1. Introduction

INTESTINAL PROTOZOA

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2. Intestinal Protozoa

Intestinal Protozoa

➤ *Giardia lamblia*
➤ *Cryptosporidium* spp. *†
➤ *Entameba histolytica* *
➤ Microsporidia†
➤ Isospora belli†
➤ Cyclospora cayetanensis†

* NIH/CDC Category B agents for Biodefense
† Opportunistic protozoa

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3. **Entameba histolytica**

**Entameba histolytica**

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4. **Entameba histolytica - Case Presentation**

**Entameba histolytica**

CASE PRESENTATION

A 65 year old man living in Kolkata, India, presented with bloody diarrhea, and abdominal pain of 10 days duration. Physical examination revealed that the patient was afebrile and had lower abdominal tenderness.

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5. **Entameba histolytica** - Case Presentation Slide 2

*Entameba histolytica*

CASE PRESENTATION

Slide 2

Sigmoidoscopy showed numerous hemorrhagic ulcers.

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6. **Entameba histolytica** - Case Presentation Slide 3

*Entameba histolytica*

CASE PRESENTATION

Slide 3

- Stool cultures were negative for bacterial pathogens.

- Microscopic examination of the stool showed the presence of numerous trophozoites and occasional cysts of *Entameba histolytica*.

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7. Entameba histolytica

**Entameba histolytica**

- Protozoan parasite that causes amebiasis
- dysentery
- liver abscess
- Transmitted by fecal-oral route

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8. Entameba histolytica - Developmental Stages

**Entameba histolytica**

**DEVELOPMENTAL STAGES**

**TROPHOZOITE**
- vegetative form, actively growing
- ameboid in shape, uninucleate
- moves by extruding pseudopodia
- does not survive in external environment

**CYST**
- dormant, infective form
- round to oval, 4 nuclei
- thick cyst wall
- highly resistant to adverse environmental conditions

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9. Entameba histolytica - Encounter

**Entameba histolytica**
**ENCOUNTER**

**INFECTIVE FORM**
- Cyst

**MODE OF TRANSMISSION**
- Fecal-oral route
  - direct person to person contact
  - ingestion of contaminated food or water

**EPIDEMIOLOGY**
- Prevalent worldwide, more so in developing countries
  - infects ~ 50 million people each year
- widely prevalent in areas of Africa, Asia and Latin America

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10. Entameba histolytica - Entry

**Entameba histolytica**
**ENTRY**

1. EXCYSTATION
   - cysts excyst in small intestine

2. ATTACHMENT
   - trophozoites attach to colonic epithelial cells and mucins via Gal/GalNAc-specific lectin

3. CONTACT-DEPENDENT LYSIS
   - attachment is followed by contact-dependent lysis of host cells

4. PHAGOCYTOSIS
   - lysed target cells phagocytosed by the trophozoite

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11. Entameba histolytica

Entameba histolytica

When a neutrophil makes contact with a virulent *Entamoeba*, the white cell dies by necrosis.

12. Entameba histolytica - Multiplication / Spread

Entameba histolytica
MULTIPLICATION/
SPREAD
LIFE CYCLE

- Cysts excyst in small intestine
- Trophozoites
  - divide by binary fission
  - invade colonic tissues
  - may disseminate via bloodstream to extra-intestinal sites
  - encyst in colon
- Cysts excreted in feces

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13. Damage and Clinical Manifestations

*Entameba histolytica*

**DAMAGE AND CLINICAL MANIFESTATIONS**

**DAMAGE**

a. Intestinal amebiasis
   - acute stage-inflammatory infiltrate (neutrophils)
   - later-tissue necrosis and formation of “flask-shaped” ulcers

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14. Entameba histolytica - Clinical Manifestations

*Entameba histolytica*

**CLINICAL MANIFESTATIONS**

a. Intestinal amebiasis
   - asymptomatic (cyst passers)
   - acute amebic dysentery
   - amebic colitis
   - AMEBIC DYSENTERY
     - Bloody diarrhea
     - Abdominal pain
     - Fever
     - Abdominal tenderness

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15. Damage and Clinical Manifestations

*Entameba histolytica*

**DAMAGE AND CLINICAL MANIFESTATIONS**

**DAMAGE**

b. Extraintestinal amebiasis

Amebic liver abscess
- necrosis of liver parenchyma
- relatively little inflammation

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16. Entameba histolytica - Clinical Manifestations

*Entameba histolytica*

**CLINICAL MANIFESTATIONS**

b. Extraintestinal amebiasis

- amebic liver abscess (most common)
- amebic lung or brain abscesses (rare)
  - AMEBIC LIVER ABCESS
    - Fever
    - Right upper quadrant pain
    - Diarrhea
    - Weight loss
    - Cough
    - Enlarged, tender liver
    - Rales or rhonchi

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17. **Entameba histolytica - Pathogenesis**

### Entameba histolytica

**PATHOGENESIS**

a. **Parasite Factors**
   - strain/isolate differences
     (non-pathogenic *E. dispar*)
   - putative virulence determinants
     - Gal/GaINAc lectin-adherence contact-dependent lysis
     - Amebapore - ionophore-like protein
     - Proteases - cysteine protease
     - Ca++ dependent phospholipase A

b. **Host Factors**
   - infection induces expression of
     proinflammatory cytokines - IL-1, IL-8, TNF-α, GROα

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18. **Entameba histolytica - Diagnosis**

### Entameba histolytica

**DIAGNOSIS**

c. **Enzyme-linked immunoassay (ELISA)**
   (detection of amebic antigens in feces)

d. **Serology** (detection of antibodies to *Entameba* in serum - useful in invasive amebiasis)
   - Indirect hemagglutination assay (IHA)
   - Immunofluorescence assay (IFA)
   - Enzyme-linked immunoassay (ELISA)

e. **PCR**

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19. Entameba histolytica - Treatment

Entameba histolytica

TREATMENT

- Asymptomatic (cyst passer)
  Luminal agents
  Diodohydroxyquin
  Paromomycin
  Diloxanide furoate

- Acute amebic colitis
  Metronidazole
  +Luminal agent

- Amebic liver abscess
  Metronidazole

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20. Entameba histolytica - Outcome - Immune Response

Entameba histolytica

OUTCOME

Immune Response

- Exposure to the parasite induces some degree of protective immunity, although protection is not always complete

- Cell-mediated responses important in limiting invasive disease

- Cytotoxic T cell responses to trophozoites

- Macrophage-mediated effector responses

- Anti-amebic antibodies (serum and sIgA) produced in natural infection, ?role

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Entameba histolytica - Outcome - Clinical

Entameba histolytica
OUTCOME
Clinical

• Acute amebic colitis
• Symptoms may subside
• May progress to chronic colitis
• Complications-
  fulminant colitis
  intestinal perforation
• Liver abscess
  • Complications-
    rupture
    pulmonary abscess

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Cryptosporidium spp.

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23. Cryptosporidium parvum - Case Presentation

**Cryptosporidium parvum**

**CASE PRESENTATION**

A 44 year old AIDS patient who had discontinued anti-retroviral therapy presented with severe watery diarrhea and weight loss over the past month. On physical examination, he was moderately dehydrated. His CD4 count was 90 cells/mm³.

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24. Cryptosporidium parvum - Case Presentation slide 2

**Cryptosporidium parvum**

**CASE PRESENTATION slide 2**

- Stool culture for bacterial pathogens was negative

- Stool examination for ova and parasites showed *Cryptosporidium* oocysts which were stained a reddish-pink color using a modified acid fast stain.

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25. Cryptosporidium spp. - Introduction

**Cryptosporidium spp.**

**INTRODUCTION**

- Protozoan (apicomplexan) parasite that causes cryptosporidiosis, characterized by watery diarrhea
- Opportunistic infection
  - mild and self limiting in immunocompetent hosts
  - severe and chronic in AIDS patients
- Transmitted by fecal-oral route

- 2 major species
  - *C. hominis* infects humans
  - *C. parvum* infects animals and humans

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26. Cryptosporidium parvum - Encounter

**Cryptosporidium parvum**

**ENCOUNTER**

**DEVELOPMENTAL FORMS OF PARASITE**

- **EXTRACELLULAR STAGES**
  - oocysts (infective)
  - sporozoites (invasive)
  - merozoites (invasive)

- **INTRACELLULAR STAGES**
  - asexual cycle
    - trophozoites
    - meronts
  - sexual cycle
    - macro and microgamonts

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Cryptosporidium spp. Encounter

Epidemiology

- prevalent worldwide
- more prevalent in developing countries (persistent diarrhea and growth faltering in children)
- opportunistic infection in immunocompromised hosts such as AIDS patients

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Cryptosporidium spp. Encounter

Infecitive Form: Oocyst

Mode of Transmission

- fecal-oral route
- direct person to person contact
- ingestion of contaminated food or water
- waterborne outbreaks due to contamination of municipal water supplies, recreational water (swimming pools, water slides)
- zoonotic transmission contact with animals (pets/farm animals)

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29. Water-borne cryptosporidiosis

Water-borne cryptosporidiosis

Milwaukee outbreak 1993
Population exposed: 1,600,000
Population infected: 403,000
Deaths > 100


30. Cryptosporidium spp. - Entry and Multiplication

Cryptosporidium spp.
ENTRY AND MULTIPLICATION
LIFE CYCLE

• Oocysts excyst in small intestine

• Sporozoites attach to and invade epithelial cells

• Undergo further intracellular development in parasitophorous vacuole

• Replicate via asexual and sexual cycles

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31. Cryptosporidium parvum

Cryptosporidium parvum

When swallowed, the cyst of this waterborne parasite is weakened by stomach acid, releasing 4 sporozoites to invade the intestinal lining

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32. Cryptosporidium spp. - Spread

Cryptosporidium spp.

SPREAD

- in immunocompetent hosts, infection is usually localized to small intestine

- in immunocompromised hosts, infection may occur throughout the gastrointestinal tract as well as in extraintestinal sites such as the biliary and pancreatic ducts and the respiratory tract

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33. Cryptosporidium spp. - Damage and Clinical Manifestations

**Cryptosporidium spp.**

**DAMAGE AND CLINICAL MANIFESTATIONS**

**DAMAGE**
- may be villus atrophy, inflammation, crypt hyperplasia in intestine

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34. Cryptosporidium spp. - Clinical Manifestations

**Cryptosporidium spp.**

**CLINICAL MANIFESTATIONS**

- in immunocompetent individuals infection is often asymptomatic or associated with mild diarrheal illness.
- in immunocompromised hosts (eg AIDS patients) infection may cause severe, persistent and possibly fatal diarrhea and wasting

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35. Symptoms

Cryptosporidium spp.

CLINICAL MANIFESTATIONS

(Many asymptomatic cases)

Symptoms

- Diarrhea
- Abdominal pain/cramps
- Nausea/vomiting
- Fever
- Anorexia
- Weight loss

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36. Cryptosporidium spp. - Pathogenesis

Cryptosporidium spp.

PATHOGENESIS

a. Host factors
   - immune status of host
   - expression of proinflammatory cytokines
     induced in intestinal epithelium

b. Parasite factors
   - isolate differences-genotypic variability
   - putative virulence determinants
     surface adhesins
     proteases

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37. Cryptosporidium gp40/15

**Cryptosporidium gp40/15**

*Cpgp40/15* gene.

encodes 40 and 15 kDa peptides present on surface of sporozoites

gp40 mucin-like protein which binds to host cells

gp15 immunodominant antigen

Human serum antibodies to gp15 are associated with protection from diarrheal symptoms

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38. Cryptosporidium spp. - Diagnosis and Identification 1

**Cryptosporidium** spp.

DIAGNOSIS AND IDENTIFICATION 1

a. Microscopic identification of oocysts in feces:

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39. Cryptosporidium spp. - Diagnosis and Identification 2

\textbf{Cryptosporidium spp.}
\textbf{DIAGNOSIS AND IDENTIFICATION 2}

\textbf{b. Immunoassays}

- Enzyme-linked immunoassay (ELISA) for detection of \textit{Cryptosporidium} antigens in feces

- Immunofluorescence assay (IFA) for detection of \textit{Cryptosporidium} oocysts in feces

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40. Cryptosporidium spp. - Diagnosis and Identification 3

\textbf{Cryptosporidium spp.}
\textbf{DIAGNOSIS AND IDENTIFICATION 3}

\textbf{c. Microscopic identification of intracellular stages in intestinal biopsy}

\textbf{d. PCR}

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41. Cryptosporidium spp. - Treatment

**Cryptosporidium spp.**

**TREATMENT**

- **SPECIFIC**
  - Limited effective specific therapy
    - Nitazoxanide recently approved by FDA for use in immunocompetent children
- **SUPPORTIVE**
  - Oral/parenteral rehydration
  - Supplemental nutrition
  - Anti-gut motility drugs
- **PREVENTIVE**
  - Improved hygiene
  - Prevention of nosocomial infection
  - Adequate water purification methods

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42. Cryptosporidium spp. - Outcome - Clinical

**Cryptosporidium spp.**

**OUTCOME - CLINICAL**

- in immunocompetent host illness is self-limiting
- in immunodeficient hosts illness may be chronic and life-threatening

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43. Cryptosporidium spp. - Outcome - Immune Response

**Cryptosporidium spp.**

OUTCOME - IMMUNE RESPONSE

- Nature of immune response poorly understood. Both cell-mediated and humoral responses important
- CD4+ve T cells important
- interferon γ important
- Serum and mucosal antibodies ? role in protection/resolution

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44. Giardia lamblia

**Giardia lamblia**

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45. Giardia lamblia - Case Presentation slide 1

**Giardia lamblia**

CASE PRESENTATION slide 1

A 2-year-old boy who attends a day care center was brought to his pediatrician’s office with a history of diarrhea (3-5 watery stools per day) for the past week. His mother stated that his appetite was decreased and that he was not as active as usual. Three other children who attended the same day care center had a similar illness in the past month. There was no history of blood in the stools and no fever or chills. Physical examination was normal.

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46. Giardia lamblia - Case Presentation slide 2

**Giardia lamblia**

CASE PRESENTATION slide 2

- Stool examination for ova and parasites revealed oval cysts with 2-4 nuclei and a refractile cyst wall with occasional flagellated, motile trophozoites of *Giardia lamblia*.

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47. **Giardia lamblia - Introduction**

**Giardia lamblia**

**INTRODUCTION**

- Protozoan parasite that causes giardiasis, a diarrheal disease
- Transmitted by fecal-oral route

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48. **Giardia lamblia - Encounter Developmental Forms**

**Giardia lamblia**

**ENCOUNTER DEVELOPMENTAL FORMS**

- **CYST**
  - infective form
  - oval, 4 nuclei
  - thick cyst wall
  - resistant to adverse environmental conditions

- **TROPHOZOITE**
  - vegetative active motile form
  - pear-shaped, 2 nuclei
  - 4 pairs of flagellae
  - attaches to mucosa by ventral adhesive (suction) disk
  - does not survive in external environment

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49. **Giardia lamblia**

*Giardia lamblia*

Intestinal parasite common in wild animals ("beaver fever") that can infect when humans drink contaminated stream or well water.

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50. **Giardia lamblia - Encounter**

*Giardia lamblia*

**ENCOUNTER**

**INFECTIVE FORM: CYST**

**MODE OF TRANSMISSION**

- fecal-oral route
- direct person to person contact
- ingestion of contaminated food or water
- waterborne outbreaks due to contamination of municipal water supplies
- hiker’s/backpacker’s diarrhea due to ingestion of water from mountain streams contaminated by animals

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51. Giardia lamblia - Encounter - Epidemiology

**Giardia lamblia**

ENCOUNTER

EPIDEMIOLOGY

- prevalent worldwide, more prevalent in developing than developed countries

- In USA most frequently identified intestinal parasite (4-7%)

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52. Giardia lamblia - Entry

**Giardia lamblia**

ENTRY

NONINVASIVE

- Cysts excyst in small intestine

- Trophozoites attach to upper small intestinal epithelial cells but do not invade

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Giardia lamblia - Multiplication - Life Cycle

**Giardia lamblia**

MULTIPLICATION 
LIFE CYCLE

- Cysts excyst in small intestine
- Trophozoites
  - divide by binary fission
  - encyst in small intestine
- Cysts excreted in feces

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Giardia lamblia - Spread

**Giardia lamblia**

SPREAD

infection localized to small intestine, does not invade or disseminate

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Giardia lamblia - Damage and Clinical Manifestations

**Giardia lamblia**

**DAMAGE AND CLINICAL MANIFESTATIONS**

- may be villus atrophy and crypt hyperplasia of intestinal mucosa
- may be increased inflammatory cell infiltrate, increased intraepithelial lymphocytes

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Giardia lamblia - Pathogenesis

**Giardia lamblia**

**PATHOGENESIS**

a. Parasite factors
   - strain/isolate genotypic differences
   - putative virulence determinants
     - variant surface proteins (VSPs)
     - proteases
b. Host factors
   - mucosal inflammation
   - structural and functional abnormalities

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57. Giardia lamblia - Clinical Manifestations

**Giardia lamblia**

**CLINICAL MANIFESTATIONS**

Wide spectrum of disease ranging from asymptomatic to mild self-limiting diarrheal illness to chronic infection with malabsorption and weight loss

- **Acute phase**
  - diarrhea
  - abdominal cramps
  - nausea
  - anorexia
  - malaise

- **Chronic phase**
  - persistent diarrhea
  - weight loss
  - debilitation

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58. Giardia lamblia - Diagnosis slide 1

**Giardia lamblia**

**DIAGNOSIS slide 1**

a. Microscopic identification of cysts in stools

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59. Giardia lamblia - Diagnosis slide 2

**Giardia lamblia**

**DIAGNOSIS slide 2**

b. Microscopic identification of trophozoites in diarrheal stool, intestinal aspirates or biopsy material

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60. Giardia lamblia - Diagnosis slide 3

**Giardia lamblia**

**DIAGNOSIS slide 3**

c. Immunoassays
- Enzyme-linked Immunoassay (ELISA) for *Giardia* antigen in stool
- Immunofluorescence assay (IFA) for cysts in stool

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Giardia lamblia - Treatment

**Giardia lamblia**

**TREATMENT**

- **SPECIFIC**
  - Metronidazole
  - Quinacrine
  - Furazolidone

- **PREVENTIVE**
  - Improved hygiene
  - Adequate water purification methods

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Giardia lamblia - Outcome - Clinical

**Giardia lamblia**

**OUTCOME CLINICAL**

- usually self-limiting illness
- responds to antimicrobial treatment
- in some cases may be chronic

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Giardia lamblia - Outcome Immune Response

Giardia lamblia
OUTCOME IMMUNE RESPONSE

- Exposure to the parasite induces some degree of protective immunity, although protection is not always complete

- Giardia-specific sIgA produced, role in protective immunity

- CD4+ T cells important in clearance

- Immune evasion due to antigenic variation
  VSPs (variant surface proteins-cysteine rich heterogeneous variant surface antigens)

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