Biology of Parasites of Potential Public Health Concern in Alberta and Northern Canada

Mike Belosevic, PhD
Professor, Department of Public Health Sciences, School of Public Health, University of Alberta
March, 2008

Entry of parasites into hosts

- Entry via mouth-
  - lumen
  - lumen/tissues
  - tissue
- Entry via skin-
  - active entry
  - vectors

Prerequisites for a successful transmission of parasites

- Low or no host specificity
- High prevalence in the environment-transmission potential
- Resistance to environmental conditions
- Zoonosis
Waterborne diseases and protection of public health

Industry/Government
- Large volume of water consumed
- Cost for detection/inactivation
- Potential for large outbreaks
- Safe water expected
- Accountability

Clinical Challenges
- Infections not reportable
- Infections self-limiting
- Non-diagnosed treatment
- Similar pathology
- Cost

Public Health Compromised

Environmental Challenges
- Pulse events (sporadic)
- Low concentration of pathogens
- Localized distributions
- Many pathogens
- Complicated sampling & detection

Waterborne protozoa

- Cryptosporidium
- Giardia
- Entamoeba
- Toxoplasma
- Naegleria
- Acanthamoeba

Environmental forms

C. parvum oocysts
- Cysts
- 2 to 5 µm

G. lamblia cysts
- Cysts
- 8 to 10 µm
Waterborne outbreaks of protozoan infections

- More than 100 *giardiasis* outbreaks in North America alone in the past 30 years, including Kelowna, Banff and Edmonton. Many outbreaks not detected/recorded.
- 71 recorded *cryptosporidiosis* outbreaks (1983-2001):
  - Milwaukee (1998) >400,000 people
  - Kelowna (1996) ~1,136
  - Cranbrook (1996) ~2,097
  - Collingwood (1998) ~182
  - North Battleford (2001) >2,000

**Cryptosporidium and Giardia:** Public Health Significance

- Prevalent in humans and animals in Alberta
- Prevalent in surface waters
- Difficult to remove and hard to kill
- Drinking water - amplifier for disease
- Up to 20% of general population may be considered at higher risk

Characteristics of Infection

- **low numbers** of parasites required to initiate infection
- **multiplication** in the host-transmission
- **self-limiting** except immunodeficient individuals
- **latent, acute & chronic phases**
- **cross-species** transmission
**Giardia: The Organism**

- Obligate intestinal parasites of all classes of vertebrates
- More than 100 described species
- Two stages in the life cycle: the motile trophozoites that inhabit the small intestine of the host, and the resistant cysts found in the feces

**Giardiasis Epidemiology**

- Worldwide prevalence about 8%
- Prevalence higher in endemic areas: Aspen, St. Petersburg, Banff, Havana
- Higher prevalence in day care centers, mental institutions, male homosexuals
- Children more susceptible than adults
- Transmitted by direct contact, food or water

**Giardiasis: The Disease**

- **Asymptomatic:** largest group
- **Symptomatic:** self-limiting infection, diarrhoea, abdominal cramps, fever, nausea and weight loss
- **Symptomatic:** chronic infection, immunodeficient individuals, malabsorption, food intolerance, chronic inflammation of the mucosa
Giardia Trophozoite - cross section (TEM)

Cryptosporidiosis: The Disease

- Serious disease in the young, pregnant women and elderly
- Potentially fatal in immunodeficient hosts
- Infectious dose in humans is low: ID$_{50}$ about 130 oocysts
- No chemotherapy available
Sporozoite
Type I Meronts
oocyst (in feces)
merozoite
Type II Meronts
gametocytes
merozoite
ingestion
zygote
Cryptosporidium
Oocyst (environment) intestinal stages
Parasites in Water
Detection in Environment ➔ Disinfection Efficacy
IFA & Vital Dyes Animal Infectivity
Measures of Viability

- **ANIMAL INFECTIVITY**: expensive, very reliable
- **EXCYSTATION**: not accurate—overestimates viability
- **CELL CULTURE**: underestimates viability, contamination
- **NUCLEIC ACID DYES**: inexpensive, convenient & rapid

Survey of Nucleic Acid Dyes

<table>
<thead>
<tr>
<th>Dye</th>
<th>% Stained</th>
<th>Dye</th>
<th>% Stained</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRG-1</td>
<td>100</td>
<td>SY756</td>
<td>92.8</td>
</tr>
<tr>
<td>BRG-2</td>
<td>100</td>
<td>SYTO-9</td>
<td>100</td>
</tr>
<tr>
<td>CY365</td>
<td>100</td>
<td>SYTO-10</td>
<td>100</td>
</tr>
<tr>
<td>CY396</td>
<td>100</td>
<td>SYTO-11</td>
<td>100</td>
</tr>
<tr>
<td>Hexidium</td>
<td>84.3</td>
<td>SYTO-12</td>
<td>100</td>
</tr>
<tr>
<td>MPR71057</td>
<td>96.7</td>
<td>SYTO-13</td>
<td>96</td>
</tr>
<tr>
<td>SYTO-59</td>
<td>100</td>
<td>SYTO-14</td>
<td>93</td>
</tr>
<tr>
<td>SYBR-1</td>
<td>100</td>
<td>SYTO-15</td>
<td>82</td>
</tr>
<tr>
<td>SY1169</td>
<td>89.9</td>
<td>SYTO-16</td>
<td>94.4</td>
</tr>
</tbody>
</table>

Double-Staining of Heat-Killed *C. parvum* Oocysts

- SYTO-59
- Antibody
- SYTO-59 + Antibody
Double-Staining of Heat-Killed *C. parvum* Oocysts: Confocal Photo

Staining of *G. lamblia* Cysts with Anti-Parasite Antibody and SYTO-59

Relative resistance to water treatment chemicals

<table>
<thead>
<tr>
<th>Oxidant</th>
<th>Cryptosporidium</th>
<th>Giardia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Free Chlorine (pH 6)</td>
<td>6300</td>
<td>40</td>
</tr>
<tr>
<td>Monochloramine</td>
<td>8300</td>
<td>700</td>
</tr>
<tr>
<td>Chlorine Dioxide</td>
<td>250</td>
<td>10</td>
</tr>
<tr>
<td>Ozone</td>
<td>5</td>
<td>0.5</td>
</tr>
</tbody>
</table>

Notes: 1: U of A studies, 2: US EPA
Infectivity and Nucleic Stain Viability Assays

- Infectivity in neonatal CD-1 mice
- Automated nucleic acid viability assay

SYTO-9 Cytometry for C. parvum Oocysts Treated with Ozone

Median SYTO-9 Fluorescence of C. parvum Oocysts Exposed to Ozone
Ozone Disinfection of *C. parvum*

- Observed kill (log-units)
- Predicted kill (log-units)
- Best fit

![Graph showing the relationship between observed and predicted kill for different temperatures.]

Medium-pressure UV for treatment of waterborne protozoa

**Advantages**
- Insensitive to temperature, pH, and water chemistry
- No chemical residual
- No chemical handling
- Few by-products
- Exposure of the population to parasite antigens—resistance (?)

**Limitations**
- No chemical residual
- Lamp fouling
- Lamp maintenance
- Lamp replacement
- Potential microorganism reactivation

Mechanism of UV germicidal effect

- UV light penetrates cell & energy absorbed by DNA
- Dimerization of adjacent thymine bases
- DNA cannot replicate
- Organism cannot reproduce
- Cannot cause infection or illness
**Bench and Pilot Scale UV apparatus**

**C. parvum inactivation using UV:**

*Bench scale*

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**Reactivation* of Giardia treated with medium pressure UV- In vivo studies**

<table>
<thead>
<tr>
<th>Days Post Infection</th>
<th>No. Mice Infected/Total No. Mice</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>≤ 25 mJ/cm²</td>
</tr>
<tr>
<td>Control</td>
<td>UV-treated</td>
</tr>
<tr>
<td>5</td>
<td>14/35</td>
</tr>
<tr>
<td>10</td>
<td>35/35</td>
</tr>
<tr>
<td>20</td>
<td>35/35</td>
</tr>
<tr>
<td>25</td>
<td>35/35</td>
</tr>
<tr>
<td>30</td>
<td>35/35</td>
</tr>
</tbody>
</table>

*3 of 7 independent experiments at ≤25 mJ/cm² showed reactivation.
Atrazine degradation by combined UV/H\textsubscript{2}O\textsubscript{2} treatment

Degradation of pesticides by UV/H\textsubscript{2}O\textsubscript{2} - Old versus new lamps

Example helminths present in Alberta and Northern Canada

**Nematodes:**
- *Trichinella spiralis* - "pork worm"
- *Enterobius vermicularis* - "pinworm"
- *Toxocara canis* (T. cati) - "arrowhead worms"

**Flatworms:**
- *Diphyllobothrium latum* - "fish tapeworm"
- *Echinococcus granulosus*/E. multilocularis
- Swimmer’s itch -schistosome infection
**Trichinella**

- common name: “pork worm”
- cosmopolitan distribution
- smallest nematode parasite of humans
- discovered in 1835 by James Paget, a medical student in London
- several species: *Trichinella spiralis*, *T. nativa*, *T. britovi*, *T. murrelli*, *T. nelsoni* and *T. pseudospiralis*
- *synanthropic* and *sylvatic* cycles

**Trichinella: Life Cycle**

**Trichinella: Muscle**
Distribution: 
*Trichinella spiralis*

Number of human trichinellosis cases in Canada: 1970 to 1997

479 cases or ~18 cases/year
## Prevalence of *Trichinella* in wildlife: BC, Alberta and NWT (%)

<table>
<thead>
<tr>
<th>Species</th>
<th>BC</th>
<th>Alberta</th>
<th>Territories</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polar bear</td>
<td>-</td>
<td>-</td>
<td>83.3</td>
</tr>
<tr>
<td>Grizzly Bear</td>
<td>35</td>
<td>51.6</td>
<td>87.5</td>
</tr>
<tr>
<td>Cougar</td>
<td>9.8</td>
<td>51.6</td>
<td>-</td>
</tr>
<tr>
<td>Wolverine</td>
<td>27.3</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>Wolf</td>
<td>16.7</td>
<td>3.5</td>
<td>-</td>
</tr>
<tr>
<td>Lynx</td>
<td>9.6</td>
<td>1.8</td>
<td>-</td>
</tr>
<tr>
<td>Marten</td>
<td>33.1</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>Black bear</td>
<td>11.9</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>Arctic or Red Fox</td>
<td>0</td>
<td>3.4</td>
<td>2.6</td>
</tr>
<tr>
<td>Wolverine</td>
<td>-</td>
<td>-</td>
<td>3.8</td>
</tr>
</tbody>
</table>


## Trichinellosis: Hot Spots

<table>
<thead>
<tr>
<th>Country</th>
<th>2003</th>
<th>2004</th>
</tr>
</thead>
<tbody>
<tr>
<td>Argentina</td>
<td>945</td>
<td>710</td>
</tr>
<tr>
<td>Bulgaria</td>
<td>195</td>
<td>88</td>
</tr>
<tr>
<td>Croatia</td>
<td>177</td>
<td>120</td>
</tr>
<tr>
<td>Georgia</td>
<td>136</td>
<td>N/A</td>
</tr>
<tr>
<td>Latvia</td>
<td>22</td>
<td>24</td>
</tr>
<tr>
<td>Poland</td>
<td>40</td>
<td>172</td>
</tr>
<tr>
<td>Romania</td>
<td>N/A</td>
<td>780</td>
</tr>
<tr>
<td>Russia</td>
<td>N/A</td>
<td>514</td>
</tr>
<tr>
<td>Spain</td>
<td>51</td>
<td>33</td>
</tr>
<tr>
<td>Thailand</td>
<td>N/A</td>
<td>212</td>
</tr>
<tr>
<td>Turkey</td>
<td>N/A</td>
<td>625</td>
</tr>
</tbody>
</table>

## Recent trichinellosis outbreaks

<table>
<thead>
<tr>
<th>Place</th>
<th>Source</th>
<th>No. Infected</th>
<th>No. Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Italy</td>
<td>hare</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Sweden</td>
<td>pork</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td>France/Fr-Canada</td>
<td>bear</td>
<td>17</td>
<td>3</td>
</tr>
<tr>
<td>Canada (BC)**</td>
<td>bear</td>
<td>43</td>
<td>N/A</td>
</tr>
<tr>
<td>Russia</td>
<td>pork, badger, bear or dog</td>
<td>71</td>
<td>N/A</td>
</tr>
<tr>
<td>Vietnam</td>
<td>pork</td>
<td>20</td>
<td>N/A</td>
</tr>
<tr>
<td>Latvia</td>
<td>pork/lacon</td>
<td>27</td>
<td>27</td>
</tr>
<tr>
<td>Latvia</td>
<td>pork</td>
<td>19</td>
<td>19</td>
</tr>
<tr>
<td>France/Fr-Algeria</td>
<td>jackal</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Argentina</td>
<td>pork/laconage</td>
<td>80</td>
<td>N/A</td>
</tr>
<tr>
<td>Thailand</td>
<td>bear</td>
<td>4</td>
<td>4 (1 Intl)</td>
</tr>
</tbody>
</table>

Source: International Commission on Trichinellosis
**Dr. A. Gajadhar, Canadian Food Inspection Agency, Saskatoon
Clinical course of trichinellosis

<table>
<thead>
<tr>
<th>Time</th>
<th>Manifestations</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Ingestion of infective larvae</td>
</tr>
<tr>
<td>5-51 days</td>
<td>Incubation period</td>
</tr>
<tr>
<td>1-8 weeks</td>
<td>Acute trichinellosis: fever, myalgia, periorbital edema, eosinophilia</td>
</tr>
<tr>
<td>Months to Years</td>
<td>Convalescence stage: recovery from disease, disappearance of symptoms</td>
</tr>
<tr>
<td>Several Years</td>
<td>Asymptomatic infection: complete recovery from illness</td>
</tr>
</tbody>
</table>

Pinworms

- Common in children - day care centers, mental institutions
- Endemic infections associated with poor hygiene
- Prevalence in children in Alberta and Northern Canada estimated at 5%
- Not life threatening
- Site of infection - colon and rectum
- Autoinfections
- Effective drug therapy

Enterobius vermicularis
**Diphyllobothrium latum:** “fish tapeworm”

- Long lasting infections
- High prevalence in Scandinavian countries—prevalence in Finland ≈ 25% in humans
- Prevalence in Northern Canada not known
- Serious pathogen: anemia, vitamin B12 deficiency, malabsorption
- Drugs available

**Diphyllobothrium latum: Life Cycle**

- **Adults** → **Eggs** → **Coracidium** → **Copepod** → **Procercoid** → **Plerocercoid** → **Fish eating mammals** → **Humans**
  - **Piscivorous Fish eat Fish** (plerocercoids accumulate)

**Diphyllobothrium**

- Images of *Diphyllobothrium* lifecycle stages
**Echinococcus**

- Long lasting infections
- Endemic cycles
  - Dingos-sheep cycle (Australia)
  - "Nurse dogs" - Turkmen tribe (Africa)
- Increased risk in people with frequent contact with wildlife-trappers, wildlife biologists
- Prevalence in Alberta not known
- Seroprevalence in select communities in Northern Canada ~8%
- Drugs not effective - treatment is surgical removal

**Echinococcus granulosus: Life Cycle**

- Adults **→** Proglottids (in canids)
- Oncosphere **→** Herbivore
- Eggs

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**Humans**

- **Hydatid Cyst**
- Human eats eggs

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**Echinococcus**

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**Echinococcus- Hydatid cyst**

E. multilocularis  
E. granulosus

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**Swimmer’s Itch**

- Parasites do make mistakes and end up in wrong hosts - result is "swimmer’s itch"
- Caused by parasites that normally infect birds called schistosomes (flatworms)
- Infection acquired by swimming in contaminated lakes
- Prevalent in Alberta and Northern Canada
- Certain schistosomes species do infect humans but in tropical and subtropical regions of the world not in North America

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**Schistosomes - life cycle**

- Adult (mammals or birds) → Eggs ↓ Snail ↓ Sporocyst (in snail) → Cercariae (free swimming) → Humans (Swimmer’s itch)
Schistosomes