**(3rd lecture) Postgraduate student Dr.Ahmed Khudhair Al-Hamairy Microbiology**

**Parasitology**

***Entamoeba gingivalis***

**Trophozoites.**

The trophozoite of *Entamoeba gingivalis* ranges in size from 8 to 20 μm and morphologically resembles that of *E. histolytica* (Fig. 3-18; Table 3-13). *E. gingivalis* trophozoites characteristically exhibit active motility. The multiple pseudopods vary in their appearance as the trophozoite moves. The pseudopods may appear long when seen at one point in time and short and blunt the next time that they are seen The single nucleus contains a central karyosome surrounded by peripheral chromatin that is for the most part fine and evenly distributed. Achromatic granules arranged in strands may be visible extending from the karyosome to the peripheral chromatin ring.

A number of inclusions are typically found in the finely granular cytoplasm including food vacuoles containing phagocytosed and partially digested white blood cells (leukocytes and epithelial cells of the host, bacteria and ingested red blood cells, although not as commonly found as in *E. histolytica* trophozoites .It is important to note that *E. gingivalis* is the only ameba that ingests white blood cells. This distinguishing characteristic is helpful when it is necessary to differentiate *E. gingivalis* from *E. histolytica.*

**Cysts.**

There is no known cyst stage of *E .gingivalis .*

**Laboratory Diagnosis**

An accurate diagnosis of *E. gingivalis* trophozoites may best be made by examining mouth scrapings , particularly from the gingival area. Material from the tonsillar crypts and pulmonary abscess, as well as sputum, may also be examined. Vaginal and cervical material may be examined to diagnose *E. gingivalis* in the vaginal and cervical areas.

|  |  |
| --- | --- |
| **TABLE 3-13 *Entamoeba gingivalis* Trophozoite : Typical Characteristics at a Glance** | |
| **Parameter**  **Size range**  **Motility**  **Number of nuclei**  **Karyosome**  **Peripheral chromatin**  **Cytoplasm**  **Cytoplasmic inclusions** | **Description**  **8-20 μm**  **Active, varying pseudopod appearance**  **One**  **Centrally**  **located Fine and evenly distributed.**  **Finely granular**  **Leukocytes Epithelial cells Bacteria** |

**Life Cycle**

*E. gingivalis*, as the name implies, typically lives around the gum line of the teeth in the tartar and gingival pockets of unhealthy mouths. In addition, *E. gingivalis* trophozoites have been known to inhabit tonsillar crypts and bronchial mucus. It is particularly important to diagnose *E. gingivalis* and *E. histolytica* correctly because both organisms may be found in the sputum and in pulmonary abscesses.

*E.* *gingivalis*  may also be found in the mouths of individuals who practice good oral hygiene. Existing as a scavenger the *E. gingivalis* trophozoites feed on disintegrated cells and multiply by binary fission .Characteristically delicate, these trophozoites will not survive following contact with stomach juices. *E. gingivalis* trophozoites have also been recovered in vaginal and cervical specimens from women who are using intrauterine devices (IUDs). Spontaneous disappearance of the trophozoites seems to occur following removal of the IUD.

**Epidemiology**

*E. gingivalis* is found in all populations that have been studied for its presence. Infections of *E .gingivalis*  are contracted via mouth-to-mouth )kissing) and droplet contamination , which may be transmitted through contaminated drinking utensils.

**Clinical Symptoms**

Infections of *E. gingivalis* occurring in the mouth and in the genital tract typically produce no symptoms. pathogenic *E. gingivalis* trophozoites are frequently recovered in patients suffering from pyorrhea alveolaris. It appears that the trophozoites thrive under disease conditions but do not produce symptoms of their own.

**Treatment**

Treatment of *E. gingivalis* is typically ( Metronidazole) indicated because the organism is generally considered a pathogen .

**Prevention and Control**

Improved oral hygiene accomplished by the proper care of the teeth and gums is necessary to prevent the spread of oral *E. gingivalis* infections .Prompt removal of IUDs in infected patients spontaneously removes *E. gingivalis* from the genital tract**.**

***- Naegleria fowleri***

Common associated disease or condition name : Primaryamebic meningoencephalitis (PAM).

**Morphology**

*Naegleria fowleri* is the only ameba with three known morphologic forms—**ameboid trophozoites** , **flagellate forms** (pertains to Protozoa that move by means of flagella), and **cysts.**

**Ameboid trophozoites. -1**

The typical ameboid trophozoite of *N. fowleri* appears elongate, measuring from 8 to 22 μm in length (Fig. 3-19 Table 3-14). The anterior end is usually broad whereas the posterior end is usually tapered. The sluglike motility of the *N. fowleri* ameboid trophozoite is accomplished by blunt pseudopodia . The single nucleus contains a large karyosome that is generally centrally located . Peripheral chromatin is absent. The cytoplasm of the *N . fowleri*  ameboid trophozoite is granular and often contains vacuoles.

**Flagellate forms**. -2

The pear-shaped flagellate form of *N. fowleri* typically measures 7 to 15 μm in size (Fig. 3-20). Two whiplike structures that assist select parasites in locomotion known as flagella extend from the broad end of the organism . The typical motility that is seen is accomplished by jerky movements or spinning. The nucleus is basically identical to that of the ameboid trophozoite, a large central karyosome minus peripheral chromatin. The flagellate trophozoites typically have granular cytoplasms that often contain vacuoles.

|  |  |
| --- | --- |
| **TABLE 3-14 *Naegleria fowleri* Ameboid Trophozoite: Typical Characteristics at a Glance** | |
| **Parameter** | **Description** |
| **Size range**  **Motility**  **Number of nuclei**  **Karyosome**  **Peripheral chromatin**  **Cytoplasm** | **8-22 μm**  **Sluglike, blunt pseudopods**  **One**  **Large and usually centrally located**  **Absent**  **Granular, usually**  **vacuolated** |

3- **Cysts.**

The cysts, measuring from 9 to 12 μm in size, are generally round and have thick cell walls (Fig. 3-21). Similar to both corresponding trophozoite stages, the *N. fowleri* cyst has only one nucleus, consisting of a large, centrally located karyosome lacking peripheral chromatin . The cytoplasm is typically granular and often contains vacuoles.

**Laboratory Diagnosis**

Microscopic examination of cerebrospinal fluid (CSF) is the method of choice for the recoveryof *N. fowleri* ameboid trophozoites. Preparing and scanning saline and iodine wet preparations of the CSF are recommended , Samples of tissues and nasal discharge may also be examined. Clinical specimens may be cultured*. N. fowleri* ameboid trophozoites show a characteristic trailing effect when placed on agar plates that have been previously inoculated with gram-negative bacilli.

**Life Cycle**

The ameboid trophozoites of  *N. fowleri* are the only form known to exist in humans. Replication of the ameboid trophozoites occurs by simple binary fission. The ameboid trophozoites transform into flagellate trophozoites *in vitro* after being transferred to water from a tissue or culture. The flagellate trophozoites do not divide but rather lose their flagella and convert back into the ameboid form, in which reproduction resumes. The cyst form is known to exist only in the external environment , It appears that the entire life cycle of *N. fowleri*, which consists of the amebic trophozoites converting to cysts and flagellates and then back to amebic trophozoites, occurs in the external environment. Humans primarily contract this ameba by swimming in contaminated water. The ameboid trophozoites enter the human body through the nasal mucosa and often migrate to the brain, causing rapid tissue destruction. Some infections may be caused by inhaling dust infected with *N. fowleri.*

**Epidemiology**

*N. fowleri* is primarily found in warm bodies of water, including lakes, streams, ponds, and swimming pools. Prevalence is higher in the summer months of the year. In addition to water sources , there have been cases of contaminated dust. One such case occurred in Nigeria, a country that has a warm climate . The ameboid trophozoites of *N. fowleri* enter the human body through the nasal mucosa. Inhalation of contaminated dust has accounted for other documented infections. There is also some evidence to suggest that sniffing contaminated water may transmit this ameba

**Clinical Symptoms**

Asymptomatic. Patients who contract *N.fowleri*  resulting in colonization of the nasal passages are usually asymptomatic . Primary amebic meningoencephalitis. Primary amebic meningoencephalitis (PAM) occurs when the ameboid trophozoites of *N. fowleri* invade the brain, causing rapid tissue destruction Patients may initially complain of fever, headache , sore throat, nausea, and vomiting. Symptoms of meningitis rapidly follow, including stiff neck and seizures. In addition, the patient will often experience smell and taste alterations blocked nose, and Kernig’s sign (defined as a diagnostic sign for meningitis, where the patient is unable to fully straighten his or her leg when the hip is flexed at 90 degrees because of hamstring stiffness). In untreated patients death usually occurs 3 to 6 days after onset Postmortem brain tissue samples of these patients reveal the typical ameboid trophozoites of *N .fowleri*

**Treatment**

Unfortunately, medications used to treat meningitis and amebic infections are ineffective against  *N. fowleri.* There is evidence, however, that prompt and aggressive treatment with amphotericin B may be of benefit to patients suffering from infections with *N. fowleri*, despite its known toxicity. In rare cases, amphotericin B in combination with rifampin or miconazole has also proved to be an effective treatment. Amphotericin B and miconazole damage the cell wall of *Naegleria*, inhibiting the biosynthesis of ergosterol and resulting in increased membrane permeability which causes nutrients to leak out of the cells. Rifampicin inhibits RNA synthesis in the amoeba by binding to beta subunits of DNA dependent RNA polymerase, which in turn blocks RNA transcription. A person can survive if signs are recognized early but, if not, PAM almost always results in death..

**Prevention and Control**

Because of the numerous bodies of water that may potentially be infected, total eradication of *N. fowleri* is highly unlikely. Posting off-limits signs around known sources of contamination as well as educating the medical community and public, may help curb infection rates. It is also important that swimming pools and hot tubs be adequately chlorinated. Cracks found in the walls of pools, hot tubs, and baths should be repaired immediately to prevent the creation of a possible source of contamination

**Notes of Interest and New Trends**

The first case of PAM was reported by Carter and Fowleri, for whom the ameba is named, in 1965 in Australia, and by Butt and Patras in 1966 in the United States. One noteworthy species of *Naegleria* that could possibly infect humans in the future is known as *N. australiensis*. This organism exists in the environment in Asia, Australia, Europe and United States. *N. australiensis* has been found to be pathogenic in mice that have been exposed to this parasite by intranasal instillation A number of methods have been studied in recent years aimed at classifying, identifying and speciating *Naegleria.* These laboratory techniques include PCR assay, monoclonal antibody testing, flow cytometry, and DNA hybridization In addition, a method designed to aid in taxonomic classification, called DNA restriction fragment length polymorphism (RFLP), has been studied. Results of all of these tests to date have been favorable. Further studies, however, have been recommended.

- ***Acanthamoeba* species**

Common associated disease or condition names Granulomatous amebic encephalitis (GAE), *Acanthamoeba Keratitis*

**Morphology (Trophozoites)**

The *Acanthamoeba*  trophozoite averages 25 μm, with a range of 12 to 45 μm in size (Figs. 3-22 and 3-23; Table 3-15). Motility is sluggish and there is little evidence of rogressive motility. Spinelike pseudopods, known as acanthopodia, project outward from the base of the organism. *Acanthamoeba* trophozoites contain one nucleus, consisting of a large karyosome similar to that of *N. fowleri*. Obvious A disorganized, granular, and sometimes vacuolated cytoplasm surrounds the nucleus .

.



***Acanthamoeba* species trophozoite showing typical thornlike acanthopodia (iron hematoxylin stain, ×1000)**).



Acanthamoeba species cyst exhibiting a typical disorganized cytoplasm and vacuoles (iron hematoxylin stain, ×1000).

|  |  |
| --- | --- |
| TABLE 3-15 *Acanthamoeba* species Trophozoite: Typical Characteristics at a Glance | |
| **Description** | **Parameter** |
| **12-45 μm**  **Sluggish, spinelike**  **pseudopods**  **One**    **Large**    **Absent**    **Granular and vacuolated** | **﻿Size range**  **Motility**    **Number of nuclei**    **Karyosome**    **Peripheral chromatin**    **Cytoplasm** |

|  |  |
| --- | --- |
| TABLE 3-16 *Acanthamoeba* species Cyst: Typical Characteristics at a Glance | |
| **Description** | **Parameter** |
| **8-25 μm**  **Roundish with ragged edges**  **One**  **Large and central**  **Absent**  **Disorganized, granular sometimes vacuolated**  **Double cell wall—smooth , inner cell wall and outer**  **jagged cell wall** | **Size range**  **Shape**  **Number of nuclei**  **Karyosome**  **Peripheral chromatin**  **Cytoplasm**  **Other features** |

**Laboratory Diagnosis**

As with *N. fowleri*, the specimen of choice for diagnosing *Acanthamoeba* spp. Trophozoites and cysts is CSF. Brain tissue may also be examined. Corneal scrapings are the specimen of choice for recovery of *Acanthamoeba* infections of the eye. Suspected corneal scrapings may be cultured on non-nutrient agar plates seeded with gram-negative bacteria ( specifically, a viable strain of *E. coli*). The bacteria serve as a source of food for the parasites. As the *Acanthamoeba* organisms feed, they produce a set of marks (known as tracks) on the agar. Histologic examination of corneal scrapings may also recover *Acanthamoeba*. Although primarily used to detect fungi in clinical specimens, calcofluor white may be used to stain *Acanthamoeba*  cysts present in corneal scrapings. Indirect immunofluorescent antibody staining is the technique of choice for speciating *Acanthamoeba*

**Life Cycle Notes**

The trophozoites and cysts of *Acanthamoeba* convert between these two morphologic forms in the external environment. Humans may acquire *Acanthamoeba* in one of two ways. One route consists of aspiration or nasal inhalation of the organisms. Trophozoites and cysts enter via the lower respiratory tract or through ulcers in the mucosa or skin. These organisms often migrate via hematogenous spread—that is, transported through the blood stream—and invade the central nervous system (CNS), causing serious CNS infections. The second route of infection consists of direct invasion of the parasite in the eye. Two groups of individuals are at risk for direct eye invasion, contact lens wearers and those who have experienced trauma to the cornea.

Contact lens wearers who use homemade nonsterile saline solutions that are contaminated with *Acanthamoeba* typically suffer serious eye infection, known as *Acanthamoeba* keratitis. It is important to note that unlike *N.fowleri*, which is associated with swimming or bathing in contaminated water, *Acanthamoeba* spp. infection is not associated with water but rather with contaminated saline .There are currently 10 species of *Acanthamoeba*  known to infect humans. *Acanthamoeba castellanii* has been identified as the species responsible for most CNS and eye infections in humans. The names of these species, as well as the type of infection with which each is associated .

**Epidemiology**

Over the years, cases of *Acanthamoeba* have been reported from many countries worldwide . Both CNS and eye infections of *Acanthamoeba* spp. have been reported in the United States CNS infections primarily occur in patients who are immunocompromised or debilitated. Contact lens wearers, particularly those wearing soft contacts, may be at risk of contracting *Acanthamoeba* eye infections. Poor hygiene practices , especially the use of homemade saline rinsing solutions, is the major risk factor that may lead to these infections Animals, including rabbits, beavers, cattle water buffalo, dogs, and turkeys, have been known to contract *Acanthamoeba* infections , Just as in humans, immunocompromised animals appear to contract fatal CNS infections.

**Clinical Symptoms**

**Granulomatous amebic encephalitis.** CNS infections with *Acanthamoeba* are also known as granulomatous amebic encephalitis (GAE Symptoms of this condition develop slowly over time and include headaches, seizures, stiff neck nausea, and vomiting. Granulomatous lesions of the brain are characteristic and may contain both *Acanthamoeba* trophozoites and cysts. On occasion *Acanthamoeba* spp. invade other areas of the body, including the kidneys, pancreas, prostate and uterus, and form similar granulomatous lesions

***Acanthamoeba* keratitis.** *Acanthamoeba* infections of the cornea of the eye are known as amebic keratitis. Common symptoms include severe ocular pain and vision problems. The infected tissue of the cornea may contain *Acanthamoeba*  trophozoites and cysts. Perforation of the cornea may result, as well as subsequent loss of vision.

**Treatment**

Because of the slow progression of GAE, most patients who suffer from it die, not only beforean accurate diagnosis may be made, but also before experimental treatments can be administered and studied. There is some evidence to suggest that sulfamethazine might be a suitable treatment. Cases of Acanthamoeba keratitis have successfully been treated with several medications that include itraconazole, ketoconazole , miconazole, propamidine isethianate, and rifampin. Of all these agents, propamidine appears to have the best documented success record. The key to successful treatment to eye infections is to begin treatment immediately once the infection has been diagnosed .

**Prevention and Control**

Strategies designed to keep individuals from contracting *Acanthamoeba* CNS infections are difficult to determine because the life cycle of this ameba is poorly understood. However, eye infections with *Acanthamoeba* may be prevented primarily by following all manufacturer-established protocols associated with the use of contact lenses. One of the most important protocols for contact lens wearers is to avoid using homemade nonsterile saline solutions.