Cyclospora cayetanensis and Cyclosporiasis Outbreaks: Why do we need genomics?

GenomeTrakr Meeting – New and Unusual Organisms
September 26th- 28th 2018

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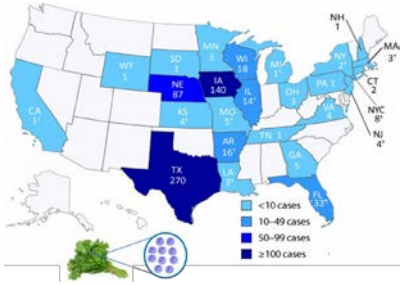
FDA/CFSAN/OARSA/DVA
Laurel, MD

Cyclospora cayetanensis

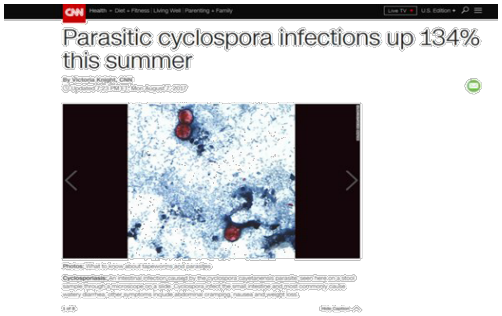
Cyclosporiasis is diarrhea illness caused by the parasite *Cyclospora cayetanensis*. People become infected by consuming food or water contaminated with sporulated oocysts of the parasite

Impact in the US



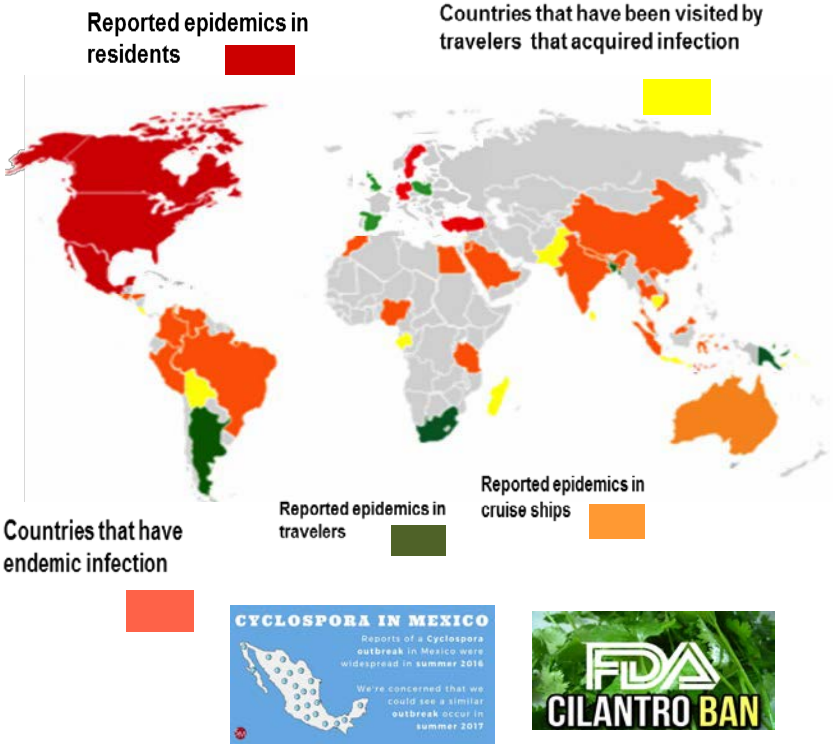
Case Count Map - 2013
A total of **631 cases** of cyclosporiasis reported from 25 states.; Some cases associated with consumption of imported **cilantro and bagged salad.**

Case Count Map - 2015
A total of **546 cases** of cyclosporiasis reported from 31 states Some cases associated with consumption of **cilantro**



Case Count Map - 2017
1,065 laboratory-confirmed cases from 40 states
At least 597 (56%) cases did not report international travel
No specific food commodity was definitively linked to cases

Global impact



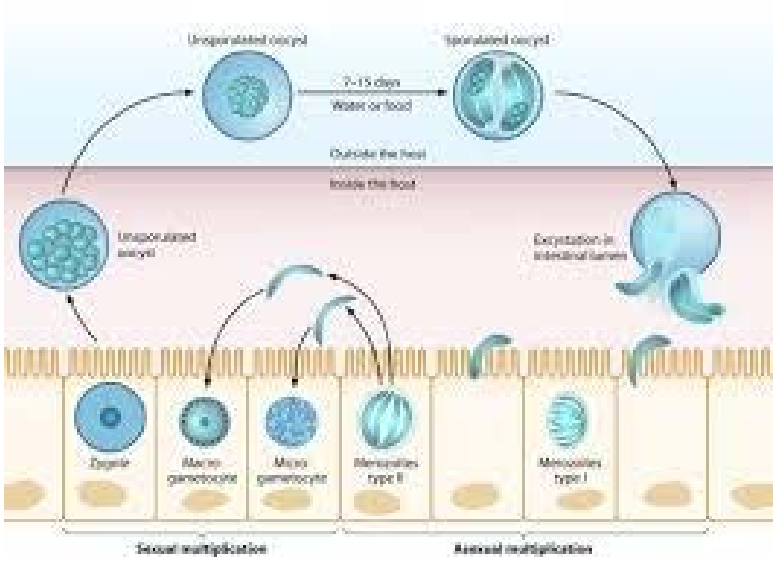
Due to its geographic distribution cyclosporiasis is considered a global public health issue.

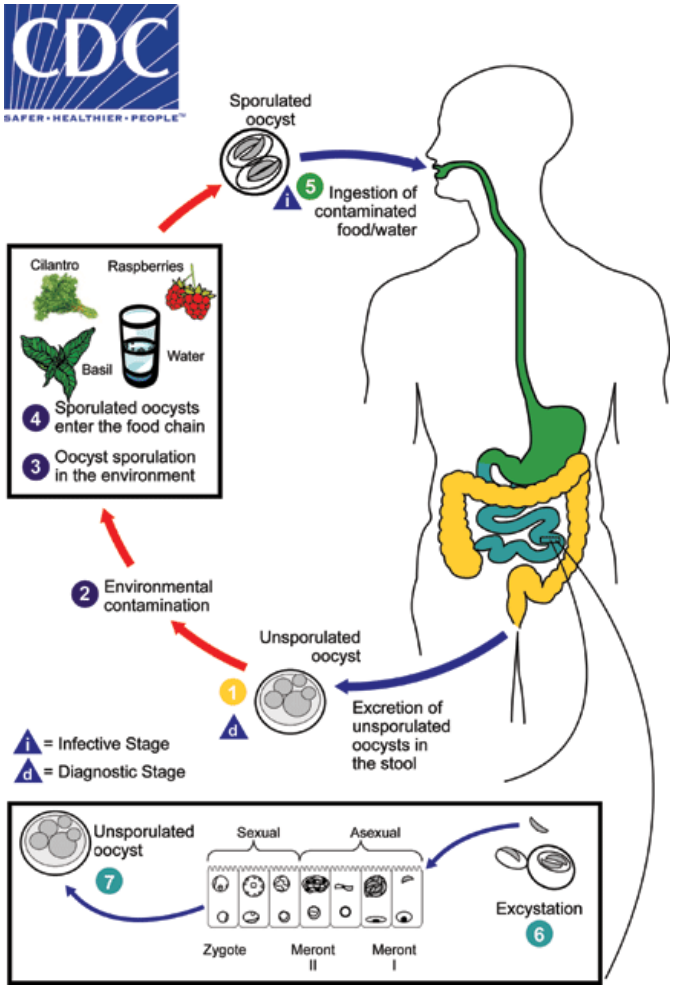
Discovering the Future To Safeguard the Present

Cyclospora cayetanensis

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

Food commodities= fresh produce

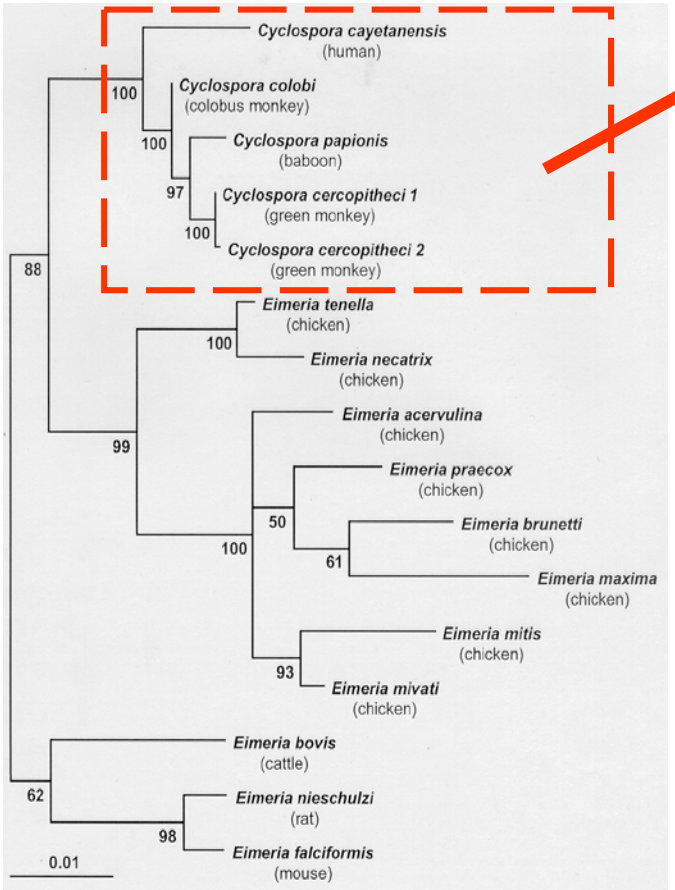




There are no *in vivo* or *in vitro* methods available to propagate *C. cayetanensis* from clinical or food samples

Source: http://www.cdc.gov/dpdx/az.html

Diversity of *Cyclospora* spp.



Morphologically identical to *C. cayetanensis*

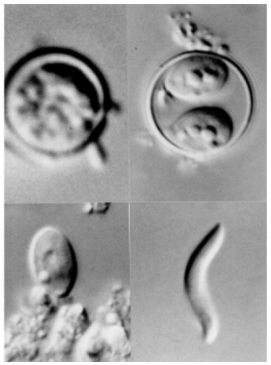
Synopses

Morphologic and Molecular Characterization of New *Cyclospora* Species from Ethiopian Monkeys: *C. cercopitheci* sp.n., *C. colobi* sp.n., and *C. papionis* sp.n.

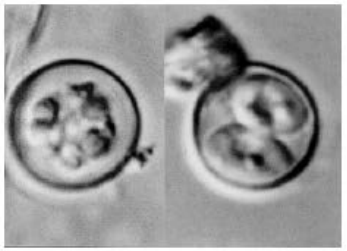
Mark L. Eberhard,* Alexandre J. da Silva,* Bruce G. Lilley,† and Norman J. Pieniazek*

*Centers for Disease Control and Prevention, Atlanta, Georgia, USA; and †University of Alabama at Birmingham, Birmingham, Alabama, USA

This report provides morphologic and molecular characterization of three parasites isolated from primates and names each isolate: *Cyclospora cercopitheci* sp.n. for a species recovered from green monkeys, *C. colobi* sp.n. for a parasite from colobus monkeys, and *C. papionis* sp.n. for a species infecting baboons.



Figures 6–9. Photomicrographs of *Cyclospora papionis* sp. n. oocysts from feces of baboons (*Papio anubis*) in Ethiopia, Africa. x 3300. 6. Unsporulated oocysts from feces. 7. Sporulated oocyst after 1 month of incubation. 8. Free sporocyst from ruptured oocyst. 9. Free sporozoite from ruptured sporocyst.



Figures 2–3. Photomicrographs of *Cyclospora cercopitheci* sp. n. from feces of African green monkeys (*Cercopithecus aethiops*) in Ethiopia, Africa. x 3300. 2. Unsporulated oocyst from feces. 3. Sporulated oocyst after 1 month of incubation.

Transmission in Mexico is seasonal; as well as in the US…

Am. J. Trop. Med. Hyg., 91(3), 2014, pp. 537–540
doi:10.4269/ajtmh.13-0535

Cyclospora cayetanensis in a Pediatric Hospital in Morelia, México

Guadalupe E. Orozco-Mosqueda*, Orlando A. Martínez-Loya, and Ynes R. Ortega
Hospital Infantil de Morelia Eva Sámano de López Mateos, Servicios de Salud de Michoacán, Michoacán, Mexico; Center for Food Safety, University of Georgia, Griffin, Georgia

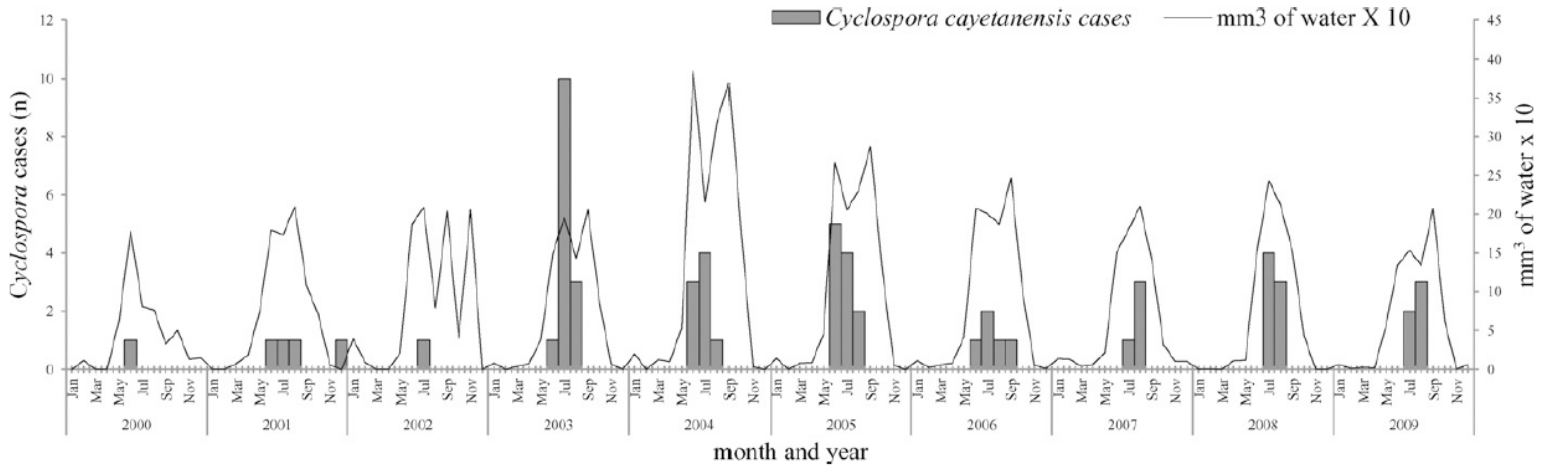
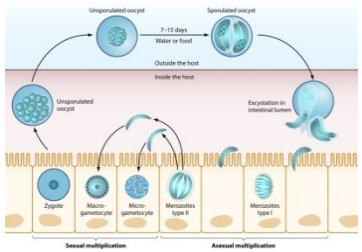
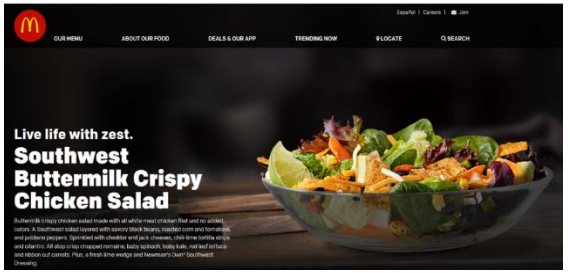


FIGURE 1. Seasonality of *Cyclospora* in children seeking medical attention at the Pediatric Hospital of Morelia, Michoacán, Mexico, 2000–2009.

* Surveys in Mexico?

Cyclosporiasis Outbreaks in US





Raspberries

1996 – 1465 cases in 20 states
978 (66.8 %) confirmed cases

RESEARCH

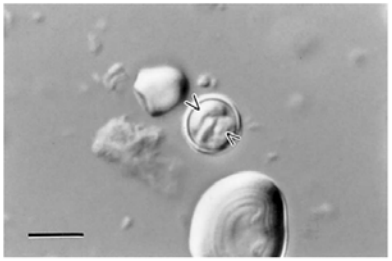
Outbreak of Cyclosporiasis Associated with Imported Raspberries, Philadelphia, Pennsylvania, 2000

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. Herwaldt‡

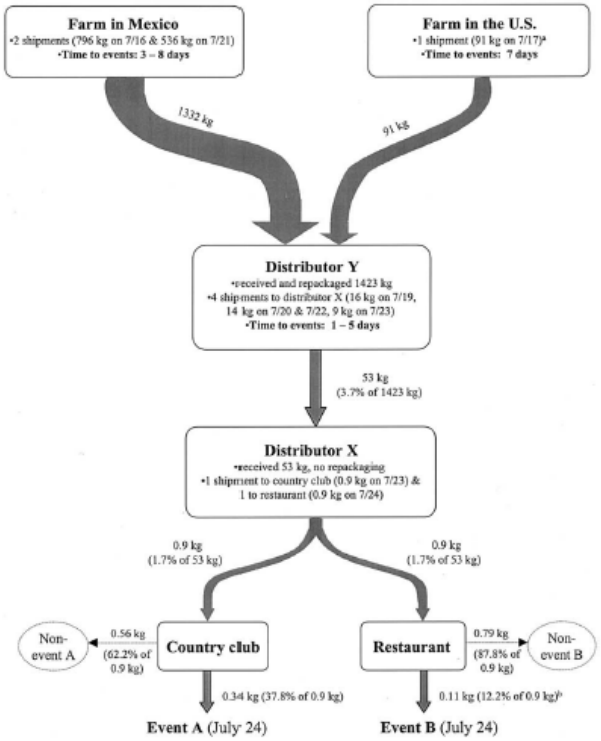
An outbreak of cyclosporiasis occurred in attendees of a wedding reception held in Philadelphia, Pennsylvania, on June 10, 2000. In a retrospective cohort study, 54 (68.4%) of the 79 interviewed guests and members of the wedding party met the case definition. The wedding cake, which had a cream filling 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.

Basil

During the summer of 1999, an outbreak of cyclosporiasis occurred among attendees of 2 events held on 24 July in different counties in Missouri. We conducted retrospective cohort studies of the 2 clusters of cases, which comprised 62 case patients. The chicken pasta salad served at one event (relative risk [RR], 4.25; 95% confidence interval [CI], 1.80–10.01) and the tomato basil salad served at the other event (RR, 2.95; 95% CI, 1.72–5.07) were most strongly associated with illness. The most likely vehicle of infection was fresh basil, which was included in both salads and could have been grown either in Mexico or the United States. Leftover chicken pasta salad was found to be positive for *Cyclospora* DNA by means of polymerase chain reaction analysis, and 1 sporulated *Cyclospora* oocyst was found by use of microscopy. This is the second documented outbreak of cyclosporiasis in the United States linked to fresh basil and the first US outbreak for which *Cyclospora* has been detected in an epidemiologically implicated food item.



Sporulated *C. cayetanensis* in leftovers of chicken pasta salad. Scale bar, 10 µm



Two possible sources of basil (Mexico and U.S.)

Cilantro

Multi-State Outbreaks of Cyclosporiasis - 2013

631 cases of cyclosporiasis in 25 states

- **A total of 270 cases in TX**
- **More than 70 clusters* of cases linked to multiple restaurants and grocery stores**
- One cluster with 25 cases (Restaurant A; with18 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.

Epidemiol. Infect. (2015), **143**, 3451–3458. © Cambridge University Press 2015
doi:10.1017/S0950268815000370

2013 multistate outbreaks of *Cyclospora cayetanensis* infections associated with fresh produce: focus on the Texas investigations

Cilantro was the most likely vehicle of infection in restaurant A, B, C, and grocery store clusters.

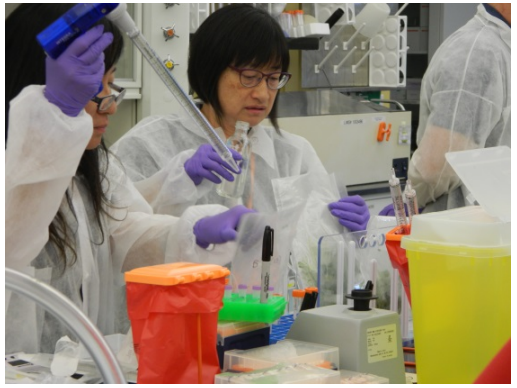
health and the produce industry. The specific challenges posed by *Cyclospora* include under-detection of cases, lack of subtyping methods to link cases to each other or to specific food items, and the absence of practical tools to detect the organism in food and potential sources of contamination in the environment (e.g. soil and insanitary irrigation water). Advances in

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.

OARSA Research Program in Foodborne Parasitology

Building Laboratory Capacity for Detection of *Cyclospora cayetanensis* in Food

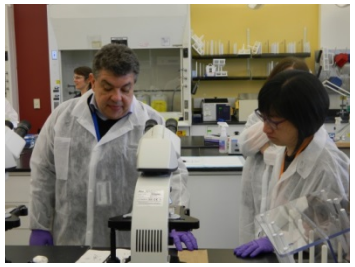
Implementation of BAM Chapter 19B Method in FDA Field Labs



LB302-Foodborne Parasites Training

Pacific Southwest Food and Feed Laboratory (PSFL), Irvine, CA

February 26th to March 2nd 2018



A total of 5 ORA Laboratories have implemented the BAM Chapter 19B Method to detect *C. cayetanensis* in Produce: The Southeast Food and Feed Laboratory (SFFL) located in Atlanta, GA; the Pacific Northwest Laboratory (PNL) Located in Bothell, WA, the San Francisco Laboratory (SANFL) located in Alameda, CA; the Arkansas Lab (ARKL) Located in Jefferson, AR. *The Pacific Southwest Food and Feed Laboratory (PSFFL) Located in Irvine, CA are in the process of implementing the method.

US Cyclosporiasis Outbreaks - 2018

A total of **2199** domestically acquired lab confirmed cases of cyclosporiasis from **33 states** with **153** hospitalizations

As of September 11, 2018, CDC was notified of **511 laboratory-confirmed cases** of *Cyclospora* infections in people from **15 states** and New York City that reported consumption salads from McDonald's restaurants in the Midwest.

Multiple sub-clusters were identified in six states. Epidemiological studies conducted (in IL, IN, MN) **identified cilantro as a vehicle of interest**.

Some cases reported consumption of meal items **including basil at two unrelated points of service in CA and MN**.

As of September 5, 2018, CDC was notified of **250 laboratory-confirmed** cases of Cyclospora infection in people from **4 states** who reported consuming pre-packaged Del Monte Fresh Produce vegetable trays containing broccoli, cauliflower, carrots, and dill dip.

"On July 26, 2018, the FDA completed final analysis of an unused package of romaine lettuce and carrot mix distributed to McDonald's by the Fresh Express processor in Streamwood, IL. **The analysis confirmed the presence of *Cyclospora* in that sample**."

https://www.cdc.gov/parasites/cyclosporiasis/outbreaks/2018/b-071318/index.html

US Cyclosporiasis Outbreaks - 2018

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FDA Statement

Statement from FDA Commissioner Scott Gottlieb, M.D., on the FDA's ongoing efforts to prevent foodborne outbreaks of *Cyclospora*

SHARE TWEET LINKEDIN PIN IT EMAIL PRINT

For Immediate Release September 18, 2018

Inquiries

Media

Peter Cassell
240-402-6537

Consumers

888-INFO-FDA

"This outbreak was linked to McDonald's salads sold in 14 states in the Midwest that contained a romaine lettuce and carrot mix supplied by Fresh Express. The FDA worked with McDonald's to quickly remove implicated salad from the stores. Testing conducted by the FDA identified the parasite in an unopened package of the bagged salad mix, supporting epidemiologic evidence that the salad mix is the source of the outbreak."

US Cyclosporiasis Outbreaks - 2018

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Statement from FDA Commissioner Scott Gottlieb, M.D., on the FDA's ongoing efforts to prevent foodborne outbreaks of *Cyclospora*

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240-402-6537

Consumers

888-INFO-FDA

"During our investigation, two samples of domestically grown romaine lettuce were also found to be positive for *Cyclospora* even though they were not sourced from locations associated with the lettuce that was linked to this outbreak. None of the romaine lettuce associated with these positive test results for *Cyclospora* went into the marketplace and all of the produce suspected of being contaminated was destroyed, preventing additional *Cyclospora* illnesses from occurring. However, these findings are important as they represent the second time that *Cyclospora* has been identified in produce grown in the U.S."

US Cyclosporiasis Outbreaks - 2018

As in **2013**, 2014, 2015 and **2017** a large percentage of the cases could not be linked to any of the outbreaks/clusters identified through epidemiologic studies.

US Cyclosporiasis Outbreaks - 2018

511 laboratory-confirmed cases

Approximately 14 cases

114 cases identified through epidemiological studies.
Additional 42 cases (by identified through interviews).

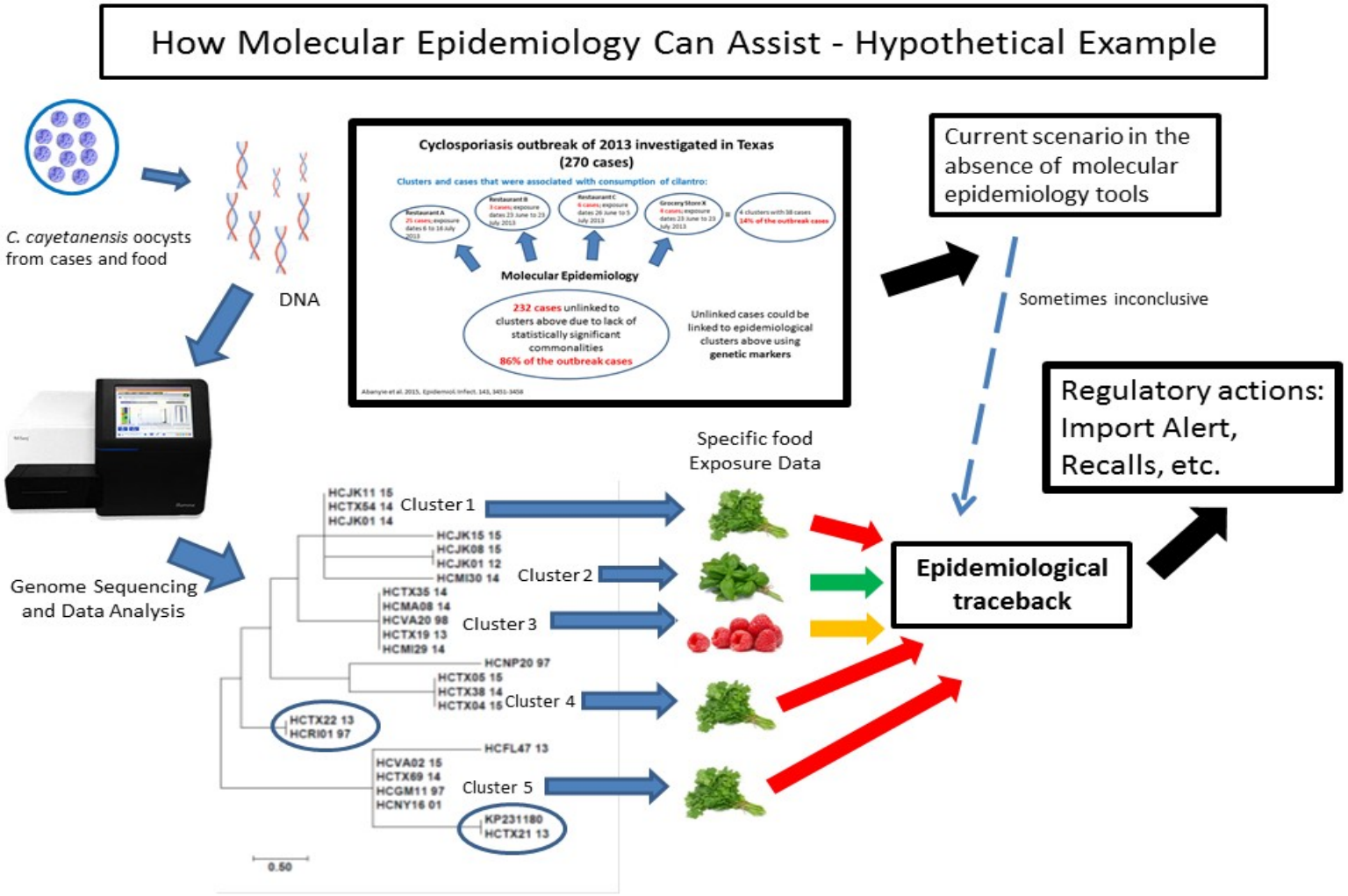
250 laboratory-confirmed

Molecular Epidemiology

1268 cases that were not linked to the clusters above due to lack of statistically significant commonalities and other surveillance issues

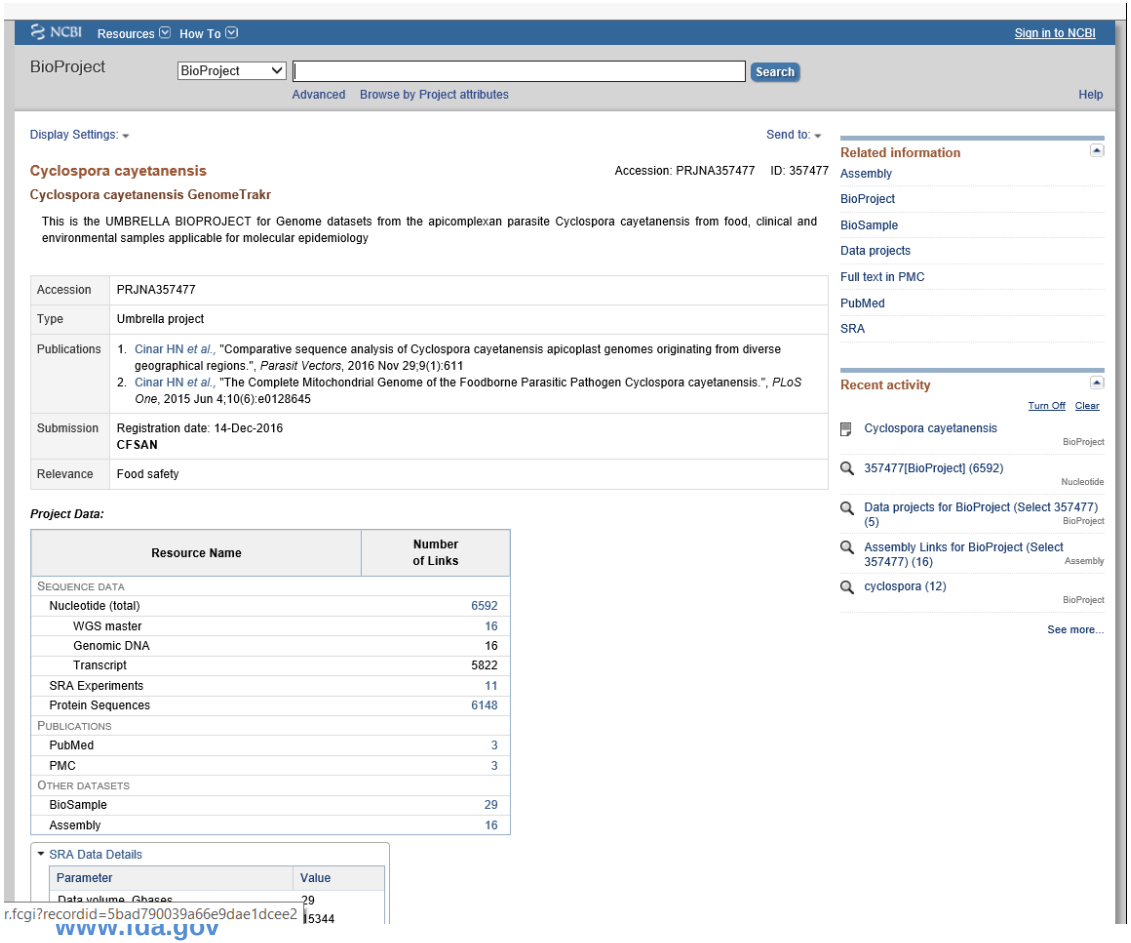
That represented approximately 57% of the 2199 domestically acquired cases reported in 2018

Genomics and Molecular Epidemiology



Genomics and Molecular Epidemiology

A database to consolidate *C. cayetanensis* genome sequences is being built. This database; the *Cyclospora cayetanensis* Genome Trakr (CycloTrakr) will provide a repository of genome sequences from *C. cayetanensis* identified worldwide. CycloTrakr enables a network model for genotyping and source tracking based on genomics

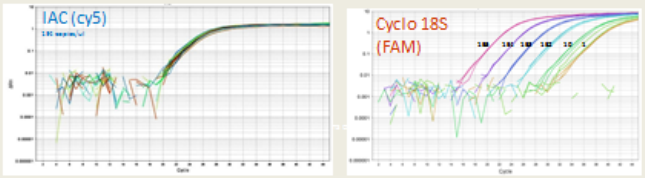


Approximately 50 *C. cayetanensis* mitochondrial genome assemblies will be uploaded in CycloTrakr by 2018

https://www.ncbi.nlm.nih.gov/bioproject/357477

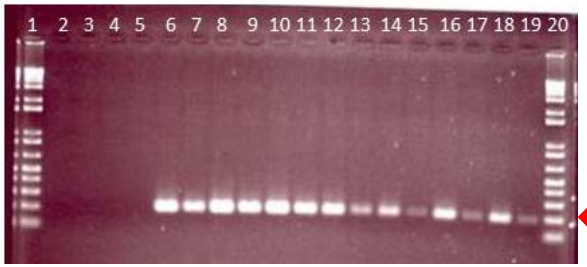
Molecular Analysis- PCR and DNA sequencing

TaqMan real-time PCR



- Amplification of a series of serial dilutions of the synthetic positive control target.
- **Limit of Detection is a SINGLE COPY** of the *C. cayetanensis* 18S rRNA gene target.

1.A- Test DNA samples with BAM Chapter 19B qPCR



Wells 6 to 19= water seeded with10,000 to 500 oocysts in 50L water samples

1.B - Test DNA samples with mit3PCR which amplifies a 182 bp fragment from *C. cayetanensis* mitochondrial genome

2.B - DNA sequencing analysis of the 182 bp amplicon produced by mit3PCR

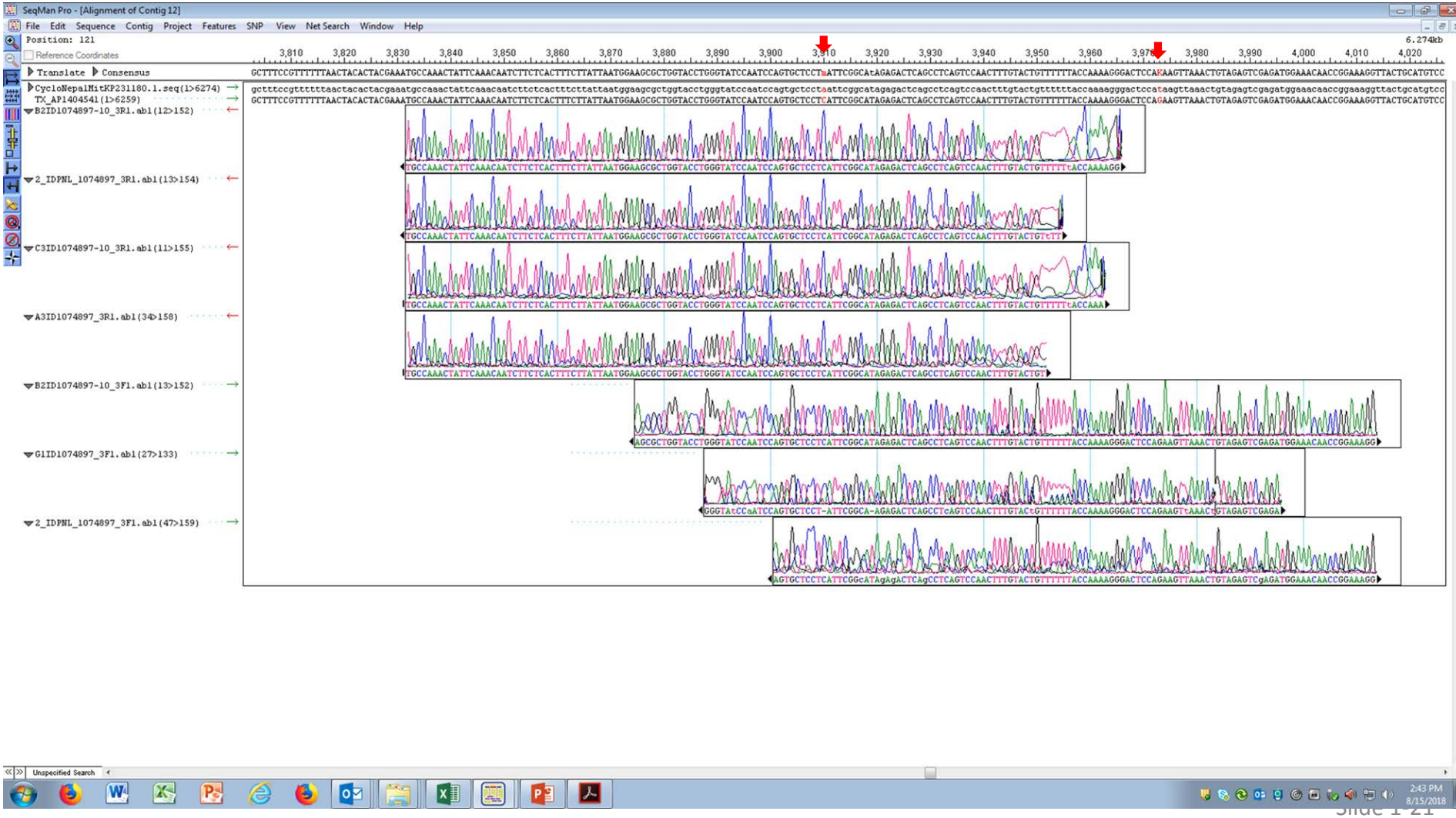
Preliminary application of mitochondrial markers in 2018 positive samples

"On July 26, 2018, the FDA completed final analysis of an unused package of romaine lettuce and carrot mix distributed to McDonald's by the Fresh Express processor in Streamwood, IL. **The analysis confirmed the presence of *Cyclospora* in that sample**." https://www.cdc.gov/parasites/cyclosporiasis/outbreaks/2018/b-071318/index.html

This sample was reported by ORA PNL in one of the subs of romaine lettuce analyzed. The sub was positive with by the BAM Chapter 19B method with a Ct of 37.9. The result indicated low concentration of oocysts in the 25g of romaine lettuce tested, e.g., less than 5 oocysts.

More than one set of amplicons had to be produced through PCR amplification for DNA sequencing analysis. Amplicons were also excised form the gels and sequenced individually. The sequences were aligned to *C. cayetanensis* sequences obtained from a sample originated from Nepal (CycloNepalmitKP231180 and a one originated from Texas US (TX_AP1404541).

Preliminary application of mitochondrial markers in 2018 positive samples



Future

- Develop the new generation of detection and genotyping techniques based on new genomic data being produced. These tools will need to be sensitive to generate sequences from samples with very low concentrations of oocysts similar to the positive food samples detected during 2018 cyclosporiasis outbreaks.

- Populate “CycloTrakr” with sequences from *C. cayetanensis* samples obtained from different geographic areas where *C. cayetanensis* has been identified as a public health issue (e.g., Guatemala, Peru, Mexico, etc.).

- Develop and validate methods for detection and genotyping of *C. cayetanensis* from agricultural water and environmental samples.

Thank you

FDA U.S. FOOD & DRUG ADMINISTRATION
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