Cyclospora cayetanensis: Epidemiology and Surveillance

Anne Straily, DVM, MPH, DACVPM

Veterinary Medical Officer, Parasitic Diseases Branch, Division of Parasitic Diseases and Malaria

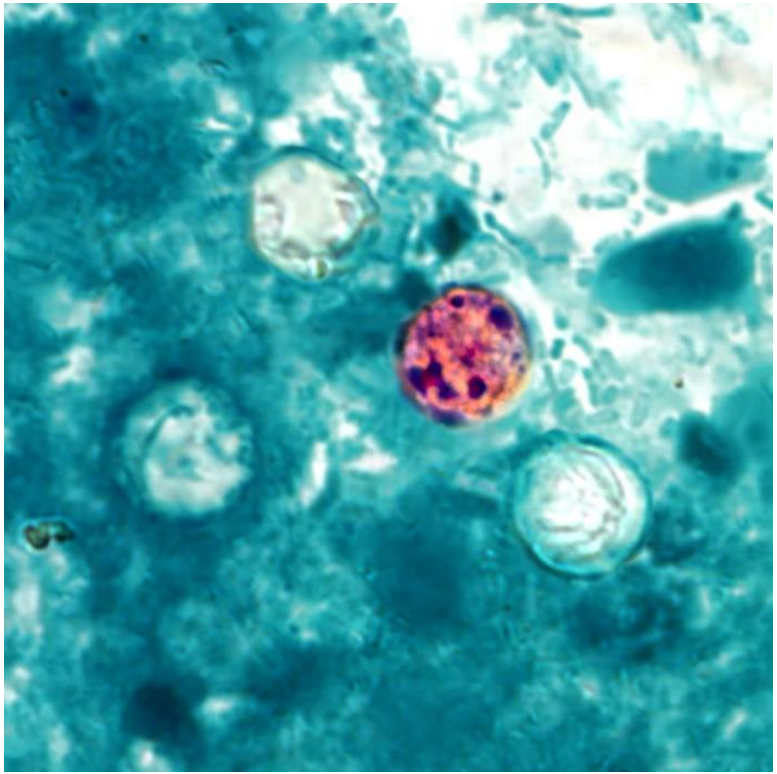
Centers for Disease Control and Prevention

AFDO Webinar

May 15, 2020

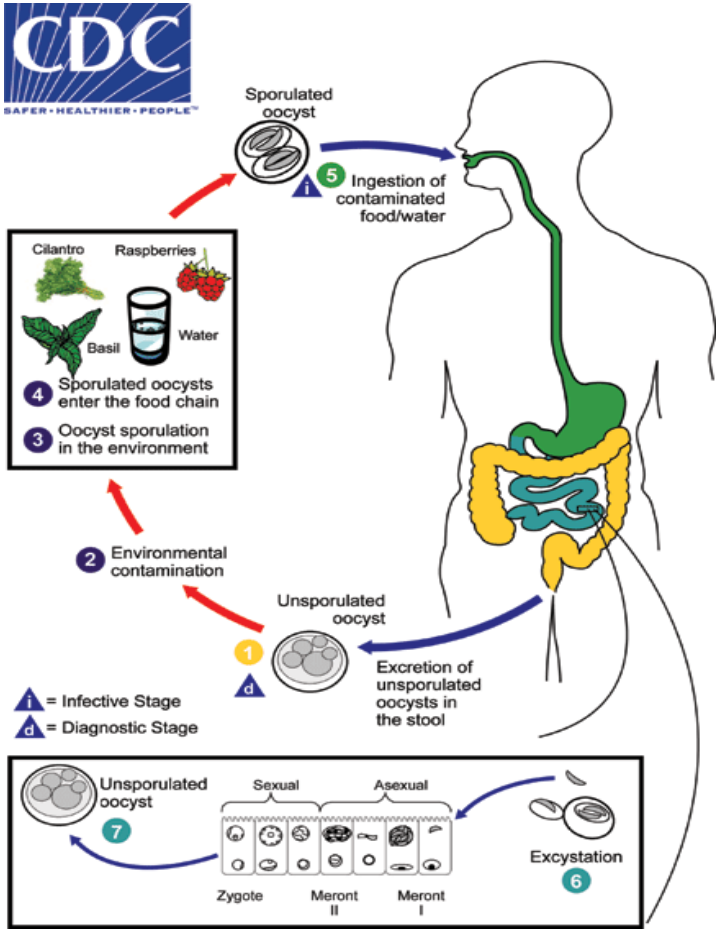
Cyclospora cayetanensis

- Coccidian protozoan parasite
- Humans are only known reservoir



Life cycle

- Fecal-oral transmission
- Unsporulated oocysts shed in stool
  - Require some time in favorable environmental conditions to sporulate
  - Direct human-to-human transmission unlikely
- Sporulated oocysts are infectious
  - Contaminate produce and/or water sources



Clinical Illness

Clinical illness

- Incubation period ~ 1 week (range: 2 to 14 days)
- Symptoms
  - Watery diarrhea (most common)
  - Loss of appetite
  - Weight loss
  - Cramping
  - Bloating
  - Increased gas
  - Nausea
  - Fatigue

Clinical illness

- Often follows remitting-relapsing course
- If untreated, symptoms can last for days to weeks to months
- Substantial weight loss and fatigue can occur in prolonged illness
- Death is very rare
- People can become infected more than once

Diagnosis

Diagnosing cyclosporiasis

- 3 methods are available:
  - Ova and Parasite examination (O&P)
  - Polymerase chain reaction (PCR)
  - Multi-pathogen molecular testing panels

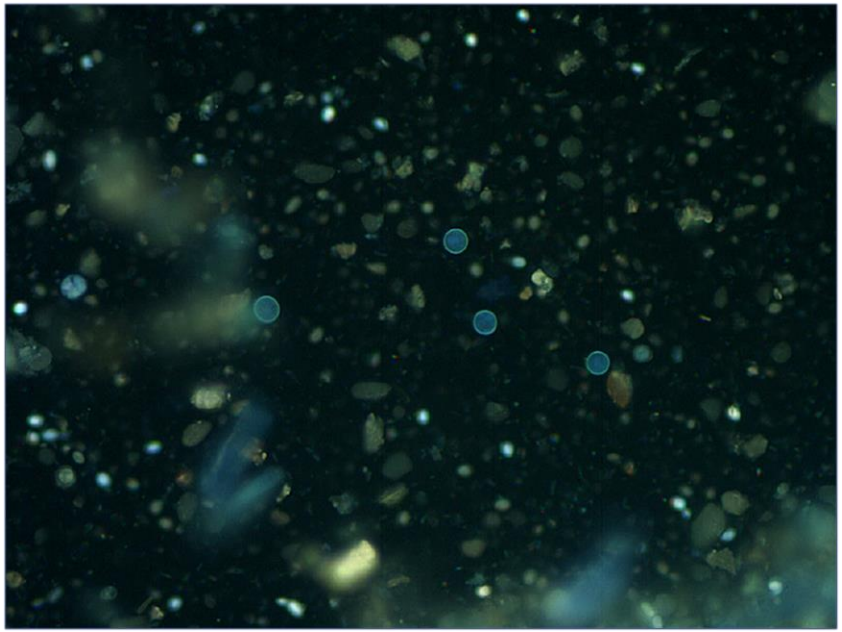
Diagnostic methods:
Ova & Parasite examination (O&P)

- Stools examined by routine O&P do not usually detect *Cyclospora*
- Requires special techniques and training
  - UV fluorescence microscopy (oocysts are autofluorescent)
  - Acid fast staining
- Physician must specifically request *Cyclospora* testing

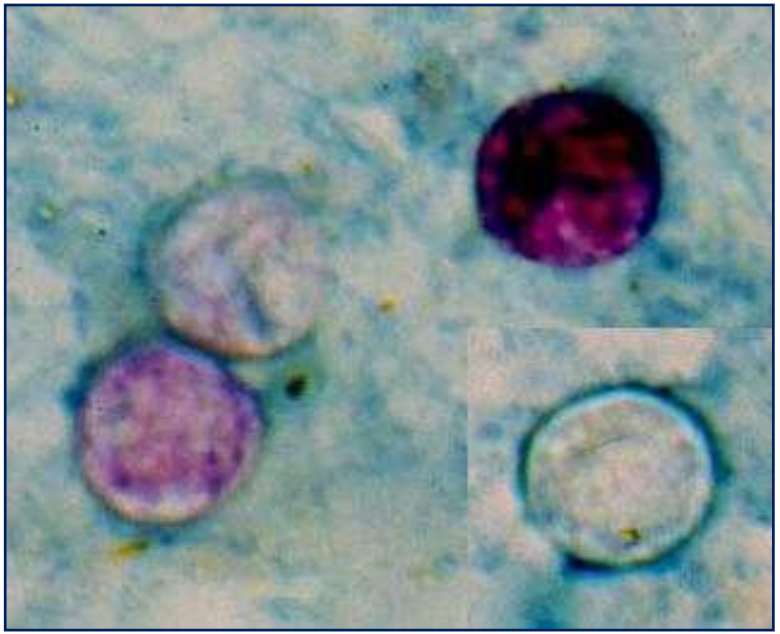
Oocysts blend in with the background on unstained slides

Photo: Dr. Barbara Herwaldt

Detection of *Cyclospora* oocysts by microscopy



UV fluorescence microscopy



Acid fast staining of oocysts (note that not all oocysts retain the stain)

Photos: Dr. Barbara Herwaldt

Diagnostic methods:
Polymerase Chain Reaction (PCR)

- Stool specimen(s) tested via PCR for *Cyclospora* DNA
- May be available at some clinical or commercial laboratories

Diagnostic methods:
Multi-pathogen molecular testing panels

- A single stool specimen tested via PCR for multiple pathogens simultaneously
- Sometimes referred to as “culture-independent diagnostic tests” (CIDTs)
- Currently, *Cyclospora* included only in one commercially-available panel
  - BioFire® FilmArray® GI Panel*
  - Became available in 2014

*The use of trade names is for identification only and does not imply endorsement by the Public Health Service or by the U.S. Department of Health and Human Services

Diagnosis can be difficult…

- If testing is done by O&P, the physician must specifically request testing for *Cyclospora*
- Even persons with profuse diarrhea might not shed enough oocysts to be readily detectable
  - Requires use of sensitive recovery and detection methods
- Parasite shed intermittently
  - May require multiple stool specimens from different days

Diagnostic methods: What's not available

- Many common tools available for diagnosing or typing other foodborne pathogens are **not** available for *Cyclospora*
  - Culture
  - Whole-genome sequencing (WGS)
  - Pulsed-field gel electrophoresis (PFGE)
  - Molecular tools for strain differentiation
  - Serologic assays

Treatment

Treatment of cyclosporiasis

- Treatment of choice is trimethoprim-sulfamethoxazole (TMP/SMX)*
- TMP/SMX is not commonly used to treat other enteric protozoan or bacterial infections
  - Highlights importance of diagnosing infection rather than treating empirically

* Treatment of cyclosporiasis is not an FDA-approved indication, thus this is an “off label use”

Epidemiology

Cyclosporiasis

- Endemic in subtropical and tropical regions
- Transmission occurs by ingestion of sporulated oocysts in contaminated water or on contaminated fresh produce
  - To date, no commercially frozen or canned produce has been implicated
- In areas where cyclosporiasis has been studied, risk of infection tends to be seasonal

Cyclosporiasis in the United States

- U.S. infections typically are associated with either:
  - International travel to areas of endemicity
  - Consumption of imported fresh produce from areas of endemicity
- In 2018, *Cyclospora cayetanensis* identified in U.S. grown produce for the first time[1,2]

[1]FDA Sampling Assignment Update Identifies Cyclospora in Herbs (August 28, 2018). https://www.fda.gov/food/cfsan-constituent-updates/fda-sampling-assignment-update-identifies-cyclospora-herbs. Accessed 9/24/2019.
[2]Interim Report: Blue-Ribbon Panel on the Prevention of Foodborne *Cyclospora* Outbreaks (June 5, 2019). https://www.freshexpress.com/sites/default/files/brp_interim_report_6.5.19-final.2_2.pdf. Accessed 9/24/2019

Previous outbreaks of cyclosporiasis

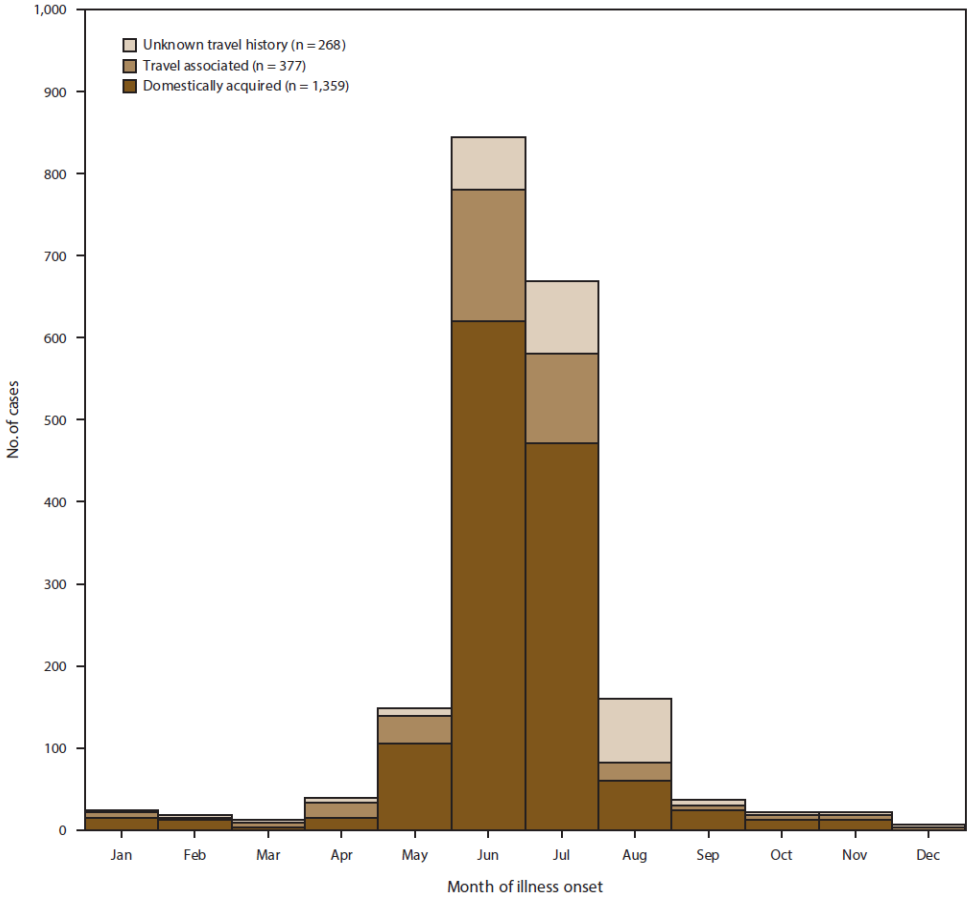
- Foodborne outbreaks of cyclosporiasis have been reported in the U.S. since the mid-1990s
- Linked to various types of imported fresh produce
  - E.g., basil, cilantro, raspberries, snow peas, mesclun lettuce



Seasonality of U.S. cyclosporiasis infections

- Infections tend to be seasonal with peaks during May to August
  - It is unknown what environmental conditions drive this seasonality

FIGURE 3. Number* of reported cases of cyclosporiasis, by month of illness onset† and international travel history§ — United States, 2011–2015



* N = 2,004.

† Data regarding month of illness onset were available for most (90.8%, or 2,004 of 2,207) of the patients whose cases were reported to CDC (2011, 205 of 237; 2012, 112 of 130; 2013, 738 of 798; 2014, 356 of 398; and 2015, 593 of 644).

§ Cases were classified as travel associated or domestically acquired on the basis of the patient's travel history during the pertinent exposure period (i.e., the 14 days before illness onset). If the patient reported having a history of international travel (i.e., travel outside the United States) during all or part of the exposure period, the patient's case was classified as travel associated. If the patient reported having no history of international travel, the patient's case was classified as domestically acquired.

Casillas, Hall, & Herwaldt (2019) *MMWR*

Surveillance –
Outbreak Detection & Monitoring

U.S. surveillance for cyclosporiasis

- Nationally notifiable since 1999
- Currently reportable in 43 states, the District of Columbia, and New York City

Side note on public health surveillance

- **Reportable** = physicians and laboratories are required to report cases to their state/local health department

- **Nationally notifiable** = states voluntarily report cases to CDC

U.S. surveillance for cyclosporiasis

- Surveillance for cyclosporiasis year round
- During the “outbreak season”, CDC and state health departments conduct enhanced surveillance
  - Cases with illness onset May 1 to August 31
  - No history of international travel in the 2 weeks preceding illness onset (domestically acquired illness)
  - Use an extended hypothesis generating questionnaire to collect produce, restaurant, and grocery store exposures

Domestically acquired vs. travel-associated

- Important to distinguish between domestically acquired and travel-associated cases
  - **Domestically acquired** = no history of travel outside the U.S. or Canada in the 14 days prior to illness onset
- Domestically acquired cases ate something in the U.S. that made them sick
  - Produce imported into the U.S.
  - Produce grown in the U.S.
  - Public health authorities can potentially take action to prevent further illness

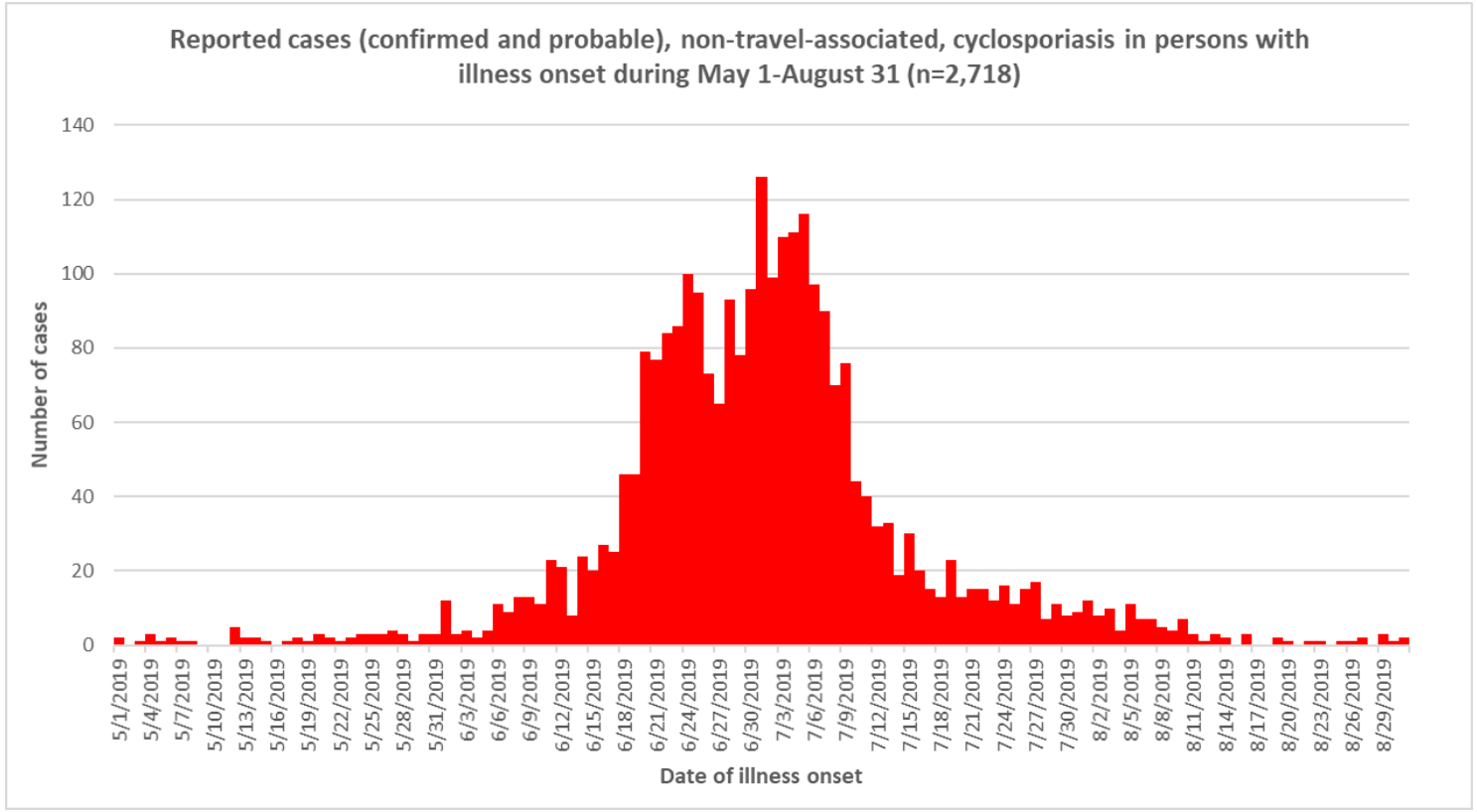
U.S. surveillance for cyclosporiasis

2019 cyclosporiasis cases

- 2,408 laboratory confirmed cases reported among persons who did not report international travel and became ill during May 1-August 31
  - Reported by 37 states, DC and NYC
  - Hospitalizations: 144
  - Median onset date: 7/1/2019
  - No deaths

*Data are preliminary and subject to change; current as of November 13, 2019

2019 cyclosporiasis cases – epi curve



2019 cluster investigations

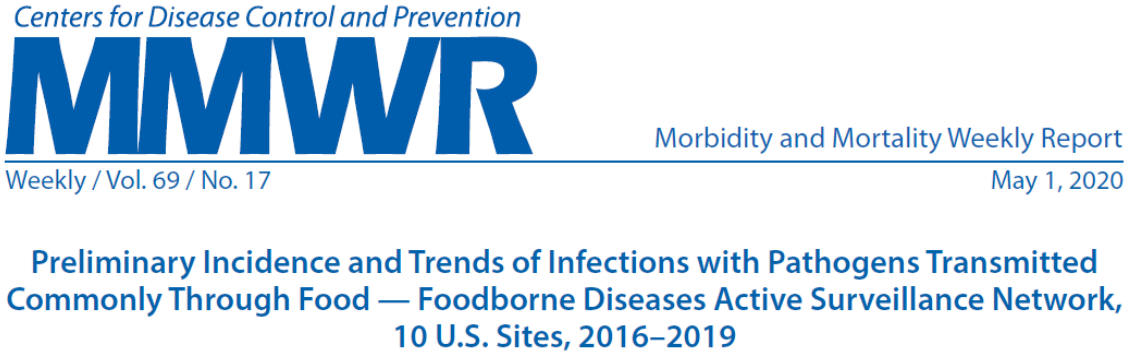
- A cluster of cases was defined as 2 or more cases in persons who shared a common food/produce exposure
- One multi-state cluster with successful traceback investigation implicated fresh basil exported by Siga Logistics de RL de CV of Morelos, Mexico

2019 cluster investigations

- One multi-state cluster with successful traceback investigation implicated fresh basil exported by Siga Logistics de RL de CV of Morelos, Mexico
  - 241 laboratory confirmed cases*
  - Reported by 11 states; exposures occurred in 5 states (FL, MN, NY, OH, WI)
  - Illness onsets June 10 – July 26, 2019
  - 6 hospitalizations
  - No deaths
- Over 80 different restaurant-associated clusters were identified and investigated

*Data are current as of 9/27/2019

2019 compared to previous years

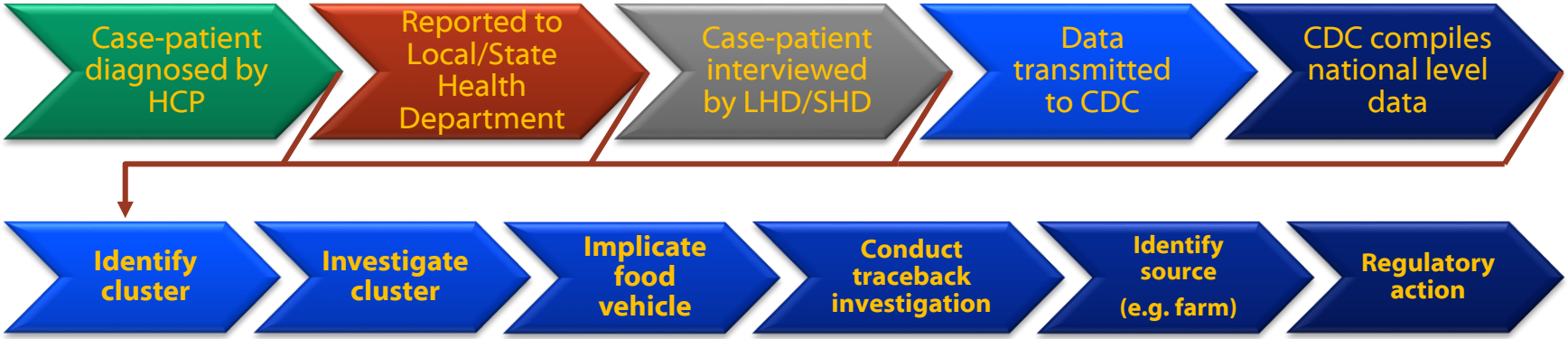


- Cyclosporiasis incidence rates increased ~1209% in 2019 compared to 2016-2018
- Figure 1 in https://www.cdc.gov/foodnet/reports/prelim-data-2019.html

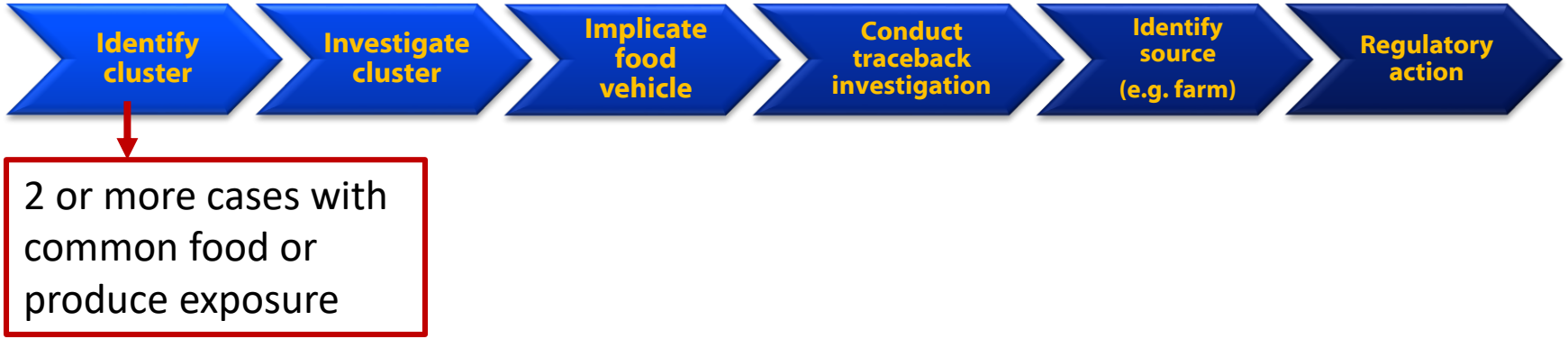
Investigation Challenges

Anatomy of a cluster investigation

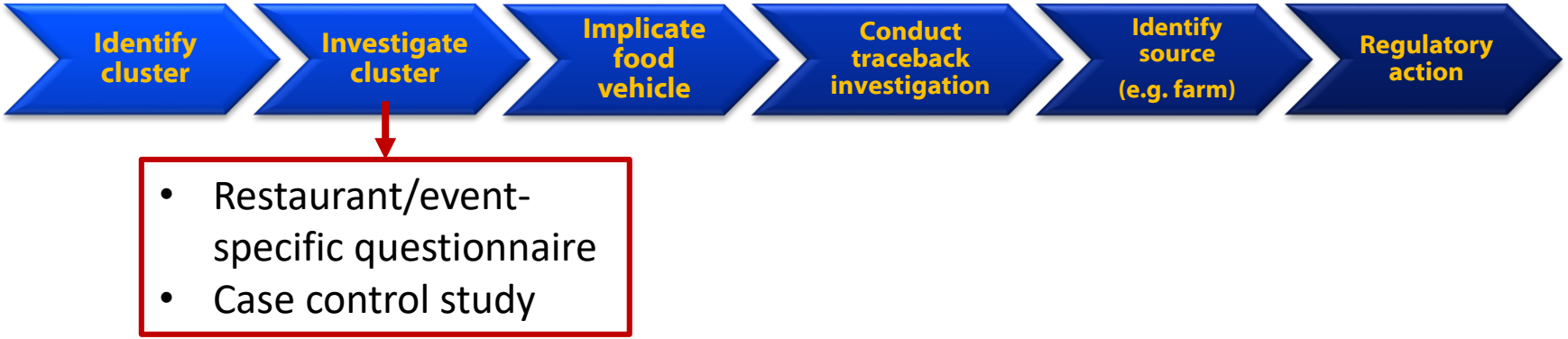
Anatomy of a cluster investigation



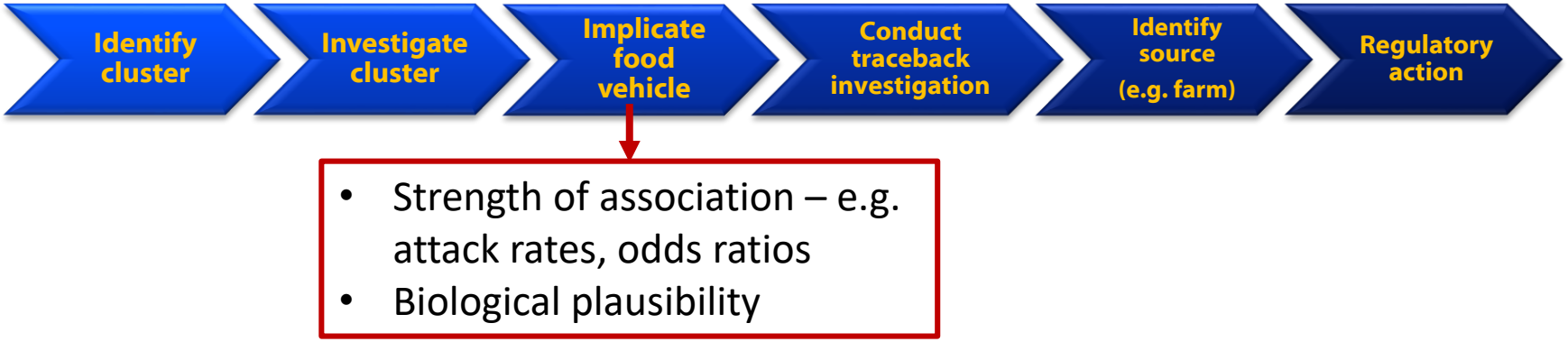
Anatomy of a cluster investigation



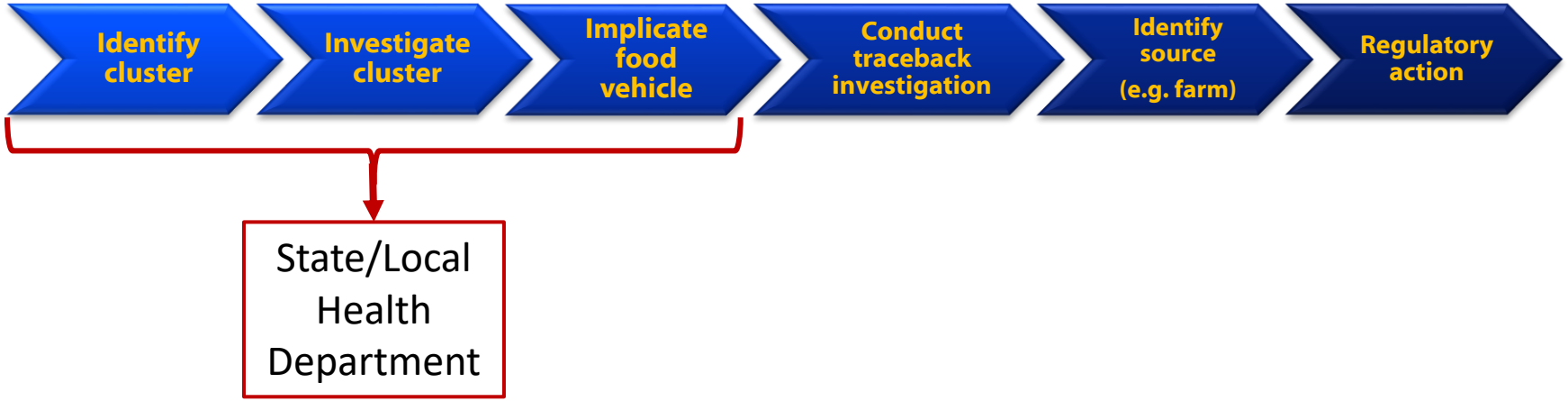
Anatomy of a cluster investigation



Anatomy of a cluster investigation

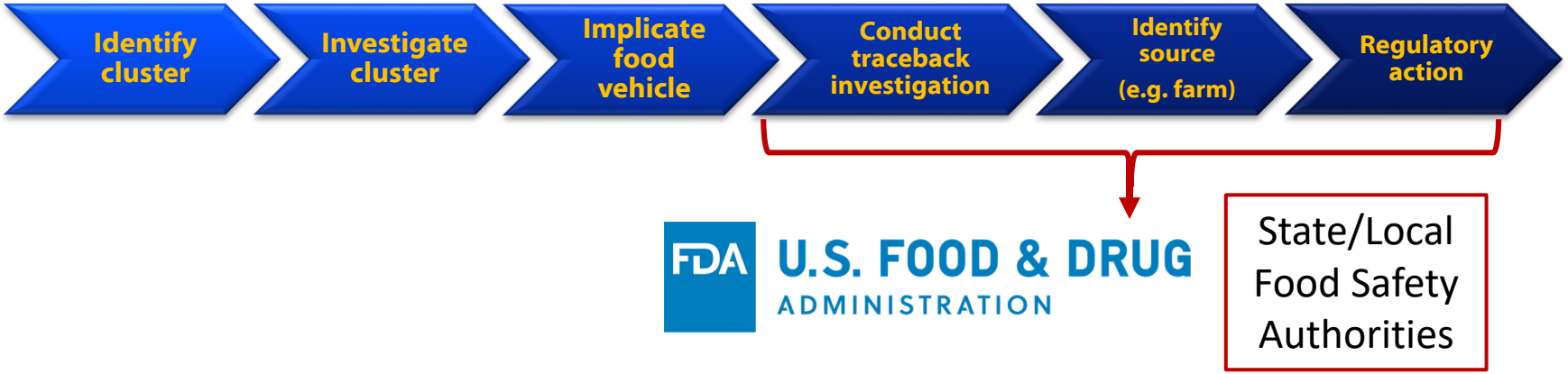


Anatomy of a cluster investigation



Anatomy of a cluster investigation

Anatomy of a cluster investigation



Challenges in investigating cyclosporiasis clusters: **Fresh produce vehicles**

- Fresh produce often served in mixtures and in inconspicuous ways (e.g. as garnishes)

Photo: Dr. Barbara Herwaldt

Challenges in investigating cyclosporiasis clusters: Case ascertainment

- Making the diagnosis
  - Routine O&P examinations do not typically include testing for *Cyclospora*
  - Physician must specifically request testing
  - Multi-pathogen panel tests: only one includes a target for *Cyclospora,* but others do not
- Case reporting
  - Physicians and laboratories need to report cases to local or state health departments

Challenges in investigating cyclosporiasis clusters: Linking cases in place and time

- No molecular subtyping tools (e.g. WGS or PFGE) have been validated for linking cases of cyclosporiasis to each other or to a particular vehicle or source

Cyclospora is **different** from bacterial foodborne pathogens

- Bacteria are haploid and reproduce asexually
  - Suitable for source-linkage analyses because the bacteria present in a case-patient would be genetically very similar to bacteria in source food
- *Cyclospora* are diploid organisms and reproduce both sexually and asexually
  - Daughter cells are a genetic blend of the parent cells
  - After multiple generations there can be significant genetic divergence
- Unable to grow *Cyclospora* in the laboratory

Challenges in investigating cyclosporiasis clusters: Linking cases in place and time

- No molecular subtyping tools have been validated for linking cases of cyclosporiasis to each other or to a particular vehicle or source
- **Exposure histories are used to link cases epidemiologically**

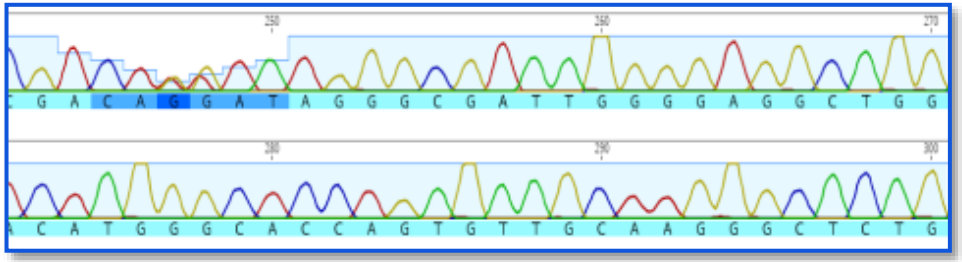
Challenges in investigating cyclosporiasis clusters: Linking cases in place and time

- No molecular subtyping tools have been validated for linking cases of cyclosporiasis to each other or to a particular vehicle or source
- **Exposure histories are used to link cases epidemiologically**
  - Case-patients may report multiple common exposures
  - Sub-optimal recall of food exposures
  - Extensive menus, overlapping ingredients make identifying actual food vehicle or ingredient difficult
  - Identifying linkages among 1,000+ cases is difficult

Genotyping Development

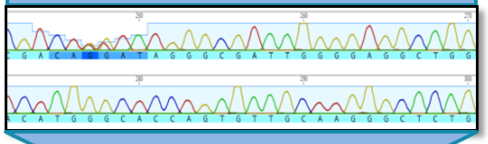
Genotyping *Cyclospora* from human specimens

- Whole genome sequencing not feasible as typing method
  - *Cyclospora* cannot be cultured
  - Most stool samples contain too few oocysts
- Amplicon sequencing of genetically variable typing markers
- Eight markers have been identified:
  - Mitochondrial markers: MSR and junction
  - Nuclear markers: GT1, GT2, GT3, GT4, 360i2 and 378
- Multiple haplotypes (haploid genotypes) possible within the same sample

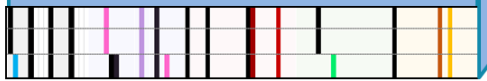


Current Workflow for *Cyclospora* Typing

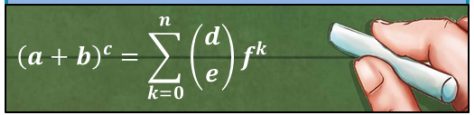
1. PCR and Deep Sequence Amplicons to Identify Haplotypes



2. Generate a list of haplotypes for each sample



3. Analysis of genetic similarity using Bayesian and Heuristic Algorithms



4. Pairwise scoring: Pairwise distances

	C_WI001_	18USWI00	18USWI03	18USWI00	C_WI005_	C_WI008_
C_WI001_	0.009008	0.700022	0.277841	0.622019	0.653151	0.728024
18USWI00	0.700022	0.014727	0.672138	0.766872	0.797414	0.624072
18USWI03	0.277841	0.672138	0.009009	0.56783	0.625186	0.700143
18USWI00	0.622019	0.766872	0.56783	0.009009	0.278844	0.719997
C_WI005_	0.653151	0.797414	0.625186	0.278844	0.009009	0.750538
C_WI008_	0.728024	0.624072	0.700143	0.719997	0.750538	0.015798
C-WI016-1	0.653151	0.797414	0.625186	0.278844	0.009009	0.750538
C-WI020-1	0.728024	0.624053	0.700143	0.719997	0.750538	0.015798

5. Visualization

6. Assign specimens to clusters: List of genetic links for downstream epidemiological comparison

Prevention & Control

Prevention and Control

- No vaccine is available
- When traveling to areas endemic for *Cyclospora*
  - Avoid food or water that may have been contaminated with feces
  - “If you can’t cook it or peel it – then forget it”
- Follow safe fruit and vegetable handling recommendations
  - Treatment of water or food by routine chemical disinfection or sanitizing methods is unlikely to kill *Cyclospora*
- Prompt diagnosis and reporting of cases can help public health authorities identify the vehicle and take steps to prevent further infections