Outline

- Introduction
- Overview of the Epidemiology of Enteric Protozoa
- Diagnostic considerations
- Environmental health considerations
  - Detection in water
  - Water treatment

Figure 3. The human hand is a common denominator in intestinal parasite transmission. Hands can act as conduits to transfer parasites from surfaces (in the home or out of the home), currency, food, animals (pets or wild), and humans.
Introduction to Enteric Protozoa

- Enteric protozoa: most commonly encountered parasitic diseases;
- Causes significant morbidity and mortality in both developed and developing regions;
- Affect millions of people annually;
- Lack access to safe drinking-water increases vulnerability of billions of people;
- The impact of water and food borne zoonoses expected to be significant.

Introduction continued

- The most common protozoa implicated in developed countries are:
  - Cryptosporidium spp.,
  - Dientamoeba fragilis,
  - Entamoeba histolytica,
  - Giardia intestinalis
- Transmitted to humans, livestock, domestic animals and pets

Public Health concerns

- Many routes of transmission: Foodborne, waterborne and zoonosis
- Persistence in the environment
- Inadequate attention given to diagnosis in developed settings;
- Lack of sensitive diagnostic techniques.
- Chronic and extra-intestinal infections.
- Disease burden greater in high risk groups:
  - children, elderly, immunocompromised; MSM, institutionalized people.
A Complex Problem!

- Meat, fruit and vegetables
- Domestic pets
- Drinking Water
- Livestock
- Sewage/estimenter
- Infection - immunity - innovation
- Disposal - house - Fletcher et al 2012, Clin Micro Review

**Enteric protozoa in children 0-12 yrs in developing regions and OECD countries**

<table>
<thead>
<tr>
<th>Pathogens</th>
<th>SAP</th>
<th>LAC</th>
<th>MENA</th>
<th>OECD</th>
<th>SAP</th>
<th>SSA</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Giardia intestinalis</strong></td>
<td>Pooled prevalence (95%CI)</td>
<td>0.1% (0.1-0.3)</td>
<td>2.0% (0.6-6.5)</td>
<td>1.2% (0.2-1.1)</td>
<td>0.5% (1.6-6.9)</td>
<td>2.6% (1.6-4.0)</td>
</tr>
<tr>
<td><strong>Entamoeba histolytica/dispar complex</strong></td>
<td></td>
<td>0.1% (0.0-0.9)</td>
<td>0.6% (0.1-6.5)</td>
<td>1.5% (0.1-7.1)</td>
<td>0.2% (0.4-2.1)</td>
<td>0.1% (0.8-2.4)</td>
</tr>
<tr>
<td><strong>Cryptosporidium spp.</strong></td>
<td></td>
<td>0.01% (0.1-2)</td>
<td>0.3% (0.03-2)</td>
<td>1.0% (0.2-4.9)</td>
<td>0.4% (0.2-0.7)</td>
<td>2.0% (1.0-3.9)</td>
</tr>
</tbody>
</table>

**Enteric parasites in adults (12-70+ yrs) in developing regions and OECD countries**

<table>
<thead>
<tr>
<th>(DEVELOPING: MENA/SSA)</th>
<th>OECD</th>
</tr>
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<tbody>
<tr>
<td><strong>Protozoa</strong></td>
<td>Pooled prevalence (95%CI)</td>
</tr>
<tr>
<td>Cryptosporidium</td>
<td>3.4% (0.5%-19.0%)</td>
</tr>
<tr>
<td>Entamoeba sp</td>
<td>2.3% (1.3%-4.1%)</td>
</tr>
<tr>
<td>Blastocystis hominis</td>
<td>2.1% (0.4%-9.9%)</td>
</tr>
<tr>
<td>Giardia intestinalis</td>
<td>1.9% (1.0%-3.6%)</td>
</tr>
<tr>
<td>Cyclospora</td>
<td>1.9% (1.0%-3.7%)</td>
</tr>
<tr>
<td>Dientamoeba fragilis</td>
<td>0.5% (0.1%-1.8%)</td>
</tr>
</tbody>
</table>
Diagnostic considerations

- Prevalence estimates affected by the lack of sensitive diagnostic techniques.
- Routine tests not done in some labs.
- Difficulty to detect some protozoa
- Consider Molecular-based techniques.
- Adv: most promising for sensitive, accurate and simultaneous detection of protozoa.
- Disadv: Molecular methods - quite costly, time-consuming and many not commercially available.

Diagnosis: Cryptosporidium spp.

- Clinically: self-limiting diarrhoea lasting weeks to months, esp in children<5 years old.
- In immunosuppressed patients: more severe, assoc with chronic diarrhoea and wasting; can be fatal.
- Traditionally diagnosis relies on special staining techniques (tinctorial/acid-fast, fluorescent/auramine phenol or immunofluorescent stains).
- Alternative techniques: ELISA and various PCR assays.
- PCR has superior sensitivity for the detection of Cryptosporidium spp. when compared to conventional staining, microscopy, and ELISA.
Diagnosis: *Dientamoeba fragilis*

- **Clinical presentation:** acute gastrointestinal disease, with chronic infections also documented.
- **Traditional diagnosis:** prompt fixation and permanent staining, (demonstrating the characteristic nuclear structure by permanent stained preparations only).
- **These techniques are time-consuming and require experienced personnel to interpret the stained smears.**
- **Molecular techniques:** conventional and real-time PCR (RT-PCR) targeting the small-subunit (SSU) ribosomal DNA (rDNA), (high sensitivity and specificity).

Diagnosis: *Entamoeba complex*

- **Clinical presentation:** asymptomatic, dysentery, colitis and invasive disease. For e.g. liver, brain, cutaneous.
- **Entamoeba histolytica** is morphologically identical to *E. dispar* and *E. moshkovskii*; (non-pathogenic spp).
- **Genetic differences** - confirmed as three separate species.
- **Stained smears** of stool specimens are insufficient for differentiation of the species.
- **PCR or ELISA**- for differentiation of the species.
- **Serological methods** are useful for detecting invasive disease. (indirect hemagglutination, latex agglutination, immunoelectrophoresis, immunofluorescence assay).

Diagnosis: *Giardia intestinalis*

- **Clinical presentation:** asymptomatic; acute and chronic gastrointestinal infections.
- **Diagnosis:** microscopy or molecular methods
  - Identifying cysts or trophozoites in stained/unstained faecal smears.
  - Enzyme immunoassays, direct fluorescence and immunochromatographic assays
  - PCR assays available.
Prevalence of Enteric Protozoa in Diarrhoeal Patients in 3 hospitals

<table>
<thead>
<tr>
<th></th>
<th>CHW Hospital</th>
<th>LPH Hospital</th>
<th>SVH Hospital **</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. positive</td>
<td>% of total</td>
<td>No. positive</td>
</tr>
<tr>
<td>Blastocystis spp.</td>
<td>37</td>
<td>2.2</td>
<td>86</td>
</tr>
<tr>
<td>Dientamoeba fragilis</td>
<td>21</td>
<td>1.3</td>
<td>6</td>
</tr>
<tr>
<td>Cryptosporidium spp.</td>
<td>10</td>
<td>0.6</td>
<td>8</td>
</tr>
<tr>
<td>Entamoeba histolytica/dispar complex</td>
<td>0</td>
<td>0</td>
<td>0.5</td>
</tr>
</tbody>
</table>

Others not routinely tested unless travel history or by special request

Comparison of Protozoa prevalence in diarrhoeal cases in three hospitals in Sydney

- **Increased risks** from use of recycled water/wastewater; biosolids, humanure;
- Difficult to detect in environmental specimen
  - Persistence of cyst forms, difficulties to separate extraneous material
- Faecal Indicators:
  - Could be useful alternative markers of faecal contamination
Detection of protozoa in water samples

- Issues: small size, dispersion in water, difficulty to concentrate oocysts/cysts from environmental samples.
- Purification and concentration techniques have addressed these problem.
- USEPA recommends four sequential steps:
  - Filtration of water retaining the (oo)cysts and extraneous materials on the filter;
  - Elution and separation process- purification and concentration of (oo)cysts by immunomagnetic separation, discarding extraneous material;
  - Staining with specific fluorescent antibodies;
  - Enumeration by fluorescence & differential interference contrast microscopy.

Water treatment considerations

- Most protozoa are resistant to chlorination and conventional water treatment methods.
- Due to their small size, unable to easily remove from water.

Water treatment considerations

- Considerations:
  - Small scale: Point-of-use water purification technologies (for e.g. chlorination + boiling with safe storage, solar UV treatment, Ceramic Filter, biosand filter.
  - Large scale: Combinations of treatments and multiple-barriers approaches to optimize water treatment.
    - Improving flocculation mixing intensities and flow distribution throughout the water treatment plant.
    - Electro-filtration process removes waterborne particles <4µ.
  - Emerging waste water treatment methods involve membrane and filtration technologies.
Summary

• Cause significant morbidity and mortality worldwide.
• The major agents are Cryptosporidium spp., Dientamoeba fragilis, Entamoeba histolytica, and Giardia intestinalis.
• Prevalence estimates affected by the lack of sensitive diagnostic techniques.
• Consider simultaneously detecting several protozoa in stool.
• Difficult to detect protozoa in environmental samples by traditional methods.
• Need for adequate standards to guide use of recycled water and biosolids.
• New and emerging water treatment technologies should be considered.

Further reading


References

Pooled Prevalence of GI Pathogens in Six (6) World Regions

OECD: 58.6 (35.3-50.7)

LAC: 61.2 (51.2-70.1)

SSA: 62.4 (45.6-71.7)

EAP: 45.0 (30.9-60.0)

SAP: 60.4 (48.2-71.5)

OECD: 40.9 (33.4-48.8)

MENA: 54.3 (43.6-64.7)

Key:
EAP=East Asia & the Pacific; MENA=Middle East and North Africa; SAP=South Asia; LAC=Latin America and the Caribbean; SSA=Sub-Saharan Africa; OECD=Developed countries excluding non-OECD.