Amebic species discussed in this handout:

1. *Entamoeba histolytica*
2. *Entamoeba coli*
3. *Entamoeba hartmanni*
4. *Entamoeba gingivalis*
5. *Iodamoeba butschlii*
6. *Endolimax nana*
7. *Naegleria fowleri*
8. *Acanthamoeba*

**INTRODUCTION**

The main pathogen of the genus *Entamoeba* is *Entamoeba histolytica*. The other entamoebic commensals usually occur in humans and they are: *E. coli, E. hartmanni, E. dispar,* and *E. moshkovskii*. The *E. polecki* is an intestinal ameba of pigs/monkeys and is sometimes seen in humans and may cause diarrhea. *Endolimax nana* and *Iodamoeba butschlii* are commensals, too.

The genus *Entamoeba* is widely distributed in both vertebrate and invertebrate animals. Main features of this genus are:

- Possession of a vesicular nucleus with a small karyosome located at or near its center
- With varying numbers of peripheral chromatin granules attached to the nuclear membrane

Many species of Entamoeba are similar in morphology but they can be distinguished from each other by:

- Isoenzyme analysis
- Restriction fragment length polymorphism
- Typing with monoclonal antibodies

*E. hartmanni* was formerly considered to be a “small race” of *E. histolytica* but now, it is a separate taxonomic entity and is distinguished from *E. histolytica* due to its smaller size

**PARADE OF THE AMOEBAE**

A. *Entamoeba histolytica*

- The principal pathogenic amebic species
- Was first described in a Russian peasant
- Prevalence rates are highest in the tropics and subtropics
  - Especially in places where there is:
    - Crowding
    - Poor sanitation
Endemic areas: Africa, Asia, Mexico, South America, and China
- In the Philippines, the prevalence of amebiasis is low (about 1.5%) and the focal areas of infection are:
  1. Baguio
  2. Iloilo
  3. Cebu
  4. Davao
  5. Cagayan De Oro

- Causative agent for:
  - Amebic dysentery
  - Multiorgan abscess formation
    - Either through:
      1. Extensive invasion
      2. Hematogenous invasion

Morphology
- Immature cyst
  - Has a vacuole
  - Few nuclei
- Mature cyst
  - With 4 nuclei (important!)—tetranucleated (tetra = four)
  - Diameter: 10-20 microns
  - With rounded or cigar-shaped chromatoidal bars
  - This is the INFECTIVE STAGE TO MAN!
- Trophozoites
  - Diameter: 12-60 microns
  - With ONLY 1 nucleus
  - With fine, even peripheral chromatin
  - The karyosome is punctuate and is usually centrally located
    - Sometimes, it can be off-center
  - Cytoplasm contains little debris with fine outer membrane
  - In preparations from stool specimen, it can be demonstrated that trophozoites are actively motile
    - They move by means of pseudopodia
      1. Pseudopodia—cytoplasmic protrusions that may be formed at any point on the surface of the organism
      2. The pseudopodium is quickly thrust out and may vary in form, it can be:
        a. Short
        b. Blunt
        c. Broad
        d. Long
        e. Finger-like
- The clear glass-like ectoplasm forms the outer layer of the body of the ameba
  - It flows out to form the pseudopodium
    1. Initially, when the pseudopods are first formed, they are characteristically hyaline
- The granular endoplasm flows slowly into the pseudopodium as the ameba moves in the direction in which it was extruded
- The motion of the trophozoite is progressive and directional
  - In other ameba, the motion is random and aimless
  - The movement may be enhanced so that it is more easily visualized by warming the slide either through the use of a:
    1. Thermostatically controlled “warm stage”
    2. A warmed copper coin placed on top of the slide
      - However, any attempt to warm the slide can NOT revive dead ameba that has been left in the slide at room temperature
- Using the scanning electron microscope, phagocytic stomata have been described on the trophozoites of *E. histolytica*
  - Phagocytic stomata AKA Endocytic food cups
  - These are used for engulfment of nutritional substances for the trophozoite
  - Two types of phagocytic stomata:
    1. Small endocytic stomata, on the other hand, is used for pinocytosis (cell drinking) for the acquisition of fluids by the trophozoite
    2. Large phagocytic stomata are involved in the phagocytosis of (1) bacteria and (2) epithelial cells
- RBCs may be ingested by the trophozoite
  - However, RBCs do not appear in chronic infections
  - In unstained preparations, the freshly ingested RBC by the trophozoite looks pale greenish
    1. The RBCs inside the trophozoite are refractile
  - Although RBC ingestion has been reported in some other ameba, for clinical purposes, we can consider that an ameba that contains RBCs is *E. histolytica*
  - Ingested RBCs stain according to the degree to which they have been digested by the ameba
    1. When stained with Hematoxylin, the following color properties are seen:
      a. Amebic cytoplasm = grayish
      b. Nuclear structures = intense bluish black
      c. Ingested RBCs = bluish black
The RBCs become progressively **paler** as they are digested by the ameba.

When stained with **Trichome**, the following color properties are seen:

- **Amebic cytoplasm** = usually **green** but can also be **pink**
  - In stained preparations, there are forms that can stain green, there are forms that can stain pink producing a **multichromatic** microscopic view of amebic trophozoites.
- **Nuclear structures** = **dark red**
- **Ingested RBCs** = **green** or **cherry-red**

- **Trophozoite death**
  - Death or **degeneration** of the parasite leads quickly to formation of **vacuoles** in the **cytoplasm** producing a “**Swiss cheese appearance**”
    1. Death of the organism makes the ameba **unidentifiable** because the **finer structures** of the **nucleus** undergo change.
      - And these structures are taxonomic keys in identifying the ameba.
  - When seen properly fixed and stained with **hematoxylin** or **trichome** stain, the nuclear details can be observed:
    - The **nuclear membrane** appears as a delicate but distinct line.
      1. On the **inner surface** of which, the **peripheral chromatin** is seen.
        - The peripheral chromatin is a **layer** of **granules** that is **uniform** and **small**.
      2. In the **center** of the nucleus is a **small mass** of **chromatin** called the **karyosome**.
      3. Now, the area between the **karyosome** and the **peripheral chromatin** are the **fibrils** of the **linin network** are located.
  - When the trophozoites prepare to enter the **resistant cyst stage**, the following events happen:
    - The trophozoites extrude all ingested material and assume a **rounded form**—the **precyst stage**
      1. The precyst is characterized by:
        - 1 **rounded nucleus**
          - The nuclear architecture of the precyst is confusing and identification of ameba is usually
done on trophozoite form or when the precyst has become a full-fledged cyst

b Absence of ingested material
c Absence of a cyst wall

- The full-fledged cyst is characterized by:
  1. A cyst wall made up of hyaline
     a. In unstained preparations, the cyst wall is highly refractile
  2. Shape: Spherical but can sometimes be ovoid or irregular in shape
  3. Diameter: 10-20 microns
  4. Nuclear number: 1-4 (rarely, more than 4)
     a. At times, the nuclei may appear as small, refractile spheres within the cytoplasm of the unstained cyst, but often, they are NOT visible
  5. Presence of chromatoidal bars (CB)
     a. CBs are so named because they stain with hematoxylin like the nuclear chromatin
     b. CBs are composed of crystalline RNA
     c. CBs, when present, are rod-shaped and are clear
  6. The iodine-stained cyst is characterized by:
     a. Cytoplasm: Light yellowish green to yellow-brown
     b. Nuclear membrane and karyosome: Distinct, light brown
     c. CBs: Do NOT stain with iodine and appear as clear spaces in the cytoplasm
     d. Glycogen: If present, they will be present in vacuoles in the cytoplasm of the iodine-stained cyst and is color dark yellow-brown
  7. The hematoxylin- or trichome-stained cyst is characterized by:
     a. The nuclear structure is similar to that seen in trophozoites
     b. The peripheral chromatin ring may appear to be thicker and less uniform in size
        o Second variant—Some strains of *E. histolytica* have eccentric karyosomes, and in some, the peripheral chromatin, instead of appearing as a layer of spherical granules, they form thin plaques on the nuclear membrane
        o A third variant of nuclear structure—The peripheral chromatin is massed in a crescent fashion at one side of the nuclear membrane
           - Presence of one or more CBs may be found in the cytoplasm
           1. They are shorter than the diameter of the cyst
Occasionally, especially in very young and usually mononucleate cysts, plenty but small CBs are seen and these CBs surround the glycogen vacuole.

The CBs generally appear in the form of elongate bars whose ends are either rounded or squared (sometimes, ovoid or cigar-shaped)

1. This CB morphology is seen in:
   a. *E. histolytica*
   b. *E. dispar*
   c. *E. hartmanni*
   d. *E. polecki*

CBs are most frequently seen in mono- or binucleate cysts but are NEVER present in tetranucleate cysts.

1. CBs, when stained with hematoxylin = bluish black
2. CBs, when stained with trichome = bright red

### Transmission and Life Cycle

> Transmission is through the ingestion of infective mature cysts through:

- Contaminated food
- Water
- Hands contaminated with feces

> Transmissibility is increased due to the following factors:

- Lack or inadequate personal hygiene among cyst carriers
- Flies feeding from feces containing cysts and subsequently contaminating food

### Life Cycle

1. Man ingests the tetranucleated mature cysts through food and drink
2. The cysts excyst in the alkaline juices of the small intestine where the tetranucleated trophozoites are liberated
3. Each of the liberated trophozoites divide into four metacystic trophozoites
4. The metacystic trophozoites pass down to the cecum and colonize the cecal crypts of Lieberkuhn
5. Upon successful colonization, they develop into normal sized trophozoites and multiply by fission
6. The normal sized trophozoites then invade the large intestines, the sigmoid colon, and the rectum
7. The trophozoites in the large intestine down to the rectum now prepare for their transformation to the precystic stage in the luminal portion of LI, sigmoid, and rectum
8. The precystic trophozoites then are passed out in diarrheic stools
9—The precystic forms develop into mononucleated cysts and they come in contact with food and water stuff and the life cycle begins ulit sa #1

Symptoms and Pathogenesis

Classification of amebiasis histolytica as per WHO (1996):
- Asymptomatic infections
- Symptomatic infections
  - Intestinal amebiasis
    - Dysenteric
    - Non-dysenteric colitis
  - Extraintestinal amebiasis
    - Hepatic
      - Acute non-suppurative
      - Liver abscess
    - Pulmonary
    - Other extraintestinal foci (very rare)

The symptoms of amebiasis are NOT well-defined and its presentation depends largely on the extent of tissue invasion and on whether the infection is confined to the intestinal tract or has spread to involve other organs.

INTESTINAL AMEBIASIS
- Most common form of amebiasis
- Can be asymptomatic
- Can present with vague and non-specific abdominal symptoms
  - Although these symptoms may improve or disappear after anti-amebic therapy, they are NOT specifically related to amebiasis alone
- A group of definite symptoms can be observed such as:
  - Diarrhea
  - Dysentery
  - Abdominal pain/cramping
  - Flatulence
  - Anorexia
  - Weight loss
  - Chronic fatigue

When we observe a patient with symptomatic intestinal amebiasis, the patient is considered to be having amebic colitis

Amebic colitis is different from amebic dysentery in such a way that the latter will present with bloody and mucoid stools

It is observed that strain differences and differences in host susceptibility are main factors that determine the virulence of the disease.

There is barrier between the immunologic surveillance (like the immune system that prevents neoplasia) and the local intestinal immunity
The destruction of this barrier can occur in patients whose amebic infection has invaded intestinal tissues

- Corticosteroid administration may provoke (and can even be fatal) severe amebic colitis
- Additionally, there is fatal necrotizing amebic enterocolitis in a severely burned patient
  - The rapid worsening of the previously asymptomatic infection was due in part to alteration of the immune response
- The susceptibility of humans to E. histolytica infection is associated with specific alleles of the HLA complex
  - Mortality is increased in patients who are pregnant and a relationship of maternal stress to the severity of the infection has been established
- There are certain genotypes of E. histolytica that are associated with amebic liver abscess formation and others with just asymptomatic colonization
- The amebic primary invasion
  - When E. histolytica succeeds in entering the intestinal mucosa, this penetration is accompanied by an inflammatory response
    - And because the local response is minimal, and so is the immune constitutional response
  - The amebae secrete proteolytic enzymes that produce a necrosis of the surrounding tissues
  - And most frequently, the following are the sites for primary invasion (from most frequent to least frequent):
    1. Cecum
    2. Ascending colon
    3. Rectosigmoid
    4. Any other part of the large intestine
- The intestinal invasion may be accompanied by little local reaction and often, with no recognizable symptoms + diffuse inflammation which is indistinguishable from the non-specific inflammatory lesion of other types of colitis
  - The diarrhea that is thus provoked may be mild with only few loose bowel movements daily
    - However, even in patients with mild diarrhea (or even with normal stool), careful examination of the feces may reveal flecks of blood-tinged mucus often containing numbers of mobile E. histolytica
- Patients in the acute phase of infection by E. histolytica may have a dozen (12!!!) or more explosive liquid stools daily containing much blood + mucus and the illness is accompanied by abdominal cramps
o Signs of rectal ulceration:
  - Tenesmus
  - Painful spasms of the rectal sphincter

o Despite extensive molecular research, the underlying molecular bases for
  pathogenicity and virulence of *E. histolytica* remain poorly understood
  However, these are the proposed mechanism for its virulence:
    1. Production of enzymes and other cytotoxic substances
    2. Contact-dependent cell killing
    3. Cytophagocytosis
  
  - The ameba kills host cells in a step-by-step fashion:
    1. Receptor-mediated adherence of amebae to target cells
    2. Amebic cytolysis of target cells
    3. Amebic phagocytosis of killed (or viable) cell

o The attachment of the trophozoites to the colonic mucosa is mediated
  by an amebal galactose-inhibitable adherence lectin (AGIAL)
  - The addition of millimolar concentrations of the following inhibits the
    attachment of the trophozoites in cultivated mammalian cells:
      1. Galactose
      2. N-acetyl-D-galactosamine
  - When the attachment of the trophozoite to the host cell is successful,
    the parasite **kills** the host cell in an extracellular process involving
    the activation of host cell Caspase-3 leading to host cell’s
    **apoptosis**
      1. The apoptosed (dead) cells are then engulfed by the ameba

o Little is known about the host-protective mechanisms in amebiasis but
  the following concepts are known:
  - **Intestinal secretory IgA** against the parasite’s Gal/GalNAc lectin
    is associated with immunity to reinfection
  - Serum antibodies can be demonstrated in human infection by a variety
    of commonly used serologic techniques
    1. It is generally assumed that serum antibodies are elicited after
      tissue invasion and that, although they are useful in serologic
      diagnosis of infection, the antibodies **DO NOT** confer immune
      protection against the ameba. To illustrate this:
        a. **Highest antibody titers** are found in symptomatic
          infections
          i. (So bakit ang daming antibodies pero
             symptomatic pa din yung patient, diba?)
        2. Much research is needed in order to determine how the serum
           antibodies participate in immune protection
  - Trophozoites of *E. histolytica* have been shown to activate the
    complement via the classical and alternative pathways
    - The complement is amebicidal
However, certain strains of *E. histolytica* may be resistant to complement-mediated lysis via inhibition of the formation of the MAC (Membrane attack complex) (Biochemistry I miss you)

- **Role of Cell-Mediated Immunity**
  - Experimental hepatic amebiasis in immunodepressed mice suggests a protective role for cell-mediated immunity
  - Conversely, TH-2 phenotype immune response appear to exacerbate amebic colitis in the murine model
  - Additional animal studies have demonstrated that host resistance is dependent on macrophages but NOT on T cell-mediated defense mechanisms
    1. In fact, during acute invasive amebiasis, the host’s T-lymphocyte responses to *E. histolytica* antigens appear to be specifically depressed by a parasite-induced serum factor

- Trophozoites of a virulent strain of *E. histolytica* are able to kill normal human polymorphonuclear neutrophils, monocytes, and macrophages in vitro
  - However, activated macrophages are able to kill the same virulent amebae through a contact-dependent, antibody-independent mechanism

- Factors that determine the ameba’s pathogenicity:
  - **Resistance of the host**
    1. Innate immunity
    2. Nutritional state
    3. Freedom from infections and other diseases
  - **Virulence and invasiveness of the amebic strain**
  - **Conditions in the intestinal tract**

- The amebae may penetrate the muscularis mucosae into the submucosa of the colon where they spread out into classic flask-shaped ulcers
  - The flask-shaped ulcers are:
    1. Crateriform in appearance
    2. With wide base and narrow opening
    3. With irregular, slightly elevated, overhanging edges
  - They can erode the blood vessels to give rise to intraluminal bleeding which is characteristic of acute infections
  - If large numbers of ulcers are formed, they may coalesce by means of intercommunicating submucosal sinus passages
    1. The compromised mucosa may remain fairly normal in appearance
      a. However, if the damage is extensive, there may be:
        i. Secondary bacterial infection
        ii. Necrosis
iii Sloughing off of large portions of intestinal wall
iv (Rarely) Intestinal casts may be seen in the stool

- Upon sigmoidoscopy, the mucosa may reveal an almost normal mucosal pattern or a mucosa that resembles that of ulcerative colitis or granulomatous colitis
  - There may be scattered ulcerations that measure up to few millimeters in diameter
    1 The ulcerations have:
      a Eryhematous border
        i Erythema—abnormal redness of the skin/mucous membrane due to capillary congestion
      b Yellowish center
  - In more advanced cases of amebiasis, the ulcerations have a diameter up to 10-12 mm often with raised edges but with normal-looking mucosa elsewhere
    1 As the amebic infection progresses, coalescence of the ulcers may produce irregularly wandering ulcer trenches
      a These ulcer trenches may sometimes be project hair-like remnants of the more resistant supportive structures producing a “Buffalo skin”/”Dyak hair” ulcers
  - The presence of a grossly normal mucosa between the ulcers serves to differentiate amebic vs bacillary dysentery on sigmoidoscopy
    1 In bacillary dysentery, the ENTIRE mucosa is involved

<table>
<thead>
<tr>
<th>Table 1. Amebic vs Bacillary Dysentery</th>
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<tbody>
<tr>
<td><strong>Point of contrast</strong></td>
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<tr>
<td>----------------------------------------</td>
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<tr>
<td><strong>Onset</strong></td>
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<tr>
<td><strong>S/Sx</strong></td>
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<tr>
<td><strong>Fecal odor</strong></td>
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<td><strong>Presence of blood/mucus in stools</strong></td>
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<tr>
<td><strong>pH</strong></td>
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<td><strong>Pus cells/Polymorphonuclear (PMN cells)</strong></td>
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<tr>
<td><strong>Cellular Exudates</strong></td>
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<tr>
<td><strong>Pyknotic residues</strong></td>
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<tr>
<td><strong>Charcot-Leyden Crystals</strong></td>
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</table>
Lecture 7: The Amebae
Parasitology: The Amebae (Lumen-dwelling protozoa)

<table>
<thead>
<tr>
<th>Presence of pathogenic amebeae (motile amebae containing RBC)</th>
<th>Present</th>
<th>Absent</th>
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<tbody>
<tr>
<td>Presence of bacteria</td>
<td>Few</td>
<td>Numerous</td>
</tr>
<tr>
<td>Presence of macrophages</td>
<td>Absent</td>
<td>Present</td>
</tr>
</tbody>
</table>

- Upon abdominal palpation, the following can present with tenderness:
  - Cecum
  - Transverse colon
  - Sigmoid
  - Liver
    - The liver can also be enlarged
      - The hepatic tenderness and enlargement do NOT mean that there is hepatic amebic invasion
- Fever is NOT characteristic of a simple amebic colitis
  - Mild leukocytosis may be seen
    - Which is probably a response to the secondary bacterial infection which is so frequently present
  - WBC count is elevated above 12k/microliter
    - In bacillary dysentery, the WBC count is usually (not always) lower than the WBC elevation produced by amebiasis
      - And sometimes, in bacillary dysentery, the WBC count can reach up to 20k/microliter
- Perforation of an amebic ulcer is a dramatic event (gaano ka-dramatic ba? Haha) which is accompanied by the usual signs of peritoneal irritation/infection (peritonitis)
  - However, slow leakage of the ulcer fluid into the abdominal cavity through a severely diseased colonic wall may be more common
    - It is marked by:
      - Distention
      - Ileus
        - Ileus—a functional obstruction of the GIT and especially the small intestine that is marked by the absence of peristalsis
        - Gas in the peritoneal cavity
  - Acute perforation is marked by board-like abdomen
    - Acute perforation can be treated with surgical intervention
      - Surgery is NOT an option for the:
        - Chronic type of perforation
        - Amebic appendicitis
          - The gut in these disease states is friable
Friable—easily broken into smaller pieces (Thanks dictionary)

Ameboma
- A chronic granulomatous lesion
- Develops most frequently in the cecal or rectosigmoid region
- It may produce a so-called napkin-ring constriction of the bowel wall indistinguishable on x-ray examination from an annular carcinoma
- It can give the cecum a conical configuration which is characteristic of but NOT specific to ameboma

In general, intestinal amebiasis upon radiologic examination mimics that of inflammatory bowel dse
- The difference is that in intestinal amebiasis, the terminal ileum is usually NOT involved

Complications of intestinal amebiasis:
- Appendicitis
- Intestinal perforation
- Hemorrhage
- Stricture
- Granulomas
- Pseudopolyposis

Table 2. Summary of symptomatic intestinal amebiasis

<table>
<thead>
<tr>
<th>Amebic disease</th>
<th>Symptomatology</th>
</tr>
</thead>
</table>
| Non-dysenteric amebic colitis        | 1. Moderate malaise  
2. Alternating diarrhea and constipation 
3. Irregular colicky abdominal pain 
4. With or without local abdominal tenderness |
| Acute dysenteric intestinal amebiasis | 1. Incubation period—1-14 weeks  
2. Severe dysentery with numerous small stools containing blood, mucus, and shreds of necrotic mucosa 
3. Acute abdominal pain and tenderness 
4. Fever at 38-39degC  
5. Dehydration, prostration (weakness/collapse), and toxemia |
| Chronic dysenteric amebiasis         | 1. Recurrent attacks of dysentery  
2. Intervening periods of mild or moderate GIT disturbances and constipation 
3. Localized abdominal tenderness 
4. Liver may be enlarged 
5. Psychoneurotic disturbances 
6. Marked weight loss and cachexia  
Cachexia—general physical wasting and malnutrition usually associated with a chronic disease |
HEPATIC AMEBIASIS

- Hepatomegaly and tenderness may occur in amebic colitis without any evidence of hepatic infection
  1. The hepatic enlargement is thought to be a toxic response to intestinal infection—unrelated to the local presence of amebae
  2. When the amebae reach the liver parenchyma, it causes a disease called amebic hepatitis and is defined as a diffuse early stage of liver infection WITHOUT abscess formation
     a. Amebic hepatitis can occur without GIT symptoms
     b. Amebic hepatitis is characterized by:
        i. RUQ tenderness
        ii. Hepatomegaly
        iii. Fever/Night sweats
           - Fever occurs daily and peaks at the afternoon at 102°F and is accompanied or followed by profuse sweating
        iv. Weight Loss
        v. Leukocytosis of 15k-35k per microliter
        vi. RUQ or Right Upper Hypochondriac Pain that is referred to the right shoulder
        vii. Sometimes, cough with evidence of pneumonitis involving the right lower lung field
  c. In amebic hepatitis, the right leaf of the diaphragm may be elevated and fixed on position
  d. The multiplication of the amebae in the liver may lead to the development of a single or multiple abscesses
     i. Although the majority of amebae that reach the liver are probably destroyed and do not produce abscesses
     ii. Single large abscesses may arise from the coalescence of multiple smaller abscesses
     iii. With abscess formation, hepatic pain becomes more severe and continuous
        - The pain can also be referred to the left or right shoulder—depending on the location of the abscess
        - Fever is common and chills may occur
        - There is mild jaundice
Liver scans reveal areas of non-visualization, most frequently **single** and in the **right lobe**, less often **multiple** or in **other locations**

- **Ultrasound, MRI, and CT** offer convenient means for evaluating the development and resolution when refined imaging techniques lead to early diagnosis and treatment

Liver function tests are of little value in the differential diagnosis amebic abscess

Aspiration of amebic abscess usually yields a **thick, reddish brown fluid** which **rarely** contains amebae

- The organisms are confined to the **hepatic tissue** of the **abscess walls**

Under all these circumstances, diagnosis by response to therapy is frequently the only practical approach, although modern techniques such as **PCR** are highly sensitive for detecting *E. histolytica* DNA in the abscess

- Results of serologic tests are usually positive in such cases

3 **Erosion** of a **hepatic abscess** through the **diaphragm** into the **lung** may lead to **PULMONARY AMEBIASIS**

**PULMONARY AMEBIASIS**

- **Pleurisy**, with or without **effusion** or **pleural rub**, or **right lower lobe pneumonitis** may signal a **subdiaphragmatic abscess** WITHOUT actual rupture into the **pleural space**

  - **Pleurisy**—inflammation of the pleura that is typically characterized by sudden onset, painful, and difficult respiration and exudation of fluid or fibrinous material into the pleural cavity

  - With rupture into the **pleural cavity**, a characteristic x-ray picture may result, with evidence of an **effusion** ascending the **greater fissure**, sometimes, followed by rupture into the **pleural space**

  - If the abscess is localized in the **left lobe** of the **liver**, it may, of course, involve the **left lung**

  - If **hepatic spread** of the infection **extends** to involve a **bronchus**, **amebae** may be found in the **sputum**

- In pulmonary amebiasis, there are chills and fever

- **Primary Pulmonary Amebiasis (PPA)**

  - Blood-borne—from an **intestinal focus** rather than arising from **hepatic spread**

  - **Amebic abscess** in **other organs** are uncommon and when they occur, it is accompanied by **hepatic amebic abscess**
Lecture 7: The Amebae
Parasitology: The Amebae (Lumen-dwelling protozoa)

- Brain
  - S/Sx of a brain tumor
  - Dx only at autopsy
- Pericardium
- Spleen
- Skin—very rare
  - Production of extensive, gangrenous ulcerations of the perineal tissues or affect the skin surrounding a colostomy or draining hepatic abscess
  - A case of cutaneous amebiasis of the face resulting in loss of vision in 1 eye but without the involvement of mucocutaneous surfaces has been described
- Vagina
- Urethra
- Clitoris
- Penis
  - Penile amebiasis occurs following intercourse with a partner who has vaginal amebiasis and also as a consequence of anal intercourse
  - In all these types of amebiasis, trophic amebae may be recovered from the affected tissues

Table 3. Amebic spread to organs

<table>
<thead>
<tr>
<th>Organ</th>
<th>Type of Spread</th>
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<tbody>
<tr>
<td>Brain</td>
<td>Hematogenous</td>
</tr>
<tr>
<td>Left Lung</td>
<td>Hematogenous</td>
</tr>
<tr>
<td>Right Lung</td>
<td>Extension (from right lobe of liver)</td>
</tr>
<tr>
<td>Liver</td>
<td>Hematogenous</td>
</tr>
<tr>
<td>Large intestine</td>
<td>Ingestion of contaminated food stuff</td>
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</tbody>
</table>

Table 4. Differential diagnosis for amebiasis

<table>
<thead>
<tr>
<th>Type of Amebias</th>
<th>Differential Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intestinal amebiasis</td>
<td>1. Diverticulitis</td>
</tr>
<tr>
<td></td>
<td>2. Ulcerative colitis</td>
</tr>
<tr>
<td></td>
<td>3. Large intestinal carcinoma</td>
</tr>
<tr>
<td>Hepatic amebiasis with abscess formation</td>
<td>1. Viral hepatitis</td>
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<tr>
<td></td>
<td>2. Bacterial hepatitis</td>
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<tr>
<td></td>
<td>3. Gallbladder infections</td>
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<td></td>
<td>4. Hepatoma</td>
</tr>
<tr>
<td></td>
<td>5. Pulmonary disease</td>
</tr>
</tbody>
</table>
Diagnosis
- IHA
  - IHA is more sensitive for extraintestinal amebiasis (100%)
    - For intestinal amebiasis, it is only 70-85% sensitive
- ELISA
- Enzyme ImmunoAssays (EIA)
  - Detects Entamoeba-specific antigen in fecal specimen
- Monoclonal ELISA
  - Detects E. histolytica Gal/GalNAc lectin in fecal specimen and distinguishes it from that of E. dispar
- DNA hybridization probe
  - Identifies E. histolytica in stool samples
- PCR
  - Detects E. histolytica and differentiates it from E. dispar
- Sigmoidoscopy-assisted acquisition of amebae-containing specimen
  - Target tissues: Colonic ulcers

Treatment
- Whenever possible, a laboratory diagnosis of E. histolytica infection, unless confirmed by visualization of ingested RBC in the trophozoite, should be substantiated by:
  - RBC in stool
  - Serum antibody titer
  - Stool E. histolytica antigen titer

- The treatment regimen varies according to the clinical stage of the infection

Table 5. Treatment regimen for different clinical stages of amebiasis

<table>
<thead>
<tr>
<th>Clinical Stage of Infection</th>
<th>Drug and Dosage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Asymptomatic intestinal amebiasis</td>
<td>1. Paromycin—25-35mg/kg/day for 7 days</td>
</tr>
<tr>
<td>2. Acute amebic colitis</td>
<td>2. Diloxanide furoate (Furamide)—500mg/3x/day for 10 days</td>
</tr>
<tr>
<td>3. Metronidazole (Flagyl)—750mg/3x/day for 10 days</td>
<td></td>
</tr>
<tr>
<td>1. Amebic dysentery</td>
<td>1. Metronidazole—750mg/3x/day for 10 days followed by a luminal agent (dose must not exceed 1.0g daily to as Metronidazole’s side effect is peripheral neuropathy)</td>
</tr>
<tr>
<td>2. Liver abscess</td>
<td></td>
</tr>
<tr>
<td>3. Ameboma</td>
<td></td>
</tr>
</tbody>
</table>

*Acute symptoms of amebic dysentery will usually be brought under control within 3-5 days; there is seldom any marked toxicity with such curtailed use of these drugs

- The drugs
  - Metronidazole—most important drug in the treatment of amebiasis at any stage (especially for invasive strains)
    - Other variant: Tinidazole
Tinidazole is better tolerated and has a shorter course of treatment but is less effective than metronidazole

- Only effective against **anaerobic** or **microaerophilic organisms**
- It is activated by reduction by **ferredoxin**, generating a **reactive radical** that attacks the organism’s cell membrane
- Side effects:
  1. Nausea
  2. Diarrhoea
  3. Metallic taste
  4. Headache
  5. Most severe is peripheral neuropathy
- Contraindicated for **pregnant women** in 1st trimester
  - 2nd and 3rd trimester, pwede na
- Amebic resistance against metronidazole/tinidazole has not been observed
- Before the standard treatment of metronidazole for amebiasis, **emetine** was used
  - However, emetine has been replaced by metronidazole because emetine elicits **cardiac toxicity** causing abnormal ECG tracings
- Metronidazole and tinidazole are **first-line agents** in the treatment of **hepatic abscess**
  - However, some reports say that metronidazole alone is NOT totally therapeutic so that **abscess aspiration** may sometimes be required
    - But in most cases, metronidazole is sufficiently therapeutic
- Along with imaging technologies such as liver scans, ultrasound, and CT, the disappearance of the abscess due to metronidazole can now be observed
  - However, drainage of larger abscesses may be necessary in exceptional circumstances
  - The introduction of a **percutaneous catheter drainage** under the guidance of CT or ultrasound has greatly facilitated the drainage of amebic abscesses that are resistant to conventional therapy and even of those that have perforated:
    - **Paromycin**
      - For treatment of intestinal (**luminal**) infection
    - **Diloxanide furoate (Furamide)**
      - Restricted for patients who pass cysts in the stool
      - Luminal amebicide
Epidemiology

- The prevalence of amebic infection, as of most enteric diseases, varies with the level of sanitation and is generally higher in the **tropics** and **subtropics** than in temperate climates
  - The severity of the disease and the incidence of complications may likewise be greater in the tropics, reflecting the higher incidence of infection
    - The severity of the disease is very dependent on the host’s **nutritional status**
  - The infection rate can reach epidemic levels in prisons, orphanages, refugee centers, and asylums
    - Outside these settings, the infection is most likely acquired through **sewage water** that is contaminated
      1. In a “**pseudo-outbreak**” in LA, California, there **38** people who were diagnosed with **intestinal amebiasis**
        a. Of these 38 people, only **2** people actually had the infection by *E. histolytica* proving that identification of this ameba is difficult

- Infection with *E. histolytica* and *E. dispar* can be acquired by men having sex with men (MSM)

- In a general hospital in **Mexico**, **7914 autopsies** were conducted and it was found out that amebiasis was the **4th leading cause of death**
  - In another report, amebiasis was identified as the **3rd most prevalent infectious disease** in **Mexico**

- From an epidemiologic standpoint, asymptomatic patients are of utmost importance in the **transmission** of the disease
  - **Food handlers** are also important in the transmission of the disease
  - Use of **human feces** as **fertilizers** contaminates the soil and can be epidemiologically important as well

- Cysts are relatively resistant but are killed by:
  - **Drying**
  - **Temperature greater than 55degC**
  - **Superchlorination** of drinking water
  - **Iodination** of drinking water

- The **Laredo strain** of *E. histolytica*
  - Now known as *E. moshkovskii*
  - **Eurythermic ameba**
    - Able to grow and multiply in **room temperature**
      1. **Optimum growth temperature: 25degC-30degC**
      2. Can survive: **0degC-41degC**
        a. *E. histolytica* can only survive within the temperature range of **20degC-43degC**
  - **Have been isolated in human feces**
E. moshkovskii is of limited pathogenicity to experimental animals and probably NOT pathogenic to humans.

Early studies involving zymodemes (patterns of electrophoretic mobility of certain parasite isoenzymes) of various isolates of what was originally thought to be all one species (that all amebic species are E. histolytica) have demonstrated a difference between invasive and non-invasive strains (Invasive strain = E. histolytica; Non-invasive strain = E. dispar).

A seroepidemiologic survey of antibody responses to zymodemes of E. histolytica showed that 94-100% of persons infected with amebae having the pathogenic zymodemes were seropositive—even though some were asymptomatic.

Whereas only 2-4% of persons infected with amebae of non-pathogenic zymodemes were seropositive.

Research using RNA/DNA probes also indicated differences between invasive and non-invasive strains.

The following technologies can also discriminate between E. histolytica vs. E dispar:
- PCR amplification of genomic DNA
- Hybridization of cDNA clones
- rRNA probes

The INABILITY to morphologically distinguish E. histolytica from non-pathogens E. dispar/E. moshkovskii underscores the importance of the modern diagnostic tests.

Prevention

Most amebiasis is acquired through fecal contamination of food and water.

And preventive measures against the transmission are designed to break the chain of transmission.

Water
- Boiling of water readily disinfects it
- Treatment of water with iodine disinfects it
- Ice cubes made with contaminated water may transmit infection
- Fruits and vegetables washed through contaminated water can transmit infection
  - In many areas, these fruits and vegetables are indirectly contaminated by human feces because human feces are used as fertilizers

The importance of food handlers in the spread of enteric diseases, including amebiasis, is brought to our attention by periodic outbreaks of hepatitis, often traced to a particular eating place and sometimes, to a single employee.

Therefore, infected food handlers must not be allowed to do the job until he/she is successfully treated in order not to infect the food eaten by others.

Treat all infections (even the asymptomatic ones) and examine contacts.

Remove carriers from the food handling occupations.
→ Institute sanitary methods of sewage disposal
→ Screening of latrines
  o Latrine—outdoor toilet that is made by digging up a hole in the ground
→ Store feces used as fertilizers for an appropriate length of time
→ Use properly safeguarded, filtered water supply
→ Screen and protect food from dust contamination
→ Control of insects with insecticides
→ Uncooked vegetables should be washed with water treated with iodine tablets or scalded at 80degC for at least 30 seconds
→ Inform the public regarding methods of avoiding infection
→ Development of a vaccine

B. Entamoeba coli
→ Non-pathogenic amoeba that verily resembles *E. histolytica*
  o The two organisms are so much alike that sometimes, diagnostic differentiation between them leads to either:
    ▪ Superfluous treatment of *E. coli* as it is mistaken as *E. histolytica*
    ▪ Omission of appropriate therapy for *E. histolytica* as it is mistaken as *E. coli*

Morphology
→ Cysts
  o Size: 12-15 microns in diameter
  o Larger than cysts of *E. histolytica*
  o Consists of 8 nuclei with very diffuse karyosomes
  o May also contain needle-like chromatoidal bodies with irregular fragmented ends
→ Trophozoites
  o Size: 18-28 microns in diameter
  o Larger than the trophozoites of *E. histolytica*
  o Has 1 nucleus containing a large diffuse karyosome
  o The peripheral chromatin is usually dense and irregular
  o The cytoplasm is usually rough and contain few to many ingested debris
  o The cytoplasm is also granular, frequently containing many vacuoles
  o Do NOT ingest RBCs except for unusual circumstances
    ▪ However, they can engulf bacteria that will be present inside vacuoles in the cytoplasm
  o Movement: Sluggish
    ▪ Movement is not progressive
      1 The movement functions more in the ingestion of food than to produce a directional movement
  o Pseudopodia: Short and blunt, never long and finger-like (*E. histolytica*)
    ▪ They are extruded slowly
• NOT made up of hyaline
  • The pseudopodial cytoplasm has NO ecto-/endoplasm
    • The nucleus is easily discerned
      • A ring of refractile granules representing the peripheral chromatin encloses another eccentric refractile mass, the karyosome
        1 Karyosome is eccentric meaning it is off center
    • When the organisms are stained, the nuclear morphology is more distinct
      • Peripheral chromatin in *E. coli* is irregular both in size and in arrangement on the nuclear membrane; it is definitely more abundant than is usual in *E. histolytica*
  • The karyosome:
    1 Large
    2 Irregular in shape
    3 Usually eccentric in position
    4 Surrounded by a halo of non-staining material
• Granules of chromatin may be seen scattered between the karyosome and the peripheral chromatin, and sometimes, a linin network is visible

→ Precystic forms
  • The precystic stage is also seen in *E. coli*
  • The identification of amebic species should not be done on these precystic forms as the structures are unclear and are not distinctive, whether the specimen is stained or not stained

→ Cyst forms
  • The cysts of *E. coli* overlap the size range of the cysts of *E. histolytica* (10-35 microns in diameter)—although, generally, the cysts of *E. coli* have larger diameter than the cysts of *E. histolytica*
  • The cyst wall is highly refractile and the cytoplasm is granular in appearance
  • Food vacuoles are absent
  • The nuclei are readily visualized with varying numbers from 1-8 (mononuclear to octonuclear)
  • The eccentrically located karyosome is easily seen even in unstained amebae
  • Chromatoidal bodies are less common than in *E. histolytica* (therefore, chromatoidal bodies are seen more frequently in *E. histolytica*) but occasionally may be observed as clear, thin lines or rods of refractile material in the cytoplasm
  • With an iodine stain:
    • Glycogen may be seen in the cysts of *E. coli*
      1 Glycogen is visualized as dark masses of darkly staining material surround the nuclei (the glycogen is therefore perinuclear, peri=around; nuclear=nucleus)
Even if the glycogen surrounds the nuclei, the nuclei do NOT become obscured.

The perinuclear characteristic of glycogen is characteristic of *E. coli*.

- Eccentric karyosomes may be observed, especially in the mononuclear and binuclear cysts because in such cysts, the karyosomes are larger.
  - Permanent stains bring out details of nuclear structure, which is similar to that of the trophozoites.
    - From one to eight nuclei are ordinarily seen; rarely, hypernucleate forms with 16 or 32 nuclei may be observed.
    - The chromatoidal are seen to be composed of splinter-shaped or rarely ribbon- or thread-like bodies.
      1. Heavier bodies with irregular ends are also frequently seen.
    - The cytoplasm of *E. coli* is very granular; areas occupied by the glycogen before fixation are marked by empty spaces in the cytoplasm of the fixed and stained cysts.

### Entamoeba hartmanni

- Formally known as the “small race” strain/variation of *E. histolytica*.
  - It was confused as a “small” *E. histolytica* because the morphological features are the same but it was, of course, smaller.
  - Tetranucleate cysts is observed in *E. hartmanni* as it is observed in *E. histolytica*.
  - The size range of trophozoites and cysts of both entamoebae overlap, and when this happens, specific differentiation between *E. histolytica* vs. *E. hartmanni* may be impossible.

- Authorities consider *E. hartmanni* as non-pathogenic and treatment is NOT required.
  - Although it is not pathogenic, it should be considered as an indicator of fecal contamination.

### Morphology

- **Trophozoites**
  - Rounded
  - Diameter: 3-12 microns
  - Nuclear features: same as *E. histolytica*
  - Nucleus-cytoplasm ratio: same as *E. histolytica*
  - The chromatoidal material assumes a similar rod- or cigar-like shape just like in *E. histolytica*.
  - Ingests bacteria but NOT RBCs.
    - *E. histolytica* ingest both bacteria + RBCs
  - Karyosome: Small and central
D. *Entamoeba gingivalis*

- The first ameba to be isolated from **humans**
- Found in all populations
- Found only in the:
  - Mouth
  - Teeth
  - Gums
  - Gingival pockets (Pyorrheal pockets)
  - Tonsillar crypts
- NO cyst forms
- Frequently occurs in **unhygienic mouths (95%)** and still can occur in healthy mouths (50%)
- Non-pathogenic (classified as of now)
  - However, it can be found in patients with **pyorrhea alveolaris**
  - Its role in **periodontal disease** is still being investigated
  - It has been found out that *E. gingivalis* can thrive in the **uterus** and it can be transferred to the mouth after **oral sex to the vagina (orogenital contact)**
- Can multiply in the **bronchial mucus**
- Can appear in the **sputum**
  - As *E. gingivalis* is morphologically resembling *E. histolytica*, the presence of *E. gingivalis* in the sputum may be mistaken as **pulmonary amebiasis with abscess** by *E. histolytica*

**Features**

- **Size**
  - Diameter: **5-35 microns**
- **Inclusions**
  - RBCs = present at times
  - Bacteria and portions of ingested leukocytes = Present, abundant
    - Only *E. gingivalis* can ingest leukocytes
      - Therefore, presence of **nuclear fragments of leukocytes** from **stained specimens** confirms that the ameba is *E. gingivalis*
  - Vacuoles = numerous
- **Pseudopodia**
  - Usually blunt
  - Made up of **hyaline**
  - Formed very rapidly
- **Motility**
  - Moderately active
Parasitology: The Amebae

- Progressive, directional

**E. Iodamoeba butschlii**
- Receives its generic name from the characteristic **glycogen vacuoles** of the **cyst stage**
  - The glycogen vacuoles are so prominent that in **iodine stain**, the glycogen vacuoles occupy much of the cytoplasm
    - While **glycogen vacuoles** occur in other amebae, it is **MOST prominent** in *I. butschlii*
- Large somewhat irregular glycogen masses are frequently seen in **iodine stains** of *E. coli* cysts—perinuclear formation of glycogen masses in **mature cysts** of *E. coli*
  - In *Iodamoeba* cysts, the **single nucleus** is seen at **one side** of the **glycogen vacuole**
    - Iodamoebae can come in **hypernucleate forms** having **2-3 nuclei**
- **Non-pathogenic to humans**
  - Although historically, 2 cases of Iodamebic infection were described, and at that time, only *E. histolytica* was the known amebic pathogen to humans
    - A Japanese soldier with disseminated amebiasis that reached the CNS
    - However, this infection is most probably caused by **Naegleria fowleri**
    - An Arizona girl who fell off a slide bruising her parietooccipital region
      - Her infection was most probably caused by **Acanthamoeba**
- **Most common ameba of swine**

**Features**
- **Trophozoites**
  - Positive identification of unstained trophozoites is difficult
  - Vary in diameter from **4-20 microns**, the majority within the range of **9-14 microns**
- **Motility**
  - Sluggish but progressive
- **Pseudopodia**
  - Made up of hyaline
  - Blunt
  - Slowly extruded
- **Inclusions**
  - **Bacteria** = present
  - **RBCs** = never present
- **Nucleus**
  - Not visible in unstained specimens
  - On permanent stain, the nuclear structure is revealed
    - The **nuclear membrane** is delicate, and if it does not take the stain, the **karyosome** will appear to be contained in a **vacuole**
The karyosome is:

- **Large**
- More or less **central** in position
- Irregularly rounded
- Surrounded by a layer of small granules
  - The granules may lie closely applied to the karyosome, in which case they are not visible unless staining and subsequent differentiation have been optimal
  - In other instances, the small **chromatin granules** form a ring at some distance from the karyosome, between it and nuclear membrane

**Cysts**

- Diameter: **6-16 microns** (ave. 9-10 microns)
- The unstained cyst is surrounded by a **refractile wall**
- Shape: Outline is **irregular**, a lot of variation in shape
  - The usual shape of amebic cysts is either **spherical** or **ovoid**
- Contains **1 nucleus** with **chromatic granules** and a **large vacuole** making identification even in the wet mount easy
- Cytoplasm: **green** in Iodine stain

**F. Endolimax nana**

- Usually encountered with about the same frequency as is **E. coli**
- Size range of both trophozoites and cysts overlap with those of **E. hartmanni** and **Dientamoeba fragilis** which may be the cause for misidentification

**Features**

**Trophozoites**

- Range from **5-12 microns** in diameter
- Pseudopodia
  - Blunt
  - Hyalinous
  - Rapidly *extruded* like pseudopodia of **E. histolytica**
- Motility
  - Sluggish and random
- Cytoplasm
  - Contains food vacuoles with **ingested bacteria**
- Nuclear structure
  - When stained, the characteristic nuclear structures are revealed
    - **Large karyosome**
2 Smaller extrakaryosomal chromatin granules
   a Mass seen against the nuclear membrane without formation of a distinct karyosome

Cysts
   o Almost the same size as the trophozoites
   o They are most frequently **void**
     ▪ Sometimes, **spherical** or **subspherical**
   o A **refractile cyst wall is present**
     ▪ Can be exhibited by the **zinc sulfate method + iodine stain**
       1 Zinc sulfate method **shrinks** the **cytoplasm** (green) of many of the cysts, which pull away from the cyst wall, leaving a clear space on one side of the organisms between the cytoplasm and the undistorted cyst wall
         ▪ This effect is also seen in *Giardia*
       1 **Hypernucleate forms**: 8 nuclei
   o **Karyosome**:
     ▪ Large
     ▪ Eccentric
     ▪ Characterizes the nucleus/nuclei of the cyst
   o Other chromatin granules and intranuclear fibrils have been reported but are seldom seen in routine stains

THE FREE-LIVING AMEBAE

Several species of ordinarily free-living amebae have been observed as human symbionts.

In some instances, the association seems to be without pathologic consequence.
And in other cases, it may result in devastating disease.

The free-living amebae comprise a large group, inhabiting fresh, brackish, and salt water, moist soil, and decaying vegetation. Some are coprozoic.

For convenience, the taxonomy of the free-living amebae can be separated into two groups on the basis of their ability to undergo transformation from an **ameba** into a **flagellated state**.

The **Vahlkampfiidae** family
   o Members of this family are characterized as **amebo-flagellates**, able temporarily to assume a **flagellate form** while being completely devoid of flagella in the **ameboid stage**
o Naegleria and Vahlkampfia belong here
  ▪ Naegleria: Is the usual cause of PAM (Primary Amebic Meningoencephalitis)
  ▪ Vahlkampfia: Cause disease in farm animals

The Acanthamoebidae family
- Amebae that can NEVER produce flagella
- Acanthamoeba belongs here
- Can cause PAM, too, but Naegleria talaga ang common cause nun

Other amebic families can cause disease as well. The family Leptomyxidae is where Balamuthia mandrillaris belongs—B. mandrillaris can cause Granulomatous Amebic Encephalitis (GAE). The ameba, Sappinia diploidea, caused a space-occupying lesion at the left posterior temporal lobe of a farmer in Texas.

G. Naegleria fowleri
- A free-living ameba that can be opportunistic and cause disease in humans
- Ameboflagellates
  - Has an ameboid stage + a flagellated stage
- In the tissues, N. fowleri are only seen as the trophozoite stage
  - For Acanthamoeba and Balamuthia, they occur in tissues as trophozoites and cysts
    - Before, Acanthamoeba was known as Hartmannella
- Naegleria infections have been reported in: Australia, USA, Americas, Asia, Europe, New Zealand, and Africa
- Main causative agent for Primary Amebic Meningoencephalitis (PAM)
  - Can also cause keratitis
  - Most infections have occurred during the hot summer months in young persons who within the preceding week, swam or dived in fresh/brackish water
    - Lakes, streams, hot springs, and swimming pools are apparent sources of infection
    - Although, NOT all infections are aquatic
      1. In Nigeria, an 8-month-old infant inhaled the organism during a dust storm
         a. The nasal mucosa and CSF were inhabited by the inhaled Naegleria
- Named after Dr. Malcom Fowler, the first person to recognize the disease this ameba caused

Morphology
- Trophozoite (the ameboid form)
  - Size: 8-22 microns
  - Motility: Slug-like but progressive
  - Nuclei: Only 1
Lecture 7: The Amebae
Parasitology: The Amebae (Lumen-dwelling protozoa)

- Karyosome: Large and centrally located
- Peripheral chromatin: Absent
- Cytoplasm: Granular and vacuolated
- Cytoplasmic inclusions: **Ingested bacteria, RBCs**
- The trophozoite is the form of *N. fowleri* that can be found in the patient’s CSF

**Flagellated form**
- Size: 7-15 microns
- Karyosome: **Central**
- Peripheral chromatin: Absent
- Number of flagella: 2

**Life Cycle and Transmission**
- In the water body, they exist as **flagellates**, and they enter as **ameboid forms** once inside the nasal cavity
- **Intranasal entry** thru bathing in stagnant water bodies (lakes, pools contaminated with sewage or other decaying matter, underchlorinated pools, etc)
- After intranasal entry, the amebae penetrate the **cribiform plate** that can cause **meningitis → encephalitis → coma → death in 3 days**

**Diagnosis**
- Diagnosis of PAM is made by **microscopic identification of living or stained amebae** in the patient’s CSF
  - The amebae can be distinguished from other cells by their “limax” (slug-like) shape and **progressive movement**
  - It is **NOT** necessary to warm the slide because these amebae are fully active at room temperature
    - Refrigeration of the CSF is **NOT** recommended
- The **flagellated form** of *N. fowleri* may be induced by suspending amebae in distilled water
  - At 37degC, maximum enflagellation occurs in 4-5 hrs
- **Peripheral blood**
  - Leukocytosis with a preponderance of neutrophils
- **CSF quality:**
  - Decreased glucose
  - Increased protein
  - Presence of neutrophils
  - (+) for culture
    - Growth in non-nutrient agar in conjunction with coliform growth

**Symptomatology and Pathogenesis**
- Prodromal symptoms of **severe frontal headache** and **fever** and is followed by the rapid onset of **nausea** and **vomiting**
With signs and symptoms of meningitis with involvement of the following brain areas:

- **Olfactory lobe**
  - Disturbances in the sense of smell or taste may be noted early in the course of the disease but are not always seen

- **Frontal lobe**
- **Temporal lobe**
- **Cerebellar area**

→ **Meningeal irritation** may be accompanied by stiff neck, generalized seizures, and Kernig’s sign

  - Kernig’s sign—severe stiffness of the hamstrings causes an inability to straighten leg when the hip is flexed to 90 degrees

→ Patients often become irrational before lapsing into coma
→ Death occurs early; the entire clinical course seldom extends beyond 3-6 days
→ There is no serologic evidence that infection in humans is caused by a single species, distinct from the non-pathogenic *Naegleria gruberi* isolated from soil and water

→ Spinal puncture reveals a cloudy to frankly purulent fluid, usually under increased pressure

  - The cell count ranges from a few hundred to >20k WBC per microliter of predominantly neutrophils
    - Failure to find bacteria in such a purulent fluid SHOULD alert physicians and MedTechs to the possibility of PAM

  - Spinal protein is generally increased (though not significantly) and glucose levels are low

  - RBCs are frequently present, and motile amebae may be found even in unstained preparations of the CSF
    - Their activity is characterized by the explosive formation of blunt pseudopodia, like those of *E. histolytica*, rather than the tapering, spiky projections (acanthopodia) as seen in *Acanthamoeba*
    - These amebae do NOT stain well with the usual bacterial staining procedures
      - Iron Hematoxylin Stain reveals the nucleus with a large karyosome that extends nearly to the delicate nuclear membrane

→ **Amebostomes**

  - Novel phagocytic structures found in *N. fowleri*
  - Used for engulfment, their role in pathogenesis is unclear though

→ On autopsy, signs of acute meningoencephalitis are seen

  - An exudate of neutrophils and monocytes is found in the subarachnoid space
  - Hemorrhage
  - An inflammatory exudate extending to the gray matter
- Rounded amebae are seen in the gray matter, ahead of the advancing margin of hemorrhage or necrosis
  1. These amebae are particularly prominent in the Virchow-Robin Spaces
  - There is focal demyelination of the white matter of the brain and spinal cord
    - Surprisingly, demyelination has occurred in the absence of amebae or cellular infiltrate
      1. It has been suggested that the demyelination occurred due to a phospholytic enzyme or enzyme-like substance produced by the actively growing amebae in the adjacent gray matter

**Treatment**

- At present, there is no satisfactory treatment for PAM
  - The antibiotics used for bacterial meningitis are ineffective as are the antiamebic drugs
- The anti-naeglerial drug Amphotericin B is considered to be the standard treatment for PAM
  - A polyene compound
  - Acts on the plasma membrane by disrupting its permeability and causing leakage of the cellular components of the ameba
  - Administered through IV or intrathecally
  - Can be co-administered with Miconazole and Rifampin

**Epidemiology**

- PAM is a rare disease but is worldwide in distribution
- Commonly affects active, healthy, young people mostly from developed nations
- Another pathogenic species, *N. australiensis* is pathogenic to mice via intranasal instillation and therefore, must be considered potentially pathogenic to humans

**Prevention**

- Because of the swimming-related nature of *Naegleria* infection, swimming areas have been subjected to intensive investigation
  - Although the ameba has been isolated from many such areas, not all sampling efforts have yielded the organism
    - Obviously, there are factors that favor the development of *N. fowleri* in swimming areas:
      1. Warm temperature
      2. Presence of adequate food supply
      3. Minimal competition from other protozoans
      4. Optimal pH and O₂ levels
- To prevent the infection, there must be awareness that such an infection, although rare, exists
Some measures to ensure safety of swimming areas:
  o Adequate chlorination (0.5mg Cl/L water)
  o Avoid hot springs as the ameba thrive in hot environments
  o Do not swim or dive in small lakes and ponds that have stagnant warm water and algal growth

H. *Acanthamoeba* spp. and *Balamuthia mandrillaris*
  ➔ Resembles *Naegleria* but these ameba do NOT have the flagellated stage
  ➔ *Balamuthia* and *Hartmanella* resemble *Acanthamoeba* but the first 2 amebae have no Acanthopodia
    o Acanthopodia—spiky pseudopodia characterizing *Acanthamoeba*
  ➔ *Acanthamoeba* was first noted as a contaminant in tissue cultures and subsequently was found to produce lethal meningoencephalitis on nasal instillation into mice and other animals
  ➔ *Acanthamoeba* may produce a chronic CNS infection which is known as Granulomatous Amebic Encephalitis (GAE) distinguishing acanthamoebic infection against *Naegleria* which causes PAM
    o GAE is not associated with swimming and is only secondary to infection elsewhere in the body—amebae reach the CNS hematogenously—most likely from the LRT or through ulcers of the skin or mucosa
      ▪ The disease tends to be chronic, with a prolonged course, and occurs most often in debilitated or immunocompromised persons
      1 However, *Balamuthia* and *Acanthamoeba* infections can also occur in immunocompetent persons
        a Immunocompetent persons get these amebae to infect their eyes via eye trauma that have resulted from improper use of contact lens
  ➔ *Acanthamoeba* may also produce Acanthamoebic keratitis
    o Keratitis of the eye stems from a corneal ulcer. Other microorganisms that can cause corneal ulcer:
      ▪ *Pseudomonas* spp
      ▪ *Herpes* simplex
  ➔ Other *Acanthamoeba* infections:
    o Granulomatous infection of the skin and other tissues
      ▪ Skin lesions are the most commonly reported clinical condition caused by *Acanthamoeba* and *Balamuthia* in patients with AIDS
    o Bony invasion that results in osteomyelitis
  ➔ There are 10 species of *Acanthamoeba* that are pathogenic to humans
    o *A. castellanii* is the species most often seen in cases of GAE and ocular infection followed by *A. culbertsonii* (for GAE) and *A. polyphaga* (for ocular infections)
Morphology

- **Trophozoite**
  - Size range: 12-45 microns
  - Motility: *Sluggish* via the **Acanthapodia** (spine-like pseudopods)
  - Nuclei: **Only 1**
  - Karyosome: **Large**
  - Peripheral chromatin: Absent
  - Cytoplasm: Granular and vacuolated

- **Cyst**
  - Cell wall: **Double cell wall** (outer wall has ragged edges)
  - Size range: 8-25 microns
  - Nuclei: **Only 1**
  - Karyosome: **Large** and **central**
  - Peripheral chromatin: Absent
  - Cytoplasm: Granular and sometimes, vacuolated

**Diagnosis**

For **GAE**:

- The laboratory diagnosis of **GAE** is made by:
  - Identifying **trophozoites** of *Acanthamoeba/Balamuthia* in **CSF**
  - Identifying **trophozoites** or **cysts** of *Acanthamoeba/Balamuthia* in **brain tissue**

- *Acanthamoeba* and *Balamuthia* are differentiated by using **indirect immunofluorescent antibody technique** and **antisera** to *Acanthamoeba/Balamuthia*

- *Naegleria* is readily culturable while *Acanthamoeba/Balamuthia* are **NOT**

For **Acanthamoeba keratitis**:

- Identify amebae cultured from **corneal scrapings** or by **histologic examination** of **infected corneal tissue**
  - As with PAM, *Acanthamoeba* may be cultivated from corneal scrapings on **non-nutrient agar** spread with **gram-negative bacteria** and later transferred to a **liquid medium** with **antibiotics** for axenic growth
  - Cultures of corneal material should be incubated at **30degC** (not 37degC)
  - Species identification is based on indirect immunofluorescent antibody staining
    - **Calcofluor white staining** has been used to identify *Acanthamoeba cysts* in corneal scrapings

- **Herpes simplex keratitis** is the disease most commonly mistaken for **Acanthamoeba keratitis**
  - The single most consistent clinical symptom of **Acanthamoeba keratitis** is **severe ocular pain**, which is **NOT** characteristic of an infection limited to the cornea and generally **NOT** of **herpes simplex keratitis**
Symptoms and Pathogenesis

➔ Although GAE usually occurs in debilitated or chronically ill persons, some of whom may be undergoing immunosuppressive therapy (Corticosteroids)
  - Not all victims of GAE have been debilitated or immunocompromised; some have been otherwise healthy

➔ GAE is NOT well-defined
  - It can be subacute, acute, chronic, lasting from weeks to months, in some instances, perhaps even years

➔ GAE
  - Characterized by focal granulomatous lesions of the brain
  - The onset of GAE, unlike PAM, is insidious, with a prolonged clinical course
  - GAE becomes GAE as the ameba traverses through the lungs or skin and mucosal ulcers and reaches the CNS hematogenously
    - Since amebae reach the CNS via the bloodstream, invasion of the CNS tends to become established first in the deeper tissues, from which it may extend toward the brain surface
  - Incubation period of GAE: Weeks to months
    - During which, single or multiple space-occupying lesions develop
  - An altered mental state is a prominent feature of GAE
  - GAE may present with:
    - Headache
    - Seizures
    - Stiff neck
    - Nausea
    - Vomiting
    - Neurologic deficits mimicking a brain tumor/brain abscess
    - Meningeal signs
  - In contrast to Naegleria infection, which is character by diffuse meningoencephalitis, GAE is focal

➔ The trophozoites and cysts occur in most infected tissues and around blood vessels

➔ Within the infected primary tissues (the site of initial invasion) occurs a chronic granulomatous reaction like that seen in the brain, with trophozoites, cysts, and multinucleate giant cells
  - Similar lesions have been described from other tissues as well, including the following probably resulting from the hematogenous dissemination of amebae from the primary focus in skin or lungs, or possibly even from a secondary CNS lesion:
    - Prostate
    - Kidneys
    - Uterus
    - Pancreas
Cases of **disseminated acanthamebiasis** have been described in patients with AIDS.

**Acanthamoebic keratitis (AK)**
- Chronic infection of the cornea
- Caused by several species of *Acanthamoeba*
- Infection is by direct contact of the cornea with amebae, which may be introduced through **minor corneal trauma** or by exposure to **contaminated water** or to **contact lenses that have become contaminated**
  - Keratitis in contact lens wearers has also been associated with infection by amebae of *Hartmannella* and *Vahlkampfia*
- Keratitis usually develops over a period of **weeks** or **months** and is characterized by **severe ocular pain**, often out of proportion to the degree of **inflammation** observed, **affected vision**, and a **stromal infiltrate** that frequently is **ring-shaped** and **composed** predominantly of **neutrophils**
- **AK** is a serious ocular infection and if not properly managed, can lead to **NOT ONLY** the loss of vision but the loss of the eye
- **Ocular infections in general**
  - Ocular infections are characterized by a **chronic progressive ulcerative keratitis**
  - Corneal ulceration may progress to **perforation**
    - A case of **endophthalmitis**, in which *Acanthamoeba* was recovered from the **aqueous** and **vitreous specimens**, has been reported in a patient with AIDS
      - Topical **propamidine, clotrimazole**, and **neomycin** could not control the infection
      - **Trophozoites** and **cyst** of *Acanthamoeba* are found in the infected corneal tissue

**Treatment**
- As with *Naegleria* infection, there is **NO satisfactory treatment** for GAE
  - Why? Because most cases of GAE are diagnosed after death (autopsy) and there has not been adequate opportunity to evaluate therapeutic regimens
- **For GAE**
  - 5-fluorocytosine and **pentamidine**—has in vitro activity
- **For *Acanthamoeba* keratitis**
  - **Propamidine, Miconazole**, and **Neomycin**—long-term application

**Epidemiology**
- Fewer cases of *Acanthamoeba/Balamuthia* infections have been reported as compared to the infections of *Naegleria*
- Greatly affects debilitated persons: AIDS patients
85% of *Acanthamoeba* keratitis cases have been reported to be linked to patients who wore contact lenses.

*Acanthamoeba/Balamuthia* can also infect animals.

**Prevention**

Proper use of contact lenses

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**References**

1. Markell and Voge’s Medical Parasitology (9th edition)
2. Lecture notes by RAsturiano from the lecturer

Downloadable for free at: [www.theelusivedoktora.wordpress.com](http://www.theelusivedoktora.wordpress.com)

For any corrections you may find, content or otherwise, email me at: ram.ustmedicine@gmail.com

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THANKS

AsturiaNOTES
By RAsturiano
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