congenital neurological birth defects; transmitted by consumption of undercooked meat or ingestion of material contaminated with cat feces.

4.7 References/Further Readings/Web Sources

Wilson, R. J. M. (2002). Progress with parasite plastids. *Journal of Molecular Biology*, 319:257-274.

Kuvardina, O. N., B. S. Leander, V. V. Aleshin, A. P. Myl'nikov, P. J. Keeling and T. G. Simdyanov (2002) The phylogeny of colpodellids (Alveolata) using small subunit rRNA gene sequeneces suggest they are the free-living sister group to Apicomplexans. *Journal of Eukaryotic Microbiology*, 49: 498-504.

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https://www.youtube.com/watch?v=fE3W1p7tMAk

https://www.youtube.com/watch?v=cZrf6RAspvY

4.8 Possible Answers to SAEs

Answers to SAEs 1

1. *A plastid organelle, known as the "apicoplast" (apicomplexan plastid)*
2. *Apicomplexa complex life cycles that are characterised by three distinct processes: sporogony, merogony and gametogony*

Answers to SAEs 2

1. *- Apicomplexans are characterised by an apical complex, being a special organelle that appears as a conical structure on the tapered (apical) end of the cell.*
- *Specialized secretory organelles, termed rhoptries and micronemes, that deploy their cargo in a coordinated fashion during parasite attachment to the host cell, invasion, establishment of the intracellular "parasitophorous vacuole" within which they reside and replicate, and modulation of the host cell.*
- *A plastid organelle, known as the "apicoplast" (apicomplexan plastid), acquired through "secondary endosymbiosis", in which an ancestral parasite ate a eukaryotic alga, and retained the algal plastid.*
- *All are obligate intracellular parasites, they must invade andreplicate within host cells in order to survive.*

2. *Once the parasite has invaded a host cell, its survival is critically linked to replication. The replicative process occurs entirely within a specialized intracellular vacuole termed the parasitophorous vacuole. As the parasite divides, the host cell swells and eventually bursts (a lytic infection), causing disease through direct tissue damage. Similarly, in malaria, Plasmodium parasites lyse (Lysis: a breakdown of a cell caused by damage to its plasma or outer membrane) infected red blood cells, causing anemia (other factors may also exascerbate anemia in severe is ease).*

Unit 5: Structure and Function of Protozoa

Unit Structure

5.1 Introduction

Protozoa, like other eukaryotic cells, have a nucleus, an endoplasmic reticulum, mitochondriaand a Golgi body and lysosomes.

5.2 Intended Learning Outcomes (ILOs)

By the end of this lecture unit, students should be able to:

- Have a good understanding on structure and function of protozoa with specific examples on *Trypanosoma brucei*, *Balantidium* and *Entamoeba*

5.3 Morphology of *Trypanosoma brucei* Protozoa

Thus locomotion, in, for example, the genus Trypanosoma (**Figure 5.3**) is facilitated by a single flagellum, and in some other protozoa by several flagella. A flagellum is a contractile fibre, arising from a structure called a basal body, and in some species is

attached to the body of the protozoan along its length, so that when the flagellum beats, the cell membrane (pellicle) is pulled up to form an undulating membrane. Sometimes, also, it projects beyond the protozoan body as a free flagellum. During movement the shape of these organisms is maintained by microtubules in the pellicle.

Self-Assessment Exercise 1

1. ***Briefly explain the morphology of Trypanosoma brucei?***
2. ***Outline the features of Balantidium sp.?***

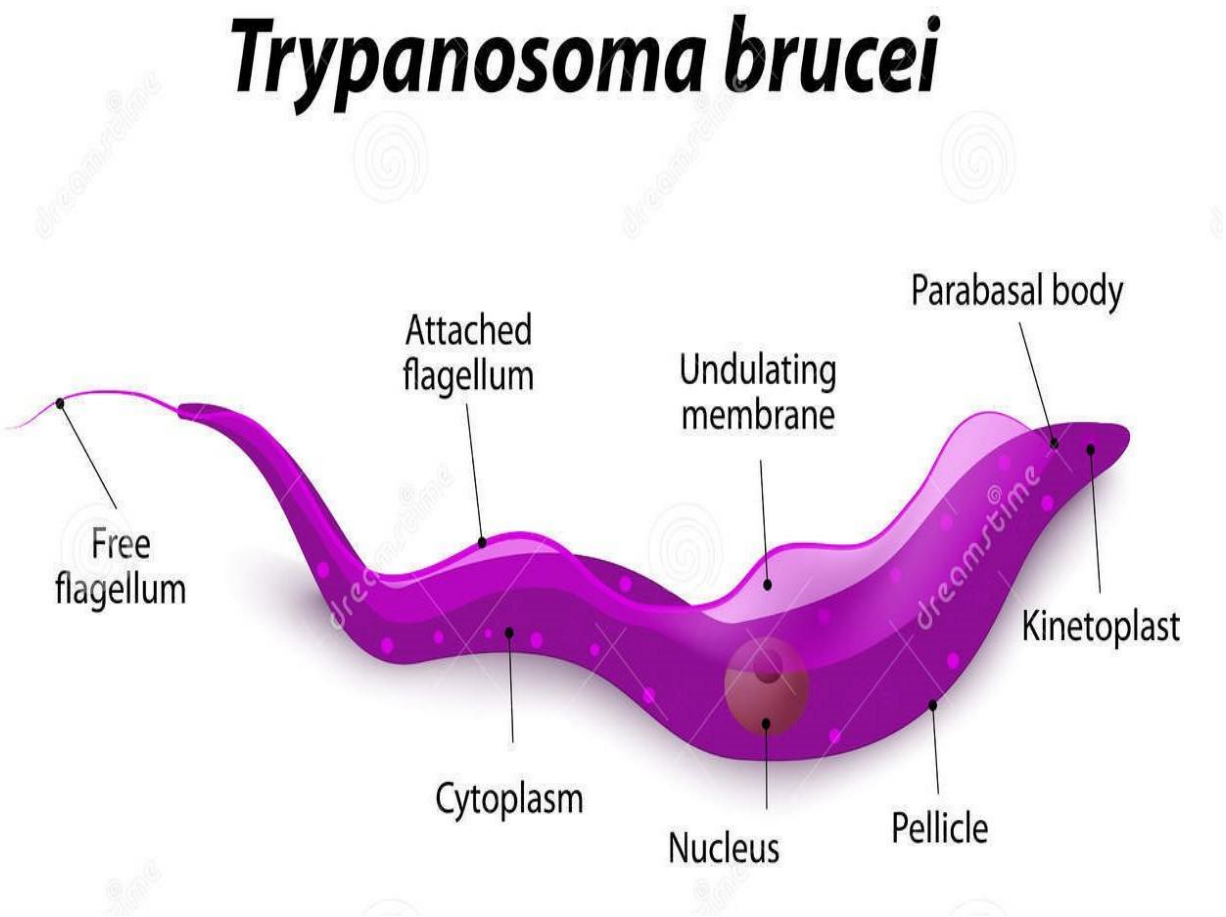

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Figure 5.3.*Trypanosoma brucei* showing the flagellum and undulating membrane

5.4 Balantidium

Other protozoa, such as *Balantidium* (**Figure 5.4**), move by means of cilia which are fine, short hairs, each arising from a basal body; these cover much of the body surface and beat in unison to effect movement. In such species a mouth or cytostome is present and the ciliary movement is also used to waft food towards this opening.

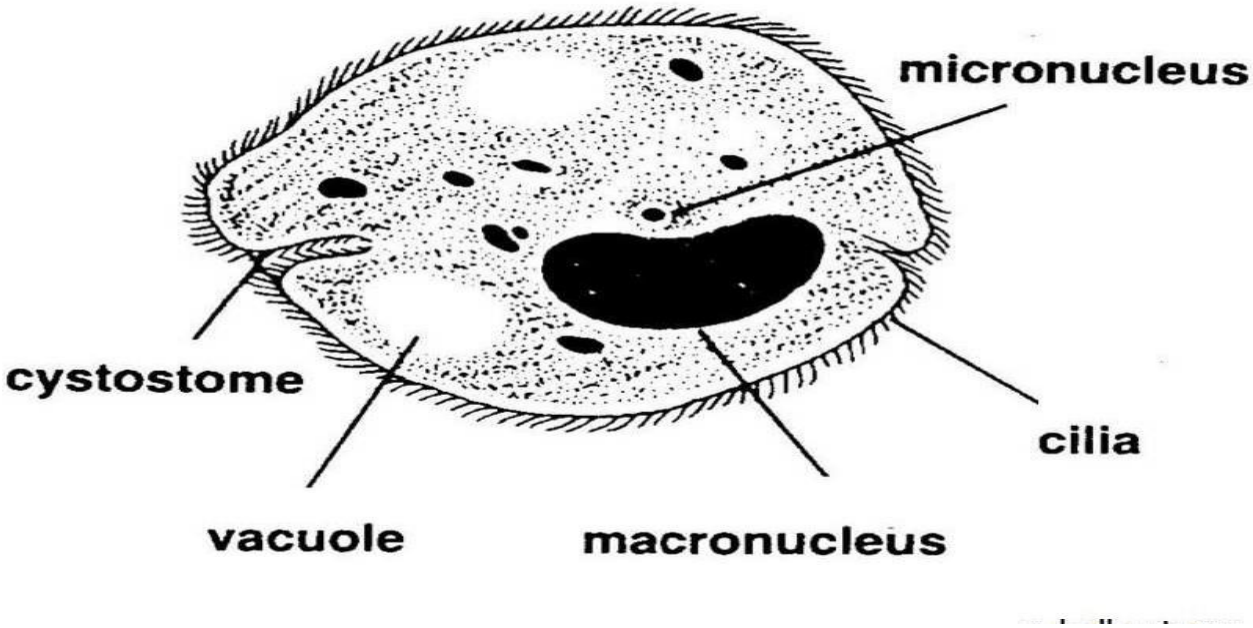

Figure 5.4. Morphology of the intestinal protozoan *Balantidium*

5.5 Entamoeba

A third means of locomotion, used by protozoa such as Entamoeba (**Figure 5.5)** are pseudopodia, which are prolongations of cytoplasm. Movement occurs as the rest of the cytoplasm flows into this prolongation.

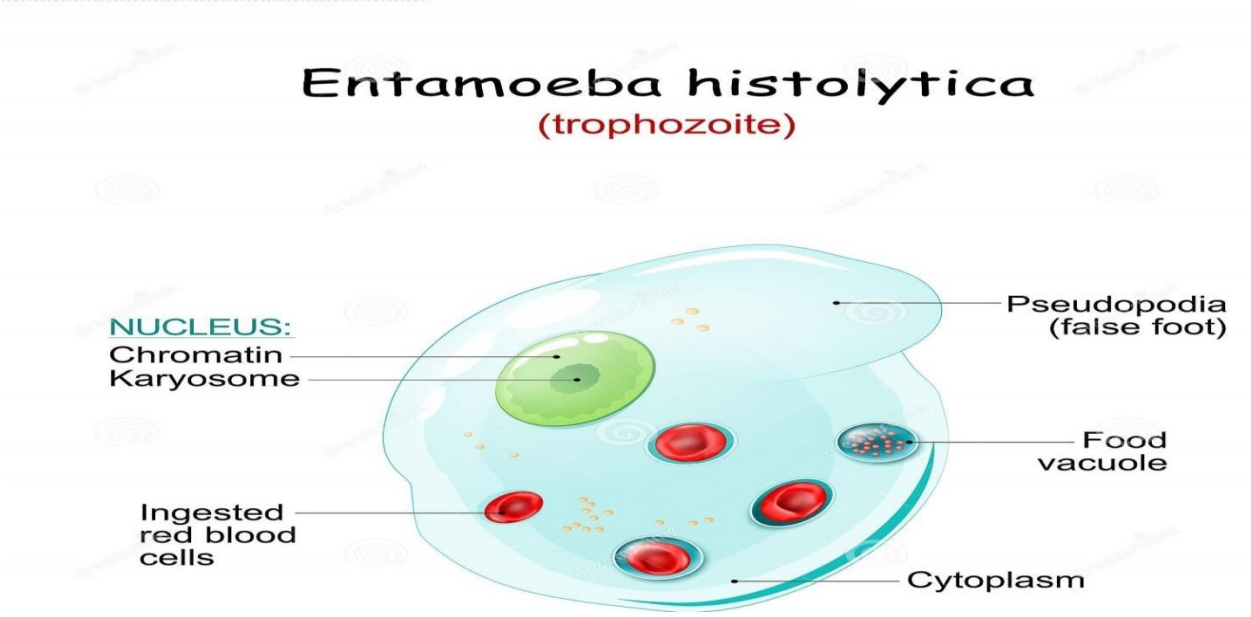

Figure 5.5. Trophozoite stage of *Enatamoeba histolytica* has an amoeboid and a non-motile cystic stage with nuclei.

The pseudopodium also possesses a phagocytic capacity and can function as a cup, which closes,enveloping particulate food material in a vacuole. Finally, some protozoa, such as the extracellular stages of the *Eimeria*, have no obvious means of locomotion but are nevertheless capable of gliding movements.

The nutrition of parasitic protozoa usually occurs by pinocytosis or phagocytosis, depending on whether tiny droplets of fluid or small objects of macromolecular dimension are taken into the cell, In both cases, the process is the same, the cell membrane gradually enveloping the dropletor object which has become adherent to its outer surface, When, this is complete, the particle is carried into the cell where fusion with lysosomes effects digestion, Finally, undigested materialis extruded from the cell. As noted above, some ciliated protozoa and also some stages of the organisms causing malaria obtain food through a cytostome; at the base of the cytostome the food enters a vacuole for digestion within the cell. Metabolic products are excreted by diffusion through the cell membrane.

The infective stage of some protozoa is called a sporozoite, while the term trophozoite is applied to that stage of the protozoa in the host, which feeds and grows, until division commences. In most protozoa, reproduction is asexual and is accomplished by binary fission or, in the case of within *Babesia* erythrocytes, by budding, Another form of asexual reproduction, which occurs in the subphylum Sporozoa is merogony (schizogony), In the latter process, the trophozoite grows to a large size while the nucleus divides repeatedly. This structure is called a meront (schizont) and, when mature, each nucleus has acquired a porrtion of the cytoplasm so that the schizont is filled with a large number of elongated separate organisms called merozoites. The meront eventually ruptures, liberating the individual merozoites.

Protozoa that only divide asexually generally have a short generation time, and since they cannotexchange genetic material, rely on mutants to provide the variants necessary for natural selection. However, most sporozoa at certain stages in their life cycle also have a sexual phase of reproduction, called gametogony, which may be followed by a free-living maturation phase, or sporogony. Sometimes, as in *Eimeria*, both asexual and sexual phases occur in vertebrate host while in others, such as *Plasmodium*, the asexual phase occurs in the vertebrate host and the sexual phase in the arthropod vector.

Discuss freely the process of locomotion in Entamoeba?

Self-Assessment Exercise 2

1. ***Briefly explain the locomotory system of Trypanosoma brucei?***
2. ***Briefly explain the locomotory system of Entamoeba?***

5.6. Summary

In this unit, you have learnt about the structure and function of protozoans as well as their locomotory system such as *Trypanosoma brucei, Balantidium and Entamoeba.* Protozoans lead an independent existence; they possess a variety of other subcellular structures or organelles with distinct organizational features and functions.

5.7. References/Further Readings/Web Sources

Wilson, R. J. M. (2002). Progress with parasite plastids. *Journal of Molecular Biology*, 319:257-274.

Kuvardina, O. N., B. S. Leander, V. V. Aleshin, A. P. Myl'nikov, P. J. Keeling and T. G. Simdyanov (2002) The phylogeny of colpodellids (Alveolata) using small subunit rRNA gene sequeneces suggest they are the free-living sister group to Apicomplexans. *Journal of Eukaryotic Microbiology*, 49: 498-504.

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https://www.youtube.com/watch?v=fE3W1p7tMAk

https://www.youtube.com/watch?v=cZrf6RAspvY

5.8 Possible Answers to SAEs

Answers to SAEs 1

1. -The genus Trypanosoma is facilitated by a single flagellum and in some other protozoa by several flagella.
- A flagellum is a contractile fibre, arising from a structure called a basal body, and in some species is attached to the body of the protozoan along its length, so that when the flagellum beats, the cell membrane (pellicle) ispulled up to form an undulating membrane.
- Sometimes, also, it projects beyond the protozoan body as a free flagellum.
- During movement the shape of these organisms is maintained by microtubules in the pellicle.
2. - *Balantidium* move by means of cilia which are fine, short hairs, each arising from a basal body; these cover much of the body surface and beat in unison to effect movement.
3. In such species a mouth or cytostome is present and the ciliary movement is also used to waft food towards this opening.

Answers to SAEs 2

1. *Trypanosoma brucei* is facilitated by a single flagellum and in some other protozoa by several flagella. A flagellum is a contractile fibre, arising from a structure called a basal body, and in some species is attached to the body of the protozoan along its length, so that when the flagellum beats, the cell membrane (pellicle) is pulled up to form an undulating membrane. Sometimes, also, it projects beyond the protozoan body as a free flagellum. During movement the shape of these organisms is maintained by microtubules in the pellicle.

2. *Entamoeba* is pseudopodia, which are prolongations of cytoplasm. Movement occurs as the rest of the cytoplasm flows into this prolongation.

Glossary

DNA = Deoxyribonucleic acid
AIDS = Acquired Immunodeficiency syndrome
ATP = Adenosine Triphosphate
STD = Sexually Transmitted Diseases
ACR = Adequate clinical response
LTF = Late treatment failure
ETF = Early treatment failure
MDR = Multi-drug resistance
ABC = ATP-binding cassette

End of the module Questions

1) A parasite is an organism that obtains food and shelter from another organism and derives all benefits from this association (**True or False**)
2) A parasite is termed obligate when it can live only in a host; it is classified as facultative when it can live both in a host as well as in free form (**True or False)**
3) A Parasites that live inside the body are termed _____ (a) ectoparasite (b) endo parasite (c) sporozoan (d) thermo parasite
4) A parasite that exist on the body surface are called _____
   (a) ecto parasite (b) endo parasite (c) sporozoan (d) thermo parasite
5) Parasites that cause harm to the host are pathogenic parasites while those that benefit from the host without causing it any harm are known as commensals (**True or False)**

Module 2: *Blood and Tissue Protozoa*

Module Structure

In this module we will discuss about the blood and Tissue Protozoa with the following units:

Unit 1: Trypanosome
Unit 2: Leishmania
Unit 3: Plasmodium
Unit 4: Babesia
Unit 5: Toxoplasma
Glossary
End of the module Questions

Unit 1: Trypanosome

Unit Structure

1.1 Introduction
1.2 Intended Learning Outcomes (ILOs)
1.3 General Morphology of Trypanosome
1.3.1 *Trypanosoma gambiense*
1.4 African trypanosomiasis (Sleeping sickness)
1.5 American trypanosomiasis (Chagas' disease) Etiology
1.6 Summary
1.7 References/Further Readings/Web Sources
1.8 Possible Answers to SAEs

1.1 Introduction

The haemoflagellates all belong to the family Trypanosomatidae, and include the trypanosomesand leishmanias

1.2 Intended Learning Outcomes (ILOs)

By the end of this lecture unit, students should be able to:

- Have a good understanding on blood and tissue protozoa such as Trypanosome

1.3 General Morphology of Trypanosome

Trypanosomes have a leaf-like or rounded body containing a vesicular nucleus, and a varyingnumber of subpellicular microtubules lying beneath the outer membrane. There is a single flagellum arising from a kinetosome or basal granule. An undulating membrane is present in some genera and the flagellum lies on its outer border. Posterior to the kinetosome is a rod- shaped or spherical kinetoplast containing DNA. Members of this family were originally parasites of the intestinal tract of insects, and many are still found in insects. Others are heteroxenous, spending part of their life cycle in a vertebrate host and part in an invertebratehost. Members of the genus *Trypanosoma* are heteroxenous and pass through amastigote, promastigote, epimastigote and trypomastigote stages in their life cycle. In some species only trypomastigote forms are found in the vertebrate host; in others, presumably more primitive species, both amastigote and trypomastigote forms are present. In the trypomastigote form, the kinetoplast and kinetosomes are near the posterior end and the flagellum forms the border of an undulating membrane that extends along the side of the body tothe anterior end.

In the **epimastigote** form, the kinetoplast and kinetosomes are just posterior to the nucleus and theundulating membrane forward from there.

In the **promastigote** form, the kinetoplast and the kinetosomes are still further anterior in the body and there is no undulating membrane.

In the **amastigote** form, the body is rounded and the flagellum emerges from the body through a wide, funnel-shaped reservoir.

1.3.1 *Trypanosoma gambiense*

Epidemiology

Trypanosoma brucei gambiense is predominant in the western and central regions of Africa, whereas *Trypanosoma brucei rhodesiense* is restricted to the eastern third of the continent. 6,000 to 10,000 human cases are documented annually. 35 million people and 25 million cattle are at risk. Regional epidemics of the disease are cause of major health and economic disaster (**Figure 1.3.1a**).

Figure 1.3.1a. Distribution of West African or Gambian Sleeping Sickness and East African orRhodesian Sleeping Sickness

Morphology

T. b. gambiense and *T. b. rhodesiense* are similar in appearance: The organism measures 10 - 30 micrometers. It has a single central nucleus and a single flagellum originating at the kinetoplast and joined to the body by an undulating membrane. The outer surface of the organism is densely coated with a layer of glycoprotein, the variable surface glycoprotein (VSG) **(Figure 1.3.1b).**

Life cycle

Metacyclic trypomastigotes (MT) in the saliva of the tsetse fly are transferred to the bloodstream of the mammalian host as the tsetse feeds. The parasite exhibits a trypomastigote morphology in the bloodstream and is extracellular. These extracellular forms undergo an antigenic variation to evade the host immune system. Within the bloodstream the trypanosome undergoes asexual replication by longitudinal binary fission. These replicating forms are generally long slender (LS) parasites. In addition to the long slender forms, intermediate and short stumpy (SS) forms are also found within the bloodstream of the mammalian host. The short stumpy forms are thought to be preadapted for the tsetse. However, *in vitro* experiments suggest that all bloodstream forms are infective for the tsetse. The tsetse can ingest trypanosomes with its blood meal. The bloodstream trypomastigote differentiates into a procyclic trypomastigote (PT) within the gut of the tsetse. Accompanying this differentiation is a loss of the VSG surface coat and changes in the mitochondria and metabolism. The environment within the gut of fly is quite different than that of the mammalian bloodstream. The mammalian bloodstream is rich in glucose and parasite exhibits a high rate of glycolysis which is carried out in a

special organelle known as the glycosome. Because of this abundance of glucose the parasite does not carry out oxidative phosphorylation within the mitochondria and consequently the mitochondria are acristate and have minimal electron transport activity. Within the vector, though, mitochondrial functions associated with aerobic metabolism return and cristae develop within the mitochondria. The procyclic trypomastigotes undergo multiple rounds of asexual replication within the midgut of the tsetse. The procyclic stage can also be cultured in vitro. The focus in medical parasitology courses tends to be on the complex interactions between the parasite and the human host which result in pathology. However, parasites also interact with and undergo complex developmental processes in the vector. Vectors are more than 'flying syringes'. (Although *T. evansi*, a trypanosome infecting horses and camels, is transmitted mechanically by horseflies.) One problem for the trypanosome is that it must move from the gut to the salivary glands of the tsetse. The exact mechanism by which the parasite migrates from the tsetse gut to the saliva glands is not known. Two routes have been proposed: 1) the classical route in which the parasite 'backtracks' through the digestive system and migrates up the salivary duct, or 2) the direct route in which the parasite penetrates the peritrophic membrane and gut epithelium to gain access to the hemolymph. After reaching the salivary glands the procyclic trypomastigotes transform into epimastigotes (E) and attach to epithelial cells via their flagella. The epimastigotes probably undergo further replication within the salivary gland. The epimastigotes are non-infective for the mammalian host and they must first mature into metacyclic trypomastigotes (MT). “During this maturation the surface coat is reformed, the mitochondria lose their cristae and the parasite detaches. These trypomastigotes are free within the lumen of the salivary gland waiting to be transferred to a vertebrate host when the tsetse feeds again, thus completing the life cycle **(Figure 1.3.1c).**

Figure 1.3.1b: Glossina (Tsetse fly) vector of Trypanosome

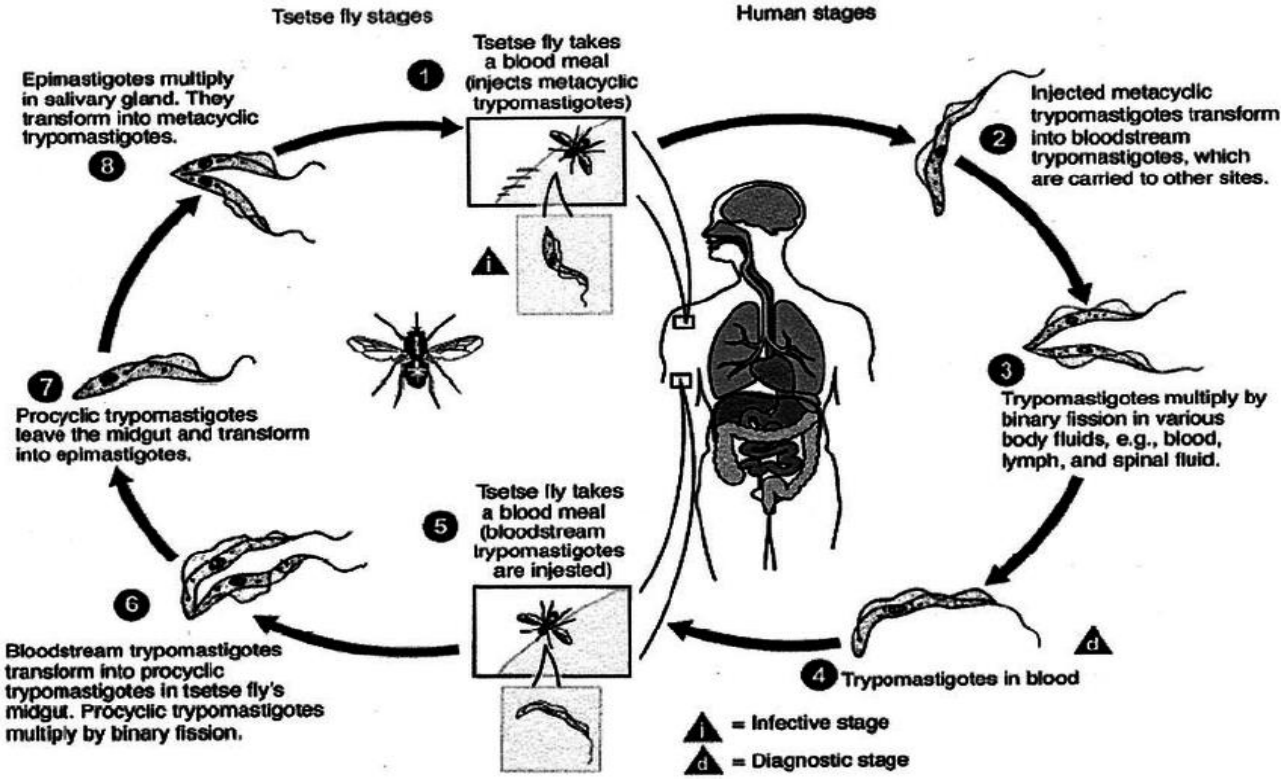

Figure 1.3.1c: Life cycle of *Trypanosoma brucei* observed in the tsetse fly and in the human blood stream

State the five blood protozoa that have major biological/medical significance.

1.4 African trypanosomiasis (Sleeping sickness)

Etiology

There are two clinical forms of African trypanosomiasis:

1. A slowly developing disease caused by *Trypanosoma brucei gambiense* and
2. A rapidly progressing disease caused by *T. brucei rhodesiense*.

Symptoms

The clinical features of Gambian and Rhodesian disease are the same. However they vary in severity and duration. Rhodesian disease progresses more rapidly and the symptoms are often more pronounced. The symptoms of the two diseases are also more pronounced in Caucasians than in the local African population. Classically, the progression of African trypanosomiasis can be divided into three stages: the bite reaction (chancre), parasitemia (blood and lymphoid tissues), and central nervous system (CNS) stage.

Bite reaction: A non-pustular, painful, itchy chancre forms 1-3 weeks after the bite and lasts 1-2 weeks. It leaves no scar.

Parasitemia: Parasitemia and lymph node invasion is marked by attacks of fever which starts 2 weeks after the bite and is accompanied by malaise, lassitude, insomnia headache and lymphadenopathy and edema. Painful sensitivity of palms and ulnar region to pressure (Kerandel's sign) may develop in some Caucasians. Very characteristic of Gambian disease is visible enlargement of the glands of the posterior cervical region (Winterbottom's sign). Febrile episodes may last few months as in Rhodesian disease or several years as in Gambian disease. Parasitemia is more prominent during the acute stage than during the recurrence episodes.

CNS Stage: The late or CNS stage is marked by changes in character and personality. They include lack of interest and disinclination to work, avoidance of acquaintances, morose and melancholic attitude alternating with exaltation, mental retardation and lethargy, low and tremulous speech, tremors of tongue and limbs, slow and shuffling gait, altered reflexes, etc. Males become impotent. There is a slow progressive involvement of cardiac tissue. The later stages are characterized by drowsiness and uncontrollable urge to sleep. The terminal stage is marked by wasting and emaciation. Death results from coma, intercurrent infection or cardiac failure.

The clinical features of Rhodesian disease are similar but briefer and more acute. The acuteness and severity of disease do not allow typical sleeping sickness. Death is due to cardiac failure within 6-9 months.

Pathology and Immunology

An exact pathogenesis of sleeping sickness is not known, although immune complexes and inflammation have been suspected to be the mechanism of damage to tissues. The immune response against the organism does help to eliminate the parasite but it is not protective, since the parasite has a unique ability of altering its antigens, the VSG. Consequently, there is a cyclic fluctuation in the number of parasites in blood and lymphatic fluids and each wave of parasite represents a different antigenic variant". The parasite causes polyclonal expansion of B lymphocytes and plasma cells and an increase in total IgM concentration. It stimulates the reticuloendothelial function. It also causes severe depression of cell mediated and humoral immunity to other antigens.

Diagnosis

Detection of parasite in the bloodstream, lymph secretions and enlarged lymph node aspirate provides a definitive diagnosis in early (acute) stages. The parasite in blood can be concentrated by centrifugation or by the use of anionic support media. Cerebrospinal fluid must always be examined for organisms. Immuno-serology (enzyme-linked immune assay, immunofluorescence) may be indicative but does not provide definite diagnosis.

Treatment and control

The blood stage of African trypanosomiasis can be treated with reasonable success with Pentamidine isethionate or Suramin. These drugs have been reported also to be effective in prophylaxis although they may mask early infection and thus increase the risk of CNS disease. Cases with CNS involvement should be treated with Melarsoprol, an organic arsenic compound. The most effective means of prevention is to avoid contact with tsetse flies. Vector eradication is impractical due to the vast area involved. Immunization has not been effective due to antigenic variation.

Self-Assessment Exercise 1

1. ***Highlight the three stages involved in African trypanosomiasis*?**

2. ***Outline the three stages involved in Chagas disease?***

1.5 American trypanosomiasis (Chagas' disease)Etiology

Chagas' disease is caused by the protozoan hemoflagellate, *Trypanosoma cruzi*.

Epidemiology

American trypanosomiasis, also known as Chagas' disease, is scattered irregularly in Central and South America, stretching from parts of Mexico to Argentina. Rare cases have been reported in Texas, California and Maryland. It is estimated that 16-18 million people are infected by the parasite and 50 million are at risk. About 50,000 people die each year from the disease.

Morphology

Depending on its host environment, the organism occurs in three different forms. The trypanosomal (trypomastigote) form, found in mammalian blood, is 15 to 20 microns long and morphologically similar to African trypanosomes (**Figure 1. 5a**). The crithidial (epimastigote) form is found in the insect intestine. The leishmanial (amastigote) form, found intracellularly or in pseudocysts in mammalian viscera (particularly in myocardium and brain), is round or oval in shape, measures 2-4 microns and lacks a prominent flagellum ((**Figure 1.5b**).

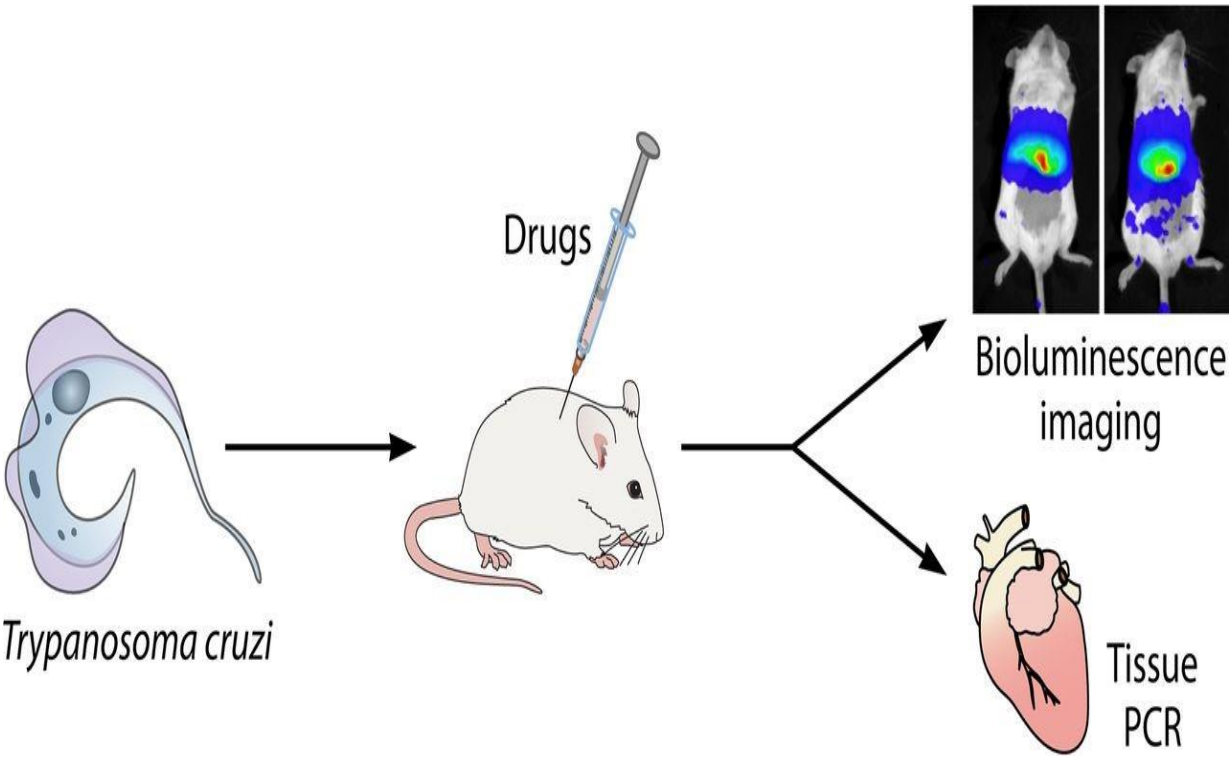

Figure 1.5a *Trypanosoma cruzi,* from experimentally infected mice

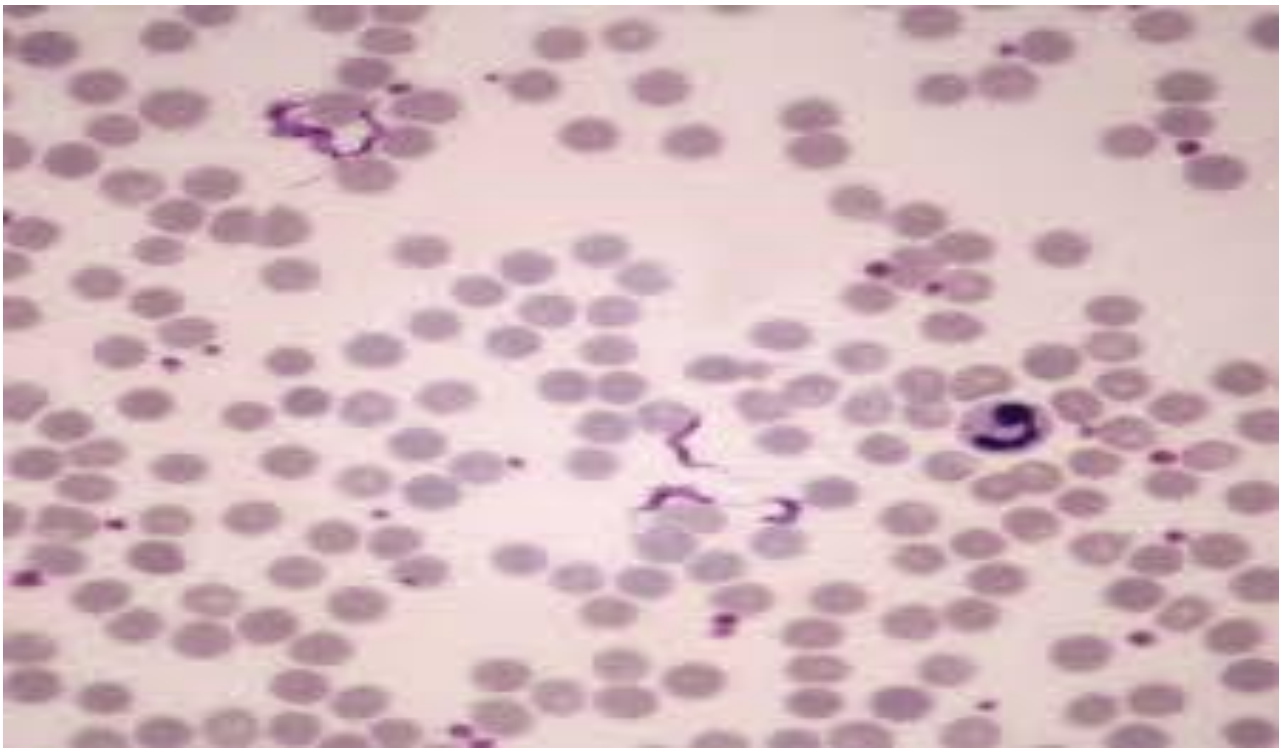

Figure 1.5b: *Trypanosoma cruzi*. As seen under the microscope X1000

Life cycle

The organism is transmitted to mammalian host by many species of kissing (riduvid) bug, most prominently by *Triatoma infestans*, *T. sordida*, *Panstrongylus megistus* and *Rhodnius prolixus*. Transmission takes place during the feeding of the bug which normally bites in the facial area (hence the name, kissing bug) and has the habit of defecating during feeding. The metacyclic trypomastigotes, contained in the fecal material, gain access to the mammalian tissue through the wound which is often rubbed by the individual that is bitten. Subsequently, they enter various cells, including macrophages, where they differentiate into amastigotes and multiply by binary fission. The amastigotes differentiate into non-replicating trypomastigotes and the cells rupture to release them into the bloodstream. Additional host cells, of a variety of types, can become infected and the trypomastigotes once again form amastigotes inside these cells **(Figure 1.5c).**

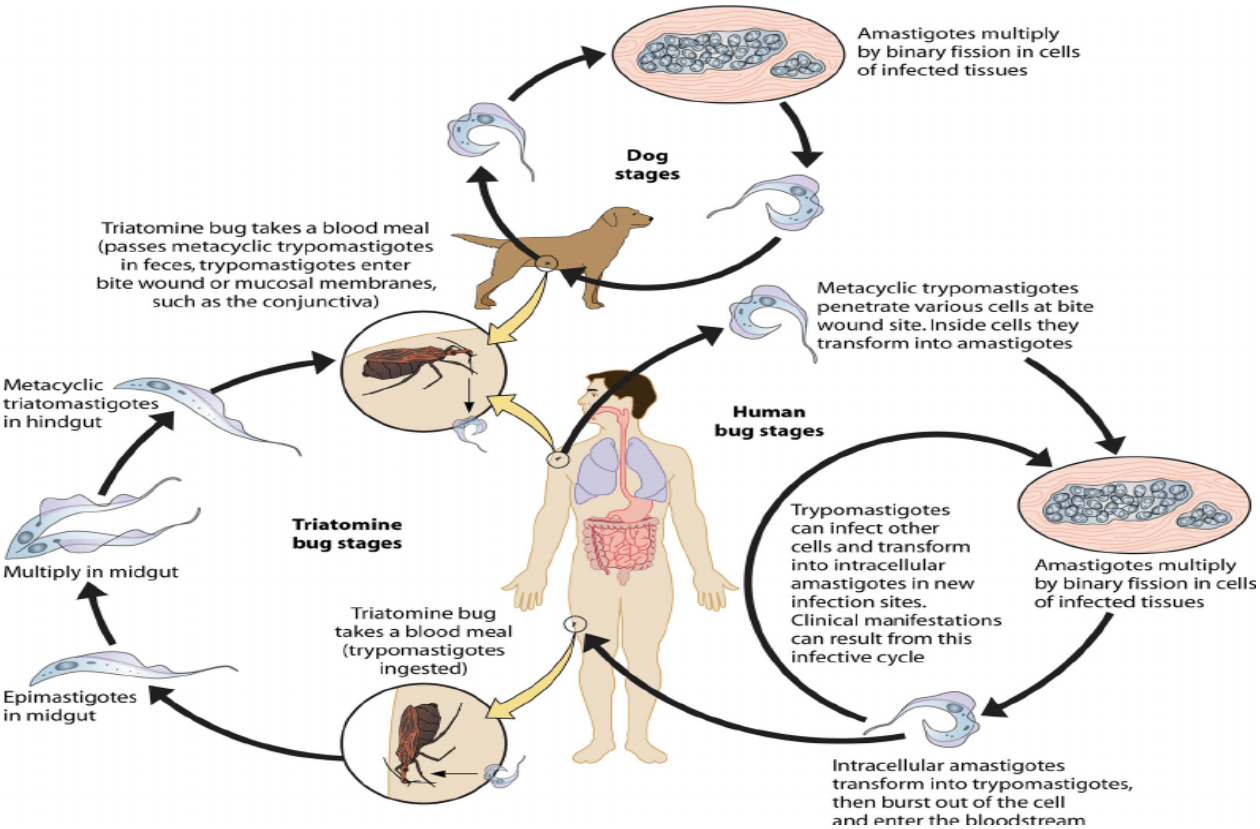

Figure 1.5c. Life cycle of *Trypanosoma cruzi*: In man and in the triatomic bug *Triatoma infestans.*

Other arthropods, bed bugs, ticks and keds can also act as vectors

Uninfected insect vectors acquire the organism when they feed on infected animals or people containing trypomastigotes circulating in their blood. Inside the alimentary tract of the insect vector, the trypomastigotes differentiate to form epimastigotes and divide longitudinally in the mid and hindgut of the insect where they develop into infective metacyclic trypomastigotes. Transmission may also occur from man to man by blood transfusion and by the transplacental route. More than one hundred mammalian species of wild and domestic animals including cattle, pigs, cats, dogs, rats, armadillo, raccoon and opossum are naturally infected by *T. cruzi* and serve as a reservoir.

Symptoms

Chagas' disease can be divided into three stages: the primary lesion, the acute stage, and the chronic stage. The primary lesion, chagoma, appearing at the site of infection,

within a few hours of a bite, consists of a slightly raised, flat non-purulent erythematous plaque surrounded by a variable area of hard edema. It is usually found on the face, eyelids, cheek, lips or the conjunctiva, but may occur on the abdomen or limbs. When the primary chagoma is on the face, there is an enlargement of the pre and post auricular and the sub maxillary glands on the side of the bite. Infection in the eyelid, resulting in a unilateral conjunctivitis and orbital edema (Ramana's sign), is the commonest finding.

Acute Stage: The acute stage appears 7-14 days after infection. It is characterized by restlessness, sleeplessness, malaise, increasing exhaustion, chills, fever and bone and muscle pains. Other manifestations of the acute phase are cervical, axillary, iliac adenitis, hepatomegaly, erythematous rash and acute myocarditis. There is a general edematous reaction associated with lymphadenopathy. Diffuse myocarditis, sometimes accompanied by serious pericarditis and endocarditis, is very frequent during the initial stage of the disease. In children, Chagas' disease may cause meningo encephalitis and coma. Death occurs in 5-10 percent of infants. Hematologic examination reveals lymphocytosis and parasitemia.

Chronic Stage: The acute stage is usually not recognized and often resolves with little or no immediate damage and the infected host remains an asymptomatic carrier. An unknown proportion (guessed at 10-20%) of victims develop a chronic disease. They alternate between asymptomatic remission periods and relapses characterized by symptoms seen in the acute phase. Cardiac arrhythmia is common. The chronic disease results in an abnormal function of the hollow organs, particularly the heart, esophagus and colon. The cardiac changes include myocardial insufficiency, cardiomegaly, disturbances of atrio-ventricular conduction and the Adams-Stoke syndrome. Disturbances of peristalsis lead to megaesophagus and megacolon.

Pathology and Immunology

The pathological effects of acute phase Chagas' disease largely result from direct damage to infected cells. In later stages, the destruction of the autonomic nerve ganglions may be of significance. Immune mechanisms, both cell mediated and humoral, involving reaction to the organism and to autologous tissues have been implicated in pathogenesis. *T. cruzi* stimulates both humoral and cell mediated immune responses. Antibody has been shown lying with the organism, but rarely causes eradication of the organism, perhaps due to its intracellular localization. Cell mediated immunity may be of significant value. While normal macrophages are targeted by the organism for growth, activated macrophages can kill the organism. Unlike *T. brucei*, *T. cruzi* does not alter its antigenic coat. Antibodies directed against heart and muscle cells have also been detected in infected patients leading to the supposition that there is an

element of autoimmune reaction in the pathogenesis of Chagas' disease. The infection causes severe depression of both cell mediated and humoral immune responses. Immunosuppression may be due to induction of suppressor Tcells and/or overstimulation of macrophages.

Diagnosis

Clinical diagnosis is usually easy among children in endemic areas. Cardiac dilation, megacolon and megaesophagus in individuals from endemic areas indicate present or former infection. Definitive diagnosis requires the demonstration of trypanosomes by microscopy or biological tests (in the insect or mice). Antibodies are often detectable by complement fixation or immunofluorescence and provide presumptive diagnosis.

Treatment and Control

There is no curative therapy available. Most drugs are either ineffective or highly toxic. Recently two experimental drugs, Benznidazol and Nifurtimox have been used with promising results in the acute stage of the disease; however their side effects limit their prolonged use in chronic cases. Control measures are limited to those that reduce contact between the vectors and man. Attempts to develop a vaccine have not been very successful, although they may be feasible.

Self-Assessment Exercise 2

1. ***Briefly explain the pathology and Immunology of Chagas disease?***

2. ***Outline the diagnosis of Chagas disease?***

1.6 Summary

In this unit, you have learnt about the medical important of blood and tissue protozoa such as Trypanosoma, their morphology, life cycle, pathology, immunology, diagnosis, treatment and control. Medically significant blood and tissue protozoans such as Trypanosoma, e.g. *T. brucei* and *T. cruzi*, they cause different disease conditions in their hosts such as sleepingsickness and Chagas' disease etc. They can be found in different parts of the world including Africa.

1.7 References/Further Readings/Web Sources

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1.8 Possible Answers to SAEs

Answers to SAEs 1

1. *The bite reaction (chancre), parasitemia (blood and lymphoid tissues) and central nervous system (CNS) stage.*

2. *The primary lesion, Acute Stage and Chronic Stage*

Answers to SAEs 2

1. *- The pathological effects of acute phase Chagas' disease largely result from direct damage to infected cells. In later stages, the destruction of the autonomic nerve ganglions may be of significance.*
- *In Immune mechanisms, both cell mediated and humoral, involving reaction to the organism and to autologous tissues has been implicated in pathogenesis. T. cruzi stimulates both humoral and cell mediated immune responses.*
- *Antibody has been shown lying with the organism, but rarely causes eradication of the organism, perhaps due to its intracellular localization.*
- *Cell mediated immunity may be of significant value. While normal macrophages are targeted by the organism for growth, activated macrophages can kill the organism. Unlike T. brucei, T. cruzi does not alter its antigenic coat.*
- *Antibodies directed against heart and muscle cells have also been detected in infected patients leading to the supposition that there is an element of autoimmune reaction in the pathogenesis of Chagas' disease.*
- *The infection causes severe depression of both cell mediated and humoral immune responses.*
- *Immunosuppression may be due to induction of suppressor Tcells and/or overstimulation of macrophages*
2. *- Clinical diagnosis is usually easy among children in endemic areas.*
- *Cardiac dilation, megacolon and megaesophagus in individuals from endemic areas indicate present or former infection.*
- *Definitive diagnosis requires the demonstration of trypanosomes by microscopy or biological tests (in the insect or mice).*
- *Antibodies are often detectable by complement fixation or immunofluorescence and provide presumptive diagnosis.*

Unit 2: Leishmania

Unit Structure

2.1 Introduction

Leishmania are ovoid organisms within the macrophage and possess a rod-shaped kinetoplast associated with a rudimentary flagellum, which, however, does not extend beyond the cell margin. The parasites are found in the amastigote stage in cells of the vertebrate host and in the promastigote stage in the intestine of the sandfly.

2.2 Intended Learning Outcomes (ILOs)

By the end of this lecture unit, students should be able to:

- Have a good understanding on blood and tissue protozoa such as Leishmania

2.3 General feature of Leishmania

In the vertebrate host Leishmania is found in the macrophages and other cells of the reticuloendothelium system in the skin, spleen, liver, bone marrow, lymph nodes and mucosa. It may also be found in leucocytes in the blood (**Figure 2.3a**; **Figure 2.3b**).

This leishmanial, or amastigote form, after ingestion by a sandfly, transforms into a promastigoteform in the insect gut in which the kinetoplast is situated at the posterior end of the body. These divide repeatedly by binary fission, migrate to the proboscis, and when the insect subsequently feeds, and are inoculated into a new host. Once within a macrophage the promastigote reverts to the amastigote form and again starts to divide (**Figure 2.3c**).

Leishmania occur primarily in mammals, although ten species have been described in

Old Worldlizards. They cause disease in man, dogs and various rodents. Leishmania have a heteroxenous life cycle, are transmitted by sandflies of the genus Phlebotomus in the Old World and Lutzomyia in the New World.

Hypopylaria are primitive species found in old world lizards, which become infected following ingestion of sandflies. Development occurs in the sandfly hindgut.

Peripylaria develop in both the hindgut and foregut of sandflies and infect both lizards and mammals. Transmission in mammals is by bite of sandflies.

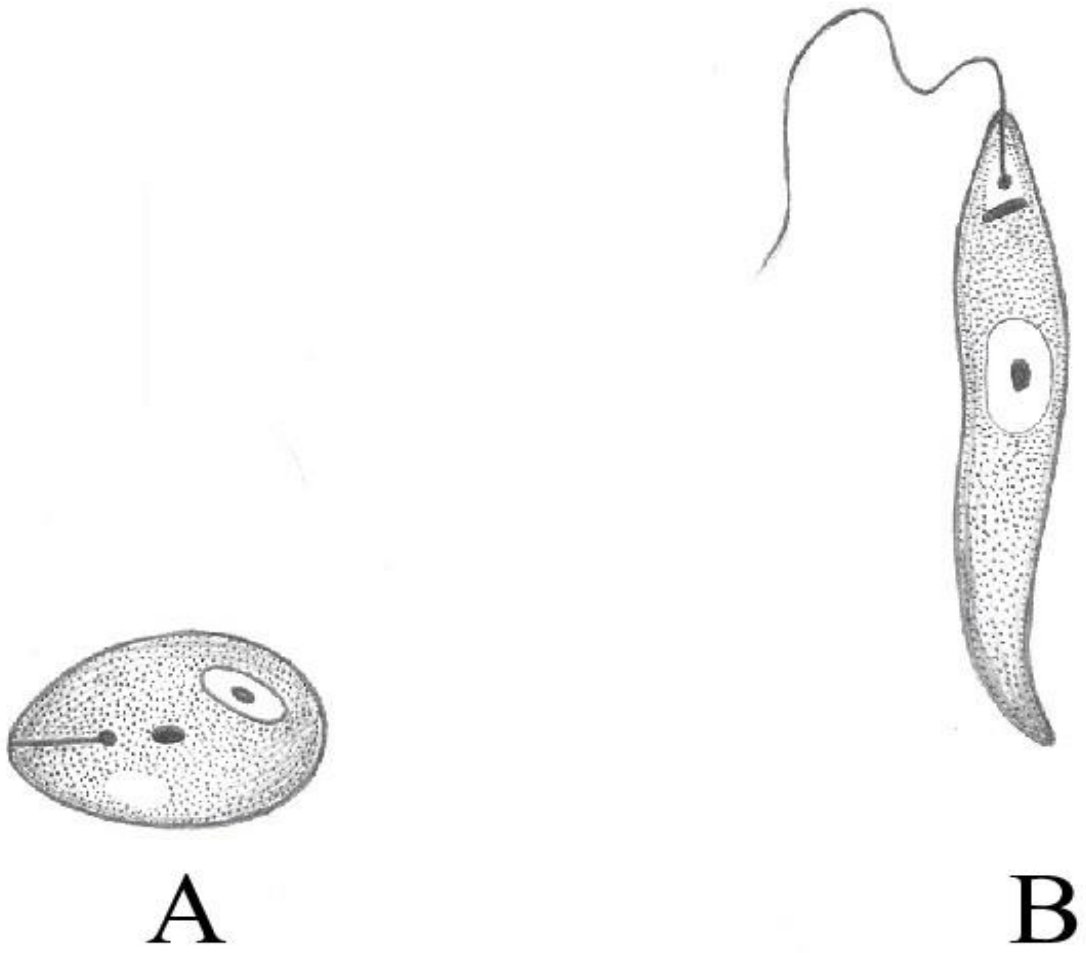


Figure 2.3a: *Leishmania* (a) Promastigote form. (b) Amastigote form

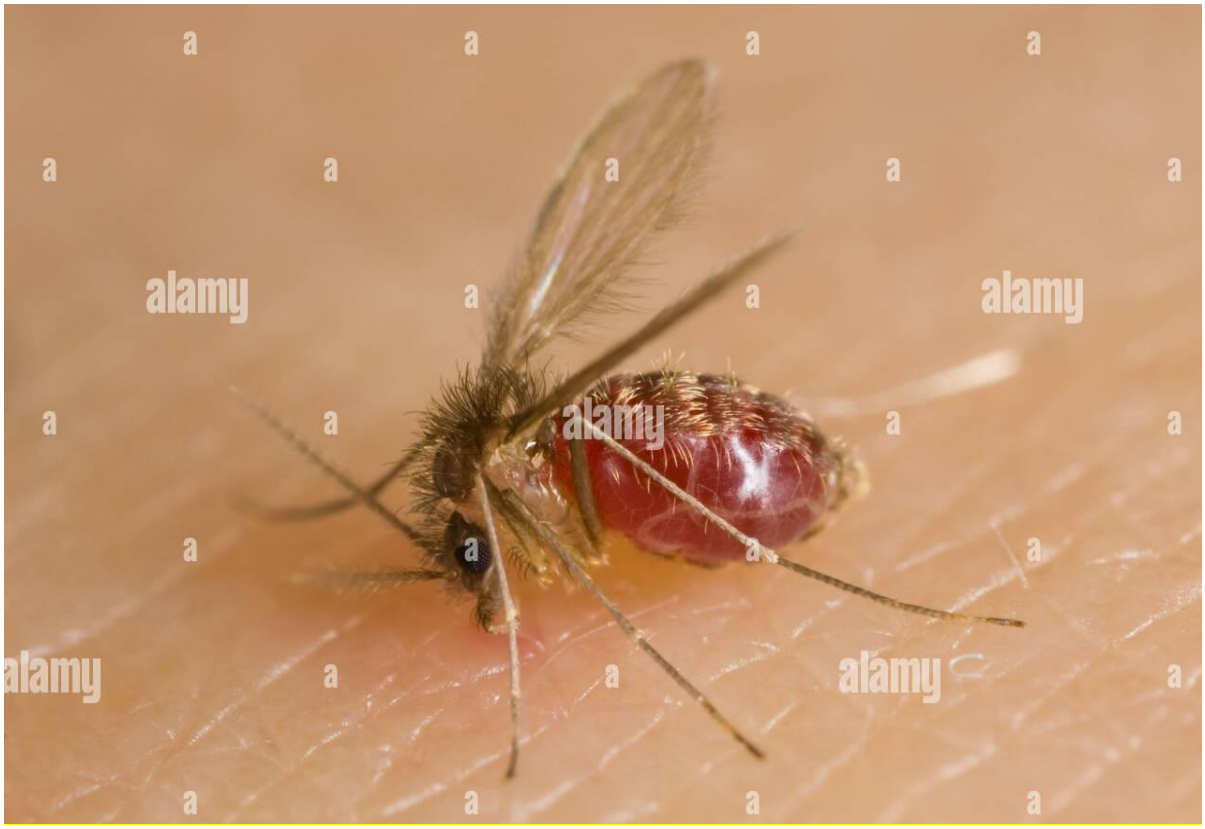


Figure 2.3b: Picture of a sand fly biting a human arm

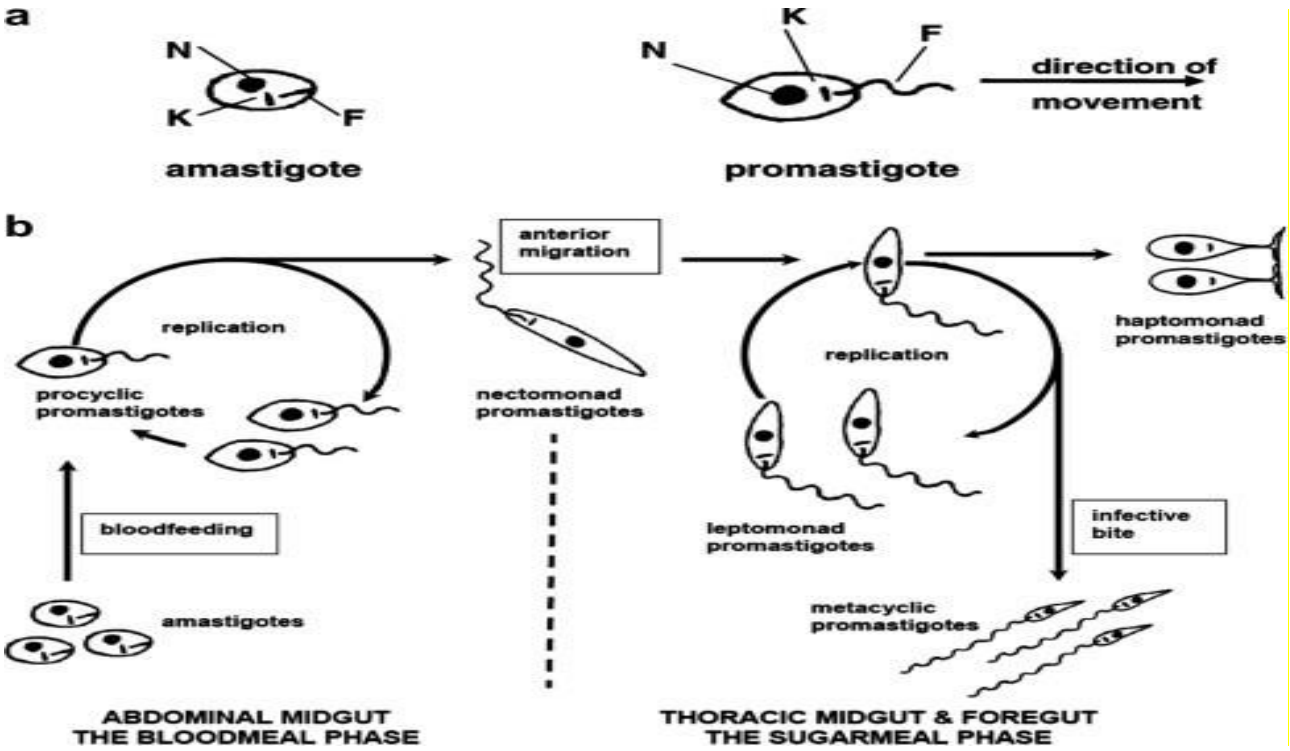


Figure 2.3c: metacyclic promastigotes of Leishmania parasite

Self-Assessment Exercise 1

1. ***Differentiate Hypopylaria from Peripylaria in Leishmaniasis?***
2. ***Briefly explain the morphology of Leishmaniasis?***

2.3.1 Leishmaniasis

Leishmaniasis is the disease caused by Leishmania

Epidemiology

Leishmaniasis is prevalent worldwide: ranging from South East Asia, Indonesia, Pakistan, Mediterranean, North and Central Africa, and South and Central America.

Morphology

In man, the parasites occur in the amastigote form only, but in the insect vector the promastigote form is assumed. The amastigote forms are small, ovoid or round bodies (often called Leishmania Donovan bodies) about 2 - 5µm in diameter by 1 - 3 µm whereas the leptomonad measures 14 - 20 microns by 1.5 - 4 microns, a similar size to trypanosomes. Under light microscopy, the cells are seen to contain a central nucleus, a rod-shaped kinetoplast and (sometimes) a basal body (from which the flagellum arises.

Life cycle

The organism is transmitted by the bite of several species of blood-feeding sand flies (*Phlebotomus*) which carry the promastigote in the anterior gut and pharynx. The parasites gain access to mononuclear phagocytes where they transform into amastigotes and divide until the infected cell ruptures. The released organisms infect other cells. The sandfly acquires the organisms during the blood meal; the amastigotes transform into flagellate promastigotes and multiply in the gut until the anterior gut and pharynx are packed. Dogs and rodents are common reservoirs (**Figure 2.3.1a)**.

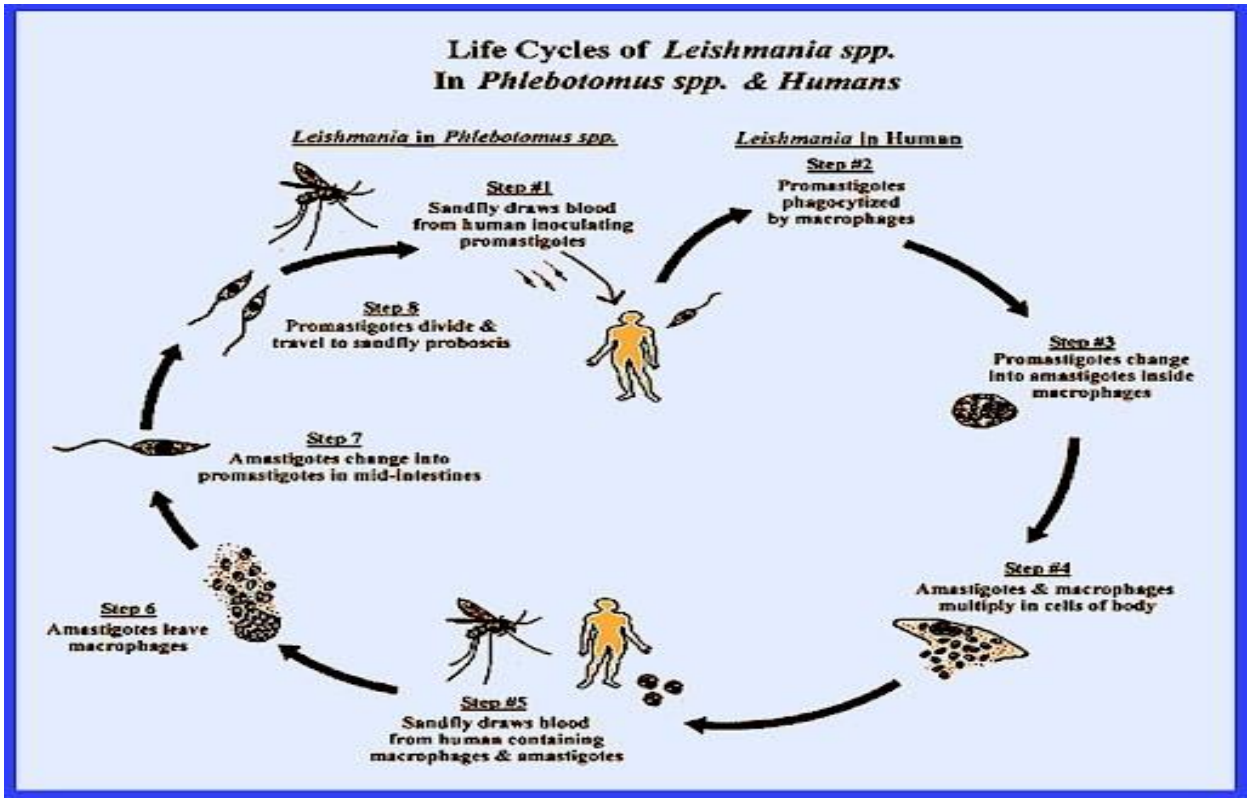


Figure 2.3.1a: Life cycle of *Leishmania donovani* and *L tropica* in man and vector, *Phlebotomusargentipes*

Etiology

Several species of Leishmania are pathogenic for man: *L. donovani* causes visceral leishmaniasis (Kala- azar, black disease, dumdum fever); *L. tropica* (*L. t. major, L. t. minor* and *L. ethiopica*) cause cutaneous leishmaniasis (oriental sore, Delhi ulcer, Aleppo, Delhi or Baghdad boil); and *L. braziliensis* (also, *L. mexicana* and *L. peruviana*) are etiologic agents of mucocutaneous leishmaniasis (espundia, Uta, chiclero ulcer).

Symptoms

Visceral leishmaniasis (kala-azar, dumdum fever)

L. donovani organisms in visceral leishmaniasis are rapidly eliminated from the site of infection; hence there is rarely a local lesion,although minute papules have been described in children. They are localized and multiply in the mononuclear phagocytic cells of spleen, liver, lymph nodes, bone marrow, intestinal mucosa and other organs. One to four months after infection, there is occurrence of fever, with a daily rise to 102-104 degrees F, accompanied by chills and sweating. The spleen and liver progressively become enlarged. With progression of the diseases, skin develops hyper pigmented granulomatous areas (kala-azar means black disease). Chronic disease renders patientssusceptible to other infections. Untreated disease results in death.

Cutaneous leishmaniasis (Oriental sore, Delhi ulcer, Baghdad boil)

In cutaneous leishmaniasis, the organism (*L. tropica*) multiplies locally, producing of a papule, 1-2 weeks (or as long as 1-2 months) after the bite. The papule gradually

grows to form a relatively painless ulcer. The center of the ulcer encrusts while satellite papules develop at the periphery. The ulcer heals in 2-10 months, even if untreated but leaves a disfiguring scar (**Figure 2.3.1b**). The disease may disseminate in the case of depressed immune function.

Mucocutaneous leishmaniasis (espundia, Uta, chiclero)

The initial symptoms of mucocutaneous leishmaniasis are the same as those of cutaneous leishmaniasis, except that in this disease the organism can metastasize and the lesions spread to mucoid (oral, pharyngeal and nasal) tissues and lead to their destruction and hence sever deformity **(Figure 2.3.1c**). The organisms responsible are *L. braziliensis*, *L. mexicana* and *L. peruviana*.

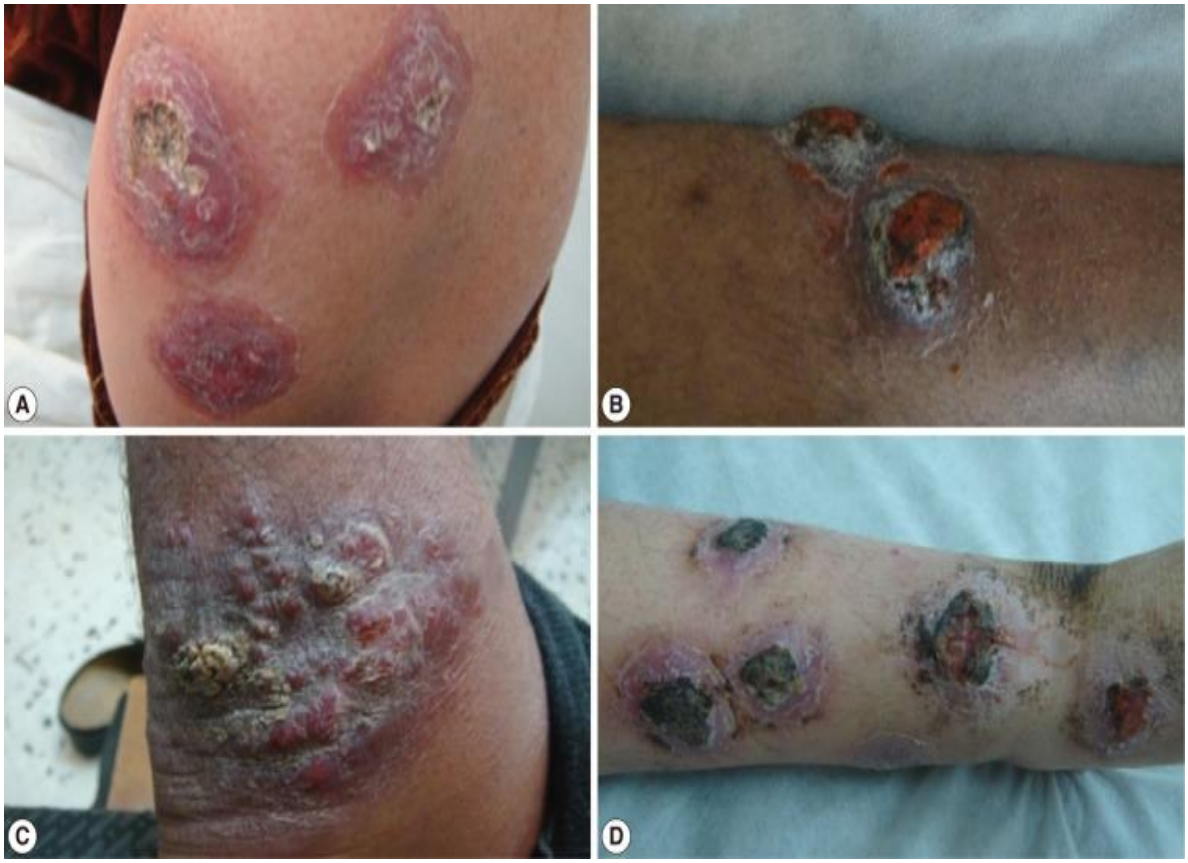
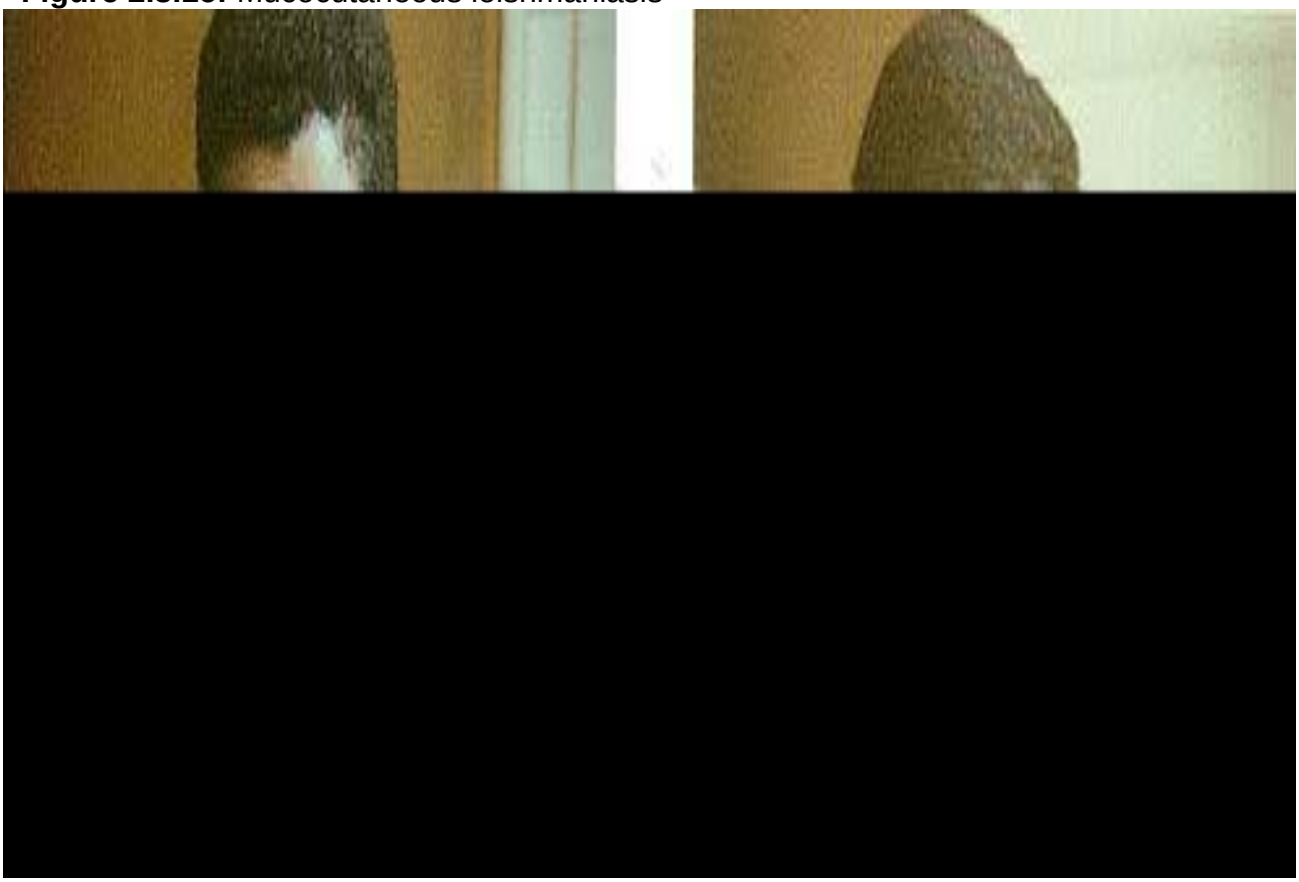


Figure 2.3.1b: Cutaneous Leishmaniasis

Figure 2.3.1c: Mucocutaneous leishmaniasis



Pathology

Pathogenesis of leishmaniasis is due to an immune reaction to the organism, particularly cell mediated immunity. Laboratory examination reveals a marked leukopenia with relative monocytosis and lymphocytosis, anemia and thrombocytopenia. IgM and IgG levels are extremely elevated due to both specific antibodies and polyclonal activation.

Diagnosis

Diagnosis is based on a history of exposure to sandfies, symptoms and isolation of the organisms from the lesion aspirate or biopsy, by direct examination or culture. A skin test (delayed hypersensitivity: Montenegro test) and detection of anti-leishmanial antibodies by immuno-fluorescence are indicative of exposure.

Treatment and Control

Sodium stibogluconate (Pentostam) is the drug of choice. Pentamidine isethionate is used as an alternative. Control measures involve vector control and avoidance. Immunization has not been effective.

Mention the three Leishmania species that are pathogenic to man.

Self-Assessment Exercise 2

1. ***Outline the species of Leishmania that are pathogenic to man?***
2. ***Briefly highlight the Diagnosis of Leishmaniasis?***

2.4 Summary

In this unit, you have learnt about the medical important of blood and tissue protozoa such as Leishmania, their etiology, morphology, life cycle, symptom, pathology, diagnosis, treatment and control. A biological significant blood and tissue protozoan such as Leishmania causes different disease conditions in their hosts. The parasites are found in the amastigote stage in cells of the vertebrate host and in the promastigote stage in the intestine of the sandfly.

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2.6 Possible Answers to SAEs

Answers to SAEs 1

1. ***Hypopylaria*** *are primitive species found in old world lizards, which become infected following ingestion of sandflies. Development occurs in the sandfly hindgut.*
   Peripylaria *develop in both the hindgut and foregut of sandflies and infect both lizards and mammals. Transmission in mammals is by bite of sandflies.*
2. *- In man, the parasites occur in the amastigote form only, but in the insect vector the promastigote form is assumed.*

- *The amastigote forms are small, ovoid or round bodies (often called Leishmania Donovan bodies) about 2 - 5µm in diameter by 1 - 3 µm whereas the leptomonad measures 14 - 20 microns by 1.5 - 4 microns, a similar size to trypanosomes.*
- *Under light microscopy, the cells are seen to contain a central nucleus, a rod-shaped kinetoplast and (sometimes) a basal body (from which the flagellum arises*.

Answers to SAEs 2

1. - *L. donovani* causes visceral leishmaniasis (Kala- azar, black disease, dumdum fever)

- *L. tropica* (*L. t. major, L. t. minor* and *L. ethiopica*) cause cutaneous leishmaniasis (oriental sore, Delhi ulcer, Aleppo, Delhi or Baghdad boil)

- *L. braziliensis* (also, *L. mexicana* and *L. peruviana*) are etiologic agents of mucocutaneous leishmaniasis (espundia, Uta, chiclero ulcer).

2. *Diagnosis is based on a history of exposure to sandfies, symptoms and isolation of the organisms from the lesion aspirate or biopsy, by direct examination or culture. A skin test (delayed hypersensitivity: Montenegro test) and detection of anti-leishmanial antibodies by immuno-fluorescence are indicative of exposure*

Unit 3: Plasmodium

Unit Structure

3.1 Introduction

Parasites of the genus Plasmodium are responsible for the disease 'malaria' in both animals and man. Although the species attacking man have been most extensively studied, considerable use has been made of species in laboratory animals, especially *P. knowlesi* and *P. cynomolgi* in monkeys, *P. relictum, P. cathemerium* and *P. gallinaceum* in birds, and *P. berghei, P. yoelii* and *P. chabaudi* in rodents. Species also occur in other mammmals such as squirrels and bats and in amphibians and reptiles, but these have not been much studied. Although it has been known for many years that some species of monkey malaria (e.g. *P. cynomolgi*) are transmissible to man, itis only comparatively recently that it has been found that human malaria can be transmitted to several species of monkeys. The Colombian night monkey, *Aotus trivirgatus*, has proved to be an especially valuable laboratory model for malarial research.

3.2 Intended Learning Outcomes (ILOs)

By the end of this lecture unit, students should be able to:

- Have a good understanding on blood and tissue protozoa such as *Plasmodium*

3.3 Plasmodium as a true malaria parasite

The genus Plasmodium contains the 'true' malarial parasites (in contrast to the haemoproteids); the recognition that profound differences exist between the various species has led to the further subdivision into seven subgenera, which are as follows:
Plasmodium: Parasitic in primates, with exo-erythrocytic schizogony in parenchymal cells of liver; erythrocytic schizonts large; gametocytes round.
Vinckeia: Parasitic in non-primate mammals with exo-erythrocytic schizogony in parenchymal cells of liver; erythrocytic schizonts usually small; gametocytes round.
Laverania: Parasitic in primates, with exo-erythrocytic schizogony in parenchymal

cells of liver;gametocytes crescentic with perinuclear pigment.
Haemamoeba: Parasitic in birds; exo-erythrocytic schizogony in reticulo-endothelial cells; erythrocytic schizonts large; gametocytes round.
Giovannolaia: Parasitic in birds, exo-erythrocytic schizogony in reticulo-endothelial cells; erythrocytic schizonts large; gametocytes elongate.
Novyella: Parasitic in birds; exo-erythrocytic schizogony in reticulo-endothelial cells; erythrocytic schizonts small; gametocytes elongate.
Huffia: Parasitic in birds; exo-erythrocytic schizonts in the haemopoietic (i.e. blood-forming) system; gametoocytes elongate.

Self-Assessment Exercise 1

1. ***Mention the four species of Plasmodium that infect man and result in four kinds of malarial fever?***
2. ***What is Premunition?***

3.3.1 Etiology of Plasmodium species

Four *Plasmodium* species are responsible for human malaria. These are *P. falciparum*, *P. vivax*, *P. ovale* and *P. malariae*.

There are four species of Plasmodium that infect man and result in four kinds of malarial fever.

- *P. vivax*: benign, simple or tertian malaria.
- *P. falciparum*: aestivo-autumnal, malignant tertian, pernicious quotidian, sub tertian or tropical malaria.
- *P. malariae*: quartan ague, or quartan malaria.
- *P. ovale*: ovale tertian malaria.

Of the above species, *P. vivax* shows the widest distribution, being prevalent throughout the tropics and many temperate regions. Vivax malaria is characterised by relapses: reappearances ofsymptoms after a latent of about up to 5 years, as is infection with P. ovale, which occurs chieflyin tropical Africa. Such relapses are due to the sudden activation of hypnozoites (sleeping merozoites) in liver cells". *P. falciparum* is most common in tropical and subtropical areas and causes the most dangerous, malignant form of malaria, which fortunately does not have relapses
P. malariae is widely distributed but is much less common than *P. vivax* or *P. falciparum*. Although falciparum malaria or malariae malaria do not show relapses, they are subjected to 'recrudescence's': repeated manifestations of infection after a relatively short latent period between 3 months and 1 year.
Human malaria can develop in certain monkey species. The pattern of the life cycles of these species follows the general life cycle already given (**Figure. 3.3.1**) but there are important physiological differences which are often reflected in the nature of the diseases produced. Many of these characteristics are sufficiently definite to be used as criteria for identification in blood films but species differentiation may not be possible in the earliest stages.
In *P. vivax* and *P. ovale* infections, erythrocytes become enlarged and paler in colour when the parasites have grown beyond the ring stage. In stained preparations,

infected corpuscles show characteristicdots called **Schuffner's dots** (*P. vivax* and *P. ovale*) **Ziemann's dots** (*P. malariae*) or Maurer's dot (*P. falciparum*).The pigment granules vary in size and shape but their appearance and colour depend on number of factors such as intensity of light, type of filter used, etc.

3.3.2 Characteristics of species of Plasmodium

***P. falciparum*:** The most important malarial parasite, the disease it produces runs an acute courseand often terminating fatally. It is a significant cause of abortion or stillbirth and even death of non-immune pregnant women. It is responsible for some 50 per cent of all the malarial cases throughout the world. Its distribution is restricted to warm and tropical countries.

The special features of its life cycle may be summarized as follows:

- It attacks erythrocytes of all ages indiscriminately so that a high density of parasites may be rapidly reached. In extreme cases up to 48 per cent of the red cells may be parasitised.
- Multiple infections (polyparasitism) resulting in several ring forms in a corpuscle are not uncommon
- The later stages in the asexual cycle, that is, the growth to schizonts, do not occur in the peripheral blood as in other forms of malaria, except in severe cases, so that only rings and crescents are found in blood films. After twenty-four hours, the ring forms and older trophozoitesshow a tendency to clump together and adhere to the visceral capillary wall and become caught up in the vessels of the heart, intestine, brain or bone marrow in which the later asexual stagesare completed. This behaviour, together with the fact that the sub tertian malaria is more toxic, is the principal reasons why this type is so dangerous.
- Sporulation is not as well synchronized as in other species so that fever paroxysms may be longer drawn out.
- EE forms do not persist in the tissues and hence relapses do not occur.

***P.* vivax:** Causes the benign tertian form of malaria which is responsible for about 43 percent of all cases in the world and has the widest geographical distribution. Although generally not life- threatening, it can cause severe, acute illness (**Figure 3.3.1**). Several points in its life cycle may be noted:

- The degree of infection is low, for only the young immature corpuscles (reticulocytes) are attacked; about 2 per cent of erythrocytes are parasitised.
- The periodicity of the asexual cycle is closely synchronized.
- Hypnozoites develop in the liver, so that relapses may occur (**Figure 3.3.1**).
- The morphological features of a sub-species *bastianellii* of the simian species *P. cynomolgi* bear a striking resemblance to those of *P. vivax*; differentiation between these two is difficult.

***P.* malariae:** A relatively rare parasite producing quartan malaria which is responsible for about seven percent of the malaria in the world (**Figure 3.3.1**). Particular points of interest are:

- Infected erythrocytes are not larger than uninfected ones and sometimes even smaller
- Mature erythrocytes are attacked and rarely reticulocytes, so that the density of parasites is very low; about 0.2 per cent of erythrocytes are parasitised.
- It is often difficult to distinguish between a large trophozoite and an immature gametocyte.

***P. ovale*:** This is a species rarely encountered; it is confined essentially to the tropics

andsubtropics although reported from many continents. The type of fever it produces (ovale tertian) is milder than the benign tertian of *P. vivax* (**Figure 3. 3.1**).

Special points of interest are:

- It morphologically resembles *P. malariae* in most of its stages.
- The changes produced in the erythrocytes in general are similar to those Produced by *P. vivax*, but Shuffer's dots appear considerably earlier in the ring stage.
- In the oocyst the pigment granules are (usually) characteristically arranged in two rows crossing each other at right angles.
- Hypnozoites develop in the liver so that relapses may occur.

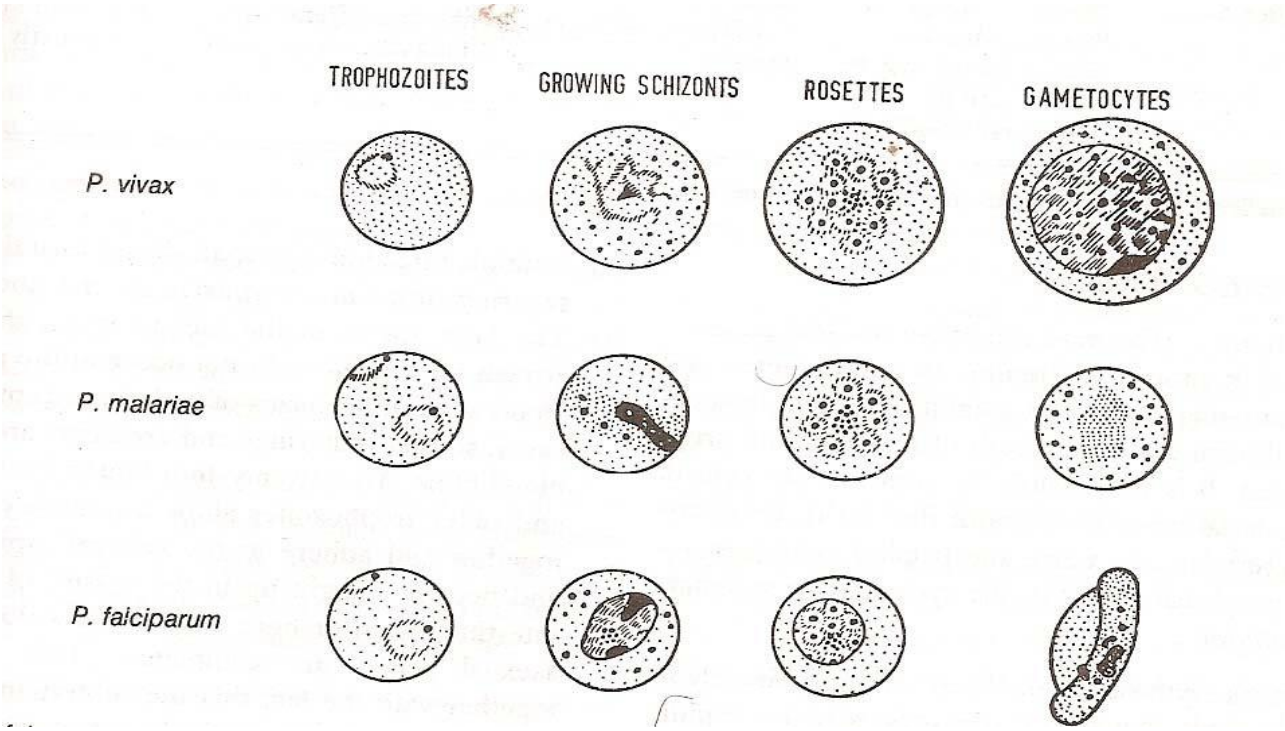

Figure 3.3.1. Comparism of various stages of the three common species of Plasmodium infecting man

3.4 General Concept of Plasmodium

Using the subgeneric designations, the correct zoological nomenclature for the four species infecting man is *Plasmodium (Plasmodium) vivax, P. (P.) ovale, P. (P.) malariae* and *P. (Laverania) falciparum*. However, as is common practice in the literature, the subgeneric name, have been omitted in this write up.

Epidemiology

There are an estimated 200 million global cases of malaria leading a mortality of more than one million people per year. *P. falciparum* (malignant tertian malaria) and *P. malariae* (quartan malaria) are themost common species of malarial parasite and are found in Asia and Africa. *P. vivax* (benign tertian malaria) predominates in Latin

America, India and Pakistan, whereas, *P. ovale* (ovale tertian malaria) is almost exclusively found in Africa.

Morphology

Malarial parasite trophozoites are generally ringed shaped, 1 - 2 microns in size; although other forms (ameboid and band) may also exist. The sexual forms of the parasite (gametocytes) are much larger and 7 - 14 microns in size. *P. falciparum* is the largest and is banana shaped while others are smaller and round. *P. vivax* causes stippling of infected red cells.

Life cycle

Malarial parasites are transmitted by the infected female anopheline mosquito which injects sporozoites present in the saliva of the insect. Sporozoites infect the liver parenchymal cells where they may remain dormant (hypnozoites) or undergo stages of schizogony to produce schizonts and merogony to produce merozoites (meronts). When parenchymal cells rupture, thousands of meronts are released into blood and infect the red cells. *P. ovale* and *P. vivax* infect immature red blood cells whereas *P. malariae* infects mature red cells. *P. falciparum* infects both. In red cells, the parasites mature into trophozoites". "These trophozoites undergo schizogony and merogony in red cells which ultimately burst and release daughter merozoites. Some of the merozoites transform into male and female gametocytes while others enter red cells to continue the erythrocytic cycle. The gametocytes are ingested by the female mosquito; the female gametocyte transforms into ookinete, is fertilized, and forms an oocyst in the gut. The oocyte produces sporozoites (sporogony) which migrate to the salivary gland and are ready to infect another host. The liver (extraerythrocytic) cycle takes 5 - 15 days whereas the erythrocytic cycle takes 48 hours or 72 hours (*P. malariae*). Malaria can be transmitted by transfusion and transplacentally **(Figure 3.4)**.

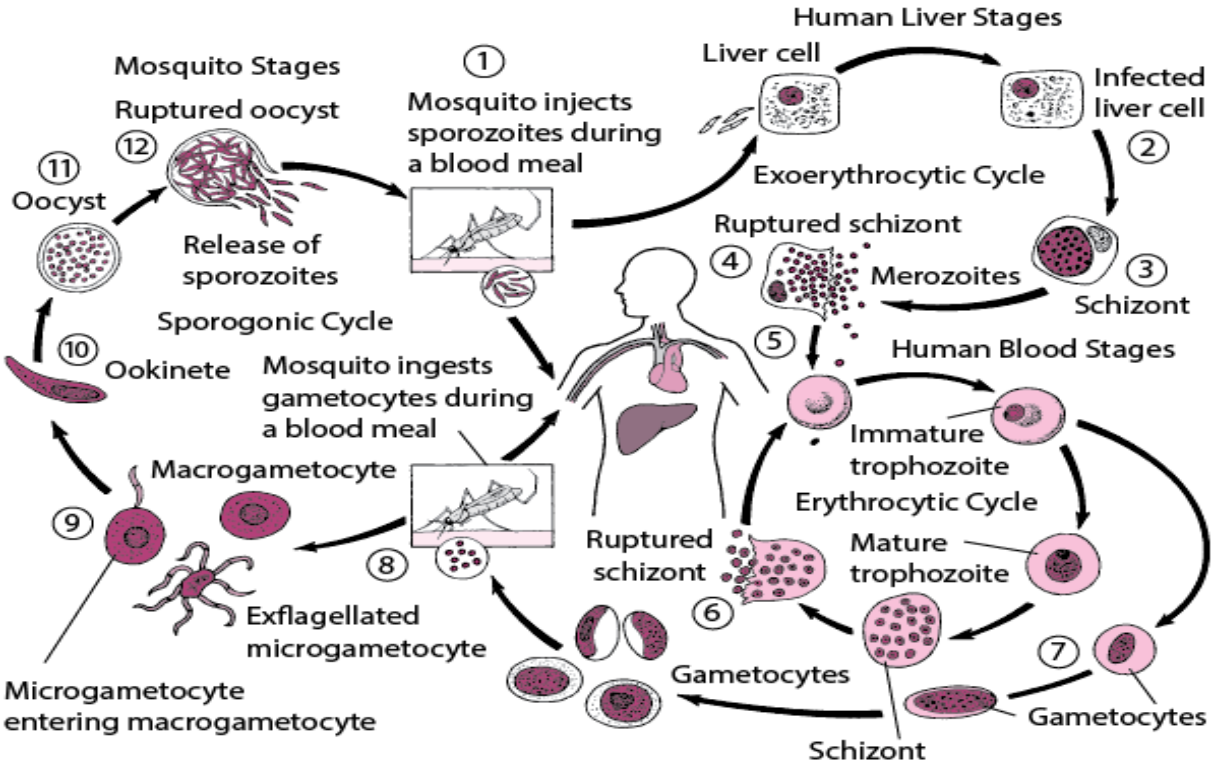

Figure 3.4: Life cycle of *Plasmodium parasite*

3.4.1 Clinical Manifestations of Plasmodium parasite

The pathology and clinical manifestations associated with malaria are almost exclusively due to the asexual erythrocytic stage parasites. Tissue schizonts and gametocytes cause little, if any, pathology. *Plasmodium* infection causes an acute febrile illness which is most notable for its periodic fever paroxysms occuring at either 48 or 72 hour intervals". "The severity of the attack depends on the *Plasmodium* species as well as other circumstances such as the state of immunity and the general health and nutritional status of the infected individual. Malaria is a chronic disease which has a tendency to relapse or recrudesce over months or even years. The most common way to obtain malaria is through the natural transmission by mosquitoes". Malaria can also be transmitted via blood transfusions or sharing syringes. "Mechanical transmission of infected blood will result in a shorter incubation period since there will be no liver stage. There is also an increased risk of fatality with mechanically-transmitted *P. falciparum*. The lack of the liver stage infection also precludes relapses in *P. vivax* or *P. ovale* infections. Congenital transmission has also been documented, but is believed to be relatively rare despite the heavy infection of the placenta.

Pathogenesis and Severe Malaria

Pathology associated with all malarial species is related to the rupture of infected erythrocytes and the release of parasite material and metabolites, hemozoin (i. e, malaria pigment) and cellular debris. In addition to the paroxysms discussed above, the deposition of hemozoin has long been known as a characteristic feature of malaria. There is an increased activity of the reticuloendothelial system, particularly in the liver and spleen and thus their enlargement, as evidenced by macrophages with ingested infected and normal erythrocytes and hemozoin. Except for *P. falciparum*, the pathology associated with malaria tends to be benign. Several severe complications can be associated with falciparum malaria with cerebral malaria being the most notable and a frequent cause of death. Cerebral malaria is characterized by an impaired consciousness (Box). The presenting symptoms are severe headache followed by drowsiness, confusion, and ultimately coma. Convulsions are also frequently associated with cerebral malaria. These neurological manifestations are believed to be due to the sequestration of the infected erythrocytes in the cerebral microvasculature. Sequestration refers to the cytoadherence of trophozoite- and schizont-infected erythrocytes to endothelial cells of deep vascular beds in vital organs, especially brain, lung, gut, heart and placenta. This sequestration provides several advantages for the parasite. The major advantage is the avoidance of the spleen and the subsequent elimination of infected erythrocytes. In addition, the low oxygen tensions in the deep tissues may provide a better metabolic environment.
Cytoadherence appears to be mediated by the electron-dense protuberances on the surface of the infected erythrocyte. These 'knobs' are expressed during the trophozoite and schizont stages and are formed as a result of parasite proteins exported to the erythrocyte membrane. Among human *Plasmodium* species, knobs are restricted to *P. falciparum* and thus suggest that the knobs play a role in cytoadherence. In addition, there is also a good correlation between animal *Plasmodium* species which express knobs and exhibit sequestration. Electron microscopy also shows that the

knobs are contact points between the infected erythrocyte and the endothelial cell. The molecular mechanisms of cytoadherence involve receptor-ligand interactions. In other words, proteins expressed on the surface of the infected erythrocyte (ligand) will bind to proteins expressed on the surface of the endothelial cells (receptor). *Pf*EMP-1 (erythrocyte membrane protein) is a parasite protein which has been implicated as the cytoadherence ligand (Box). In contrast to the usually highly conserved nature of receptor-ligand interactions, *Pf*EMP-1 is a member of a highly variable (=*var*) gene family with 40-50 different genes. Several host proteins which possibly function as receptors have been identified (see box below). Many of these host proteins function in cell-cell interactions and are involved in cellular adhesion. Several studies have indicated that the expression of different *Pf*EMP-1 genes is correlated with different receptor-binding phenotypes. This antigenic variation associated with the surface exposed *Pf*EMP-1 allows the parasite to evade the immune system. However, the cytoadherence function is preserved through its ability to recognize multiple receptors. This antigenic variation may also account for different disease outcomes. For example, intercellular adhesion molecule-1 (ICAM-1) is usually implicated in cerebral pathology.

Symptoms

The symptomatology of malaria depends on the parasitemia, the presence of the organism in different organs and the parasite burden. The incubation period varies generally between 10 - 30 days. As the parasite load becomes significant, the patient develops headache, lassitude, vague pains in the bones and joints, chilly sensations and fever. As the disease progresses, the chills and fever become more prominent. The chill and fever follow a cyclic pattern (paroxysm) with the symptomatic period lasting 8 - 12 hours. In between the symptomatic periods, there is a period of relative normalcy, the duration of which depends upon the species of the infecting parasite. This interval is about 34 - 36 hours in the case of *P. vivax* and *P. ovale* (tertian malaria), and 58 - 60 hours in the case of *P. malariae* (quartan malaria). Classical tertian paroxysm is rarely seen in *P. falciparum* and persistent spiking or a daily paroxysm is more usual. The malarial paroxysm is most dramatic and frightening. It begins with a chilly sensation that progresses to teeth chattering, overtly shaking chill and peripheral vasoconstriction resulting in cyanotic lips and nails (cold stage). This lasts for about an hour. At the end of this period, the body temperature begins to climb and reaches 103 -106 degrees F (39 - 41degrees C). Fever is associated with severe headache, nausea (vomiting) and convulsions. The patient experiences euphoria, and profuse perspiration and the temperature begin to drop. Within a few hours the patient feels exhausted but symptom less and remains symptomatic until the next paroxysm. Each paroxysm is due to the rupture of infected erythrocytes and release of parasites.

Without treatment, all species of human malaria may ultimately result in spontaneous cure except with *P. falciparum* which becomes more severe progressively and results in death. This organism causes sequestration of capillary vasculature in the brain, gastrointestinal and renal tissues. Chronic malaria results in splenomegaly, hepatomegaly and nephritic syndromes.

Diagnosis

Malaria is suspected in persons with a history of being in an endemic area and presenting symptoms consistent with malaria. These symptoms, especially in the early stages of the infection, are non-specific and often described as flu-like. As the disease

progresses, the patient may exhibit an enlarged spleen and/or liver and anemia. Diagnosis is confirmed by microscopy. Thick blood smears are generally more sensitive for the detection of parasites, whereas thin smears are preferable for species identification. (See blood-stage morphology of *Plasmodium* species.) If parasites are not found on the first blood smear it is recommended to make additional smears every 6 -12 hours for as long as 48 hours. A tentative diagnosis of *P. falciparum* (numerous and exclusively ring stages) could constitute a medical emergency, especially in a non-immune person. Rapid immunochromatographic tests (i.e., dipsticks) based on antigen detection are also available.

Prevention and Control

Strategies for preventing and controlling malaria involve three different approaches. Prevention of malaria in individuals will generally involve the reduction of human-mosquito contact through the use of bednets, repellents, etc. Chemoprophylaxis can also be used, especially in travelers. However chemoprophylaxis only suppresses parasitemia and does not prevent infection. Control activities at the community level can utilize approaches which directly reduce human-mosquito contact as well as approaches which reduce the total number of mosquitoes in an area. Such approaches include the reduction in mosquito breeding grounds (e.g. environmental modification); target the larva stages with chemical or biological agents and massive insecticide spraying for the adult mosquitoes. Biological control methods include the introduction of fish which eat the mosquito larvae or bacteria (e.g. *Bacillus thuringiensis*) which excrete larval toxins. Case detection and treatment is another potential control method. Identifying and treating infected persons, especially asymptomatic individuals, will reduce the size of the parasite reservoir within the human population and can lower transmission rates. However, this can be a relatively expensive approach. These approaches are not mutually exclusive and can be combined. Many of the successful control programs include both measures to control mosquitoes and treatment of infected individuals. There is no standard method of malaria control that has proven universally effective. The epidemiologic, socioeconomic, cultural and infrastructural factors of a particular region will determine the most appropriate malaria control. Some of the factors which need to be considered include:

- infrastructure of existing health care services and other resources
- intensity and periodicity (e g, seasonality) of transmission
- Mosquito species (ecological requirements, behavioral characteristics, insecticide sensitivity, etc.)
- parasite species and drug sensitivities
- cultural and social characteristics of the population
- presence of social and ecological change

The control of malaria in tropical Africa has been particularly problematic because of the high transmission rates and the overall low socio-economic level. Several studies have shown that insecticide treated bednets (ITBN) reduce the morbidity and mortality associated with malaria. In most areas the introductions of bednets do not require large promotional programs and their use is readily accepted. This may be in part due to the reduction in mosquito nuisance biting. Some questions have been raised in regards to the economic sustainability of bednet programs. It is necessary to re-treat

the bednets with insecticide periodically and the bednets need to be repaired and replaced as they become torn and wear out. In addition, some have raised concerns about the long-term benefits of bednets since they reduce exposure, but do not eliminate it. This reduction in exposure may delay the acquisition of immunity and simply postpone morbidity and mortality to older age groups.

3.5. Malaria Immunity and treatment

As far as is known, the plasmodia of man will develop only in female mosquitoes of the genus *Anopheles* and not in any other arthropod. Almost any species can be infected with plasmodia in the laboratory, but many species are poor vectors and not natural ones. For a mosquito to be efficient vector, it must present certain characteristics: (a) it must be susceptible to infection and present physico-chemical and nutritional characteristics suitable for the development of plasmodia; (b) it must bite man in preference to animals; (c) it must not be shy of human habitation; (d) its span of life must be sufficiently long to permit sexual development of the plasmodium. Persons living in endemic areas do develop immunity against malaria. Almost always a person will exhibit symptoms during their initial exposures to malaria. Symptoms associated with subsequent exposures to malaria are usually less severe, though. The immunity against malaria is slow to develop and requires multiple exposures. In highly endemic areas only young children are at a high risk of developing severe falciparum malaria whereas older children and adults are essentially protected from severe disease and death. However, this immunity is not a sterilizing immunity in that persons can still become infected. In addition the immunity is short lived and in the absence of repeated exposure the level of immunity decreases. For example, previously semi-immune adults will often develop severe malaria upon returning to an endemic area after being in a non-endemic area for 1-2 years". "This state of partial immunity in which parasitemia is lowered, but not eliminated, and parasitemia is better tolerated is sometimes referred to as '**Premunition'**. **Premunition** refers to an immunity that is contingent upon the pathogen being present. The immune response could be directed at either the pre-erythrocytic or erythrocytic stages of the parasite's life cycle. However, the erythrocytic stage of the life cycle is probably the most important in terms of clearing the parasite and lessening the disease. Due to the lack of HLA molecules on the surface of the parasite or the erythrocyte it is usually assumed that antibody will play a key role in blood-stage immunity". Possible effecter mechanisms for antibody include: blocking erythrocyte invasion by merozoites, antibody-dependent cellular killing mediated by cytophilic antibodies, or increased clearance of infected erythrocytes due to binding of antibodies to parasite antigens exposed on the erythrocyte surface. All of these will result in lower parasitemia. "The relative importance of these various mechanisms is not clear and probably immunity probably requires the generation of antibodies against numerous targets. This, along with antigenic variation and polymorphisms in many *Plasmodium* antigens, could explain the slow development of immunity. The observation that asymptomatic individuals can exhibit high levels of parasitemia has led to the concept of 'anti-disease immunity'. This would be in addition to the 'anti-parasite' immunity discussed above which results in lower parasitemia. Severe malaria and death are correlated with TNF-α and other proinflammatory cytokines. As discussed for the paroxysms and cerebral malaria, antigens or toxins released by the infected erythrocyte could stimulate the production of proinflammatory cytokines. Antibodies

against these exo-antigens could possibly neutralize their toxic effects and thus lead to an anti-disease immunity.

Human Genetics and Innate Resistance

Several inherited erythrocyte disorders are found predominantly in malaria endemic areas and at frequencies much higher than expected. This has led to speculation that these disorders confer some protection against malaria. For example, Southeast Asian ovalcytosis is due to a mutation in an erythrocyte membrane protein called band 3. This mutation causes the erythrocyte membrane to become more rigid and more refractory to merozoite invasion". "The mechanism(s) by which the other diseases might confer protection against malaria are not known. In most cases it is presumed or speculated that the combination of the defect and infection leads to premature lysis or clearance of the infected erythrocyte. For example, glucose-6-phosphate dehydrogenase (G6PD) deficient erythrocytes would have an impaired ability to handle oxidative stress. The additional oxidants produced as a result of parasite metabolism and the digestion of hemoglobin may overwhelm the infected erythrocyte and lead to its destruction before the parasite is able to complete schizogony. Sickle cell anemia and thalassemia are also speculated to make the infected erythrocyte more susceptible to oxidative stress.

Chemotherapy

Several antimalarial drugs are available. Many factors are involved in deciding the best treatment for malaria. These factors include the parasite species, the severity of disease (e.g. complicated), the patient's age and immune status, the parasite's susceptibility to the drugs (i.e. drug resistance), and the cost and availability of drugs. Therefore, the exact recommendations will often vary according to geographical region. In addition, the various drugs act differentially on the different life cycle stages. Fast-acting blood schizontocides, which act upon the blood stage of the parasite, are used to treat acute infections and to quickly relieve the clinical symptoms". Chloroquine is generally the recommended treatment for patients with *P. vivax*, *P. ovale*, *P. malariae*, and uncomplicated chloroquine-sensitive *P. falciparum* infections. Chloroquine is safe and usually well tolerated. Side effects may include pruritus (i.e., itching), nausea, or agitation. Patients infected with either *P. vivax* or *P. ovale*, and that are not at a high risk for reinfection, should also be treated with primaquine (a tissue schizontocide). Primaquine is effective against the liver stage of the parasite, including hypnozoites (see relapses), and will prevent future relapses. The combination of chloroquine and primaquine is often called **'Radical Cure'**.
Severe, or complicated, falciparum malaria is a serious disease with a high mortality rate and must be regarded as life threatening, and thus requires urgent treatment. Treatment typically requires parenteral drug administrations (i.e. injections) since the patients are often comatose or vomiting, and thus cannot take the drugs orally. Parenteral formulations are available for chloroquine, quinine, quinidine and artemisinin derivatives. The artemisinin derivatives are generally the preferred choice, but are not yet approved everywhere. For example, in the United States quinine and quinidine are the approved drugs for severe malaria. Patients need to be continuously monitored for hematocrit, parasitemia, hydration levels, hypoglycemia, and signs of drug toxicity and other complications during the course of treatment. A switch to oral administration should be made as soon as the patient is able. Most deaths due to

severe malaria occur at or close to home in situations where the patients cannot be taken to the hospital. Artemisinin suppositories which can be administered by village health workers have also been developed and have proved to be safe and effective.
The efficacy of chloroquine is greatly diminished by the wide spread chloroquine resistance of *P. falciparum* and the emergence of chloroquine-resistant *P. vivax*. If chloroquine therapy is not effective, or if in an area with chloroquine-resistant malaria, common alternative treatments include: mefloquine, quinine in combination with doxycycline, or Fansidar®. Derivatives of artemisinin (dihydroartemisinin, artesunate and artemether) are increasingly used in Asia and Africa and are now recommending as the first line of treatment by the World Health Organization". These drugs were originally derived from the wormwood plant (*Artemesia annua*) and have been used for a long time in China as an herbal tea called quinhaosu to treat febrile illnesses. To prevent the high recrudescence rates associated with artemisinin derivatives and to slow the development of drug resistance it is recommended that treatment be combined with an unrelated anti-malarial. Drugs used in combination with artemisinin include mefloquine, lumefantrine, Fansidar®, and amodiaquine.
Chemoprophylaxis is especially important for persons from non-malarious areas who visit areas endemic for malaria. Such non-immune persons can quickly develop a serious and life-threatening disease. As in the case of treatment there is no standard recommendation and the choices for chemoprophylaxis are highly dependent upon the conditions associated with the travel and the individual person. Chemoprophylaxis requires the use of non-toxic drugs since these drugs will be taken over extended periods of time. Generally the patient will start to take the drug before traveling and then continue taking the drug during the stay in the endemic area and continue taking the drug after returning". This is to insure the drug is maintained at sufficient levels throughout out the visit and to protect against any infection obtained during the visit. Unfortunately, many of the effective and non-toxic drugs (e.g. chloroquine, pyrimethamine, proquanil) are of limited use because of drug resistance. Another strategy is presumptive (or 'standby') treatment to be used in conjunction with prophylaxis. In this case a person either forgoes prophylaxis or takes chloroquine or another relatively non-toxic drug for prophylaxis and carries a drug like Fansidar, mefloquine, or quinine, which they will take if they start to exhibit symptoms associated with malaria.
The use of mefloquine for malaria chemoprophylaxis is somewhat controversial. Mefloquine is efficacious at preventing malaria with a single does per week, thus offering advantages to drugs that need to be administered daily. At this dosage mefloquine is tolerated by most individuals. However, some people experience neuropsychiatric adverse effects such as sleep disturbances and nightmares. This could be exacerbated by international travel which is a stressful event. Randomized, blinded and controlled trials indicate that neuropsychiatric adverse effects are only slightly higher with mefloquine than with other anti-malarial. Killing the exoerythrocytic stage (i.e. liver) would prevent the blood infection and is known as causal prophylaxis. This is highly desirable in that it limits the amount of time the prophylactic drug needs to be taken before and after travel to an endemic area. The only currently available drug for causal prophylaxis is primaquine. However, malaria prophylaxis is not an approved use of primaquine and should only be prescribed for prophylaxis on a case-by-case basis". For example, for persons who frequently have trips of short duration to highly endemic areas and that the person does not exhibit glucose-6-phosphate dehydrogenase deficiency. Tafenoquine is currently undergoing field evaluation for its use in causal prophylaxis.

Drug Resistance

Drug resistance, and in particular, chloroquine resistance is a major public health problem in the control of malaria. Drug resistance is defined by a treatment failure and can be graded into different levels depending on the timing of the recrudescence following treatment (Figure). Traditionally these levels of drug resistance have been defined as sensitive (no recrudescence), RI (delayed recrudescence), RII (early recrudescence), and RIII (minimal or no anti-parasite effect)". A modified protocol based on clinical outcome was introduced by WHO in 1996. In this protocol the level of resistance is expressed as adequate clinical response (ACR), late treatment failure (LTF), or early treatment failure (ETF) as defined by the following:

- ACR, absence of parasitemia (irrespective of fever) or absence of clinical symptoms (irrespective of parasitemia) on day 14 of follow-up
- LTF, reappearance of symptoms or the presence of parasitemia during days 4-14 of follow-up
- ETF, persistence of clinical symptoms in the presence of parasitemia during the first 3 days of follow-up

Either protocol can be used to determine drug resistance, but the clinical outcome protocol is more practical in areas of intense transmission where it may be difficult to distinguish re-infection from recrudescence and where parasitemia in the absence of clinical symptoms is common. Drug resistance by either protocol is determined with in vivo tests in which patients are hospitalized and monitored during and following standard drug treatment". "There are also in vitro tests that can estimate the level of drug resistance by determining the efficacy of the drugs against *P. falciparum* grown in culture. The in vivo and in vitro tests do not always correspond since host immunity and other factors can affect the in vivo outcomes. The identification of specific mutations which might be associated with drug resistance may also lead to the development of tests based on molecular markers.

Drug resistance develops when parasites with decreased sensitivities to antimalarial drugs are selected under drug pressure. Decreased drug sensitivity can be conferred by several mechanisms and reflects genetic mutation(s) or polymorphisms in the parasite population". The drug-resistance parasites will have a selective advantage over the drug-sensitive parasites in the presence of drug and will be preferentially transmitted. Major factors in the development of drug resistance are the use of subtherapeutic doses of drugs or not completing the treatment regimen. The lower drug levels will eliminate the most susceptible parasites, but those which can tolerate the drug will recover and reproduce. "Over time this will lead to a continued selection for parasites which can tolerate even higher doses of the drug. It is crucial to maintain an adequate concentration of the drug for a sufficient time to completely eliminate the parasites from any given individual.

Chloroquine resistance: After its introduction near the end of World War II, chloroquine quickly became the drug of choice for the treatment and prevention of malaria. Not only is chloroquine an effective drug--probably due to its site of action in the food vacuole and its interference with hemozoin formation (see drug action)--but it is also relatively non-toxic and cheap. Two foci of chloroquine resistant *P. falciparum* were detected in Colombia and at the Cambodia-Thailand border during the late 1950's. During the 1960's and 1970's, resistant parasites spread through South America, Southeast Asia, and India. Resistance was first reported in east Africa in 1978 and spread throughout the continent during the 1980's. Chloroquine resistant *P. vivax* was not reported until 1989 in Papua New Guinea and is now found

in several foci in Southeast Asia and perhaps South America. The basis of chloroquine resistance is reduced chloroquine accumulation in the parasite's food vacuole. Furthermore, chloroquine resistance can be partially reversed with inhibitors of P-glycoprotein (an ABC transporter) which are responsible for multi-drug resistance (MDR) in tumor cell lines, thus suggesting a similar phenomenon may occur in *Plasmodium*. Mutations in a MDR-like gene from *P. falciparum* (*Pf*mdr1) were implicated in chloroquine resistance. However, these mutations are not predictive of chloroquine resistance in all geographical areas. *Pf*MDR1 appears to contribute to the degree of chloroquine resistance, but alone it is insufficient to confer resistance. However, *Pf*MDR1 does appear to play a role in resistance to mefloquine and halofantrine and influences the sensitivity to artemisinin.

Another candidate for the genetic locus of chloroquine resistance was identified through a genetic cross and mapping experiment. A 400 kb region on chromosome 7 was found to segregate with chloroquine resistance and further analysis suggested that a single gene, called '***Pf*crt'**, was responsible for chloroquine resistance. Out of a total of 10 polymorphisms identified in this gene, only a single mutation is perfectly associated with the chloroquine resistance phenotype. This mutation results in a lysine at residue 76 being changed to a threonine (K76T). Several field studies have demonstrated an association between *Pf*crt-K76T and chloroquine resistance using both in vivo and in vitro methods. It has been recently suggested that there have been at least 4 founder mutations in the *Pf*crt gene associated with different geographical regions: Asia/Africa, Papua New Guinea, Brazil/Peru, and Colombia. Presumably the use of chloroquine resulted in the subsequent selection and spread of the resistant phenotype.

Malaria is a disease cause by which parasite?

Self-Assessment Exercise 2

1. ***Outline the factors which need to be considered for appropriate malaria control?***
2. ***Briefly discuss the diagnosis of malaria?***

3.6 Summary

In this unit, you have learnt about the medical important of blood and tissue protozoa such as Plasmodium, their etiology, morphology, life cycle, symptom, pathology, diagnosis, treatment and control. A biologically/medically significant blood and tissue protozoan includes members of the Plasmodium (*P. falciparum*, *P. ovale*, *P. malariae* and *P. vivax*). They cause different disease conditions in their hosts such as malaria etc. They can be found in different parts of the world including Africa.

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3.8 Possible Answers to SAEs

Answers to SAEs 1

1. P. vivax: benign, simple or tertian malaria.

- P. falciparum: aestivo-autumnal, malignant tertian, pernicious quotidian, sub tertian or tropicalmalaria.

- P. malariae: quartan ague, or quartan malaria.

- P. ovale: ovale tertian malaria.

2. *Premunition refers to an immunity that is contingent upon the pathogen being present. The immune response could be directed at either the pre-erythrocytic or erythrocytic stages of the parasite's life cycle*

Answers to SAEs 2

1. *-Infrastructure of existing health care services and other resources*
- *Intensity and periodicity (e.g. seasonality) of transmission.*

- *Mosquito species (ecological requirements, behavioral characteristics, insecticide sensitivity, etc.)*
- *Parasite species and drug sensitivities.*
- *Cultural and social characteristics of the population.*
- *Presence of social and ecological change*

2. *- Malaria is suspected in persons with a history of being in an endemic area and presenting symptoms consistent with malaria.*
- *These symptoms, especially in the early stages of the infection, are non-specific and often described as flu-like. As the disease progresses, the patient may exhibit an enlarged spleen and/or liver and anemia.*
- *Diagnosis is confirmed by microscopy. Thick blood smears are generally more sensitive for the detection of parasites, whereas thin smears are preferable for species identification. (See blood-stage morphology of Plasmodium species.) If parasites are not found on the first blood smear it is recommended to make additional smears every 6 -12 hours for as long as 48 hours.*
- *A tentative diagnosis of P. falciparum (numerous and exclusively ring stages) could constitute a medical emergency, especially in a non-immune person.*
- *Rapid immunochromatographic tests (i.e., dipsticks) based on antigen detection are also available.*

Unit 4: Babesia

Unit Structure

4.1 Introduction

Babesiosis is a rare zoonotic infection transmitted by ticks. *Babesia* species are blood parasites which infect a wide variety of wild and domestic animals throughout the world. *Babesia* and *Theileria* form a group called the piroplasms, in reference to intraerythrocytic forms that are pear-shaped in some species. Piroplasms cause tremendous losses of livestock in endemic areas.

4.2 Intended Learning Outcomes (ILOs)

By the end of this lecture unit, students should be able to:

- Have a good understanding on blood and tissue protozoa such as *Babesia*

4.3 Babesia as a parasite

Babesiosis is the disease caused by Babesia parasite. The trophozoite is very similar to the ring form of the *Plasmodium* species. *Babesia*, are more closely related to *Theileria*. Consistent with this molecular data, none of the small *Babesia*-in contrast to the large *Babesia* - appears to be transmitted transovarially in ticks, suggesting a need for some re-evaluation of piroplasm classification.

Self-Assessment Exercise 1

1. ***Briefly explain the Symptom of Babesia?***
2. ***Briefly explain the Diagnosis of Babesia?***

Life cycle

Babesia exhibits a typical apicomplexan life cycle characterized by merogony, gametogony, and sporogony. The organism (sporozoite) is transmitted by a tick and enters the red cell where it undergoes mitosis and the organisms (merozoite) are released to infect other red cells. Ticks acquire the organism during feeding on an

infected individual. In the tick, the organism divides sexually in the gut and migrates into the salivary gland.

Etiology

Babesia microti is the only member of the genus that infects man.

Symptoms

Babesiosis is associated with hemolytic anemia, jaundice, fever and hepatomegaly, usually 1-2weeks after infection.

Diagnosis

Diagnosis is based on symptoms, patient history and detection of intraerythrocytic parasite in thepatient or transfer of blood in normal hamsters which can be heavily parasitized.

Treatment and control

Drugs of choice are clindamycin combined with quinine. The patient may recover spontaneously.One should avoid tick exposure and, if bitten, remove the tick from the skin immediately.
Outline at least four symptoms of Babesiosis.

Self-Assessment Exercise 2

1. ***Briefly explain the treatment and control of Babesia?***
2. ***Briefly explain the Life cycle of Babesia?***

4.4 Summary

In this unit, you have learnt about the medical and Biological important of blood and tissue protozoa such as Babesia, their etiology, morphology, life cycle, symptom, pathology, diagnosis, treatment and control. Babesia species belong to the phylum Apicomplexa, which includes the protozoan parasites causing malaria, toxoplasmosis, and cryptosporidiosis.

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4.6 Possible Answers to SAEs

Answers to SAEs 1

1. *Babesiosis is associated with hemolytic anemia, jaundice, fever and hepatomegaly, usually 1-2weeks after infection.*
2. *Diagnosis is based on symptoms, patient history and detection of intraerythrocytic parasite in thepatient or transfer of blood in normal hamsters which can be heavily parasitized.*

Answers to SAEs 2

1. *Drugs of choice are clindamycin combined with quinine. The patient may recover spontaneously.One should avoid tick exposure and, if bitten, remove the tick from the skin immediately.*
2. *Babesia exhibits a typical apicomplexan life cycle characterized by merogony, gametogony, and sporogony. The organism (sporozoite) is transmitted by a*

tick and enters the red cell where it undergoes mitosis and the organisms (merozoite) are released to infect other red cells. Ticks acquire the organism during feeding on an infected individual. In the tick, the organism divides sexually in the gut and migrates into the salivary gland.

Unit 5: Toxoplasma

Unit Structure

5.1 Introduction

This remarkable species 'Toxoplasma' was known as a potential parasite of man for many years but its true nature as a coccidian was only discovered not too long ago. It was known to occur in all warm- blooded animals - mammals and birds - but its nature and means of transmission remained a mystery.

5.2 Intended Learning Outcomes (ILOs)

By the end of this lecture unit, students should be able to:

- Have a good understanding on blood and tissue protozoa such as *Toxoplasma*

5.3 Toxoplasma as a Parasite

The two stages known were the trophozoite and the cyst, also known as a pseudocyst (seebelow). The trophozoites were intracellular parasites which could invade almost any nucleated cell within the host **(Figure 5.3a)**.The cysts had well-formed walls and contained organisms which multiplied within the cysts, as many as 1000-3000 organisms being found within a single cyst. The trophozoite and the cystic form appeared to be almost identical in morphology, but they exhibited a striking physiological difference in that the trophozoites were rapidly killed in acid pepsin whereas the cystic forms could survive 1hour of acid pepsin digestion. This physiological difference should have provided a clue to the life cycle as it suggested that there was a further development of an intestinal phase inthe life cycle. This, in fact, proved to be the case when workers in a number of differentcountries, almost simultaneously, pieced together the puzzle and showed that *T gondii* developeda sexual stage in the intestine of a cat and was, in fact, a coccidian closely related to (but not identical to) an Isospora species. This discovery revolutionized this area of protozoology and opened the door to the solution of the taxonomy of several others.

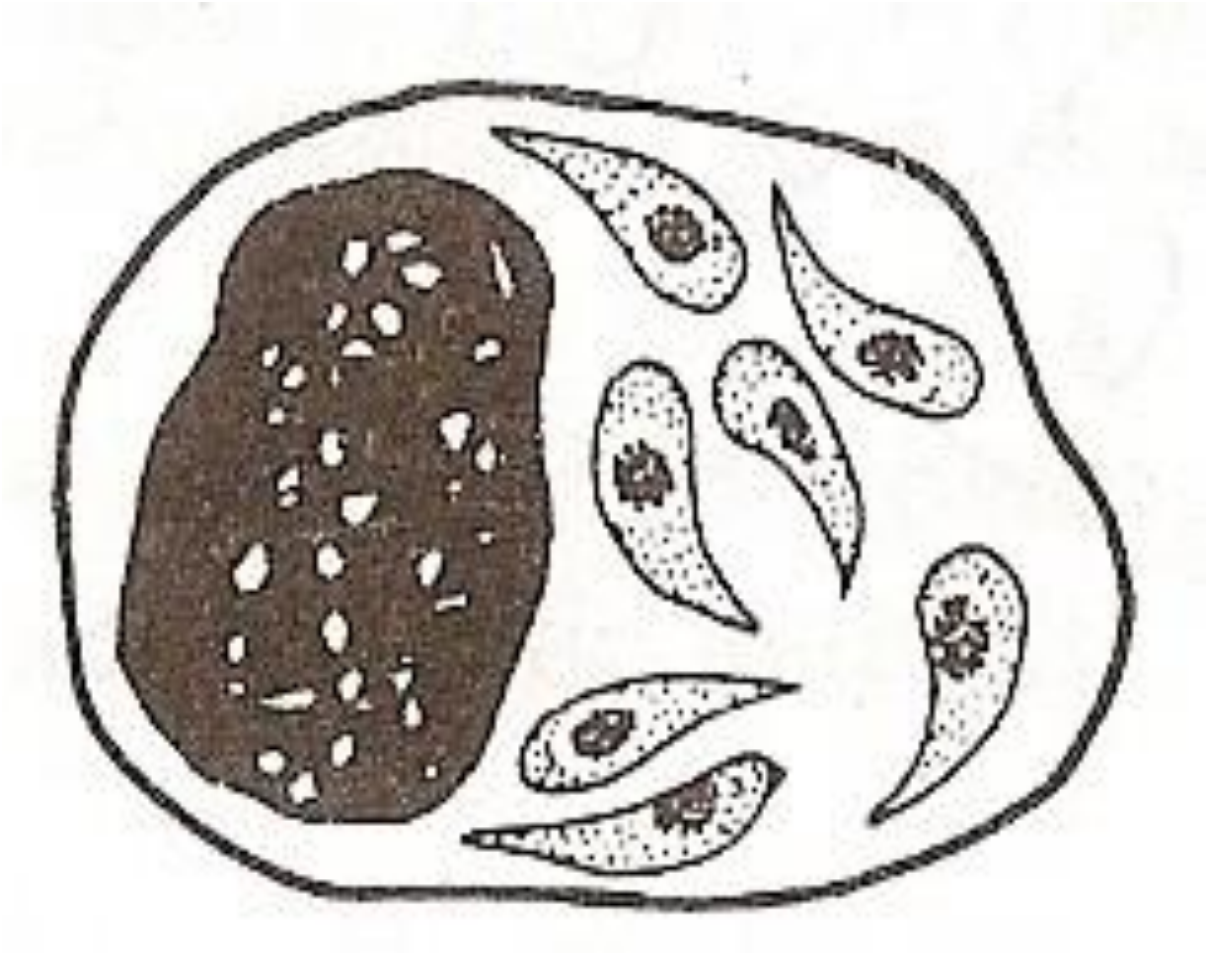

Figure 5.3a: Toxoplasma *gondii*, trophozoites in macrophage

Epidemiology

Toxoplasma has worldwide distribution and 20%-75% of the population is seropositive without any symptomatic episode. However, the infection poses a serious threat in immunosuppressed individuals and pregnant females.

Morphology

The intracellular parasites (tachyzoite) are 3x6 microns, pear-shaped organisms that are enclosed in a parasite membrane to form a cyst measuring 10-100 microns in size. Cysts in cat feces (oocysts) are 10- 13 microns in diameter.

Life cycle

The natural life cycle of *T. gondii* occurs in cats and small rodents, although the parasite can grow in the organs (brain, eye, skeletal muscle, etc.) of any mammal or birds. Cats get infected by ingestion of cysts in flesh. Decystation occurs in the small intestine, and the organisms penetrate the sub mucosal epithelial cells where they undergo several generations of mitosis, finally resulting in the development of micro- (male) and macro- (female) gametocytes. Fertilized macro-gametocytes develop into oocysts that are discharged into the gut lumen and excreted. Oocysts sporulate in

the warm environment and are infectious to a variety of animals including rodents and man. Sporozoites released from the oocyst in the small intestine penetrate the intestinal mucosa and find their way into macrophages where they divide very rapidly (hence the name tachyzoites) and form a cyst which may occupy the whole cell. The infected cells ultimately burst and release the tachyzoites to enter other cells, including muscle and nerve cells, where they are protected from the host immune system and multiply slowly (bradyzoites). These cysts are infectious to carnivores (including man). Unless man is eaten by a cat, it is a dead-end host (**Figure 5.3b**).

Self-Assessment Exercise 1

1. ***Briefly explain the* Epidemiology *of Toxoplasma?***
2. ***Briefly explain the Morphology of Toxoplasma?***

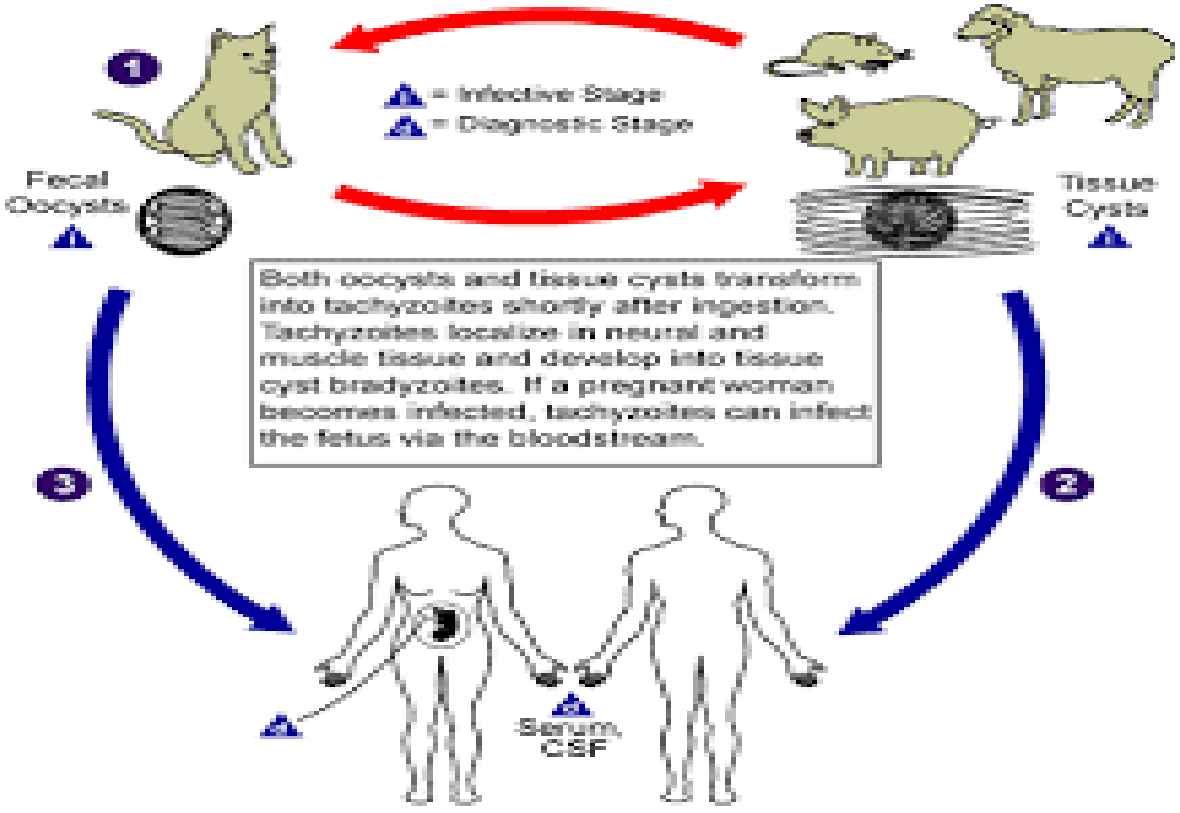

Figure 5.3b: Life cycle of *Toxoplasma gondii*

Etiology

Toxoplasmosis is the disease caused by Toxoplasma parasite. *Toxoplasma gondii* is the organism responsible for toxoplasmosis

Symptoms

Although Toxoplasma infection is common, it rarely produces symptoms in normal individuals. Its serious consequences are limited to pregnant women and immunodeficient hosts. Congenital infections occur in about 1-5 per 1000 pregnancies of which 5-10% result in miscarriage and 8-10% result in serious brain and eye damage to the fetus. 10-13% of the babies will have visual handicaps. Although 58-70% of infected women will give birth to a normal offspring, a small proportion of babies will develop active retino-chorditis or mental retardation in childhood or young adulthood. In immunocompetent adults, toxoplasmosis may produce flu-like symptoms, sometimes associated with lymphadenopathy. In immunocompromised individuals, infection results in generalized parasitemia involvement of brain, liver lung and other organs, and often death.

Immunology

Both humoral and cell mediated immune responses are stimulated in normal individuals. Cell-mediated immunity is protective and humoral response is of diagnostic value.

Diagnosis

Suspected toxoplasmosis can be confirmed by isolation of the organism from tonsil or lymph gland biopsy.

Treatment

Acute infections benefit from pyrimethamine or sulphadiazine. Spiramycin is a successful alternative. Pregnant women are advised to avoid cat litter and to handle uncooked and undercooked meat carefully.

Organism responsible for Toxoplasmosis is called?

Self-Assessment Exercise 2

1. ***Briefly explain the* Symptoms *of Toxoplasma?***
2. ***Briefly explain the Immunology of Toxoplasma?***

5.4 Summary

In this unit, you have learnt about the medical and Biological important of blood and tissue protozoa such as Toxoplasma, their etiology, morphology, life cycle, symptom,

pathology, diagnosis, treatment and control. *Toxoplasma gondii* is a coccidian parasite which infects humans as well as a wide variety of mammals and birds. It exhibits a predator-prey type life cycle and felines are the only definitive host. Toxoplasmosis is found throughout the world (except extremely cold or dry climates) and tends to be more prevalent in tropical climates.

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5.6 Possible Answers to SAEs

Answers to SAEs 1

1. *Toxoplasma has worldwide distribution and 20%-75% of the population is seropositive without any symptomatic episode. However, the infection poses a serious threat in immunosuppressed individuals and pregnant females.*
2. *The intracellular parasites (tachyzoite) are 3x6 microns, pear-shaped organisms that are enclosed in a parasite membrane to form a cyst measuring 10-100 microns in size. Cysts in cat feces (oocysts) are 10- 13 microns in diameter.*

Answers to SAEs 2

1. *Although Toxoplasma infection is common, it rarely produces symptoms in normal individuals. Its serious consequences are limited to pregnant women and immunodeficient hosts. Congenital infections occur in about 1-5 per 1000 pregnancies of which 5-10% result in miscarriage and 8-10% result in serious brain and eye damage to the fetus. 10-13% of the babies will have visual handicaps. Although 58-70% of infected women will give birth to a normal offspring, a small proportion of babies will develop active retino-chorditis or mental retardation in childhood or young adulthood. In immunocompetent adults, toxoplasmosis may produce flu-like symptoms, sometimes associated with lymphadenopathy. In immunocompromised individuals, infection results in generalized parasitemia involvement of brain, liver lung and other organs, and often death.*
2. *Both humoral and cell mediated immune responses are stimulated in normal individuals. Cell-mediated immunity is protective and humoral response is of diagnostic value.*

Glossary

DNA = Deoxyribonucleic acid
AIDS = Acquired Immunodeficiency syndrome
ATP = Adenosine Triphosphate
STD = Sexually Transmitted Diseases
ACR = Adequate clinical response
LTF = Late treatment failure
ETF = Early treatment failure
MDR = Multi-drug resistance
ABC = ATP-binding cassette

End of the module Questions

1. There are two clinical forms of African trypanosomiasis **(True or False)**
2. Leishmaniasis is the disease caused by Leishmania **(True or False)**
3. Four *Plasmodium* species are responsible for human malaria **(True or False)**
4. *Babesia microti* is the only member of the genus that infects man **(True or False)**
5. Toxoplasmosis is the disease caused by Toxoplasma parasite **(True or False)**

Module 3: Luminal Protozoa

Module Structure

In this module we will discuss about the blood and Tissue Protozoa with the following units:

Unit 1: Amebae
Unit 2: Giardia
Unit 3: Trichomoniasis
Unit 4: *Blastocystis hominis*
Unit 5: *Dientamoeba fragilis*
Glossary
End of the module Questions

Unit 1: Amebae

Unit Structure

1.1 Introduction

Species of Entamoeba have been found in a number of invertebrate and vertebrate hosts. Five species of the genus Entamoeba infect man: *E. histolytica*, a species which is sometimes pathogenic in the caecum and colon; *E. hartmanni*, a harmless species closely related to *E. histolytica* and not regarded as a separate species by some workers; *E. coli*, a harmless species; *E. gingivalis*, in the mouth; and *E. polecki*, a species rare in man and probably normallyoccurring in pigs. The relationship between these species is complicated by the discovery of numerous strains' of some species.

Infections of *Entamoeba histolytica* commonly result in amoebiasis, the term 'histolytica' literallymeaning 'tissue-dissolving, referring to the potential carnivorous habits of this organism. The qualification potentially is stressed, for a high percentage of individuals infected with entamoebae show no symptoms of disease. Where clinical symptoms result, the disease isreferred to as invasive amoebiasis; the non-invasive infection is sometimes called lumenalamoebiasis.

1.2 Intended Learning Outcomes (ILOs)

At the end of this lecture unit, students should:

1. Be acquinted with genera and species of protozoans that constitutes luminal protozoans
2. Know protozoans parasitizing other organs aside from blood and intestines
3. Know their epidemiology, morphological, characteristics and life cycle.

1.3 Amebae as a parasite

Several members of the genus *Entamoeba* infect humans. Among these only *E. histolytica* is considered pathogenic and the disease it causes is called amebiasis or amebic dysentery. *E. dispar* is morphologically identical to *E. histolytica* and the two were previously considered to be the same species. The two species are found throughout the world, but like many other intestinal protozoa, they are more common in tropical countries or other areas with poor sanitary conditions. It is estimated that up to 10% of the world's population may be infected with either *E. histolytica* or *E. dispar* and in many tropical countries the prevalence may approach 50%. There are an estimated 50 million cases of amebiasis per year and up to 100,000 deaths.

Epidemiology

The population of about 0.5 to 50% worldwide harbors *E. histolytica* parasites with the higher rates of infection being in underdeveloped countries. 1 to 3% of the populations of the USA are infected. Infection is associated with poor hygiene. Humans are the principal host, although dogs, cats androdents may be infected.

Morphology

Trophozoite: This form has an ameboid appearance and is usually 15-30 micrometers in diameter, although more invasive strains tend to be larger. The organism has a single nucleus with a distinctive small central karyosome. The fine granular endoplasm may contain ingestederythrocytes. The nuclear chromatin is evenly distributed along the periphery of the nucleus (**Figure 1.3a**).

Cyst

Entamoeba histolytica cysts are spherical, with a refractile wall; the cytoplasm contains darkstaining chromatoidal bodies and 1 to 4 nuclei with a central karyosome and evenly distributed peripheral chromatin.

Life cycle

Infection occurs by ingestion of cysts on fecally contaminated food or hands. The cyst is resistantto the gastric environment and passes into small intestine where it decysts. The metacyst divides into four and then eight amoebae which move to the large intestine". The majority of the organisms are passed out of the body with the feces but, with larger bolus of infection, some amebae attach to and invade the mucosal tissue forming "flask-shaped" lesions (bomb craters). The organisms encyst for mitosis and are passed through with feces. There is no intermediate or reservoir hosts (**Figure 1.3b**).

Self-Assessment Exercise 1

1. ***Briefly explain the Immunology of Amebae parasite?***
2. ***Briefly explain the treatment and control of Amebae parasite?***

AMEBIASIS (amebic dysentery, amebic hepatitis) is the disease caused by Entamoeba parasite

Etiology

E. histolytica is the major cause of amebic dysentery.

Symptoms

Acute: Frequent dysentery with necrotic mucosa and abdominal pain.

Chronic: Recurrent episodes of dysentery with blood and mucus in the feces. There are intervening gastrointestinal disturbances and constipation. Cysts are found in the stool. The organism may invade the liver, lung and brain where it produces abscesses that result in liver dysfunction, pneumonitis, and encephalitis.

Pathology

Intestinal ulcers (craters/flasks) are due to enzymatic degradation of tissue. The infection may result in appendicitis, perforation, stricture granuloma, pseudo-polyps, liver abscess; sometimes brain, lung and spleen abscesses can also occur. Strictures and pseudo-polyps result from thehost inflammatory response

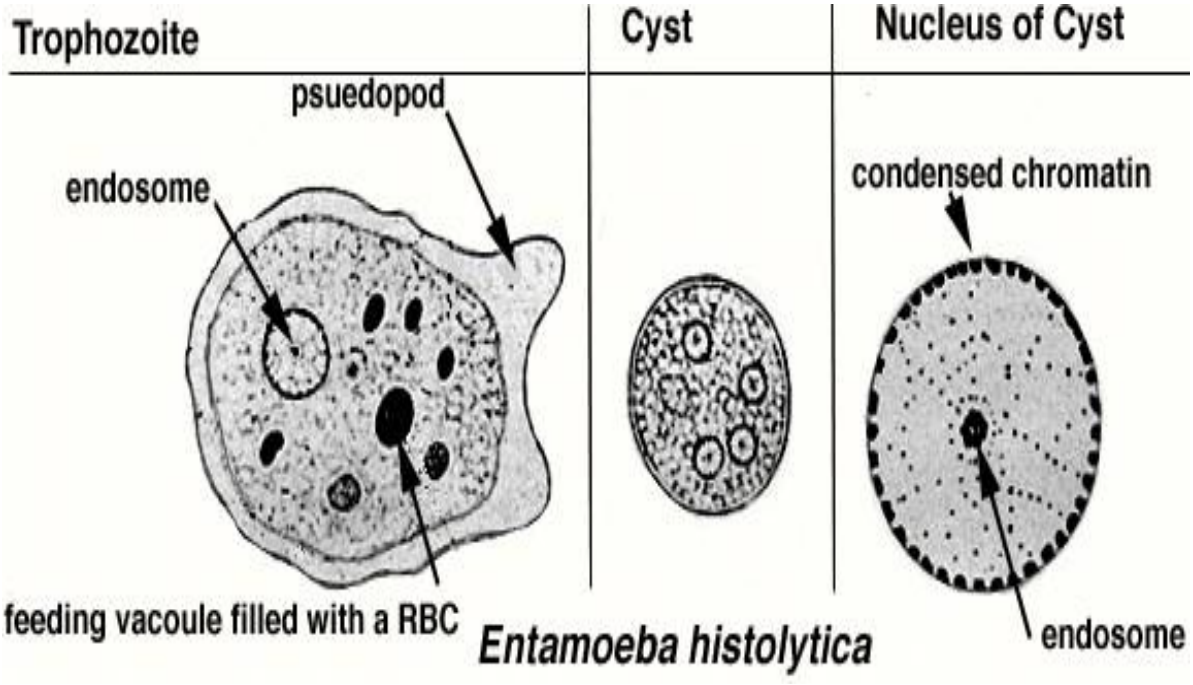

Figure 1.3a: Trophozoite and Cyst of *E. histolytica*

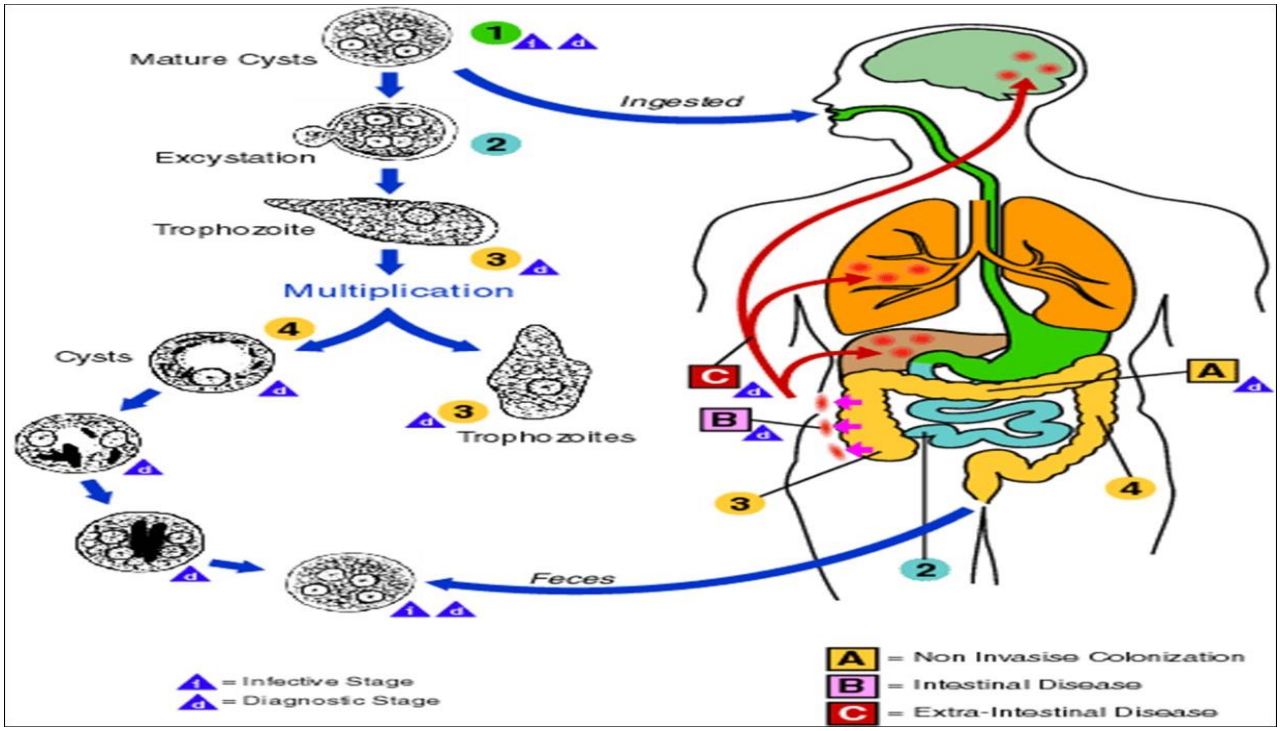

Figure 1.3b: Life cycle of *E. histolytica*

Immunology

There is an antibody response after invasive infection (liver abscess or colitis) but it is of questionable significance in immunity, as there is recurrence of enteric episodes in these patients.

Diagnosis

Symptoms, history and epidemiology are the keys to diagnosis. In the laboratory, the infection is confirmed by finding cysts in the stool. *E. histolytica* infection is distinguished from bacillary dysentery by the lack of high fever and absence PMN leukocytosis. Distinction must be made from other non-pathogenic intestinal protozoa (*e.g. Entamoeba coli, Entamoeba hartmanni, Dientamoeba fragilis, Endolimax nana, Iodamoeba buetschlii, etc.*).

Treatment

Iodoquinol is used to treat asymptomatic infections and metronidazole is used for symptomaticand chronic amebiasis, including extra-intestinal disease.
Highlight at least four Luminal protozoa that are significant to human health.

1.4 Other species of Entamoeba:

Entamoeba coli

This is a non-pathogenic species whose distribution is world-wide, occurring in some 30 per cent of the world's population. It is found in the large intestine and distinguished from *E. histolytica*by a number of features, chief of which are: (a) it has slower movement; (b) its pseudopodium is mainly coarser and not clear like that of

E. histolytica; (c) its nucleus is coarser and with an eccentric karyosome (d) it has a larger number of food vacuoles. The precystic stages are difficult to distinguish from those of *E. histolytica*, critical observationof the nucleus being necessary. The cysts, however, are easily differentiated from those of *E. histolytica* by having eight nuclei and the chromatoid bodies, which, when present, have splintered, but never rounded ends. *E. coli* is entirely a scavenger, feeding on bacteria and detritus in the large intestine. It is not capable of eroding the intestinal mucosa, but, as an indiscriminate feeder, may phagocytose blood cells if these are available.

Entamoeba gingivalis

A common parasite of the human mouth; related species occur in the mouths of dogs, horses and donkeys. The spaces between the teeth and the soft pits of the gums offer ideal surfaces for amoebae, because in these sites bacteria and detritus abound. *E. gingivalis* resembles *E. histolytica*; it has a crystal-clear ectoplasm and moves, actively. Its food vacuoles are usually numerous and contain bacteria, leucocytes and occasionally red cells. The pathogenicity of this species has long been a matter of dispute and has never been established.

Entamoeba polecki

This is a species which normally occurs in pigs. It forms uninucleate cysts and shows some morphological differences from the cyst of *E. histolytica*. It is generally considered to be a rare parasite of man but may be more common than supposed.

Entamoeba moshkovskii

This species is not an animal parasite, but it may conveniently be discussed with the other forms. It is a free-living species, which was first discovered in sewage:-disposal plant in Moscow and has since been shown be of world-wide occurrence. A large number of strains have been identified. Morphologically it closely resembles *E. histolytica* throughout life cycle, including encystment and metacystic development, differing from it only in details. Yet all experiments to establish this species in rats or amphibians, animals which might act as hosts in sewage beds, have failed, and there seems no doubt that it is a e free-living form.

Entamoeba invadens

This species is increasingly being used as an experimental model for the study of amoebiasis. This is partly on account of its resemblance to *E. histolytica* and partly because it causes invasiveamoebiasis in reptiles. It is probably relatively harmless in turtles, which may have been the original hosts,' but is highly pathogenic to snakes, which may die within two weeks.

Entamoeba ranarnm

The morphology of this species is indistinguishable from that of *E. histolytica*. It is commoner in tadpoles than adult frogs.

Mention the drugs used in treating Amebiasis.

Self-Assessment Exercise 2

1. ***Briefly explain Entamoeba invadens?***
2. ***Briefly Explain the Diagnosis of Entamoeba parasite?***

1.5 Summary

In this unit, you have learnt about the protozoans parasitizing other organs aside from blood and intestines, their epidemiology, morphological characteristics and life cycle. The major difference is that humans are the only host for *E. histolytica* and there is no possibility of zoonotic transmission. Control is based on avoiding the contamination of food or water with fecal material. Health education in regards to improving personal hygiene, sanitary disposal of feces, and hand washing are particularly effective. Protecting water supplies will lower endemicity and epidemics.

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https://www.youtube.com/watch?v=cpu9PRIEYWY

https://www.youtube.com/watch?v=CQma1Ff1YYU

 **1.7 Possible Answers to SAEs**

Answers to SAEs 1

1. *There is an antibody response after invasive infection (liver abscess or colitis) but it is of questionable significance in immunity, as there is recurrence of enteric episodes in these patients.*
2. *Iodoquinol is used to treat asymptomatic infections and metronidazole is used for symptomatic and chronic amebiasis, including extra-intestinal disease.*

Answers to SAEs 2

1. *This species is increasingly being used as an experimental model for the study of amoebiasis. This is partly on account of its resemblance to E. histolytica and partly because it causes invasiveamoebiasis in reptiles. It is probably relatively harmless in turtles, which may have been the original hosts,' but is highly pathogenic to snakes, which may die within two weeks.*
2. *Symptoms, history and epidemiology are the keys to diagnosis. In the laboratory, the infection is confirmed by finding cysts in the stool. E. histolytica infection is distinguished from bacillary dysentery by the lack of high fever and absence PMN leukocytosis. Distinction must be made from other non-pathogenic intestinal protozoa (e.g. Entamoeba coli, Entamoeba hartmanni, Dientamoeba fragilis, Endolimax nana, Iodamoeba buetschlii, etc.).*

Unit 2: Giardia

Unit Structure

 **2.1 Introduction**

A flagellate of this genus is quite unlike any of the other species in shape or habits. It has been described in front view as looking like a 'tennis racquet without a handle' and it has a comical, face-like appearance. “The body is a 'tear-drop shaped' with a convex dorsal surface and a concave ventral one. The latter possesses two depressions, sometimes termed *adhesive discs* (suckers) which make contact with the intestinal cells of the host. A single or double *median body*, unique to this genus, is found just below the adhesive discs”. Species of Giardia are confined in their distribution to the small intestine, particularly theduodenum, occasionally invading the bile ducts. In severe infections they may carpet large areas of the mucosa. Nutritionally the duodenum is the richest habitat in the alimentary canal and, as mentioned above, the organism makes very close contact with the mucosa.

2.2 Intended Learning Outcomes (ILOs)

At the end of this lecture unit, students should:

1. be acquinted with the genera and species of protozoans that constitutes luminal protozoans such as Giardia
2. know protozoans parasitizing other organs aside from blood and intestines
3. Know their epidemiology, morphology, characteristics and life cycle.

2.3 Giardia as a parasite

Giardiasis (lambliasis) is the disease caused by Giardi parasite. Giardia exhibits perfect bilateral symmetry, and there are double sets of nuclei, flagella and kinetosomes. Each nucleus has been shown to contain a haploid number of chromosomes. Ultrastructural studies show that the so-called adhesive disc is in fact a structure composed of supportive, rather than contractile, elements which remain a fixed shape; the term 'striated' disc has been suggested. The median body superficially resembles the axostyle of trichomonads, but the organisation of the microtubules which compose it, and the fact that the body is not always present, make it a distinctive structure. No structures identifiable as mitochondria, smooth endoplasmic reticulum or Golgi complex have been identified in Giardia. The central flagella are'ribbon-like', and thus morphologically adapted to their suggested pumping function. Studies with ferritin have demonstrated that particulate material can be ingested by pinocytosis.

Etiology

Giardia lamblia (a flagellate)

Epidemiology

Giardia has worldwide distribution and is not uncommon in South Carolina. It is the most frequent protozoan intestinal disease in the US and the most common identified

cause of water- borne disease associated with breakdown of water purification systems, drinking from contaminated streams, travel to endemic areas (Russia, India, Rocky Mountains, etc.) and day care centers.

Morphology

Trophozoite: *Giardia* is a 12 to 15 micrometer, half pear-shaped organism with 8 flagella and 2 axostyles arranged in a bilateral symmetry. There are two anteriorly located large suction discs. The cytoplasm contains two nuclei and two parabasal bodies.
Cyst: *Giardia* cysts are 9 to 12 micrometer ellipsoidal cells with a smooth well-defined wall. The cytoplasm contains four nuclei and many of the structures seen in the trophozoite (**Figure 2.3a**).

Life cycle

Infection occurs by ingestion of cysts, usually in contaminated water. Decystation occurs in the duodenum and trophozoites (trophs) colonize the upper small intestine where they may swim freely or attach to the sub-mucosal epithelium via the ventral suction disc. The free trophozoites encyst as they move down stream and mitosis takes place during the encystment. The cysts are passed in the stool. Man is the primary host although beavers, pigs and monkeys are also infected and serve as reservoirs (**Figure 2.3b**).

In-Text Question (ITQ)

Outline atleast four gastro-intestinal disturbances associated with giardiasis?

Answer

Flatulence, bloating, anorexia, cramps, and foul sulfuric belching

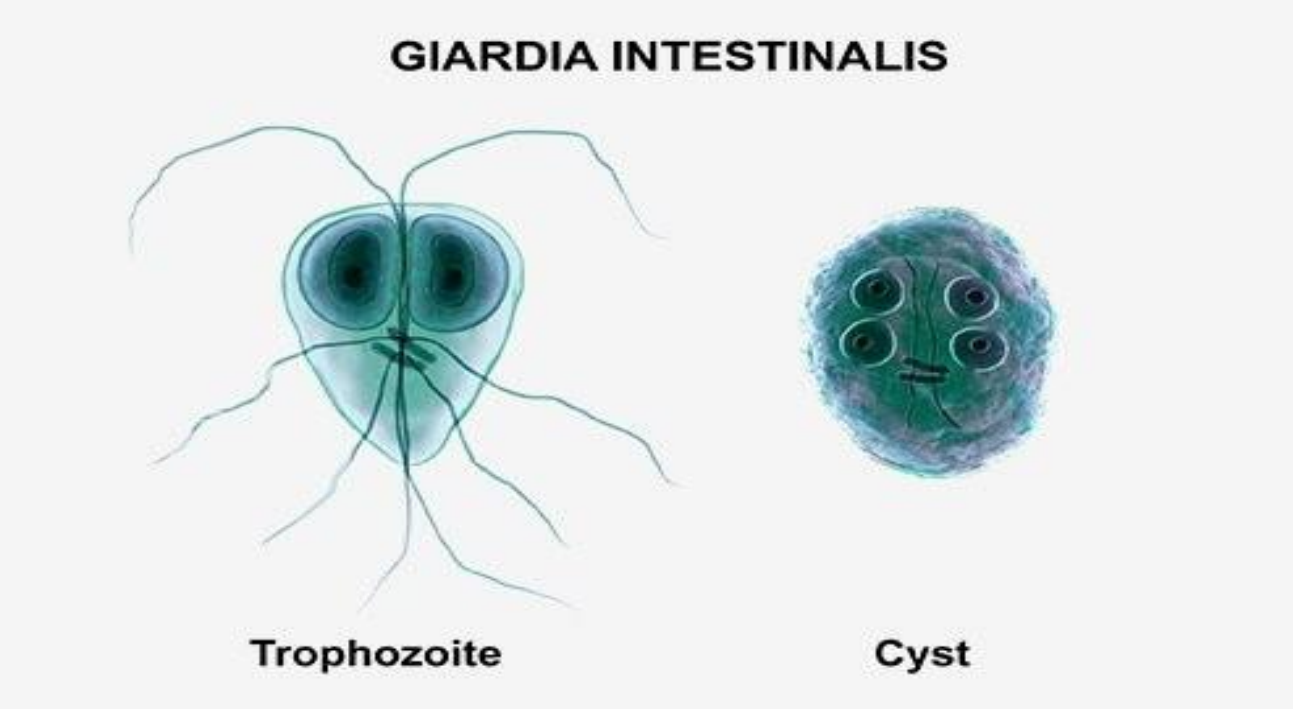

Figure 2.3a: Trophozoite and Cyst of *Giardia lamblia*

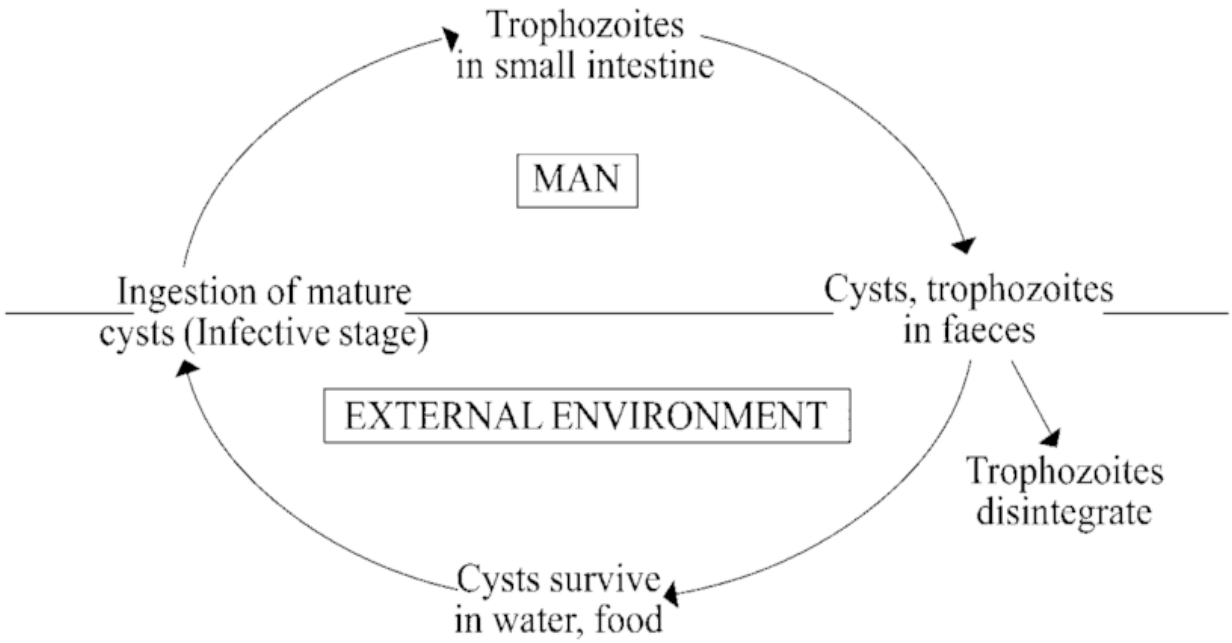

Figure 2.3b: Life cycle of *Giardia lamblia*

Self-Assessment Exercise 1

1. ***Outline the clinical features associated with Giardia infection?***
2. ***Giardia is distributed worldwide and is not uncommon _____ country?***

Symptoms and Pathogenesis

The clinical features associated with Giardia infection range from total latency (ie, asymptomatic), to acute self-resolving diarrhea, to chronic syndromes associated with nutritional disorders, weight loss and failure to thrive. Children exhibit clinical symptoms more frequently that adults and subsequent infections tend to be less severe than initial infections. The incubation period is generally 1-2 weeks, but ranges of 1-75 days have been reported. The first signs of acute giardiasis include nausea, loss of appetite and an upper gastro-intestinal uneasiness. These signs are often followed or accompanied by a sudden onset of explosive, watery, foul-smelling diarrhea. Stools associated with Giardia infection are generally described as loose, bulky, frothy and/or greasy with the absence of blood or mucus, which may help distinguish giardiasis from other acute diarrheas. Other gastro-intestinal disturbances associated with giardiasis include: flatulence, bloating, anorexia, cramps, and foul sulfuric belching (sometimes called 'purple burbs'). The acute stage usually resolves spontaneously in 3 - 4 days and is often not recognized as being giardiasis. Occasionally, though, an acute infection will persist and lead to malabsorption, steatorrhea (excessive loss of fat in the feces), debility (loss of strength) and weight loss. Some of the individuals who resolve the acute symptoms do not clear the infection, but become asymptomatic cyst passers without clinical manifestations, whereas others may have a few sporadic recurrences of the acute symptoms. Acute infections can also develop into long-standing subacute or chronic infections which in rare cases last for years. The typical chronic stage patient presents with recurrent brief episodes of loose foul stools which may be yellowish, frothy and float, accompanied by intestinal gurgling, abdominal distention and flatulence. Between episodes the stools are usually mushy, but normal stools or constipation can also occur. Cramps are uncommon during chronic infections, but sulfuric belching is frequent. Anorexia, nausea, and epigastric uneasiness are additional frequent complaints during chronic infections. In the majority of chronic cases the parasites and symptoms spontaneously disappear.

The specific mechanisms of *Giardia* pathogenesis leading to diarrhea and intestinal malabsorption are not completely understood and no specific virulence factors have been identified. Attachment of trophozoites to the brush border could produce a mechanical irritation or mucosal injury. In addition, normal villus structure is affected in some patients. For example, villus blunting (atrophy) and crypt cell hypertrophy and an increase in crypt depth have been observed to varying degrees. The increase in crypt cells will lead to a repopulation of the intestinal epithelium by relatively immature enterocytes with reduced absorptive capacities. An increased inflammatory cell infiltration in the lamina propria has also been observed and this inflammation may be associated with the pathology. *Giardia* infection can also lead to lactase deficiency (see lactose intolerance below) as well as other enzyme deficiencies in the microvilli. This reduced digestion and absorption of solutes may lead to an osmotic diarrhea and

could also explain the malabsorption syndromes. Thus far, no single virulence factor or unifying mechanism explains the pathogenesis of giardiasis.

Immunology

There is some role for IgA and IgM and there is increased incidence of infection inimmunodeficient patients (*e.g.* AIDS).

Diagnosis

Diagnosis is confirmed by finding cysts or trophozoites in feces or in duodenojejunal aspirates or biopsies. Detection of the parasites can be difficult since *Giardia* does not appear consistently in the stools of all patients. Some patients will express high levels of cysts in nearly all the stools, whereas others will only exhibit low parasite counts in some of the stools. A mixed pattern, in which periods of high cyst excretion alternate with periods of low excretion, has also been observed. In addition, parasites are easier to find during acute infections than chronic infections. Aspiration and biopsy may also fail to confirm the infection due to patchy loci of infection, and some question the usefulness of these invasive procedures. Stool examination is the preferred method for *Giardia* diagnosis. Three stools taken at intervals of at least two days should be examined. Watery or loose stools may contain motile trophozoites which are detectable by the immediate examination of wet smears. Otherwise the specimen should be preserved and stained due to trophozoite liability. The hardier cysts are relatively easy to recognize in either direct or stained smears. In addition, diagnostic kits based on immunofluorescence or the detection of copro-antigens is also available. Diagnosis can also be made by examining duodenal fluid for trophozoites. Duodenal fluid is obtained by either intubation or the Enterotest® (also called 'string test'). The Enterotest® consists of a gelatin capsule containing a nylon string of the appropriate length. The free end of the string is taped to the patient's face and the capsule is swallowed. After four hours to overnight the string is retrieved and the bile-stained mucus on the distal portion of the string is scraped off and examined by both wet mount and permanent staining. A small intestinal biopsy, preferably from multiple duodenal and jejunal sites, may also reveal trophozoites attached to the intestinal epithelium. The small intestine is divided into 3 sections: the duodenum (first or proximal portion after the stomach); the jejunum (the middle portion); and the ileum (the distal or last portion before the large intestine).

Treatment and Control

Infected individuals should be treated since *Giardia* can persist and lead to severe malabsorption syndromes and weight loss. Treatment is effective at reducing morbidity and there are no sequelae. Metronidazole (Flagyl®), although not licensed in the United States for giardiasis, effectively clears the parasite (cure rates approximately 85%) and is the drug of choice. The recommended dosage is 750 mg three times per day for five days (or at least >3 days). For children 15 mg/kg/d in three doses is recommended. Other effective drugs include: quinacrine (Atabrine®), tinidazole (Fasigyn®), furazolidone (Furoxone®), and paramomycin (Humatin®). Tinidazole is effective as a single two gram dose; paramomycin is not absorbed and may be useful during pregnancy. The widespread distribution of *Giardia* and the infectivity of the cysts make it unlikely that human infection will be completely eliminated. Control measures to prevent or reduce *Giardia* infection will depend on the specific circumstances of transmission, but in general involve measures which prevent the

ingestion of substances contaminated with fecal material. Health promotion and education aimed at improving personal hygiene, and emphasizing hand washing, sanitation and food handling, are effective control activities for the reduction of person-to-person transmission. Special attention to personal hygiene in high-risk situations such as day-care centers and other institutions is needed". Treatment of asymptomatic household members prevents reinfection in non-endemic areas. However, the value of treating asymptomatic carriers in hyper endemic communities is questionable since reinfection rates are high. The socio-economic situation in many developing countries makes it difficult to prevent infection. Public health measures to protect water supplies from contamination are required to prevent epidemics and to reduce endemicity. Tourists should not drink tap water without additional treatment in places where purity is questionable. Boiling or iodine treatment kills *Giardia* cysts, but standard chlorination does not. There are no safe or effective chemoprophylatic drugs for giardiasis.

2.4. Post-*Giardia* Lactose Intolerance

Some patients may present with lactose intolerance during active *Giardia* infections which can persist after parasite clearance. This clinical manifestation is due to the parasite-induced lactase deficiency and is most common in ethnic groups with a predisposition for lactase deficiency. Lactase is an enzyme that breaks down lactose, a sugar found in milk, to monosaccharide's which can be absorbed. This lactose intolerance syndrome should be considered in persons who still present mushy stools and excessive gas following treatment, but have no detectable parasites.

Giardiasis (lambliasis) is the disease caused by which parasite?

Self-Assessment Exercise 2

1. ***Outline atleast three diagnosis associated with Giardia infection?***
2. ***Highlight the usefulness of lactose in Giardia?***

2.5 Summary

In this unit, you have learnt about Giardia, their epidemiology, morphology, characteristics, life cycle, symptoms, pathogenesis, immunology, treatment and control. *Giardia lamblia* is a protozoan parasite that colonizes the upper portions of the

small intestine. It has a worldwide distribution and is the most common protozoan isolated from human stools. The incidence is estimated at 200 million clinical cases per year. In fact, it was probably the first symbiotic protozoan ever observed.

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2.7 Possible Answers to SAEs

Answers to SAEs 1

1. *The clinical features associated with Giardia infection range from total latency (i.e. asymptomatic), to acute self-resolving diarrhea, to chronic syndromes associated with nutritional disorders, weight loss and failure to thrive.*
2. *South Carolina*

Answers to SAEs 2

1. *- Diagnosis is confirmed by finding cysts or trophozoites in feces or in duodenojejunal aspirates or biopsies.*
- *Detection of the parasites can be difficult since Giardia does not appear consistently in the stools of all patients. Some patients will express high levels of cysts in nearly all the stools, whereas others will only exhibit low parasite counts in some of the stools.*
- *A mixed pattern, in which periods of high cyst excretion alternate with periods of low excretion, has also been observed. In addition, parasites are easier to find during acute infections than chronic infections.*

2. *Lactase is an enzyme that breaks down lactose, a sugar found in milk, to monosaccharide's which can be absorbed. This lactose intolerance syndrome should be considered in persons who still present mushy stools and excessive gas following treatment, but have no detectable parasites.*

Unit 3: Trichomoniasis

Unit Structure

3.1 Introduction

The trichomonads are a group of flagellated protozoa. Most of the members of this group are parasitic and only a few free-living species have been identified. Generally the trichomonads are non-pathogenic commensals and only a few species are of importance in animals and humans. Four species of trichomonads infect humans. Among these only *Trichomonas vaginalis* is clearly pathogenic and it is usually of low virulence. The others exhibit a questionable pathogenicity.

3.2 Intended Learning Outcomes (ILOs)

At the end of this lecture unit, students should:

3. be acquinted with the genera and species of protozoans that constitutes luminal protozoans such a Trichomoniasis
4. Know their epidemiology, morphology, characteristics, life cycle, symptoms, diagnosis, treatment and control.

3.3 Trichomoniasis as a parasite

Trichomoniasis is the disease caused by this organism. The trichomonads of humans inhabit different anatomical locations. *T. vaginalis* is a common sexually transmitted disease found in the uro-genital tract. *T. tenax*, also called *T. buccalis*, is a commensal of the human oral cavity, found particularly in patients with poor oral hygiene and advanced periodontal disease. *T. tenax* or an organism with similar morphology is also occasionally found in the lungs. Such cases have reported mainly in patients with underlying cancers or other lung diseases or following surgery. *Pentatrichomonas hominis*, formerly known as *Trichomonas hominis*, is a non-pathogenic commensal of the large intestine. Some authors divide the trichomonads into three genera based on the number of free flagella. Species with three flagella are called *Tritrichomonas*, those with four are called *Trichomonas*, and *Pentatrichomonas* refers to trichomonads with five free anterior flagella. *Dientamoeba fragilis* was originally believed to be an ameba. Now, it is known to be a flagellate - however without flagella - related to the trichomonads.

Etiology

Trichomonas vaginalis (a flagellate)

Epidemiology

Trichomonas vaginalis was first described from purulent vaginal discharges in 1836 and by the early part of the twentieth century was recognized as an etiological agent of vaginitis. Trichomoniasis is a common sexually transmitted disease with a worldwide distribution and an estimated 167 million people becoming infected per year worldwide and 5 million new infections per year in the United States. Trichomoniasis is believed to be the most common non-viral sexually transmitted disease. Despite the frequency of trichomoniasis it has in the past been considered more of a nuisance parasite rather than a major pathogen. However it is now recognized a factor in promoting HIV infection causing low-weight and premature births, and predisposing women to substantial discomfort and stress. *Trichomonas vaginalis* has a world-wide distribution; incidence is as low as 5% in normal females and as high as 70% among prostitutes and prison inmates.

Morphology

The trophozoite form is 15 to 18 micrometers in diameter and is half pear shaped with a single nucleus, four anterior flagella and a lateral flagellum attached by an undulating membrane. Two axostyles are arranged asymmetrically. The organism does not encyst (**Figure 3.3**).

Life cycle

T. vaginalis colonizes the vagina of women and the urethra (sometimes prostate) of men.Infection occurs primarily via sexual contact, although non-venereal infections are possible. The organism does not encyst and divides by binary fission which is

favored by low acidity (pH > 5.9; the normal pH is 3.5 to 4.5). There is no non-human reservoir.
T. vaginalis causes different clinical manifestations in _____and _____***?***

Self-Assessment Exercise 1

1. ***Symptoms of T vaginalis commonly occur during or immediately after _____?***
2. ***Outline atleast three Common symptoms of T vaginalis?***

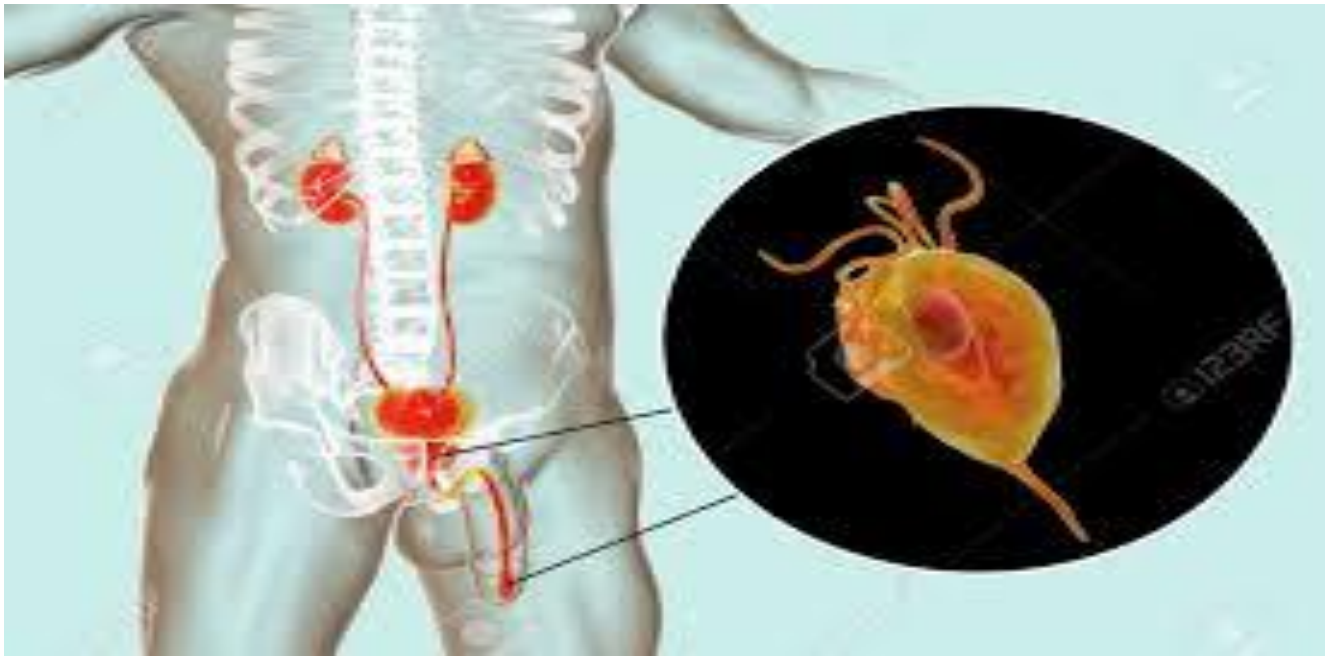

Figure 3.1: *Trichomonas vaginalis* from man

Symptoms and Pathogenesis

T. vaginalis causes different clinical manifestations in men and women and women are more likely to exhibit symptoms which tend to persist longer. The incubation period typically ranges from 4-28 days. In females the infection can present as a mild vaginitis, an acute or chronic vulvovaginitis, or urethritis. The onset or exacerbation of symptoms commonly occurs during or immediately after menstration. The most common complaint associated with *T. vaginalis* infection is a persistent mild vaginitis associated with a copious, foul-smelling discharge that is often accompanied by burning or itching. This discharge is most often gray, but can be yellow or green and is occasionally frothy or blood tinged. The discharge diminishes as the infection becomes more chronic. Many women also experience painful or difficult coitus. Urethral involvement occurs in a large number of cases and is characterized by dysuria (painful urination) and frequent urination. The vaginal epithelium is the primary site of infection. Thus the vaginal walls are usually erythematous (i.e. red) and may show petechial (a small non-raised spot) hemorrhages. Punctate hemorrhages of the cervix,

called strawberry cervix, are observed in approximately 2% of the cases. This strawberry cervix is a distinctive pathological observation associated with trichomonasis not seen with other sexually transmitted diseases. Males are likely to be asymptomatic (50-90%) and the infection tends to be self-limiting". The urethra and prostate are the most common sites of infection. Common symptoms include: urethral discharge (ranging from scant to purulent), dysuria, and urethral pruritus (itching). Some men experience burning immediately after coitus. Little is known about the pathophysiology associated with *T. vaginalis* infection, but is presumably due to interactions between the parasite and host epithelial cells. In vitro studies indicate that *T. vaginalis* can destroy cells in a contact dependent manner. Therefore adhesion of the trophozoites to the epithelium is believed to be a major factor in the pathogenesis. Several adhesion proteins have been identified on the surface of the trophozoites. In addition, secreted proteases that could play a role in pathogenesis have also been identified.

Diagnosis

Diagnosis is confirmed by the demonstration of trophozoites in vaginal, urethral, prostatic secretions, or urine sediment (following prostate massage). Microscopic examination of wet mounts of fresh vaginal discharge, preferably collected with a speculum on a cotton-tipped applicator, is the most practical method of diagnosis. Specimens should be diluted in saline and examined immediately. *T. vaginalis* is recognized by its characteristic morphological features and its rapid jerky motility. Specimens can also be fixed and stained with Giemsa or fluorescent dyes. However, the organism may be difficult to recognize on stained slides. "The sensitivity of direct observation ranges from 40-80%. Therefore, in vitro culture is considered the gold standard for diagnosis despite some limitations. For example, access to facilities is needed and organisms require 2-7 days of growth before they are detected. The accessibility issue is partly resolved by the InPouch™TV culture system (Biomed Diagnostics)". This is a commercially available self-contained system for the detection of *T. vaginalis* in clinical specimens. Antibody and DNA-based tests with high sensitivity and specificity are being developed.

Treatment and Control

Metronidazole (Flagyl®) and other nitroimidazoles, such as tinidazole, are highly effective against trichomoniasis. The metronidazole is activated by the hydrogensome to a nitro radical ion intermediate. Either a single two gram dose (85-92% cure rate) or 250 mg three time daily for 7-10 days (>95% cure rate) can be used. Sexual partners should be treated at the same time to prevent reinfection. Some drug resistance has been reported, but this is not a wide-spread problem. Treatment failures are generally due to noncompliance or reinfection. The epidemiology of trichomonasis exhibits features similar to other sexually transmitted diseases and incidence correlates with the number of sexual partners. In addition, co-infection with other STDs is common. It is estimated that up to 25% of sexually active women will become infected at some point during their lives and the disease will be transmitted to 30-70% of their male partners. Measures used in the control of other STD, such as limiting number of sexual partners and use of condoms, are also effective in preventing trichomoniasis.

3.3.1 Distinctive feature of the Trichomonads

- A distinctive feature of the trichomonads is an axostyle (ax) which runs the length of the organism and appears to protrude from the posterior end.

- The axostyle is a cytoskeletal element composed of concentric rows of microtubules and is believed to function in the attachment of the parasite to epithelial cells.

- Trichomonads are also characterized by 4-6 flagella (fg) emerging from the anterior end. One of the flagella is attached to the body of the organism and forms a posteriorly-directed undulating membrane (um), whereas the remaining flagella are free.

- The combined basal bodies (bb) and the base of the undulating membrane, called the costa (cs), are often seen is stained preparations. Less frequently seen is the cytostomal groove (cy). A single nucleus (nu) is found at the anterior end of the parasite.

- The trichomonads, like many other intestinal protozoa, exhibit an anerobic metabolism and lack mitochondria. Part of energy metabolism of trichomonads involves a unique organelle called the hydrogenosome.

- The hydrogenosome has a double membrane and is distantly related to the mitochondrion. However, it lacks DNA, cytochromes and many typical mitochnondrial functions such as enzymes of the tricarboxylic acid cycle and oxidative phosphorylation.

- The primary function of the hydrogenosome is the metabolism of pyruvate, produced during glycolysis within the cytosol, to acetate and carbon dioxide with the concomitant production of ATP.

- The electrons release from the oxidation of pyruvate are transferred to hydrogen ions to produce molecular hydrogen, hence the name hydrogenosome.

Briefly explain the following:

- *Tritrichomonas*
- *Trichomonas*
- *Pentatrichomonas*

Self-Assessment Exercise 2

1. ***Outline at least two the feature of trichomonads?***
2. ***Mention atleast two measures that can be used to control Trichomonas vaginalis?***

3.4 Summary

In this unit, you have learnt about Trichomoniasis, their epidemiology, morphology, characteristics, life cycle, symptoms, pathogenesis, immunology, treatment and control. *T. vaginalis*, despite its name, infects both men and women. In females the organism primarily inhabits the vagina, and in males it is usually found in the urethra, prostate or epididymis.

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3.6 Possible Answers to SAEs

Answers to SAEs 1

1. *Menstration*
2. *Urethral discharge (ranging from scant to purulent), dysuria, and urethral pruritus (itching)*

Answers to SAEs 2

1 *- A distinctive feature of the trichomonads is an axostyle (ax) which runs the length of the organism and appears to protrude from the posterior end. - The axostyle is a cytoskeletal element composed of concentric rows of microtubules and is believed to function in the attachment of the parasite to epithelial cells.*

2 *Limiting number of sexual partners and use of condoms.*

Unit 4: *Blastocystis hominis*

Unit Structure

4.1 Introduction

Blastocystis hominis is a common organism found in human stools. Since its initial description approximately 100 years ago, it has been variously classified as an ameba, yeast, a sporozoan, and the cyst stage of a flagellate. Analysis of the small subunit rRNA sequence indicates that *Blastocystis* is most closely related to the stramenopiles, a complex assemblage of unicellular and multicellular protists. Other stramenopiles include diatoms, brown algae, and water molds. Many of the characteristics of *Blastocystis* are unknown or controversial. The mode of transmission, mechanism of cell replication, and other features of the life cycle have not conclusively demonstrated. Similarly, the status of *Blastocystis* as a pathogen, commensal, or opportunistic organism is unknown.

4.2 Intended Learning Outcomes (ILOs)

At the end of this lecture unit, students should:

5. Know their epidemiology, morphology, characteristics, life cycle, symptoms, diagnosis, treatment and control.

4.3 *Blastocystis hominis* as a parasite

Blastocystis is also found in a wide range of animals, including mammals, birds, reptiles, amphibians and even insects, and exhibits a wide range of molecular diversity. The genetic distance between *Blastocystis* isolates is greater than the genetic distance between *E. histolytica* and *E. dispar*. This complicates the designation of species and historically human isolates have been designated as *B. hominis* and isolates for other hosts as *Blastocystis* sp. However, phylogenetic analysis reveals that there are no exclusively human clades and human isolates are found in all of the clades.

Epidemiology

Blastocystis hominis is the most common intestinal protozoan, with a wide geographic distribution that has unclear clinical significance.

Morphology

Blastocystis is polymorphic in that a variety of morphological forms are found in feces and in vitro culture. The most widely recognized form is spherical 10-15 µm in diameter with a large central vacuole. This large vacuole pushes the nuclei and other organelles to the periphery of the cell. The vacuole is sometimes filled with a granular material. Small resistant cyst-like forms have been identified from in vitro cultures and occasionally observed in feces. These presumed cysts are approximately 5 µm and surround by a multilayered wall. Furthermore, the cysts do not lyse when placed in water suggesting that they are resistant to environmental conditions. Presumably *Blastocystis* is transmitted via a fecal-oral route. However, this has not been conclusively demonstrated.

Blastocystis is also found in a wide range of animals including _____, _____, _____, _____ and _____.

Self-Assessment Exercise 1

1. ***Blastocystis* is transmitted through _____?**
2. **The drug used against Blastocystis is called _____?**

Symptoms

Diarrhea, cramps, nausea, vomiting and abdominal pain have been associated with large numbers of organisms in the stool.

Treatment

However, the drugs used against *Blastocystis* (e.g. metronidazole) also work against many other intestinal protozoa and bacteria. The inability to rule out other organisms as the source of symptoms and the observation that many infected persons exhibit no symptoms makes it difficult to draw any definitive conclusions about the pathogenesis of *Blastocystis*.
Mention the Symptoms of Blastocystis hominis.

Self-Assessment Exercise 2

1. ***Blastocystis* is not host specific and can be transmitted _____?**
2. ***Outline at least three morphology of Blastocystis hominis?***

4.4 Summary

In this unit, you have learnt about *Blastocystis*, their epidemiology, morphology, characteristics, life cycle, symptoms, pathogenesis, immunology, treatment and control. *Blastocystis* is not host specific and can be transmitted zoonotically. In addition, the wide range of genetic diversity might explain the controversy concerning the pathogenicity of *Blastocystis* in that some genotypes may be more virulent than others. However, studies addressing this issue suggest that this is not the case. Resolution of the confusion about the taxonomy, transmission and virulence of *Blastocystis* will require additional studies.

4.5 References/Further Readings/Web Sources

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4.6 Possible Answers to SAEs

Answers to SAEs 1

1. *A fecal-oral route*

2. *Metronidazole*

Answers to SAEs 2

1. *Zoonotically*

2. *- Blastocystis is polymorphic in that a variety of morphological forms are found in feces and in vitro culture.*

- *The most widely recognized form is spherical 10-15 µm in diameter with a large central vacuole. This large vacuole pushes the nuclei and other organelles to the periphery of the cell.*
- *The vacuole is sometimes filled with a granular material. Small resistant cyst-like forms have been identified from in vitro cultures and occasionally observed in feces.*

Unit 5: *Dientamoeba fragilis*

Unit Structure

5.1 Introduction

Dientamoeba fragilis is a species of single-celled excavates found in the gastrointestinal tract of some humans, pigs and gorillas. It causes gastrointestinal upset in some people, but not in others. It is an important cause of traveller's diarrhoea, chronic diarrhoea, fatigue and, in children, failure to thrive. Despite this, its role as a commensal, pathobiont, or pathogen is still debated. *D. fragilis* is one of the smaller parasites that are able to live in the human intestine. *Dientamoeba fragilis* cells are able to survive and move in fresh feces but are sensitive to aerobic environments. They dissociate when in contact or placed in saline, tap water or distilled water.

5.2 Intended Learning Outcomes (ILOs)

At the end of this lecture unit, students should:

1. Know their epidemiology, morphology, characteristics, life cycle, symptoms, diagnosis, treatment and control.

5.3 *Dientamoeba fragilis* as a parasite

Dientamoeba fragilis was originally described as an ameba based upon its morphology. However, later it was recognized to exhibit morphology more similar to the turkey parasite *Histomonas meleagridis*, except for the lack of flagella. Ultrastructural studies also suggest similarities to the trichomonads, including the possession of hydrogenosomes and molecular studies have confirmed a close phylogenetic relationship between *Dientamoeba* and *Histomonas* and a possible more distal relationship to *Trichomonas*.

Epidemiology

Dientamoeba fragilis has an estimated prevalence throughout the United States. Unlike majority of parasitic infections, *D. fragilis* is more prevalent in well-developed countries as opposed to disadvantaged and resource poor nations. The parasite is also endemic in crowded communities (i.e. institutions), populations with unsatisfactory sanitation conditions, and individuals who travel to underprivileged countries. Globally, the prevalence of D. fragilis ranges from 0.3% to 90%, occurring in multiple countries including many urbanized cities such as Los Angeles, California and Sydney, Australia. Recently, *D. fragilis* was considered to be more prevalent than Giardia, thus leading to better diagnostics.

Life cycle

As with other trichomonads, *Dientamoeba* only exhibits a trophozoite stage (**Figure 5.3)**. This raises some questions about the mode of transmission in that a cyst stage is usually involved in fecal oral transmission. In addition, the trophozoites of *Dientamoeba* survive outside of the body for a very short time. *H. meleagridis* also lacks a cyst stage and has been demonstrated to be transmitted via the eggs of a nematode. Due to the close relationship between *Histomonas* and *Dientamoeba*, it is proposed that *Dientamoeba* is also transmitted via helminthes eggs. Epidemiological and experimental evidence tends to incriminate the pinworm *Enterobius vermicularis* as the carrier for *Dientamoeba*. More recently, pigs have been shown to be a natural host for *D. fragilis* of the same genotype as found in humans, thus raising the possibility of a zoonotic transmission.

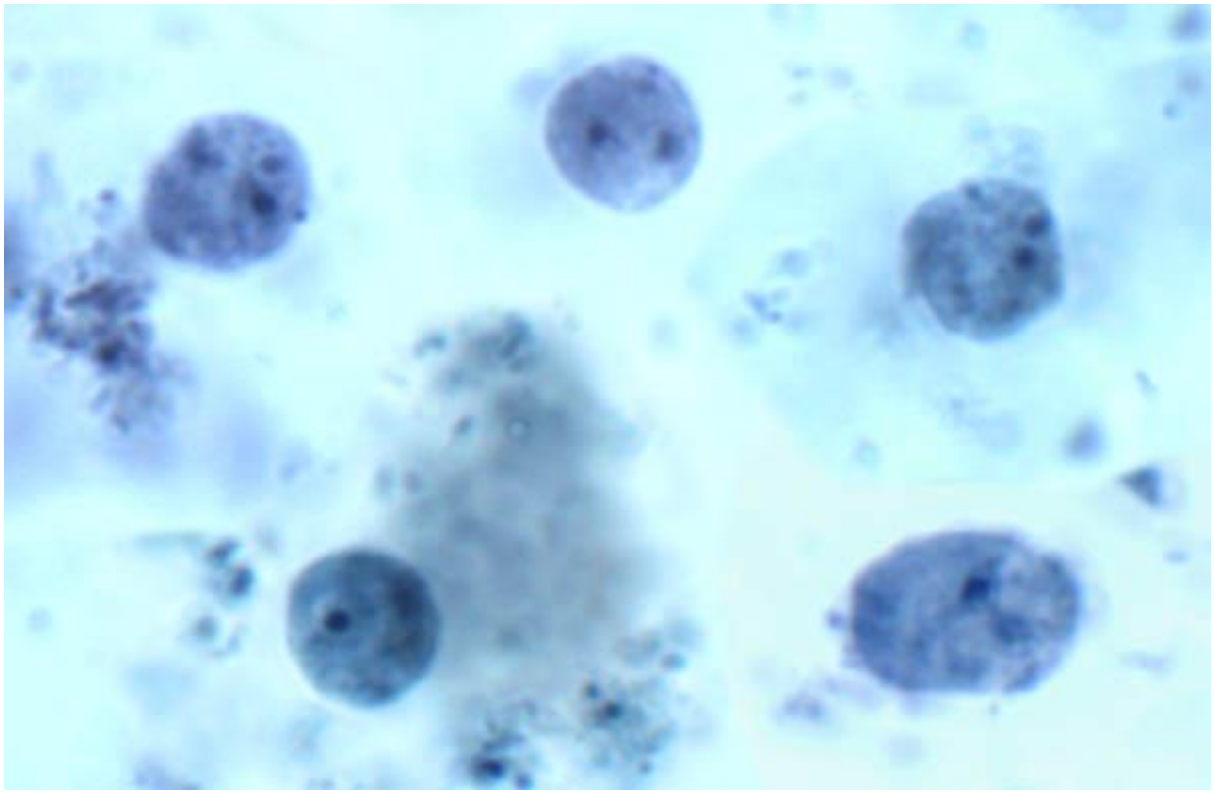

Figure 5.3: Morphology of *Dientamoeba fragilis* from a stool sample. Trophozoites exhibit an ameba-like morphology and are often bi-nucleated.

Symptoms

Historically *Dientamoeba* has been considered as a non-pathogenic commensal. However, clinical symptoms often correlate with the presence of large numbers of trophozoites and treatment of the infection resolves the symptoms. The incidence of symptoms is estimated at 15 - 30% of infected individuals. Clinical symptoms associated with *Dientamoeba* include intermittent diarrhea, abdominal pain, flatulence, nausea and fatigue. Little is known about the pathogenesis and *Dientamoeba* probably acts as a low-grade irritant of intestinal mucosal surfaces that may lead to some inflammation.

Diagnosis

In order to diagnose the parasite, patients are required to provide (multiple) fresh stool samples that have been preserved for parasite examination. The multiple samples are required because of parasite detection being difficult; therefore, a sample might be obtained each day to help increase the sensitivity. Patients can also be tested for *E. vermicularis* since the two parasites are known to coincide.

Treatment

Iodoquinol is generally the drug of choice for the treatment of *Dientamoeba*. Paromomycin and metronidazole are also effective.
Dientamoeba fragilis was originally described as_____based upon its morphology.

Self-Assessment Exercise 1

1. ***D. fragilis is one of the smaller parasites that are able to live in the _____?***
2. ***Globally, the prevalence of D. fragilis ranges from _____ to _____ occurring in multiple countries including many urbanized cities such as Los Angeles, California and Sydney, Australia?***

5.4 Phylogenetics of *Dientamoeba fragilis*

Dientamoeba fragilis is a type of trichomonad. Trichomonads are flagellated organisms but *D. fragilis* lacks flagella, having secondarily lost them over evolutionary time. Thus, it is an amoeba of flagellate ancestry. In point of ultrastructural and antigenic view, Dientamoeba is reclassified as a flagellate. The lifecycle of this parasite has not yet been completely determined, but some assumptions have been made based on clinical data. A cyst stage has been reported, although it is yet to be independently confirmed as of 2013. If true, *D. fragilis* is probably transmitted by the fecal-oral route. Prior to the report of this cyst stage in the lifecycle of *Dientamoeba*, transmission was postulated to occur by helminthes eggs (e.g., *Ascaris*, *Enterobius* spp.). "The rationale for this suggestion was that *D. fragilis* is closely related to the turkey parasite *Histomonas*, which is known to be transmitted by the eggs of the helminthes Heterakis. Since *D. fragilis* is known to frequently coinfect with *E. vermicularis*, this leads to the assumption that *E. vermicularis* is a possible vector and mode of transmission. When inside the host, the parasite infects the mucosal crypts of the large intestine. They primarily affect the cecum and proximal colon. It is assumed that when *D. fragilis* is inside the colon, it reproduces asexually by binary fission. From there, the trophozoites are in the lumen of the colon, and are excreted as wastes. *D. fragilis* is not considered to be invasive nor cause cell or tissue damage.

State the drugs used in the treatment of Dientamoeba.

Answer

Iodoquinol, Paromomycin and Metronidazole

Self-Assessment Exercise 2

1. ***Briefly explain the Diagnosis of Dientamoeba fragilis?***
2. ***Briefly explain the Treatment of Dientamoeba fragilis?***

5.5 Summary

In this unit, you have learnt about *D. fragilis,* their epidemiology, morphology, life cycle, symptoms, diagnosis treatment and control. There is a continuous debate whether *D.*

fragilis is considered to be a harmless organism or a pathogenic parasite. Infection with *D. fragilis*, called dientamoebiasis, is associated variously with symptoms of abdominal pain, diarrhea, weight loss, nausea, fatigue and fever. In one study, *D. fragilis* was identified in 0.9% of patients observed. Its coincidence with enterobiasis, caused by pinworm (*Enterobius vermicularis*), has been reported. In another study, eosinophilia was present in half of the infected children participating in the case. *D. fragilis* does not penetrate the host tissue directly; therefore, some of these symptoms may be caused from irritation which then leads to colonic motility. Infection can occur at any age; however, the most common ages that have been reported are children 5 - 10 years old.

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http://www.blastocystis.net/search/label/PCR

5.7 Possible Answers to SAEs

Answers to SAEs 1

1. *Human intestine*
2. *0.3% to 90%,*

Answers to SAEs 2

1. *In order to diagnose the parasite, patients are required to provide (multiple) fresh stool samples that have been preserved for parasite examination. The multiple samples are required because of parasite detection being difficult; therefore, a sample might be obtained each day to help increase the sensitivity. Patients can also be tested for E. vermicularis since the two parasites are known to coincide.*
2. *Iodoquinol is generally the drug of choice for the treatment of* Dientamoeba*. Paromomycin and metronidazole are also effective.*

Glossary

DNA = Deoxyribonucleic acid
AIDS = Acquired Immunodeficiency syndrome
ATP = Adenosine Triphosphate
STD = Sexually Transmitted Diseases
ACR = Adequate clinical response
LTF = Late treatment failure
ETF = Early treatment failure
MDR = Multi-drug resistance
ABC = ATP-binding cassette
ART = Active antiretroviral therapy
GAE = Granulomatous amebic encephalitis
CNS = Central Nervous System
PAM = Primary amebic meningoencephalitis

End of the module Questions

1. *E. histolytica* is considered pathogenic and the disease it causes is called amebiasis or amebic dysentery (**True or False**)
2. _____ is morphologically identical to *E. histolytica* and the two were previously considered to be the same species. (a) *E. dispar* (b) *E. lipar* (c) *E. dilaris* (d) *E. coli*
3. Iodoquinol is used to treat asymptomatic infections and metronidazole is used for symptomatic infection and chronic amebiasis, including extra-intestinal disease (**True or False**)
4. Lactase is an enzyme that breaks down lactose, a sugar found in milk, to monosaccharide's which can be absorbed (**True or False**)
5. Infection with *D. fragilis*, called _____ (a) Dientamoebiasis (b) Ameobiasis (c) Laptobiasis (d) Debrisbiasis

Module 4: *Other Intestinal/Free Living Protozoa*

Module Structure

In this module we will discuss about other intestinal/free living protozoa with the following units:

Unit 1: *Balantidium coli*
Unit 2: *Cryptosporidium parvum*
Unit 3: *Isospora belli*
Unit 4: *Acanthamoeba* sp.
Unit 5: *Naegleria fowler*
Glossary
End of the module Questions

Unit 1: *Balantidium coli*

Unit Structure

1.1 Introduction
1.2 Intended Learning Outcomes (ILOs)
1.3 *Balantidium coli* as a parasite
1.4 Summary
1.5 References/Further Readings/Web Sources
1.6 Possible Answers to SAEs

1.1 Introduction

Balantidium coli are parasitic species of ciliate alveolates that causes the disease balantidiasis. It is the only member of the ciliate phylum known to be pathogenic to humans.

1.2 Intended Learning Outcomes (ILOs)

At the end of this lecture unit, students should:

1. Know their epidemiology, morphology, life cycle, symptoms, diagnosis, treatment and control.

1.3 *Balantium coli* as a parasite

This is a parasite primarily of cows, pigs and horses. The organism is a large (100 x 60micrometer) ciliate with a macro- and a micro-nucleus. The infection occurs mostly in farm workers and other rural dwellers by ingestion of cysts in fecal material of farm animals. Man-to- man transmission is rare but possible (**Figure 1.3**).

Epidemiology

Balantidiasis in humans is common in the Philippines, but it can be found anywhere in the world, especially among those that are in close contact with swine. The disease is considered to be rare and occurs in less than 1% of the human population. The disease poses a problem mostly in developing countries, where water sources may be contaminated with swine or human feces.

Morphology

Balantidium coli have two developmental stages, a trophozoite stage and a cyst stage. In trophozoites, the two nuclei are visible. The macronucleus is long and sausage-shaped, and the spherical micronucleus is nested next to it, often hidden by the macronucleus. The opening, known as the peristome, at the pointed anterior end leads to the cytostome, or the mouth. Cysts are smaller than trophozoites and are round and have a tough, heavy cyst wall made of one or two layers. Usually only the macronucleus and sometimes cilia and contractile vacuoles are visible in the cyst, however, both nuclei are present because nuclear multiplication does not occur when the organism is a cyst. Living trophozoites and cysts are yellowish or greenish in color.
Balantidium coli have two developmental stages namely:

Self-Assessment Exercise 1

1. ***Balantidiasis in humans is common in the _____?***
2. ***In Balantidium, _____ is smaller than _____ and are round and have a tough, heavy cyst wall made of one or two layers?***

Symptoms and pathogenesis

Symptoms and pathogenesis of balantidiasis are similar to those seen in entamoebiasis, including intestinal epithelial erosion. However, liver, lung and brain abscesses are not seen.

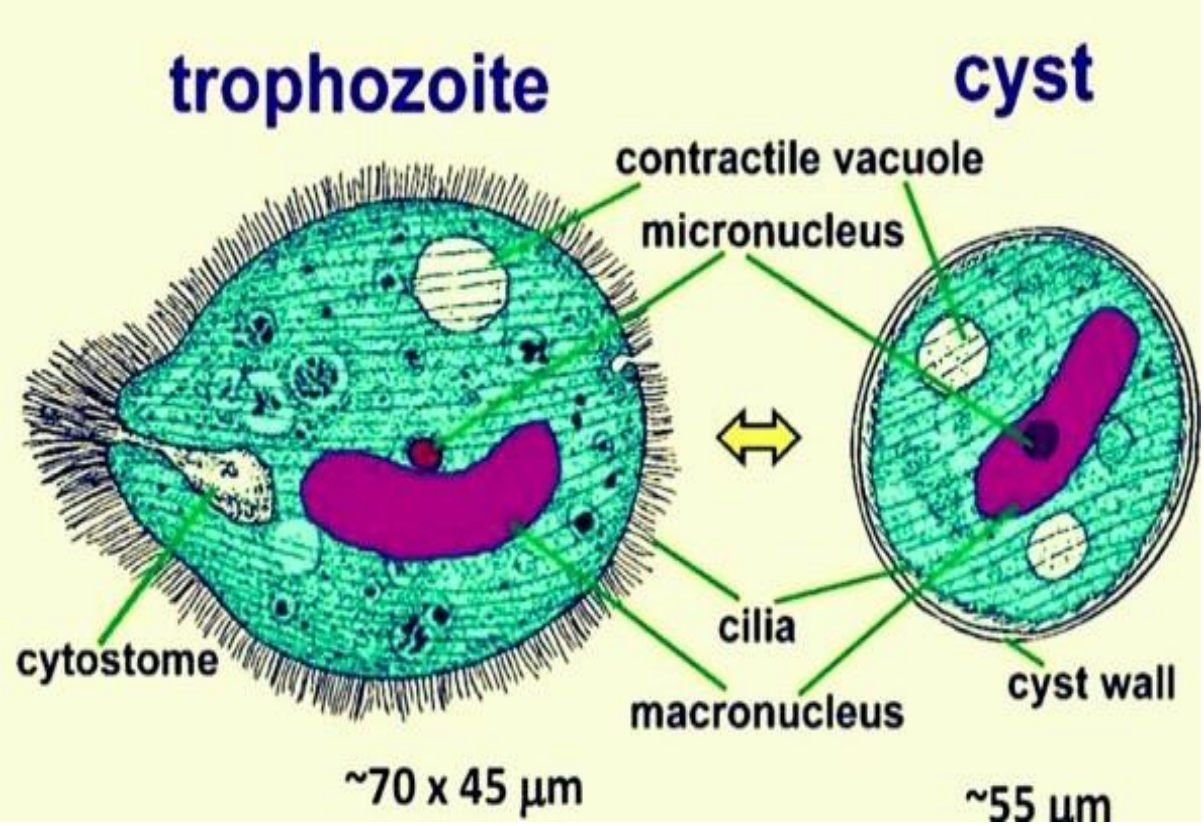

Figure 1.3: Trophozoites and Cyst of *Balantidium coli*. These are characterized by: their large size(45 µm to more than 70 µm) the presence of cilia on the cell surface a cytostome (arrows) a bean shaped macronucleus which is often visible - see (A), and asmaller, less conspicuous micronucleus.

Treatment and control

Metronidazole and iodoquinol are effective

Mention at least two zoonotic protozoan intestinal infections with some health significance.

Self-Assessment Exercise 2

1. ***Mention the drugs used to treat and control the person infected with Balantidium parasite?***
2. ***Briefly outline the epidemiology of Balantidium coli?***

1.4 Summary

In this unit, you have learnt about the *Balantidium coli,* their epidemiology, morphology, life cycle, symptoms, pathogenesis, treatment and control. *Balantidium coli* are they

only ciliated protozoan known to infect humans. Balantidiasis is a zoonotic disease and is acquired by humans via the feco-oral route from the normal host, the domestic pig, where it is asymptomatic. Contaminated water is the most common mechanism of transmission.

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1.6 Possible Answers to SAEs

Answers to SAEs 1

1. *Philippines*

2. *Cysts and trophozoites*

Answers to SAEs 2

1. *Metronidazole and iodoquinol are effective*

2. *- Balantidiasis in humans is common in the Philippines, but it can be found anywhere in the world, especially among those that are in close contact with swine.*

- *The disease is considered to be rare and occurs in less than 1% of the human population.*
- *The disease poses a problem mostly in developing countries, where water sources may be contaminated with swine or human feces.*

Unit 2: *Cryptosporidium parvum*

Unit Structure

2.1 Introduction

Cryptosporidium parvum (*C. parvum*) is a small round parasite measuring 3 to 5 micrometers which is found in the gastrointestinal tract of many animals and causes epidemics of diarrhea in humans via contaminated food and water.

2.2 Intended Learning Outcomes (ILOs)

At the end of this lecture unit, students should:

1. Know their life cycle, symptoms, treatment and control.

2.3 *Cryptosporidium parvum* as a Parasite

Cryptosporidium parvum is one of several species that cause cryptosporidiosis, a parasitic disease of the mammalian intestinal tract. Primary symptoms of *C. parvum* infection are acute, watery, and non-bloody diarrhea. *C. parvum* infection is of particular concern in immunocompromised patients, where diarrhea can reach 10–15 times per day. Other symptoms may include anorexia, nausea/vomiting, and abdominal pain. Extra-intestinal sites include the lung, liver, and gall bladder, where it causes respiratory cryptosporidiosis, hepatitis, and cholecystitis, respectively. Infection is caused by ingestion of sporulated oocysts transmitted by the faecal-oral route. In healthy human hosts, the median infective dose is 132 oocysts. The general *C. parvum* lifecycle is shared by other members of the genus. Invasion of the apical tip of ileal enterocytes by sporozoites and merozoites causes pathology seen in the disease. Infection is generally self-limiting in immunocompetent people. In immunocompromised patients, such as those with AIDS or those undergoing immunosuppressive therapy, infection may not be self-limiting, leading to dehydration and, in severe cases, death.

In AIDS patients, Cryptosporidium parvum may cause _____.

Answer

Prolonged, severe diarrhea

Self-Assessment Exercise 1

1. ***Cryptosporidium parvum* is one of several species that cause _____?**
2. ***Mention at least three primary symptom of Cryptosporidium parvum?***

Life cycle

Humans are infected by ingestion of *C. parvum* oocysts containing many sporozoites. The sporozoites are released in the upper GI tract and attach to the gut mucosal cells where they divide to produce merozoites. The merozoites invade other mucosal cells and further multiply asexually. Some of the merozoites differentiate into male and female gametocytes and form an oocyst in which they multiply and differentiate into sporozoites. The mature oocyst is excreted with fecal material and infects other individuals".

Symptoms

When a large number of humans in a community have diarrhea, the most likely cause is *C. parvum*. A small bolus of infection may cause mild diarrhea, whereas a larger intake of organisms may cause more pronounced symptoms including copious watery diarrhea, cramping abdominal pain, flatulence and weight loss. Severity and duration of symptoms are related to immuno-competence. In AIDS patients, the organism may cause prolonged, severe diarrhea and the organisms may invade the gallbladder,

biliary tract and the lung epithelium.

Treatment and Control

There is no approved effective treatment for cryptosporidiasis, although paromycin is used as an investigational drug. There are a variety of antibody tests for detection but many of these detect other species of *Cryptosporidium* than *C. parvum*. Sensitive polymerase chain reaction tests are available for *C. parvum* detection in environmental and animal samples.
Outline the symptoms of Cryptosporidium parvum.

Self-Assessment Exercise 2

1. ***Briefly explain the Treatment and Control of Cryptosporidium parvum?***
2. ***Briefly outline the life cycle of Cryptosporidium parvum?***

2.4 Summary

In this unit, you have learnt about *Cryptosporidium parvum*, their life cycle, symptoms, treatment and control. *C. parvum* is considered to be the most important waterborne pathogen in developed countries. The protozoa also caused the largest waterborne-disease outbreak ever documented in the United States, making 403,000 people ill in Milwaukee, Wisconsin, in 1993. It is resistant to all practical levels of chlorination, surviving for 24 hours at 1000 mg/L free chlorine.

2.5 References/Further Readings/Web Sources

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2.6 Possible Answers to SAEs

Answers to SAEs 1

1. *Cryptosporidiosis*
2. *Acute, watery, and non-bloody diarrhea*

Answers to SAEs 2

1. *- There is no approved effective treatment for cryptosporidiasis, although paromycin is used as an investigational drug.*
- *There are a variety of antibody tests for detection but many of these detect other species of Cryptosporidium than C. parvum. Sensitive polymerase chain reaction tests are available for C. parvum detection in environmental and animal samples.*
2. *- Humans are infected by ingestion of C. parvum oocysts containing many sporozoites.*
- *The sporozoites are released in the upper GI tract and attach to the gut mucosal cells where they divide to produce merozoites.*
- *The merozoites invade other mucosal cells and further multiply asexually. Some of the merozoites differentiate into male and female gametocytes and form an oocyst in which they multiply and differentiate into sporozoites.*
- *The mature oocyst is excreted with fecal material and infects other individuals.*

Unit 3: *Isospora belli*

Unit Structure

3.1 Introduction

Cystoisosporiasis, which was previously known as isosporiasis, is an uncommon diarrheal illness caused by the protozoan. *Cystoisospora belli* (formerly known as *Isospora belli*). *C belli* was first described by Virchow in 1860. The genus Cystoisospora is related closely to the genera*Cryptosporidium, Cyclospora,* and *Toxoplasma.* However, *Cystoisospora* infe ction is not as common as infection with *Cryptosporidium* or *Toxoplasma*. The first case of human infection with *C. belli* was described in 1915.

3.2 Intended Learning Outcomes (ILOs)

At the end of this lecture unit, students should:

1. Know their epidemiology, life cycle, symptoms, diagnosis, treatment and control.

3.3 *Isospora belli* as a Parasite

Isospora belli is a protozoan parasite in the Phylum Apicomplexa. These groups of parasites are referred to as coccidia. This organism can be acquired by the ingestion of sporulated oocysts found in contaminated food or water. The oocyst stages is 23 - 36 by 12-17 µm and are much larger than the oocysts of related coccidial species such as *Cryptosporidium parvum*. *Isospora belli* is a rare infection of normal humans, although it is being seen in increasing numbers in AIDS patients. The infection occurs via the oro-fecal route. The infective stage of the organism is an oval oocyst which, upon ingestion, follows the same course as C. parvum. The disease produces symptoms similar to those of giardiasis.
The first case of human infection with *C. belli* was described in _____.

Self-Assessment Exercise 1

1. ***Cystoisosporiasis, which was previously known as _____?***
2. ***C. belli was first described by _____ in 1860?***

Epidemiology

Isospora belli infections are essentially cosmopolitan in distribution but are more common in tropical and subtropical regions, especially Haiti, Mexico, Brazil, El Salvador, tropical Africa, Middle East, and Southeast Asia. For example, *Isospora belli* has been reported as the most common protozoan parasite in HIV infected patients with acute or chronic diarrhea in India. In French HIV patients with *I. belli* a significant risk factor was being from sub-Saharan Africa. An outbreak of *I. belli* infections was reported in Antofagasta City Chile in 1977. It was associated with ingestion of vegetables contaminated with irrigation water from a sewage treatment plant. Approximately 90 people were infected.

Symptoms

The disease produces symptoms similar to those of giardiasis. Early symptoms include flatulence, abdominal distension, nausea and foul-smelling bulky, explosive, often watery, diarrhea. The stool contains excessive lipids but very rarely any blood or necrotic tissue. The more chronic stage is associated with vitamin B_{12} malabsorption, disaccharidase deficiency and lactose intolerance.

Pathogenesis

I.belli can cause marked villous atrophy, and crypt hyperplasia in the small intestine. Inflammatory infiltrates in the lamina propria include eosinophils, neutrophils, lymphocytes and plasma cells. The precise mechanism causing these changes is unknown, but they result in steatorrhea and malabsorption. Infection of the biliary tract by *I. belli* is also possible. The parasite can complete its life cycle in the biliary tract and oocysts can be observed in bile. Stages are located in the bile duct epithelium.

Treatment and Control

Combination therapy with oral trimethoprim (160 mg)-sulfamethoxazole (800 mg) 4 times a day for 10 days results in a decrease in diarrhea and abdominal pain within 1 to 6 days (mean = 2.5 days) after treatment. Stool samples examined after 10 days usually do not contain oocysts. Combination therapy with oral trimethoprim (320mg)-sulfamethoxazole (1,600 mg) 2 times a day for 10 to 14 days is as effective and may be an easier course of therapy for some patients. It is important to note here that HIV patients have a very high rate of relapse and should receive secondary prophylaxis, until they are immune reconstituted by active antiretroviral therapy (ART).

How can a patient that is infected with Isospora bellii be treated?

Self-Assessment Exercise 2

1. ***Briefly highlight the symptoms of Isospora bellii?***
2. ***Briefly outline the pathogenesis of Isospora bellii?***

3.4 Summary

In this unit, you have learnt about *Isospora bellii,* their epidemiology, symptoms, pathogenesis, treatment and control. Humans are the only known hosts for *C belli,* which has no known animal reservoir. Cystoisosporiasis has a worldwide distribution, although it is more common in tropical and subtropical climates.

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3.6 Possible Answers to SAEs

Answers to SAEs 1

1. *Isosporiasis*
2. *Virchow*

Answers to SAEs 2

1. *- The disease produces symptoms similar to those of giardiasis.*
- *Early symptoms include flatulence, abdominal distension, nausea and foul-smelling bulky, explosive, often watery, diarrhea.*
- *The stool contains excessive lipids but very rarely any blood ornecrotic tissue.*
- *The more chronic stage is associated with vitamin B_{12} malabsorption, disaccharidase deficiency and lactose intolerance.*

2. *- I.belli can cause marked villous atrophy, and crypt hyperplasia in the small intestine.*
- *Inflammatory infiltrates in the lamina propria include eosinophils, neutrophils, lymphocytes and plasma cells.*
- *The precise mechanism causing these changes is unknown, but they result in steatorrhea and malabsorption.*
- *Infection of the biliary tract by I. belli is also possible.*
- *The parasite can complete its life cycle in the biliary tract and oocysts can be observed in bile. Stages are located in the bile duct epithelium*

Unit 4: *Acanthamoeba sp.*

Unit Structure

4.1 Introduction

Acanthamoeba is a genus of amoebae that are commonly recovered from soil, fresh water, and other habitats. *Acanthamoeba* has two evolutive forms, the metabolically active trophozoite and a dormant, stress-resistant cyst. Trophozoites are small, usually 15 to 25µm in length and amoeboid in shape.

4.2 Intended Learning Outcomes (ILOs)

At the end of this lecture unit, students should:

1. Know their epidemiology, morphology, life cycle, symptoms, diagnosis, treatment and control.

4.3 *Acanthamoeba* as a Parasite

Acanthamoeba species are ubiquitous in soil and water. Their pathogenic potential was first recognized in 1958 by Culbertson who produced encephalitis in mice following inoculation with an *Acanthamoeba*-contaminated cell culture. The first definitive human cases were reported in the early 1970's. In contrast to PAM, *Acanthamoeba* infections are usually associated with chronically ill, immunocompromised, or other debilitated patients. *Acanthamoeba*, like *Naegleria*, is neurotropic and causes encephalitis like disease. However the disease, known as granulomatous amebic encephalitis (GAE), is more slowly progressing and chronic. *Acanthamoeba* infections are also associated with lesions in the cornea (*Amebic keratitis*), lungs and skin.

Morphology

The portal of entry for *Acanthamoeba* is not known but believed to the either the respiratory tract via inhalation of cysts or through wounds in the skin that become contaminated by soil. Presumably the trophozoites disseminate by a hematogenous route (i.e. via the circulatory system) to the central nervous system (CNS) (**Figure 4.3a**).
Acanthamoeba species was first recognized in 1958 by _____.

Self-Assessment Exercise 1

1. ***Acanthamoeba infections are usually associated with _____, _____ and _____?***
2. ***Acanthamoeba, like Naegleria, is neurotropic which causes disease known as _____?***

Life Cycle

Acanthamoeba exibits a typical protozoan life cycle consisting of an ameboid trophozoite stage and a cyst stage (see figure legend of Naegleria life cycle for explanation of trophozoites and cysts). In contrast to *Naegleria*, both cyst and trophozoite stages can be found in histological specimens. The cysts have a three-layered wall, a wrinkled appearance and are extremely resistant to desiccation. In histological preparations the trophozoites of *Acanthamoeba* are very similar to *Naegleria* trophozoites and cannot be distinguished on morphological criteria. However, in culture *Acanthamoeba* trophozoites can be distinguished by their spike-like pseudopodia (**Figure 4.3b**)

Symptom

The onset of symptoms is often insidious and the clinical manifestations include subtle headache, personality changes and slight fever. GAE has a prolonged clinical course and can take weeks to months to progress to coma and death.

Diagnosis

Diagnosis is difficult and usually done at autopsy. Diagnosis is confirmed by detecting the amoebas in corneal scrapings or biopsies. Some success in treating amebic keratitis has been obtained with poly-hexa-methylene biguanide or propamidine isethionate (Brolene). Surgery is often needed to correct the loss of vision. Clinical manifestations of amebic keratitis include severe ocular pain and corneal lesions refractory.

Treatment

Drugs such as antiviral, antibacterial and antimycotic drugs can be used for treatment.
Briefly explain the diagnosis of Acanthamoeba species.

Self-Assessment Exercise 2

1. ***Briefly outline the life cycle of Acanthamoeba sp***.?
2. ***Mention at least three symptoms of Acanthamoeba sp***.?

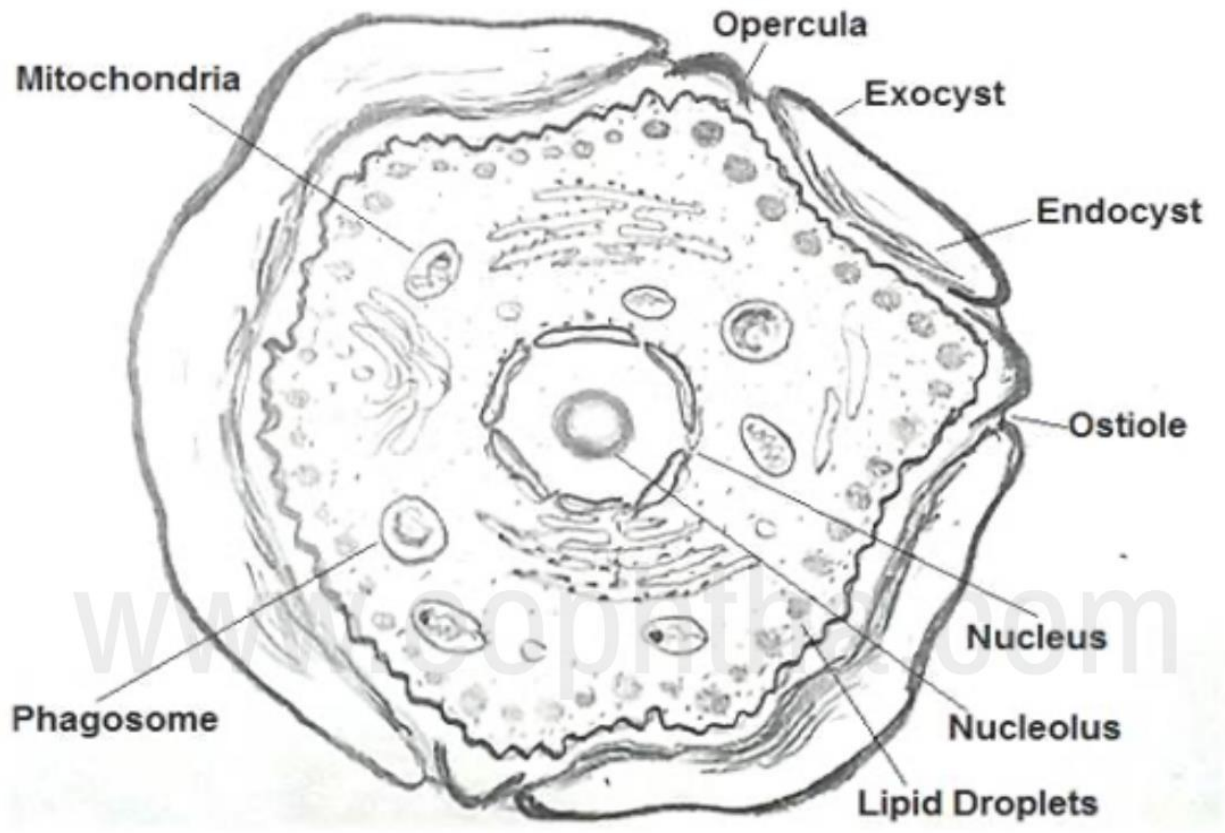

Figure 4.3a: Morphology/Structure of *Acanthamoeba sp*.

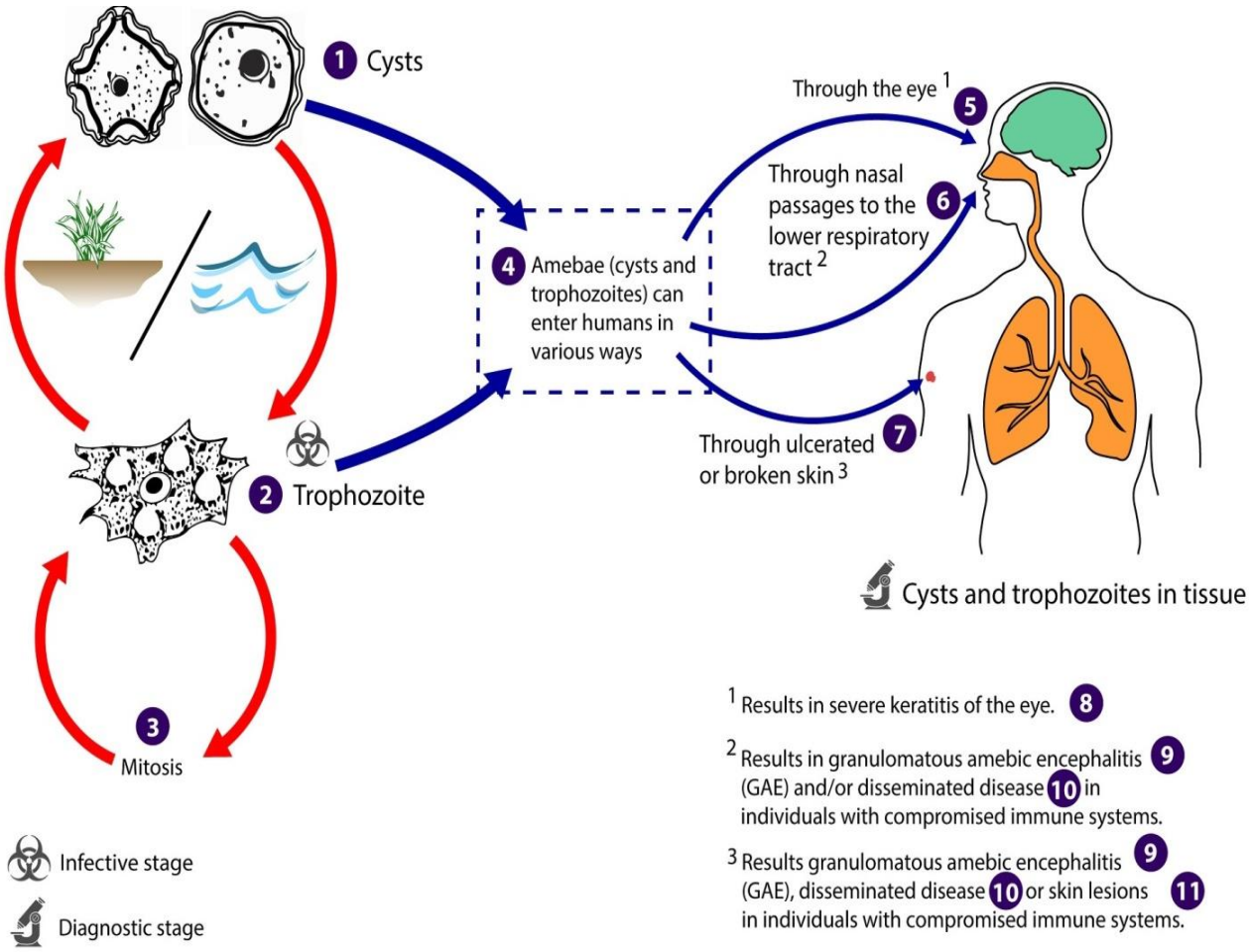

Figure 4.3b: Life cycle of *Acanthamoeba sp*.

4.3.1 Role as a model organism

Because *Acanthamoeba* does not differ greatly at the ultrastructural level from a mammalian cell, it is an attractive model for cell-biology studies; it is important in cellular microbiology, environmental biology, physiology, cellular interactions, molecular biology, biochemistry, and evolutionary studies, due to the organisms' versatile roles in the ecosystem and ability to capture prey by phagocytosis, act as vectors and reservoirs for microbial pathogens, and to produce serious human infections. In addition, *Acanthamoeba* has been used extensively to understand the molecular biology of cell motility and cancer cell dormancy by in-depth exploration of the process of encystation.

4.3.2 Role in disease

Diseases caused by *Acanthamoeba* include keratitis and granulomatous amoebic encephalitis (GAE). The latter is often but not always seen in immunosuppressed patients. GAE is caused by the amoebae entering the body through an open wound and then spreading to the brain. The combination of host immune responses and

secreted amoebal proteases causes massive brain swelling resulting in death in about 95% of those infected.

4.4 Summary

In this unit, you have learnt about *Acanthamoeba* sp., their morphology, life cycle, symptoms, diagnosis, treatment and control. In nature, *Acanthamoeba* species are free-living bacterivores, but in certain situations, they can cause infections (Acanthamebiasis) in humans and other animals.

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https://www.microbiologyresearch.org/content/journal/jmm/10.1099/00222615-47-1-5

4.6 Possible Answers to SAEs

Answers to SAEs 1

1. *Chronically ill, immunocompromised and other debilitated patients*
2. *Granulomatous amebic encephalitis (GAE)*

Answers to SAEs 2

1. *- Acanthamoeba exibits a typical protozoan life cycle consisting of an ameboid trophozoite stage and a cyst stage (see figure legend of Naegleria life cycle for explanation of trophozoites and cysts).*
   - *In contrast to* Naegleria*, both cyst and trophozoite stages can be found in histological specimens.*
   - *The cysts have a three-layered wall, a wrinkled appearance and are extremely resistant to desiccation.*
   - *In histological preparations the trophozoites of Acanthamoeba are very similar to Naegleria trophozoites and cannot be distinguished on morphological criteria.*
   - *However, in culture Acanthamoeba trophozoites can be distinguished by their spike-like pseudopodia*
2. *Subtle headache, personality changes and slight fever.*

Unit 5: *Naegleria fowleri*

Unit Structure

5.1 Introduction

Naegleria species are found in freshwater habitats and moist soil throughout the world. Primary amebic meningoencephalitis (PAM) was first recognized by Fowler in 1965 and *N. fowleri* is the only *Naegleria* species known to be pathogenic to humans. As of 1996, approximately 175 cases have been reported worldwide and 81 of these are in the U.S.

5.2 Intended Learning Outcomes (ILOs)

At the end of this lecture unit, students should:

6. Know their epidemiology, morphology, life cycle, symptoms, diagnosis, treatment and control.

5.3 *Naegleria fowleri* as a parasite

Naegleria fowleri, colloquially known as a "brain-eating amoeba", is a species of the genus Naegleria, belonging to the phylum Percolozoa, which is technically not classified as true amoeba, but a shape shifting amoeboflagellate excavate. It is a free-living, bacteria-eating microorganism that can be pathogenic, causing an extremely rare, sudden, severe and usually fatal brain infection called naegleriasis or primary amoebic meningoencephalitis (PAM). This microorganism is typically found in bodies of warm freshwater, such as ponds, lakes, rivers, hot springs, warm water discharge from industrial or power plants, geothermal well water, poorly maintained or minimally chlorinated (under 0.5 mg/m^3 residual) swimming pools, water heaters, soil, and pipes connected to tap water. It can be seen in either an amoeboid or temporary flagellate stage. The naegleriasis infection has been documented in Australia in 1965, Czechoslovakia in 1962 to 1965, the United States in 2003, 2011, 2013, 2020, and 2021, and Pakistan in 2008.

Life Cycle

The life cycle consists of trophozoite and cyst stages and the trophozoite stage can be either ameboid or flagellated. The ameboid trophozoite feeds on bacteria and other organic matter and undergoes asexual replication. The ameboid form transforms into a pear-shaped flagellated form with two flagella at the broad end when placed in distilled water or deprived of nutrients. Flagellated trophozoites are non-feeding and do not replicate, but will convert back into the ameboid form when nutrients are restored. The ameboid forms can also encyst resulting in a stage resistant to desiccation. All stages are characterized by a single nucleus with a large karyosome and no peripheral chromatin. Only the ameboid form is found in tissue. *Naegleria* produces a fulminant, and almost always fatal, acute meningoencephalitis in children and young adults who were previously in excellent health. Almost all reported cases have been associated with swimming in warm or heated waters a few days prior to the onset of symptoms. The portal of entry appears to be the olfactory neuroepithelium in the nasal cavity. The trophozoites probably migrate along the olfactory nerves into the brain and CNS **(Figure 5.3)**.
Primary amebic meningoencephalitis (PAM) was first recognized by which scientist and in what year?

Self-Assessment Exercise 1

1. *Naegleria fowleri*, colloquially known as a _____?
2. *Naegleria fowleri* causes an extremely rare, sudden, severe and usually fatal brain infection called_____?

-
-

Symptoms

The symptoms of PAM resemble bacterial meningoencephalitis and PAM is generally characterized by a sudden onset of headache and fever. Nausea, vomiting and other symptoms related to increase intracranial pressure may also be evident. There is a rapid progression from headache and fever to coma, with occasional seizures, and death.

Diagnosis

Diagnosis is almost always post-mortem and the prognosis is not good.

Treatment

Drugs such as Amphotericin B can be used for a known survivor.

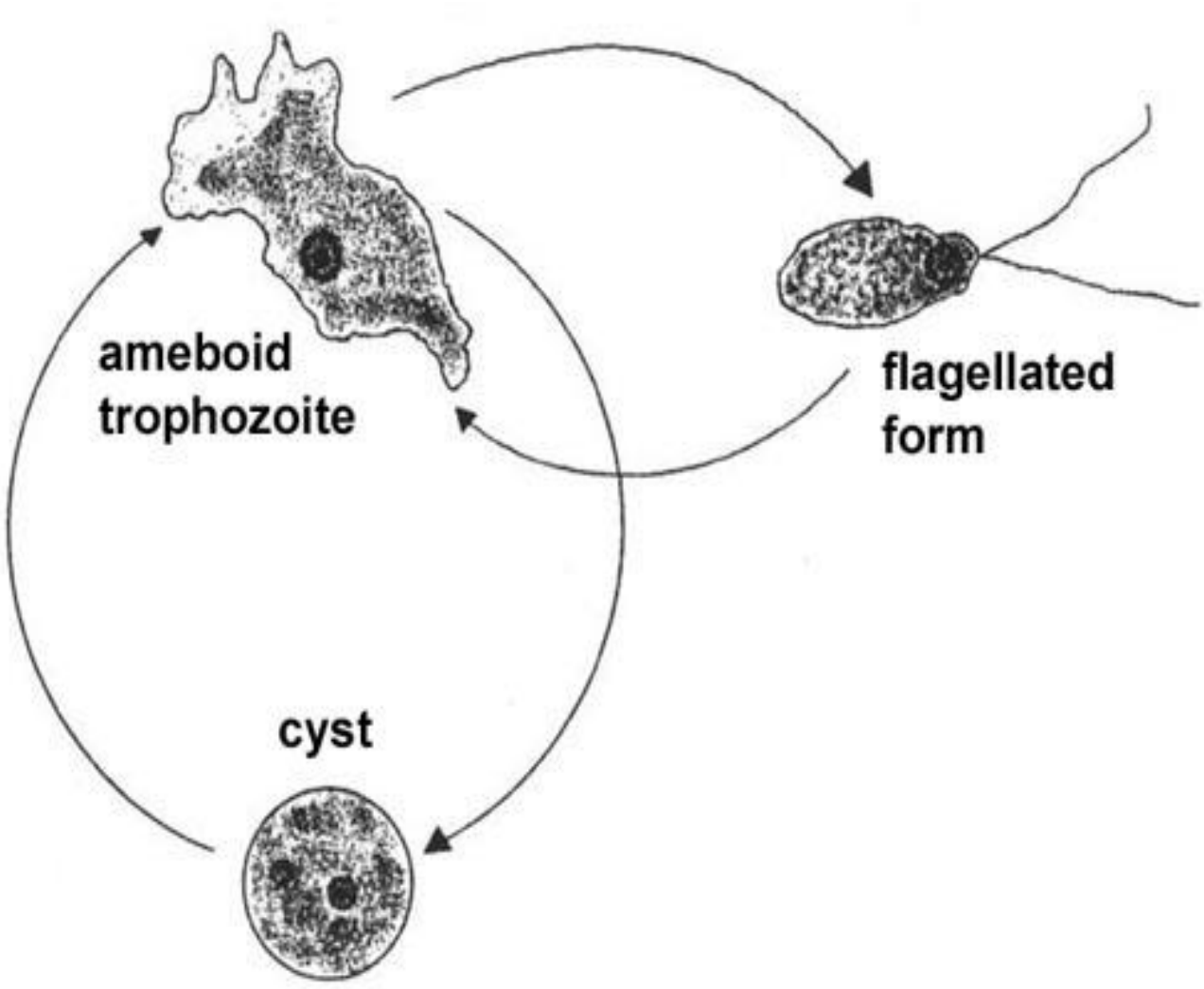

Figure 5.3.: Life cycle of *Naegleria fowleri* consists of cysts and trophozoites

5.3.1 Ecology of *Naegleria fowleri*

Naegleria fowleri are excavates that inhabit soil and water. *N. fowleri* is sensitive to drying and acid. It cannot survive in sea water. This amoeba is able to grow best at moderately elevated temperatures making summer month cases more likely. *N. fowleri* is a facultative thermophile and is able to grow at temperatures up to 46 °C (115 °F). Warm, fresh water with a sufficient supply of bacterial food provides a habitat for amoebae. Man-made bodies of water, disturbed natural habitats, or areas with soil and unchlorinated/unfiltered water are locations where many amoebic infections have occurred. *N. fowleri* seems to thrive during periods of disturbance; the flagellate-empty hypothesis explains that Nagleria's success may be due to decreased competition from a depleted population of the normal, thermosensitive protozoal fauna. In other words, *N. fowleri* thrives in the absence of other predators consuming its food supply. This hypothesis suggests that human disturbances such as thermal pollution increase *N. fowleri* abundance by removing their resource competitors. Ameoboflagellates have a motile flagellate stage that is evolved for dispersal, which is advantageous when an environment has been cleared of competing organisms.

N. fowleri is a facultative thermophile and is able to grow at temperatures up to _____.

Self-Assessment Exercise 2

1. ***Mention the drugs used in treatment of Naegleria fowleri?***
2. ***Briefly explain the symptoms of Naegleria fowleri?***

5.4 Summary

In this unit, you have learnt about *Naegleria fowleri*, their life cycle, symptoms, diagnosis, treatment and control. *Naegleria fowleri* is a thermophilic, free-living amoeba. It is found in warm and hot freshwater ponds, lakes and rivers, and in the very warm water of hot springs. As the water temperature rises, its numbers increase. The amoeba was identified in the 1960s in Australia but appears to have evolved in the United States.

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5.6 Possible Answers to SAEs

Answers to SAEs 1

1. *Brain-eating amoeba*
2. *Naegleriasis*

Answers to SAEs 2

1. *Amphotericin B*
2. *- The symptoms of PAM resemble bacterial meningoencephalitis and PAM is generally characterized by a sudden onset of headache and fever.*

- *Nausea, vomiting and other symptoms related to increase intracranial pressure may also be evident.*

- *There is a rapid progression from headache and fever to coma, with occasional seizures, and death.*

Glossary

DNA = Deoxyribonucleic acid
AIDS = Acquired Immunodeficiency syndrome
ATP = Adenosine Triphosphate
STD = Sexually Transmitted Diseases
ACR = Adequate clinical response
LTF = Late treatment failure
ETF = Early treatment failure
MDR = Multi-drug resistance
ABC = ATP-binding cassette
ART = Active antiretroviral therapy
GAE = Granulomatous amebic encephalitis
CNS = Central Nervous System
PAM = Primary amebic meningoencephalitis

End of the module Questions

1. *Balantidium coli* are parasitic species of ciliate alveolates that causes the disease balantidiasis **(True or False)**
2. It is the only member of the ciliate phylum known to be pathogenic to humans **(True or False)**
3. *Cryptosporidium parvum* (*C. parvum*) causes epidemics of diarrhea in humans through contaminated food and water **(True or False)**
4. Cystoisosporiasis was previously known as isosporiasis and is an uncommon diarrheal illness caused by the protozoan **(True or False)**
5. *Acanthamoeba* has two evolutive forms, the metabolically active trophozoite and a dormant stress-resistant cyst **(True or False)**
6. *Naegleria fowleri* are excavates that inhabit soil and water **(True or False)**