Challenges with Foodborne Parasites

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The contents of this presentation are my own, and do not necessarily reflect the view and/or policies of the Food and Drug Administration or its staff.
Foodborne parasitology is a complex field

<table>
<thead>
<tr>
<th>Foods</th>
<th>Protozoa</th>
<th>Nematodes</th>
<th>Cestodes</th>
<th>Trematodes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beef</td>
<td><em>Toxoplasma gondii</em></td>
<td><em>Trichinella spp.</em></td>
<td><em>Taenia saginata</em></td>
<td><em>Fasciola hepatica</em></td>
</tr>
<tr>
<td>Pork, Other meat</td>
<td><em>Toxoplasma</em></td>
<td><em>Trichinella spp.</em></td>
<td><em>Taenia solium/asiatica</em></td>
<td><em>Paragonimus</em> (wild boar)</td>
</tr>
<tr>
<td>Milk</td>
<td><em>Toxoplasma</em></td>
<td><em>Anisakis spp.</em></td>
<td><em>Diphyllobothrium</em></td>
<td><em>Clonorchis</em></td>
</tr>
<tr>
<td>Fish/squid</td>
<td><em>Cryptosporidium</em></td>
<td><em>Gnathostoma</em></td>
<td><em>Opisthorchis</em></td>
<td><em>Paragonimus</em></td>
</tr>
<tr>
<td>Crabs, shrimps</td>
<td><em>Giardia lamblia</em></td>
<td><em>Gnathostoma</em></td>
<td><em>Echinostomes</em></td>
<td></td>
</tr>
<tr>
<td>Shell fish</td>
<td><em>Cryptosporidium gondii</em></td>
<td><em>Gnathostoma</em></td>
<td><em>Clonorchis</em></td>
<td></td>
</tr>
<tr>
<td>Snails/slugs</td>
<td><em>Cyclospora</em></td>
<td><em>Angiostrongyulus</em></td>
<td><em>Echinococcus</em></td>
<td></td>
</tr>
<tr>
<td>Fruit/vegetables (raw)</td>
<td><em>Cryptosporidium</em></td>
<td><em>Angiostrongyulus</em></td>
<td><em>Fasciola hepatica</em></td>
<td></td>
</tr>
<tr>
<td>Water</td>
<td><em>Balantidium coli</em></td>
<td><em>Ascaris</em></td>
<td><em>Fasciolopsis</em></td>
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A large number of parasites are foodborne….
Foodborne Parasitology Challenges at the U.S. FDA

Lack of molecular epidemiology tools to assist in outbreaks investigations and link cases with source of contamination

Lack of streamlined detection methods to implement high-throughput screening/field testing in food commodities that are regulated by the FDA

Lack of laboratory-based surveillance to identify food commodities contaminated with parasites
Parasitology Program Challenges
CFSAN/OARSA

- Implement a science-based framework to address food safety issues associated with foodborne parasites
- Coordinate the scientific activities with FDA’s programmatic objectives and goals to maximize research outcomes
- Provide the U.S. FDA with practical solutions to minimize or eliminate the public health issues caused by foodborne parasites
Some parasites (e.g., *Trypanosoma cruzi*) may be foodborne, but are more frequently associated with vector-borne transmission.

**Health Benefits of Acai berry**

- Improves Digestion
- Great de-tox agent
- Reduces effects of Aging
- Improves stamina
- Rich in Antioxidant
- Sources of Anthocyanins
- Ensures a Healthy heart
- Lowers cholesterol
Oral Transmission of Chagas Disease

Maria Aparecida Shikanai-Yasuda\textsuperscript{1,2} and Noemí Barbosa Carvalho\textsuperscript{2,3}

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Chagas disease is now an active disease in the urban centers of countries of nonendemicity and endemicity because of congenital and blood and/or organ transplantation transmissions and the reactivation of the chronic disease in smaller scale than vectorial transmission, reported as controlled in countries of endemicity. Oral transmission of Chagas disease has emerged in unpredictable situations in the Amazon region and, more rarely, in areas of nonendemicity where the domiciliary triatomine cycle was under control because of exposition of the food to infected triatomine and contaminated secretions of reservoir hosts. Oral transmission of Chagas disease is considered when >1 acute case of febrile disease without other causes is linked to a suspected food and should be confirmed by the presence of the parasite after direct microscopic examination of the blood or other biological fluid sample from the patient.

Only a few autochthonous cases have been reported in the United States, although the most important triatomine vectors were found in large areas: in the eastern United States (Triatoma sanguisuga), central Texas and also in Mexico (Triatoma gerstaeckeri), and Arizona and California (Triatoma protacta and Triatoma rubida)

Although many of these recommendations have been implemented, difficult access to rural areas, late diagnosis, detection of parasites in the food, their inactivation in the food handling at home, and implementation of good handling practices of food processing in Amazon and the country in general remain as challenges to control oral acute Chagas
CFSAN/OAO is pursuing studies in collaboration with various groups with different objectives:

1. Assessing *T. gondii* prevalence in oysters by effluent proximity (with Dauphin Island)
2. Evaluating the association between *T. gondii* seropositivity and obesity (using NHANES data; with OAO Stats Staff)
3. Examining the relationships between *T. gondii* seropositivity and selected biomarkers of chronic disease and food exposures among the Amish (with UMD-School of Medicine)
C. cayetanensis

The only species of genus *Cyclospora* known to infect humans

Food commodities = fresh produce

Source: http://www.cdc.gov/dpdx/az.html
**Infective Stage** – is the stage in the life cycle of a parasite during which it is capable of producing infection.

**Protozoa (Apicomplexa)**

**Oocyst** - The stage of a coccidian protozoa that is evacuated with the feces. **Sporocysts** develop within the oocyst and, as the oocyst matures.

**Sporocyst** - In protozoa, a cyst that develops within an oocyst of a coccidian protozoa in which **sporozoites** develop.

**Sporozoite** - The structure (infective unit) that develops within an oocyst that, when freed from the oocyst after ingestion by a suitable host, penetrates the intestinal wall initiating infection.

**Sporulation and excystation in C. cayetanensis**
Multi-State Outbreaks of Cyclosporiasis - 2013

631 cases of cyclosporiasis in 25 states

- A total of 270 cases
- More than 70 clusters* of cases linked to multiple restaurants and grocery stores
- One cluster with 25 cases (Restaurant A; with 18 confirmed and 7 probable) associated with one restaurant.
- The only ingredient to which all 25 case-patients above were exposed was cilantro.

*A cluster of illnesses was defined as more than one unrelated ill person (i.e.: individuals that do not know each other) who report eating at the same restaurant location, attending a common event, or shopping at the same location of a grocery store before becoming ill.
2013 multistate outbreaks of Cyclospora cayetanensis infections associated with fresh produce: focus on the Texas investigations

The outbreaks of cyclosporiasis in 2013 underscore the need for molecular subtyping to complement evidence from epidemiological investigations, potentially assisting in identifying the number of outbreaks in a given season and suggesting links between clusters and facilitating source tracking.

The specific challenges posed by Cyclospora include under-detection of cases, lack of subtyping methods to link cases to each other or to specific food items, and the absence of practical tools to detect the organism in food and potential sources of contamination in the environment (e.g., soil and irrigation water). Advances in restaurant, A, B, C, and grocery store clusters.

Cilantro was the most likely vehicle of infection in...
* Surveys in Mexico?
2014 cyclosporiasis outbreaks:
304 ill persons in 19 states/133 cases from TX
2015?
GASTROINTESTINAL PANEL

The most comprehensive FDA-cleared GI test on the market.

Learn More »

GASTROINTESTINAL (GI) PANEL

The FilmArray Gastrointestinal (GI) Panel tests for common gastrointestinal pathogens including viruses, bacteria and parasites that cause infectious diarrhea. The integrated FilmArray system brings sample to results in about an hour, with only 2 minutes of hands-on time.

- Simple: 2 minutes of hands-on time
- Easy: No precise measuring or pipetting required
- Fast: Turnaround time of about 1 hour
- Comprehensive: 22 target GI panel

Download Product Sheet

Bacteria
- Campylobacter (jejuni, coli and upsaliensis)
- Clostridium difficile (Toxin A/B)
- Plesiomonas shigelloides
- Salmonella
- Yersinia enterocolitica
- Vibrio (parahaemolyticus, vulnificus and cholerae)
- Vibrio cholerae

Diarrheagenic E.coli/Shigella
- Enterotoxigenic E. coli (ETEC)
- Enteropathogenic E. coli (EPEC)
- Shiga-like toxin-producing E. coli (STE C) stx1/stx2
- Shigella O157
- Shigella/Enteroaggregative E. coli (EIEC)

Parasites
- Cryptosporidium
- Entamoeba histolytica
- Cyclospora cayetanensis
- Giardia lamblia

Viruses
- Adenovirus F40/41
- Rotavirus A
- Astrovirus
- Sapovirus (I, II, IV and V)
- Norovirus GI/GII

Panel Specifications

<table>
<thead>
<tr>
<th>Sample Handling</th>
<th>Performance Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample Type: Stool in Cary Blair</td>
<td>Hands-on time: Approx. 2 minutes</td>
</tr>
<tr>
<td>Sample Volume: 200 μL</td>
<td>Run turnaround time: About 1 hour</td>
</tr>
</tbody>
</table>
BioFire Film array – Gastro intestinal panel

- Detects 22 intestinal pathogens including the parasites:
  - *Cyclospora cayetanensis*
  - *Cryptosporidium* spp.
  - *Giardia intestinalis* (lamblia)
  - *Entamoeba hystolitica*
Findings - Environmental Assessment (May 2015)

“Raw sewage is overflowing from the firm’s septic system onto exterior grounds that employees walk and delivery vehicles drive, a dead dog is along a fence immediately outside the facility…”

“…no sanitary controls in place for processing water; the water is trucked in from an unknown source and no chlorine or other treatment is utilized.”

“…multiple restroom toilets leak sewage from the toilet / floor juncture, restroom hand washing sinks are non-operational and so community drums of water are shared for hand washing…”
As of September 14, 2015 (3pm EDT), CDC had been notified of 546 ill persons with confirmed Cyclospora infection from 31 states in 2015. Most of these persons—319 (58%) of 546—experienced onset of illness on or after May 1, 2015, and did not have a history of international travel within two weeks before illness onset. These 319 persons were from the following 23 states: Arkansas (3), California (2), Connecticut (5), Florida (13), Georgia (26), Iowa (1), Illinois (9), Kansas (2), Massachusetts (12), Maryland (1), Michigan (2), Missouri (1), Montana (3), Nebraska (1), New Jersey (7), New Mexico (2), New York (excluding NYC) (10), New York City (22), North Carolina (1), Texas (179), Utah (1), Virginia (3), Washington (2), and Wisconsin (11).

Note the disparity regarding number of cases by states
9 states + DC do not report cyclosporiasis (as of August 2015; in red)
Walmart has also issued recalls based on traceback investigations…
Mexican Cilantro Source of Outbreak

by Derek Merman in Cyclospora

The Texas Department of State Health Services has reported that 205 people have contracted Cyclosporiasis after eating cilantro contaminated with the bacterium Cyclospora. Nine Texas counties have been affected by the contaminated cilantro. Travis County has reported the most cases with 77, followed by 17 cases in Dallas County, 9 cases in Collin County, 9 cases in Tarrant County, and 8 cases in Denton County. Johnson, Kaufman, Parker, and Rockwall counties have all reported one case of Cyclospora poisoning. Wisconsin has also reported eight cases of the illness.

This is not the first time that cilantro from the Puebla region of Mexico has caused a Cyclospora outbreak. In fact, this is the third year in a row that contaminated cilantro has caused an outbreak. In 2013, 270 people were sickened because of cilantro from the Puebla region of Mexico. In 2014, 133 Texans contracted Cyclospora. Because of the recurring outbreaks, the FDA launched an investigation regarding the cilantro farms. Investigators found human feces and toilet paper in some of the cilantro fields, and uncovered that the fields were being irrigated with water that was contaminated with sewage. These conditions are conducive for Cyclospora growth.

Cyclosporiasis is an illness caused by the bacterium Cyclospora. Once someone is exposed to the bacterium, it will take between 2 and 11 days before symptoms will appear. A Cyclospora infection will generally cause symptoms including watery diarrhea, loss of appetite, stomach cramps, vomiting, fever, and fatigue. The illness will last a few days for those with healthy immune systems, although it may last over a month if left untreated. If you or a loved one has persistent diarrhea over a few days, and it either does not go away or comes back, contact your doctor. Cyclospora infections are treated with antibiotics that will help end the infection.
2015 Outbreaks – Preliminary Epi curve

Reported U.S. Cases of Lab-confirmed, Non-travel-associated Cyclosporiasis with Onset of Illness since May 1, 2015 (N=253*)

*From completed case data received at CDC as of 5PM on 8 Aug 2015. Data are preliminary and subject to change.
†Diagnosis date is used in those cases for which onset date is unknown or cannot be approximated (n=4)
‡Illnesses that occurred after 4 July 2015 might not yet be reported due to the time it takes between when a person becomes ill and when the illness is reported.
Testing of food samples during the 2015 outbreaks investigation

All samples being tested by the Southeast Regional Laboratory (SRL) - ORA

Techniques used to test samples include an improved sample preparation method for fresh produce (this method is currently under multi-lab-validation and it works for cilantro and raspberries) and nested conventional PCR.

Type of samples being tested include produce and mixed foods such as salsa, pico de gallo, guacamole and a leftover burrito.

Photos provided by Lacresha Chatman, FDA/ORA/SRL

Alice Y. Ho,* Adriana S. Lopez,†‡ Michael G. Eberhart,* Robert Levenson,* Bernard S. Finkel,* Alexandre J. da Silva,‡ Jacquelin M. Roberts,‡ Palmer A. Orlandi,§ Caroline C. Johnson,* and Barbara L. Herwald‡

An outbreak of cyclosporiasis occurred in attendees of a wedding reception held in Philadelphia, Pennsylvania, on June 10, 2000. In a retrospective cohort study, 54 (68.4%) of the 79 interviewed guests and members of the wedding party met the case definition. The wedding cake, which had a cream filling that included raspberries, was the food item most strongly associated with illness (multivariate relative risk, 5.9; 95% confidence interval, 3.6 to 10.5). Leftover cake was positive for Cyclospora DNA by polymerase chain reaction analyses. Sequencing of the amplified fragments confirmed that the organism was Cyclospora cayetanensis. The year 2000 was the fifth year since 1995 that outbreaks of cyclosporiasis definitely or probably associated with Guatemalan raspberries have occurred in the spring in North America. Additionally, this is the second documented U.S. outbreak, and the first associated with raspberries, for which Cyclospora has been detected in the epidemiologically implicated food item.

Emerging Infectious Diseases • Vol. 8, No. 8, August 2002
A prototype of the gelified assay for *Cyclospora cayetanensis* was tested with DNA extracted from food.
Genomics of *C. cayetanensis*

Collaboration between FDA and CDC towards whole genome sequencing of *C. cayetanensis*

- Sequencing of different “samples” from different geographic origins to identify strain-level variations
- Sharing data, protocols and harmonization of techniques
- Development of database with genome-level annotations and improve expertise in genomics of eukaryotic microorganism

Mitochondrial and apicoplast genomes sequenced

Focus on development of metagenomics approaches for molecular epidemiology
Light at the end of the tunnel…?
Status of *C. cayetanensis* Research

- Development of database with genome-level annotations and improve expertise in genomics of eukaryotic microorganism: This will allow the development of robust metagenomics approaches for molecular epidemiology. Database will include the first draft of *C. cayetanensis* genome to be published in 2016 (CDC publication).

- Mitochondrial and apicoplast genomes were sequenced/annotated: To be published in 2016.

- Superior standards for detection of *C. cayetanensis* in water are being developed: Preliminary results to be evaluated by January of 2016.

- An improved sample preparation method for detection of *C. cayetanensis* produce is being validate: The method went through a multi-laboratory validation study which is going to be accomplished in the beginning of 2016.

- Novel qPCR methods are being developed for detection of *C. cayetanensis*: This includes the gelified PCR assay (ready to use) for detection of *C. cayetanensis* in produce. The qPCR methods are scheduled for a multi-laboratory validation study in 2016.

- Anti-*C. cayetanensis* monoclonal antibodies: Development of antibody-based detection methods, methods to better purify *C. cayetanensis* oocysts from clinical samples (for whole genome sequencing) food commodities and water (enrichments process to improve sensitivity of detection).
Thank you!

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