The Changing Landscape of Stool Parasite Diagnosis and Surveillance

Ann Valley- Advanced Microbiologist
Tim Monson- Microbiologist
Supervisor
Communicable Disease Division
Wisconsin State Laboratory of Hygiene
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Objectives

• Discuss intestinal parasites seen in WI and some resources available to aid in their diagnosis
• Discuss continued use of traditional diagnostic methods for parasite detection
• Describe multi-target diagnostic assays and rapid antigen tests that are currently available for parasite detection and their effect on reporting and disease surveillance

Let’s talk about coccidian parasites:

• Cryptosporidia
• Cyclospora cayetanensis
• Cystoisospora

Cryptosporidium spp.

• Most common cause of waterborne disease in the US.
• Most common parasite infection in WI
• Spread through contaminated food or water or direct or indirect contact with human or animal feces.
• Produces watery diarrhea 2-10 days post exposure which may last up to 2 weeks
Cryptosporidium spp.

- Each person has an average 0.14 grams of fecal material on their perianal surface if they do not take a pre-swim shower with soap.
- A single diarrheal accident can introduce $10^7$-$10^8$ Cryptosporidium oocysts into the water—enough to cause infection with a single mouthful of pool water.
- Crypto survives up to 11 days at chlorine concentrations found in most pools.

Cyclospora cayetanensis

- Summer 2013
  - June 15-29: 86 Nebraska and 153 Iowa cases associated with salad consumption at 2 national restaurant chains
  - July-August: 278 Texas cases associated with cilantro consumption at local restaurant

Cystoisospora belli

- Worldwide distribution
- More common in immunocompromised individuals
- Institutional outbreaks have occurred in the US
- Presents with non-bloody diarrhea which can last for weeks; more severe in children and elderly

The Flagellates: Giardia and D.fragilis
**Giardia spp.**

- Global distribution
- Diarrhea, malabsorption (1-2 weeks)
- Risk factors:
  - Travel to endemic areas
  - Backpackers, campers
  - Consumption of contaminated drinking water or water from lakes, rivers
  - Child-care workers

**Dientamoeba fragilis**

- Worldwide distribution
- Diarrhea and abdominal pain.
- Possible association with pinworm infections
- Fragile and may not survive well outside host
- Increased risk for those living under poor sanitary conditions

**E.histo/dispar and B. hominis**

- Entamoeba histolytica/dispar
  - More common in tropical areas
  - Illness can range from asymptomatic to amebic dysentery
  - Complications may include organ invasion
  - Important to differentiate species

- Blastocystis hominis
  - Clinical significance is questionable.
  - Disease may be associated with parasite load or with specific subtypes.
  - Suggestion of correlation of B. hominis infection with Irritable Bowel Disease (IBS)
  - Traveler’s diarrhea.

**Balantidium coli**

- Infection occurs when the cysts are ingested by eating contaminated food or water.
- Approximately 1% of people worldwide are infected.
- Occurs mainly in developing countries.
- Higher risk among pig farmers
- Diarrhea, weight loss, dysentery

**Microsporidium**

- Worldwide distribution
- 1200 species (13 known to infect humans)
- More common in immunocompromised patients (HIV)
- Many clinical manifestations including diarrhea, corneal and muscular infection

**WORMS!!!!!**

- Nematodes
- Cestodes
- Trematodes
**Nematodes**

- **Enterobius vermicularis**: Pinworm
  - Most common worm infection in the US
  - Occurs in young children and may spread to family members
  - Eggs may survive 2-3 weeks on clothing and surfaces.

- **Ascaris**
- **Whipworm**
- **Hookworm**

**Diphyllobothrium latum** (Freshwater fish tapeworm)
- Largest tapeworm that can infect humans
- Consumption of raw or undercooked fish
- Predominantly in Northern hemisphere
- Most cases asymptomatic but may cause diarrhea, intestinal obstruction, gall bladder disease

**Taenia spp.**
- Consumption of undercooked beef and pork
- Digestive issues with weight loss
- Cysticercosis (T. solium)
- Recognized as one of the 5 Neglected Parasitic Diseases in the US by CDC.

**Liver Flukes**
- Ingestion of the metacercarial stage of the parasite.
- Adult flukes develop in ~3 months
- Intestinal obstruction and accumulation of abdominal fluids due to the blockage of bile ducts.

**Trichrome Stain**

- E. histolytica/dispar
Trichrome Stain

E. histolytica/dispar
Entamoeba coli

Modified Acid Fast and Hot Safranin

Cryptosporidium
Cyclospora
Cystoisospora belli

Images from CDC DPDX

Wet Mount

Giardia trophozoite stained using Trichrome
Giardia cyst stained using Trichrome
Giardia cyst from wet mount using Iodine

Epifluorescence

Cyclospora cayetanensis
Cystoisospora belli

Images from CDC DPDX

Identification of worms
Non-Traditional Parasite Dx Tests

- Serology
- Microplate EIA
- Direct Fluorescent Antibody
- Rapid Cartridge Assays
  - Optical immunoassay
  - Lateral flow immunoassay
- Polymerase Chain Reaction (PCR)- Single target
- Multi-target Assays
  - PCR- based
  - Film array
  - Bead-based technology

Serology- Stool Parasites

- No serology tests available at WSLH for diagnosis of stool parasite infections
- CDC offers a limited menu of test options for stool parasite detection in serum
  - Antibody detection
- Reference laboratories might offer serology testing for the more common (to the U.S.) systemic stool parasites

Serology Testing- CDC

<table>
<thead>
<tr>
<th>Parasite</th>
<th>Test</th>
<th>Specimens</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. histolytica</td>
<td>EIA</td>
<td>Serum or CSF</td>
</tr>
<tr>
<td>Cryptosporidium</td>
<td>Immunoblot</td>
<td>Serum or CSF</td>
</tr>
<tr>
<td>Echinococcus</td>
<td>EIA/ Blot</td>
<td>Serum</td>
</tr>
<tr>
<td>Paragonimus</td>
<td>Blot Serum</td>
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<tr>
<td>Schistosoma</td>
<td>Fast-ELISA/ Blot</td>
<td>Serum</td>
</tr>
<tr>
<td>Strongyloides</td>
<td>EIA Serum</td>
<td></td>
</tr>
<tr>
<td>Toxocara</td>
<td>EIA Serum or Vitreous</td>
<td>Serum</td>
</tr>
</tbody>
</table>
**Direct Fluorescent Antibody**

- Many consider this a gold standard test
- Stool concentration recommended prior to testing to increase sensitivity*
- Read using different wavelength filter than that used for epifluorescence
- Easily adapted to a lower throughput lab
- Generally easy to read and interpret
  - Background minimal with brightly fluorescing cysts or oocysts
  - Note size and morphology of cysts/ oocysts

* Most antigen detection tests do not recommend concentration prior to testing

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**Rapid Cartridge Assays**

- Concentration not recommended prior to use
- Very easy to use; Must strictly adhere to the procedure times in the package insert
- In general, stool antigen flows across a membrane containing antibody against the targeted parasite(s); Ab-Ag reaction leads to an immunochromatographic (colorimetric) reaction within a defined time period
- Limited to *Cryptosporidium* and *Giardia* with exception of BioSite Triage® which also detects *E. histolytica*

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**2012-2013 Multi-site RCA Study**

- WSLH participated in a CDC-sponsored study looking at the efficacy of the two major *Cryptosporidium* RCA’s on the market at the time (Meridian Immunocard STAT! and Remel Xpect)
- Stools positive for *Cryptosporidium* by RCA at the clinical laboratory were submitted to WSLH; DFA (gold standard) and both RCA methods above were performed in-house
2012-2013 Multi-site RCA Study

- 176 stool specimens evaluated at WSLH
- Meridian ImmunoCard STAT!
  - Sensitivity 94%
  - Specificity 73%
- Remel Xpect Cryptosporidium
  - Sensitivity 74%
  - Specificity 87%
- Similar data generated by the two other study sites

Single Target PCR Tests

- Many reference laboratories, state/regional public health laboratories and CDC have developed single target PCR assays to detect stool parasites
- Common targets are Cryptosporidium and E. histolytica (histolytica/dispar)
- High complexity, high costs to test and limitations of lab developed tests (LDT’s) are some of the main reasons these tests did not become widely available

Multi-Target Stool Pathogen Tests

- Last two years has seen a major movement by numerous commercial test developers to get tests approved that will detect multiple stool pathogens simultaneously
- These rapid, multi-target tests have begun to re-shape the clinical testing and public health surveillance landscape
- Accurate and comprehensive testing is available without the need for traditional test methods on which current surveillance was defined/built

Luminex xTAG GPP

- Both ASR and RUO kits available
- Besides detecting multiple viral and bacterial stool pathogens, has the ability to detect Cryptosporidium, Giardia and E. histolytica
- Bead-based technology; high throughput but labor intensive
- Next generation platform in development that will be more hands-off and efficient

BioFire FilmArray® GI Panel

- 22-target diagnostic GI panel; detects common bacterial, viral and the following parasitic agents:
  - Cryptosporidium
  - Cyclospora
  - E. histolytica
  - Giardia
- Multiplex PCR system
- Minimal hands-on time; results in approximately one hour; low throughput

BD Max™ Enteric Parasite Panel

- Currently under development/not launched*
- Will detect three common human stool parasites:
  - Cryptosporidium
  - E. histolytica
  - Giardia
- Flexible panel testing options; will complement enteric bacteria panel and enteric virus panel (also in development)
**Cryptosporidiosis Case Definition Changes - 2015**

Updated CSTe case definition relies on laboratory diagnostic test used to diagnose infection.

**Confirmed case** = detection of organisms or DNA by:
- Direct fluorescent antibody (DFA).
- Polymerase Chain Reaction (PCR).
- Enzyme Immunoassay (EIA - microplate only).
- Light microscopy.

**Probable case** = detection of antigen by:
- Enzyme Immunoassay (EIA for antigen, microplate not specified).
- Immunochromatographic card test (i.e. ImmunoCard STAT!, some labs call these as EIAs).
- Rapid card test (some labs also call these EIAs).
- Unknown method.

**Other Potential Impacts on Stool Parasite Surveillance**

- Potential for improved surveillance due to detection of more parasites
  - Parasite testing may not be routinely ordered by clinicians in cases of GI illness
  - Expanded use of multi-target assays that include parasites will increase likelihood of their detection (Cyclospora, Crypto, Giardia; "syndromic" testing
  - Significance of results in patients with multiple pathogens detected will have to be weighed

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**Molecular epidemiology - CryptoNet**

- CDC launched molecular subtyping network for Cryptosporidium isolates.
- Differentiation/connection of outbreaks.
- Source tracking.
  - Linkage between cases and environmental source.
  - Identification of common zoonotic exposure.
- Improved epidemiologic understanding.
  - Geographic distribution
  - Common subtypes

*Subtyping can only be performed on specimens NOT fixed in formalin.

**Stool Specimen Submission - Cryptosporidium**

- Stool specimens positive for Cryptosporidium are asked to be sent to WSLH for genotyping and surveillance testing
  - Fee exempt confirmation of positive specimens
  - Analysis of specimens unable to be confirmed or whose test results are in question
  - Genotyping performed for surveillance of clusters of illness
  - Can use Dunham Express courier service

**Stool Specimen Submission - E. histolytica/ dispar**

- If E. histolytica/ dispar is detected in a stool wet preparation or permanent stained smear, a clinician may inquire about the confirmation of the pathogenic E. histolytica
  - Some of the multi-target assays will differentiate
  - Reference laboratory performing the E. histolytica microplate EIA may differentiate
  - Submit unfixed stool (Raw, enteric culture transport, PVA) along with stool in formalin to WSLH for referral to CDC for PCR testing
Resources

Wisconsin State Laboratory of Hygiene
- Contact WSLH CDD Customer Service

Dunham Express Courier
- (800)236 – 7127
- Account 7271
- Next day delivery except on Sat/Sun
- Call WSLH customer service (800)862-1013 during normal work hours or the WSLH pager service (800)263-3280 after hours or weekends to discuss STAT testing

Wisconsin or national reference laboratories
- Many reference laboratories maintain the ability to perform comprehensive ova & parasite examinations of stool specimens
- Many may also be implementing multi-target PCR-based assays that will cover the common human parasitic pathogens that might be acquired and or detected locally

Centers for Disease Control and Prevention- Division of Parasitic Disease
- DPDx Site maintained by the Division of Parasitic Diseases and Malaria (DPDM)
  - http://www.cdc.gov/dpdx/
  - Can submit digital images to DPDx via email for telediagnosis; Fill out specimen submission form 50.34 (available from their web site or link from WSLH)
  - Testing is fee exempt; STAT testing should be worked out directly with CDC contacts

Information regarding which tests CDC offers can be acquired from the CDC web site:
  - http://www.cdc.gov/laboratory/specimen-submission/list.html
  - If submitting directly to CDC, we ask that you fill out a WSLH req form (A) and fax it along with the CDC form 50.34 (if filled out) to WSLH (608-890-2548); All reports come back through WSLH; if already in the WSLH system, reporting will be expedited

Summary
- Traditional test methods are still utilized and effective for the detection of human stool parasites
- While not seen often, clinical laboratory parasitologists need to be able to recognize and identify human parasitic pathogens when they are present
- Numerous diagnostic resources are available via online (web-based), telediagnosis or via specimen referral.
Summary

- There are numerous commercial tests available for the detection of stool parasites in humans; **WSLH does not endorse any available test** but encourages laboratories to look at test performance, sensitivity, specificity and test validation when deciding to implement any such test.
- Case definitions and surveillance are subject to change due to shifting landscape of diagnostic parasitology in WI and the U.S.

Contact Information

WSLH
- Ann Valley - ann.valley@slh.wisc.edu
- Tim Monson - timothy.monson@slh.wisc.edu
- WSLH Customer Service - (800)862-1088

WDPH
- Rachel Klos (608)267-7422
- Communicable Epi Section (608)267-9003

CDC Division of Parasitic Diseases
- (404) 718-4100 / dpd@cdc.gov