C. Urine Specimens - *T. vaginalis*.

D. Sputum Specimens.

E. Aspirates and Biopsies.

F. Abscess aspirates - usually for extra-intestinal amoebiasis - wall of abscess is best area to examine.

Procedures for Detecting Blood Parasites:

A. Collection of Blood Samples

B. Examination of Blood Samples

1. Wet Mounts - screening for motile organisms (trypanosomes & filariae)
2. Permanent Stained Smears

a.Stains used -

1) Wright's - alcohol based.
2) Giemsa - water based, preferred stain, all things considered.

b.Thick Blood Films

1) Used in the identification of malaria parasites, trypanosomes, and microfilariae.

c.Thin Blood Films

1) Used in the identification of malaria parasites, trypanosomes, and microfilariae.

Concentration Techniques - Thick Blood smears

The Intestinal Protozoa

Introduction

The Phylum Protozoa is classified into four major subdivisions according to the methods of locomotion and reproduction.

a. ***The amoebae (Superclass Sarcodina***, ***Class Rhizopodea****:* move by means of pseudopodia and reproduce exclusively by asexual binary division.
b. ***The flagellates (Superclass Mastigophora, Class Zoomasitgophorea)*** typically move by long, whiplike flagella and reproduce by binary fission.
c. ***The ciliates (Subphylum Ciliophora, Class Ciliata)*** are propelled by rows of cilia that beat with a synchronized wavelike motion.
d. ***The sporozoans (Subphylum Sporozoa)***lack specialized organelles of motility but have a unique type of life cycle, alternating between sexual and asexual reproductive cycles (alternation of generations).

Number of species - there are about 45,000 protozoan species; around 8000 are parasitic, and around 25 species are important to humans.

General Features

– The single protozoal cell performs all functions.
– Most of the protozoa are completely nonpathogenic¬ but few may cause major diseases such as malaria, leishmaniasis, and sleeping sickness.

– Protozoa exhibit wide range of size (1–150 μm)

Structures

1. Cytoplasm

It has 2 portions:

A. Ectoplasm: Outer homogeneous part that serves as¬ the organ for locomotion and for engulfment of foodby producing pseudopodia.

B. **Endoplasm**: The inner granular portion of cytoplasm that contains **Chromatoid Body** :

Extranuclear chromatin material is called chromatoid body (e.g., as found in Entamoeba histolytica cyst).

- **Karyosome** :It is a DNA containing body, situated peripherally or centrally within the nucleus and found in intestinal amoeba, e.g. E. histolytica, E. coli.
- **Kinetoplast** :Non-nuclear DNA present in addition to nucleus is called kinetoplast. It is seen in trypanosomes. Flagellum orginates near the kinetoplast. Point of origin of agellum is called as basal body
- nucleus is called endoplasm

2. Nucleus

The nucleus is usually single but may be double or multiple; some **species** having as many as hundred nuclei in a single cell. The nucleus contains one or more nucleoli or a central¬ karyosome.

Reproduction

Reproduction can be: **Asexual reproduction**¬

Sexual reproduction.¬ Reproduction usually occurs asexually in protozoans; however, sexual reproduction occurs in cillates and sporozoas.

Life Cycle

– **Single Host**: Protozoa like intestinal agellates and¬ cillates require only 1 host, within which they multiply asexually .

– **Second host**: In some protozoa like Plasmodium,¬ asexual method of reproduction occurs in one host (man) and sexual method of reproduction in another host (mosquito).

1. ***trophozoite*** - the motile vegetative stage; multiplies via binary fission; colonizes host.
2. ***cyst*** - the inactive, non-motile, infective stage; survives the environment due to the presence of a cyst wall.

diagnostic features

a. **size** - helpful in identifying organisms; must have calibrated objectives on the microscope in order to measure accurately.

b. ***type of motility*** - directional or non-directional; sluggish or fast.

c. ***cytoplasmic inclusions*** - chromatoid bars (coalesced RNA); red blood cells; food vacuoles containing bacteria, yeast, etc.

d. ***appearance of cytoplasm*** - smooth & clean or vacuolated.

e. ***nuclear structure*** - important in the identification of organisms and species differentiation.

f. ***endosome*** - also called the "karyosome," this is a mass of chromatin within nucleus. The size, shape, and location of this structure are helpful in identification of organisms.

g. ***chromatin -*** nuclear DNA

h. ***chromatoid*** **body or "bar"** - coalesced RNA within the cytoplasm in the cyst stage. This is not always present, but when it is, its size and shape are helpful in determining species identification.

1. Class: Sarcodina ***- The Amoebae***

A. Life cycle -

a) The definitive host ingests the infective cyst stage from fecal contamination in environment.

b) The cyst passes into the small intestine & excystation occurs with transformation to the trophozoite stage.

c) Trophozoites in the large intestine colonize the host by multiplying asexually via binary fission. They can remain in the lumen or invade the wall of the intestine (pathogenic species only) & multiply, from here they can be transported via the circulation to other organs (liver, lungs, etc.).

d) Cysts and trophozoites are passed in the feces of the infected host.

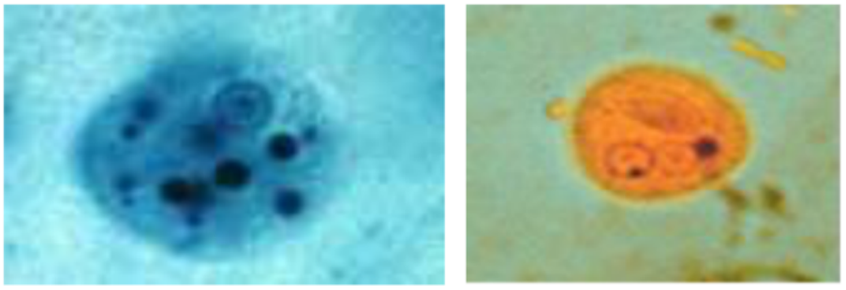
e) ***Infective stage*** - the mature cyst.

f) ***Diagnostic stage -*** the trophozoite or cyst in stool or tissue specimens.

2. ***Genus Entamoeba***

contains the most important of the amoebae causing disease in humans.

Entamoeba histolytica



Entamoeba histolytica trophozoite with ingested red blood cells

Morphological features

Trophozoite

Viable trophozoites vary in size from about 10-60μm in diameter. Motility is rapid, progressive, and unidirectional, through pseudopods. The nucleus is characterized by evenly arranged chromatin on the nuclear membrane and the presence of a small, compact, centrally located karyosome. The cytoplasm is usually described as finely granular with few ingested bacteria or debris in vacuoles. In the case of dysentery, however, RBCs may be visible in the cytoplasm, and this feature is diagnostic for *E.histolytica*.

The cyst ranges between 10 and 30 microns in diameter and contains four nuclei when mature. Cigar-shaped chromatoid bars may be present in some cysts.