Wisconsin State Laboratory of Hygiene
UNIVERSITY OF WISCONSIN–MADISON

The Changing Landscape of Stool Parasite Diagnosis and Surveillance

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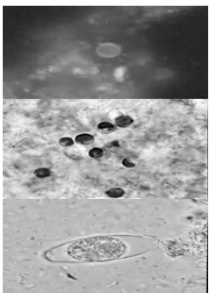
WCLN Audioconference- Feb 11, 2015

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*

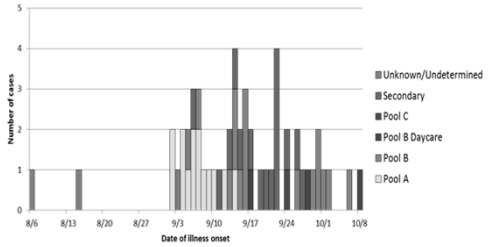


Cryptosporidium spp.

- 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.

Cryptosporidium spp. Community Pool Outbreak 2013

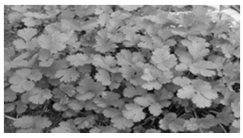


Cryptosporidium spp.

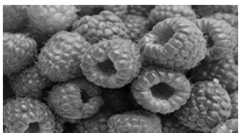
- Each person has an average 0.14 grams of fecal material on their perianal surface if they do not take a pre-swim shower with soap.
- A single diarrheal accident can introduce 10^7-10^8 *Cryptosporidium* oocysts into the water- enough to cause infection with a single mouthful of pool water.
- Crypto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

Cyclospora cayetanensis

- Found in tropical and subtropical regions
- Outbreaks in the US are associated with contaminated fresh produce.



- Incubation: ~ 1 week
- Watery diarrhea, cramping, low-grade fever
- Symptoms may last for several weeks
- Treatment with SXT



Cyclospora cayetanensis

Summer 2013

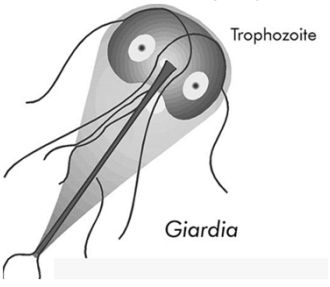
- June 15-29: 86 Nebraska and 153 Iowa cases associated with salad consumption at 2 national restaurant chains
- July-August: 278 Texas cases associated with cilantro consumption at local restaurant.

http://www.fda.gov/food/recallsoutbreaksemergencies/outbreaks/ucm361637.htm

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*



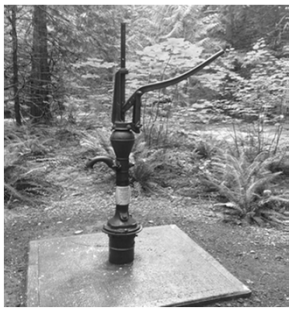
Giardia spp.

- 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

Giardia spp.

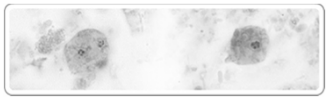
Giardiasis associated with transient well contamination:

August 2014



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

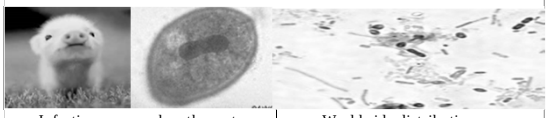


E.histo/dispar and *B. hominis*

- *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 ***Microsporidium***

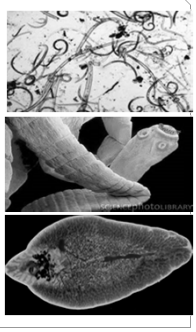


- Infection occurs when the cysts are ingested by eating contaminated food or water.
- Approximately 1% of people worldwide are infected.
- Occurs mainly in developing countries.
- Higher risk among pig farmers
- Diarrhea, weight loss, dysentery

- Worldwide distribution
- 1200 species (15 known to infect humans)
- More common in immunocompromised patients (HIV)
- Many clinical manifestations including diarrhea, corneal and muscular infection

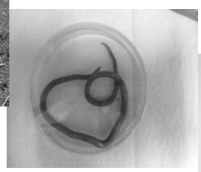
WORMS!!!!!

- Nematodes
- Cestodes
- Trematodes



Nematodes

Whipworm



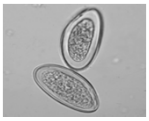
Ascaris

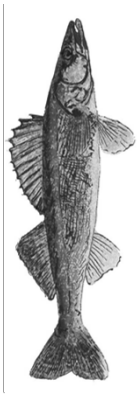
Hookworm

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.



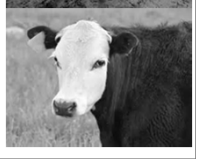


Diphyllobothrium latum
(Freshwater fish tapeworm)

- 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.

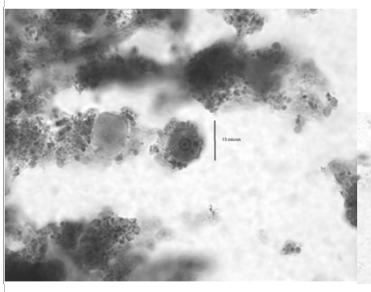
- Consumption of undercooked beef and pork
- Digestive issues with weight loss
- Cysticercosis (*T. solium*)
- Recognized as one of the 5 Neglected Parasitic Diseases in the US by CDC.



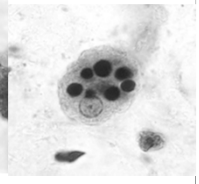
Liver Flukes

- Ingestion of the of 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.

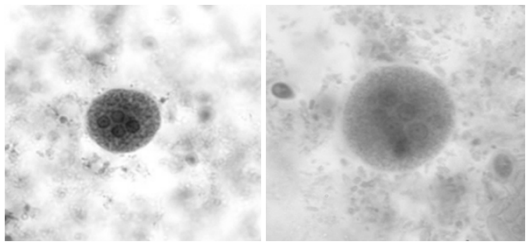
Trichrome Stain



E. histolytica/dispar

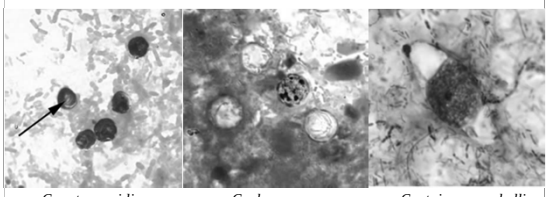


Trichrome Stain



E. histolytica/dispar *Entamoeba coli*

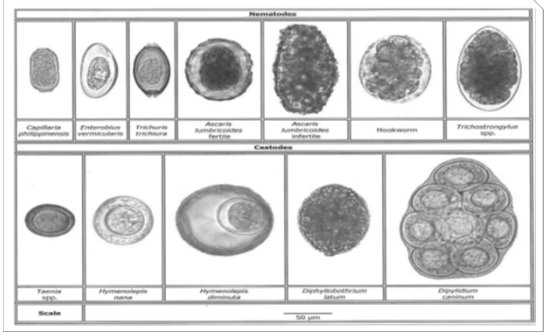
Modified Acid Fast and Hot Safranin



Cryptosporidium *Cyclospora* *Cystoisospora belli*

Images from CDC DPDX

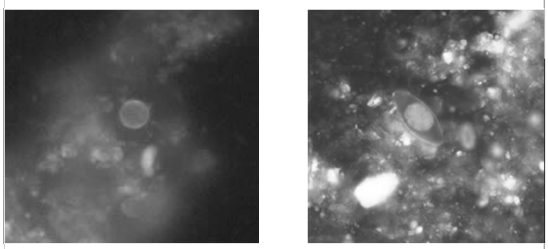
Wet Mount



Wet Mount

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

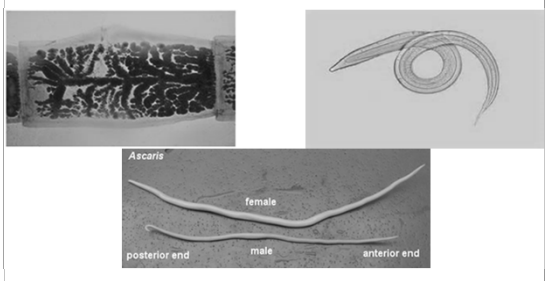
Epifluorescence



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

Antibody Detection

Parasite	Test	Specimen
E. histolytica	EIA	Serum
Baylisascaris	Immunoblot	Serum or CSF
Cysticercosis (T. solium)	Immunoblot	Serum or CSF
Echinococcus	EIA/ Blot	Serum
Paragonimus	Blot	Serum
Schistosoma	Fast-ELISA/ Blot	Serum
Strongyloides	EIA	Serum
Toxocara	EIA	Serum or Vitreous
Trichinella	EIA	Serum

Antigen Detection

- Microplate EIA
  - High throughput
  - May cost more in long run if kits not used up
  - Generally very good sensitivity and specificity
- DFA
  - Very good sensitivity and specificity
  - Requires fluorescent microscope
- Rapid cartridge assays
  - Easy to perform
  - Assess performance, PPV/NPV before implementation

http://www.cdc.gov/dpdx/diagnosticProcedures/stool/antigendetection.html

Organism	Kit name	Manufacturer - distributor*	Type of Test*
Cryptosporidium spp.	Crypto CELISA	Cellabs	EIA
	PARA-TECT™ Cryptosporidium Antigen 96	Medical Chemical Corporation	EIA
	ProSpecT Rapid	Remel	EIA
	ProSpecT	Remel	EIA
	Cryptosporidium	TechLab	EIA
	Cryptosporidium	Wampole	EIA
	Crypto CEL	Cellabs	IFA
	XPect Crypto	Remel	Rapid
Cryptosporidium spp./Giardia duodenalis	PARA-TECT™ Cryptosporidium/Giardia DFA 75	Medical Chemical Corporation	DFA
	Merifluor	Meridian	DFA
	ProSpecT	Remel	EIA
	Crypto/Giardia CEL	Cellabs	IFA
	ColorPAC*	Becton Dickinson	Rapid
	ImmunoCard STAT!*	Meridian	Rapid
	XPect	Remel	Rapid
Cryptosporidium spp./Giardia duodenalis/Entamoeba histolytica/dispar	Triage	BioSite	Rapid
Entamoeba histolytica	Entamoeba CELISA	Cellabs	EIA
	E. histolytica	Wampole	EIA
	E. histolytica II	TechLab	EIA
Entamoeba histolytica/E. dispar	ProSpecT	Remel	EIA
Giardia duodenalis	Giardia CELISA	Cellabs	EIA
	PARA-TECT™ Giardia Antigen 96	Medical Chemical Corporation	EIA
	ProSpecT	Remel	EIA
	Giardia II	TechLab	EIA
	Giardia	Wampole	EIA
	GiardiaEIA	Antibodies, Inc.	EIA
	Giardia CEL	Cellabs	IFA
	ProSpecT	Remel	Rapid
	Simple-Read Giardia	Medical Chemical Corporation	Rapid
Wuchereria bancrofti	Filariasis CELISA	Cellabs	EIA

Microplate EIA

- Many commercial kits on market for *Crypto* and *Giardia*
- Few kits available for *E. histolytica/ dispar* (only Techlab EIA assay will differentiate *histolytica/dispar*)

Google Images

Direct Fluorescent Antibody

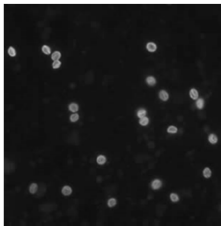
- Many consider this a gold standard test
- Stool concentration recommended prior to testing to increase sensitivity*
- Read using different wavelength filter than that used for epifluorescence
- Easily adapted to a lower throughput lab
- Generally easy to read and interpret
  - Background minimal with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

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

Direct Fluorescent Antibody

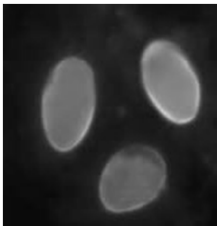
***Cryptosporidium* oocysts**

image from CDC



***Giardia* cysts**

Image from CDC



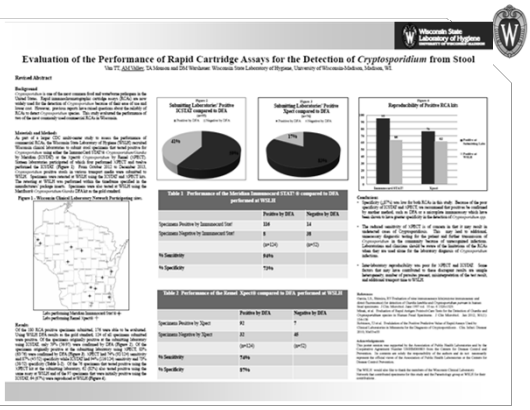
Rapid Cartridge Assays

- 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 *Crypto* and *Giardia* with exception of BioSite Triage® which also detects *E. histolytica*

Rapid Cartridge Assays

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



Evaluation of the Performance of Rapid Cartridge Assays for the Detection of *Cryptosporidium* from Stool

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.

Cryptosporidiosis Case Definition Changes- 2015

DPH survey of clinical laboratories to collect required information:

- Test kits used
- Testing protocols
- Referral practices

Development of classification algorithm to help local health departments with classification.

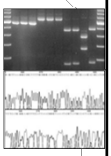
Benefits:

- More accurate confirmed vs. probable case numbers.
- Improved knowledge of how many labs are using rapid card tests.
  - Rapid card tests have a low positive predictive value and give many false positives.

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

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**.

CDC

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

Resources

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

Resources

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

Resources

- 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

CDC Test Directory


CDC Centers for Disease Control and Prevention
CDC 24/7: Saving Lives, Protecting People™
Submitting Specimens to CDC

Test Directory

CDC's Infectious Diseases Laboratories provides an online Test Directory that allows you to identify the right test for your needs. The searchable Test Directory features an up-to-date list of orderable tests and provides information on specimen requirements, contact information, test turnaround times, and other supplemental information. Access the directory here or while completing a Specimen Submission Form.

You may also download a copy of the entire Test Directory.

Effective December 5th 2014, an updated test directory is available. View the major list of changes here.

Search
Narrow the results with a keyword, test title, test synonym, or point of contact:
Go

A | B | C | D | E | F | G | H | I | J | K | L | M | N | O | P | R | S | T | V | X | Y

Showing 334 of 334 tests.

Test Name	Test Code
Acanthamoeba Molecular Detection	CDC-10471
Actinomyces – Anaerobic ID	CDC-10483
Actinomycetes-Aerobic ID	CDC-10548

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

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WDPH

Rachel Klos(608)267-7422

Communicable Epi Section(608)267-9003

CDC Division of Parasitic Diseases

(404) 718-4100/ dpd@cdc.gov

Thank You!

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Questions?

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