Challenges and Evolving Methods to Detect and Respond to Outbreaks

(a focus on Food-borne Disease Outbreaks)

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Disclosure Information

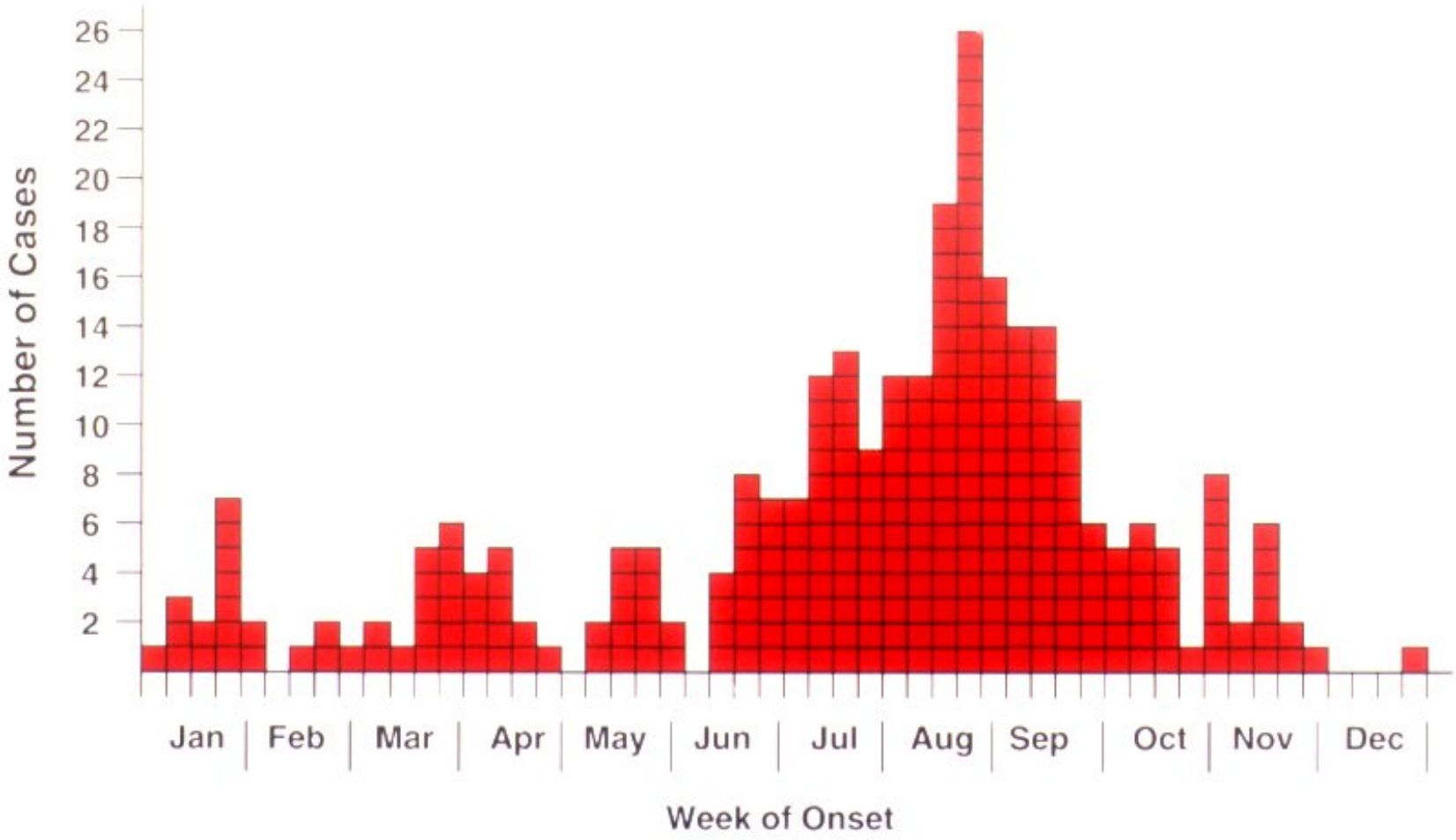
- I have no industry financial relationships to disclose
- My current research is supported by the CDC and the University of Minnesota
- I will not discuss any current off label or investigational medications

Presentation Overview

- Outbreak detection and PFGE
- Syndromic panel testing
  - Culture Independent Diagnostic Tests (CIDT)
- Whole Genome Sequencing (WGS)
- Issues and challenges
- Future considerations

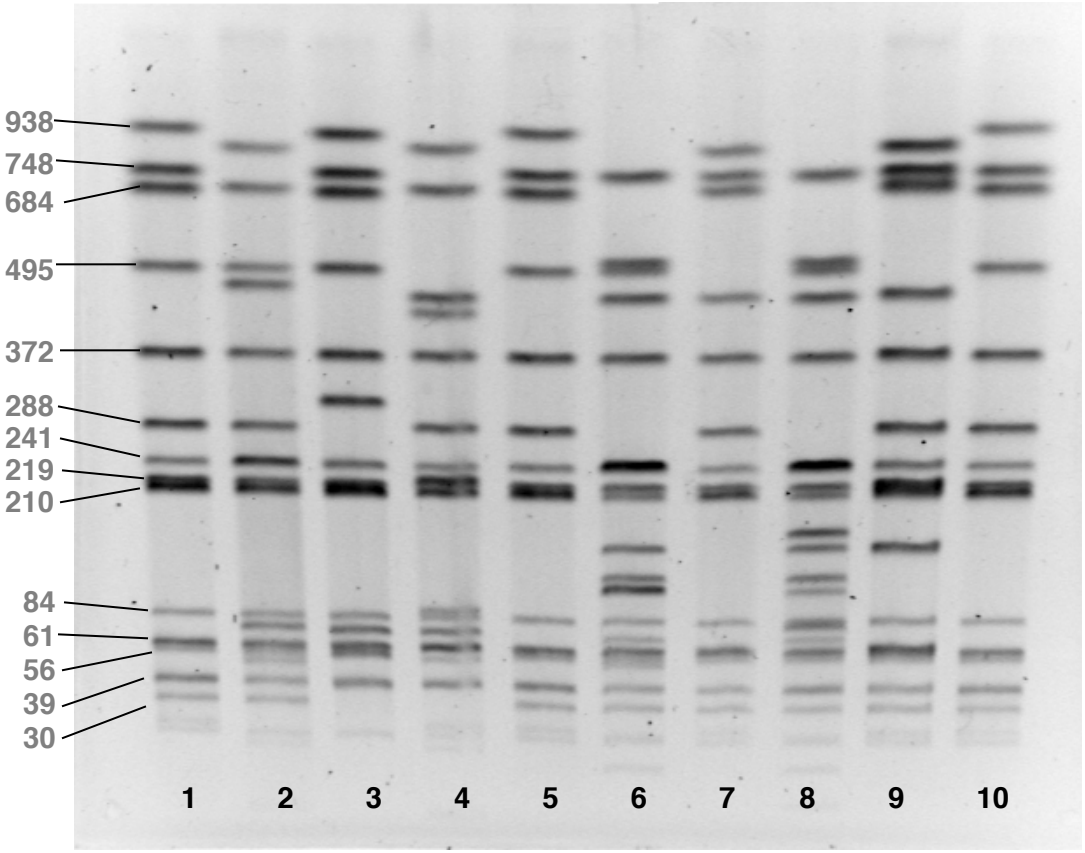
SOLVING COMPLEXITY

Cases of *Salmonella typhimurium* Infection by Week of Onset, Minnesota, 1995



Bender NEJM 2001

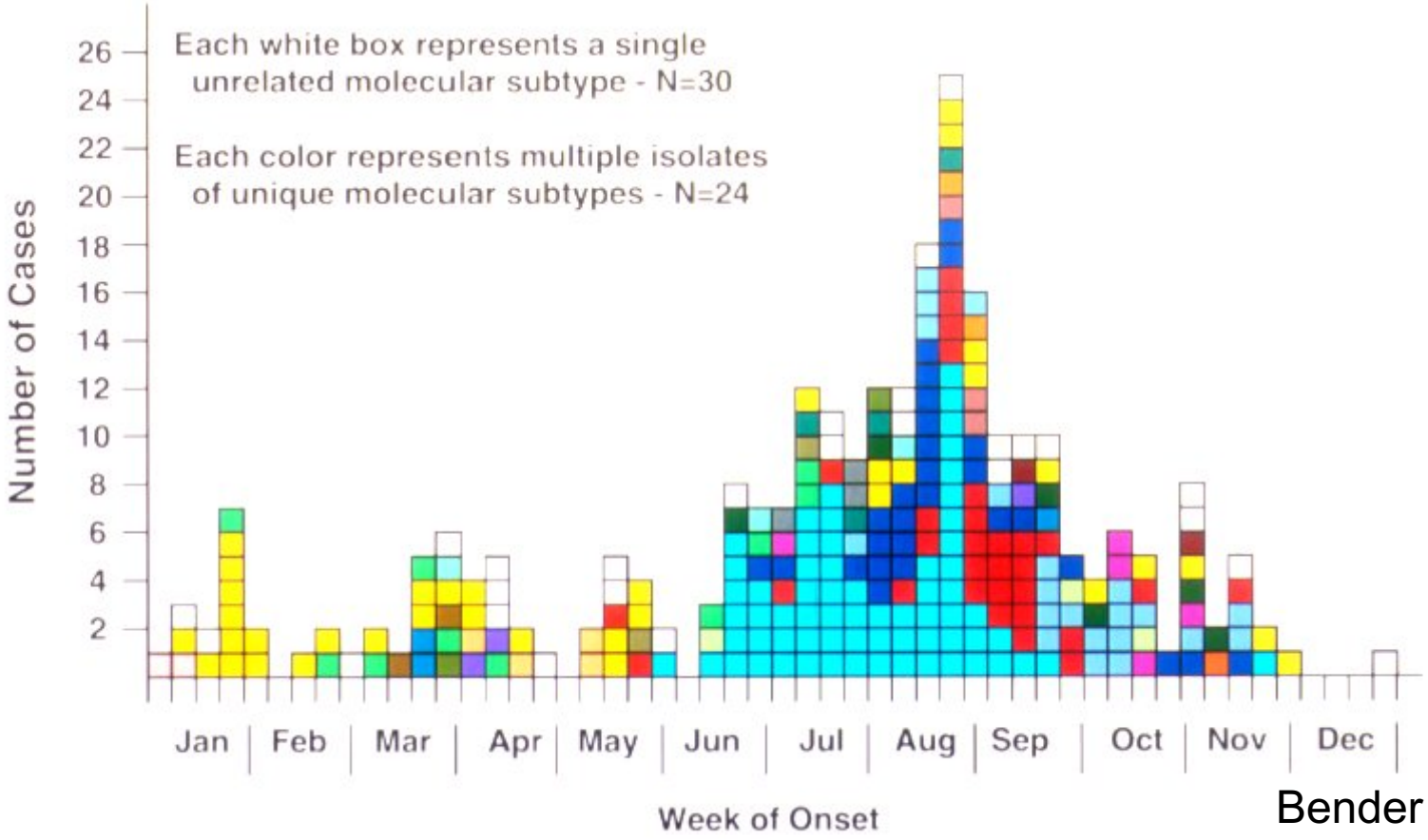
PFGE Patterns of Selected *Salmonella typhimurium* Isolates



Lanes 1, 5, and 10 show standard strain to characterize molecular weight. Lanes 2,3, and 7, show isolates from the three separate outbreaks highlighted in Figure 1. Lane 4 shows a common PFGE pattern coinciding with R-type ACSSuT. Lane 6 shows the most common PFGE pattern associated with R-type AKSSuT. Lanes 8 and 9 are sporadic strains.

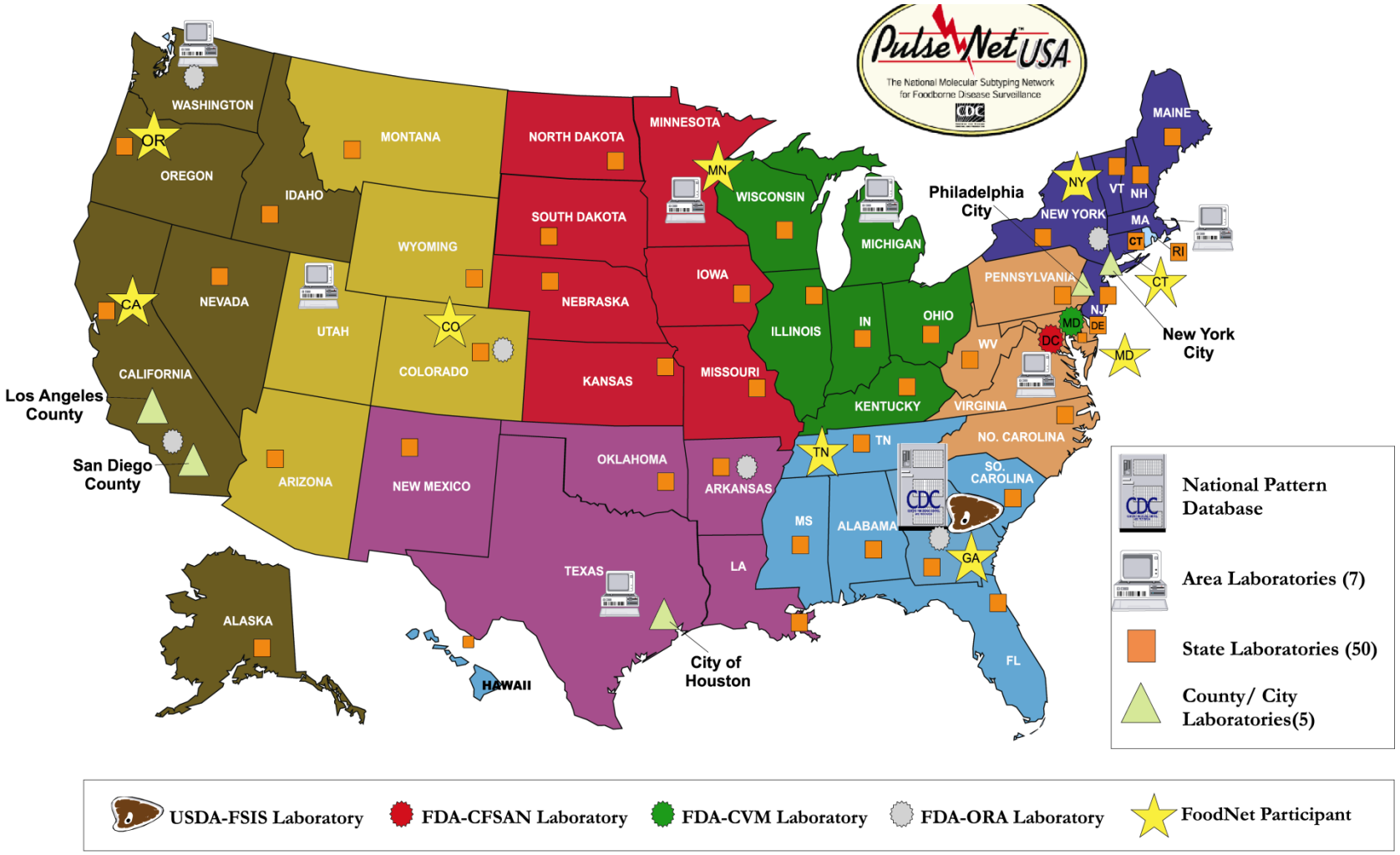
Bender NEJM 2001

Cases of *Salmonella typhimurium* Infection by Week of Onset and Pulsed-Field Gel Electrophoresis Subtype Minnesota, 1995 (n=276)



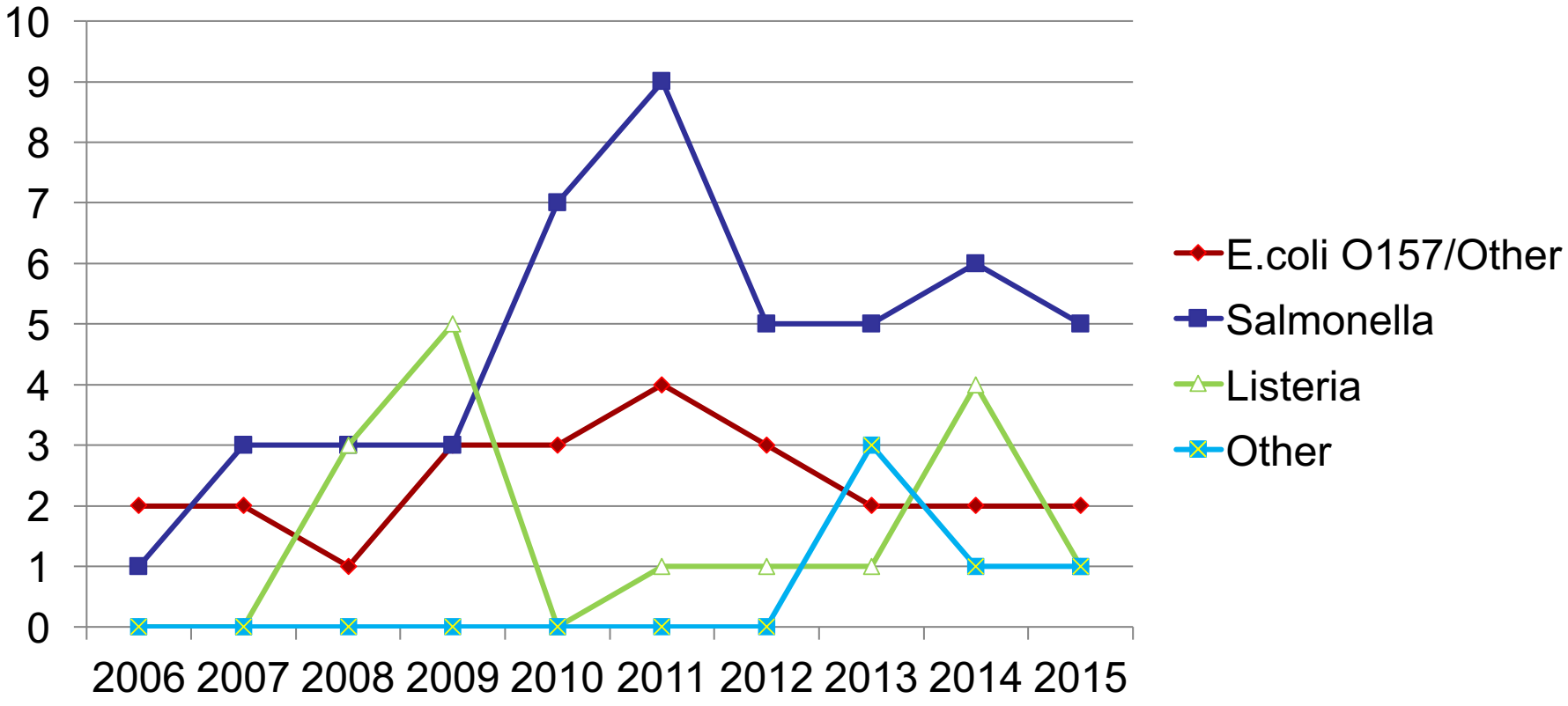
Bender NEJM 2001

The Most Important Foodborne Disease *Epidemiology* Tool Developed in My Lifetime

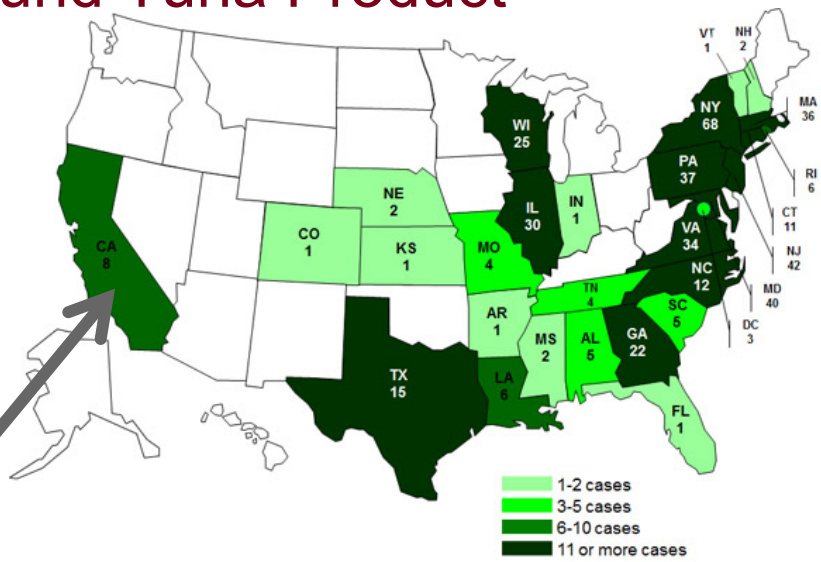


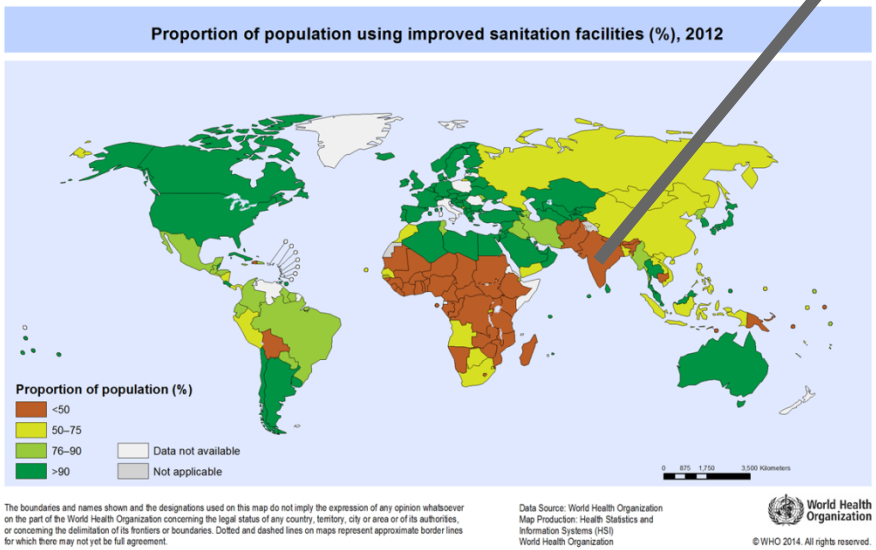
August 2002

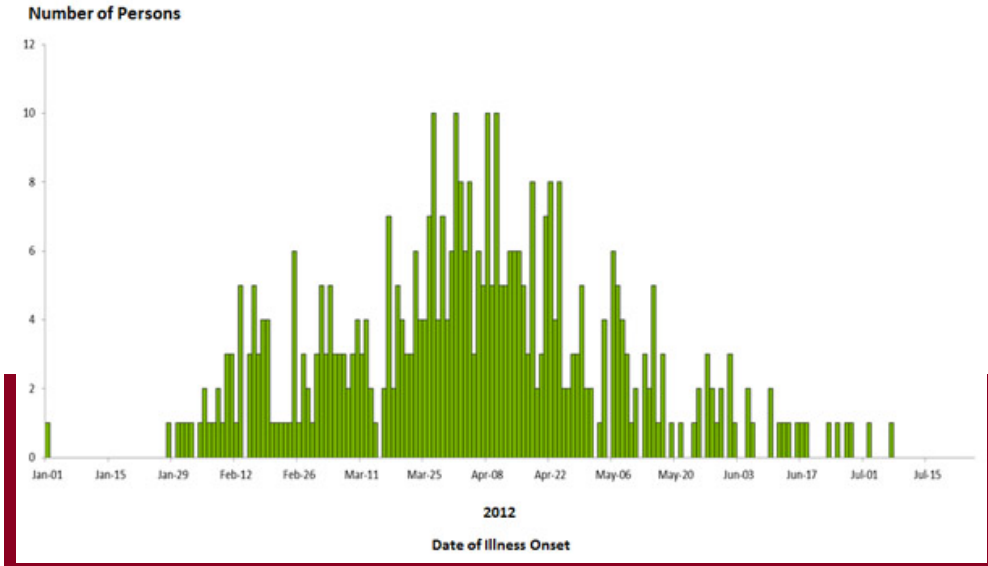
Multi-State Foodborne Outbreaks (U.S. 2006-2015)



Multistate Outbreak of Salmonella Bareilly and Salmonella Nchanga Infections Associated with a Raw Scraped Ground Tuna Product







New Tools for Outbreak Detection

- Whole Genome Sequencing (WGS)
- MALDI-TOF MS
- Syndromic Panel-Based Testing or Culture Independent Diagnostic Tests (CIDT)
- Big Data Analytics

FDA Approved/Cleared Multiplex Assays

- FilmArray (BCID) panel
  - (BioFire Diagnostics, LLC)
- Verigene (BC-GP)
  - (Luminex Corporation)
- Accelerate Pheno System
  - (Accelerate Diagnostics)

General Reported Clinical Benefits

Highly automated system resulted in:

- Decrease time to organism identification (1 to 5 hours)
- Decrease unnecessary antibiotic therapy
- Decrease length of hospitalization stays

TABLE 3 FDA-approved/cleared multiplex respiratory panels[a]

Parameter	FilmArray	Verigene	x-TAG RVP	x-TAG RVP Fast	NxTAG-RPP	eSensor RVP	ePlex
Analysis platform	FilmArray system or FilmArray Torch	Verigene system	Luminex 100/200	Luminex 100/200	Luminex Magpix	eSensor	ePlex system
No. of targets	20	16	12	8	20	14	17
Ability to detect pathogen							
Viruses							
Adenovirus	✓	✓	✓	✓	✓	✓ (differentiates subgroup B/E from C)	✓
Coronavirus							✓
Coronavirus HKU1	✓				✓		
Coronavirus NL63	✓				✓		
Coronavirus 229E	✓				✓		
Coronavirus OC43	✓				✓		
Human bocavirus					✓		
Human metapneumovirus	✓	✓	✓	✓	✓	✓	✓
Influenza A virus	✓	✓	✓	✓	✓	✓	✓
Subtype H1	✓	✓	✓	✓	✓	✓	✓
Subtype H3	✓	✓	✓	✓	✓	✓	✓
Subtype 2009 H1N1	✓					✓	✓
Influenza B virus	✓	✓	✓	✓	✓	✓	✓
Parainfluenza virus 1	✓	✓	✓		✓	✓	✓
Parainfluenza virus 2	✓	✓	✓		✓	✓	✓
Parainfluenza virus 3	✓	✓	✓		✓	✓	✓
Parainfluenza virus 4	✓	✓			✓		✓
Respiratory syncytial virus	✓			✓			
Respiratory syncytial virus A		✓	✓		✓	✓	✓
Respiratory syncytial virus B		✓	✓		✓	✓	✓
Rhinovirus/enterovirus	✓	✓	✓	✓	✓	✓	✓
Bacteria							
Chlamydophila pneumoniae	✓				✓		✓
Mycoplasma pneumoniae	✓				✓		✓
Bordetella pertussis	✓	✓					
Bordetella parapertussis-Bordetella bronchiseptica		✓					
Bordetella holmesii		✓					
Time to result (h)	~1	~2–3	~8	~6	~4	~6	~1.5

[a]The acceptable specimen type for all panels is a nasopharyngeal swab. RVP, respiratory virus panel; RPP, respiratory pathogen panel.

Ramanan P et. al. Clinical Micro Reviews 2018

Clinical Benefits of Multiplex Respiratory Testing

Observed Benefits	Value
Decrease time to Dx of influenza	1.7 vs. 7.7 hrs
Decrease time to Dx non-influenza viruses	1.5 vs. 13.5 hrs
Lower odds for admissions	P=0.046
Lower number of chest radiographs	P=0.005
Shorter duration of hospital stay	P=0.04
Shorter durations of antimicrobial use	P=0.03

Rappo U et. al. 2016. J Clin Microbiol 54:2096 –2103

TABLE 4 FDA-approved/cleared multiplex gastrointestinal panels[a]

Parameter	Verigene EP	Luminex GPP	BioFire GIP
Analysis platform	Verigene system	Magpix or Luminex 100/200 system	FilmArray system or FilmArray Torch
Acceptable specimen type	Stool in Cary-Blair medium	Fresh stool or stool in Cary-Blair medium	Stool in Cary-Blair medium
No. of targets	9	14	22
Ability to detect pathogen			
Bacteria			
Campylobacter species	✓	✓	✓
Salmonella species	✓	✓	✓
Shigella species/enteroinvasive *E. coli*[b]	✓	✓	✓
Vibrio species	✓		✓
Vibrio cholerae		✓	✓
Yersinia enterocolitica	✓		✓
Escherichia coli O157		✓	✓
Enterotoxigenic *E. coli*		✓	✓
Enteropathogenic *E. coli*			✓
Enteroaggregative *E. coli*			✓
Plesiomonas shigelloides			✓
Shiga toxin-producing *E. coli* (stx_1-stx_2)	✓[c]	✓	✓
Clostridium difficile (toxin A/B)		✓	✓
Viruses			
Norovirus GI/GII	✓	✓	✓
Rotavirus A	✓	✓	✓
Astrovirus			✓
Adenovirus 40/41		✓	✓
Sapovirus			✓
Parasites			
Cryptosporidium species		✓	✓
Entamoeba histolytica		✓	✓
Giardia lamblia		✓	✓
Cyclospora cayetanensis			✓
No. of samples (throughput)	1–32 (scalable)	24	1–12 (scalable)
Time to result (h)	<2	~5	~1

[a]EP, enteric pathogens; GPP, gastrointestinal pathogen panel; GIP, gastrointestinal panel.
[b]The Verigene EP and Luminex GPP do not specifically target enteroinvasive *E. coli*.
[c]The Verigene EP has separate targets for stx_1 and stx_2.

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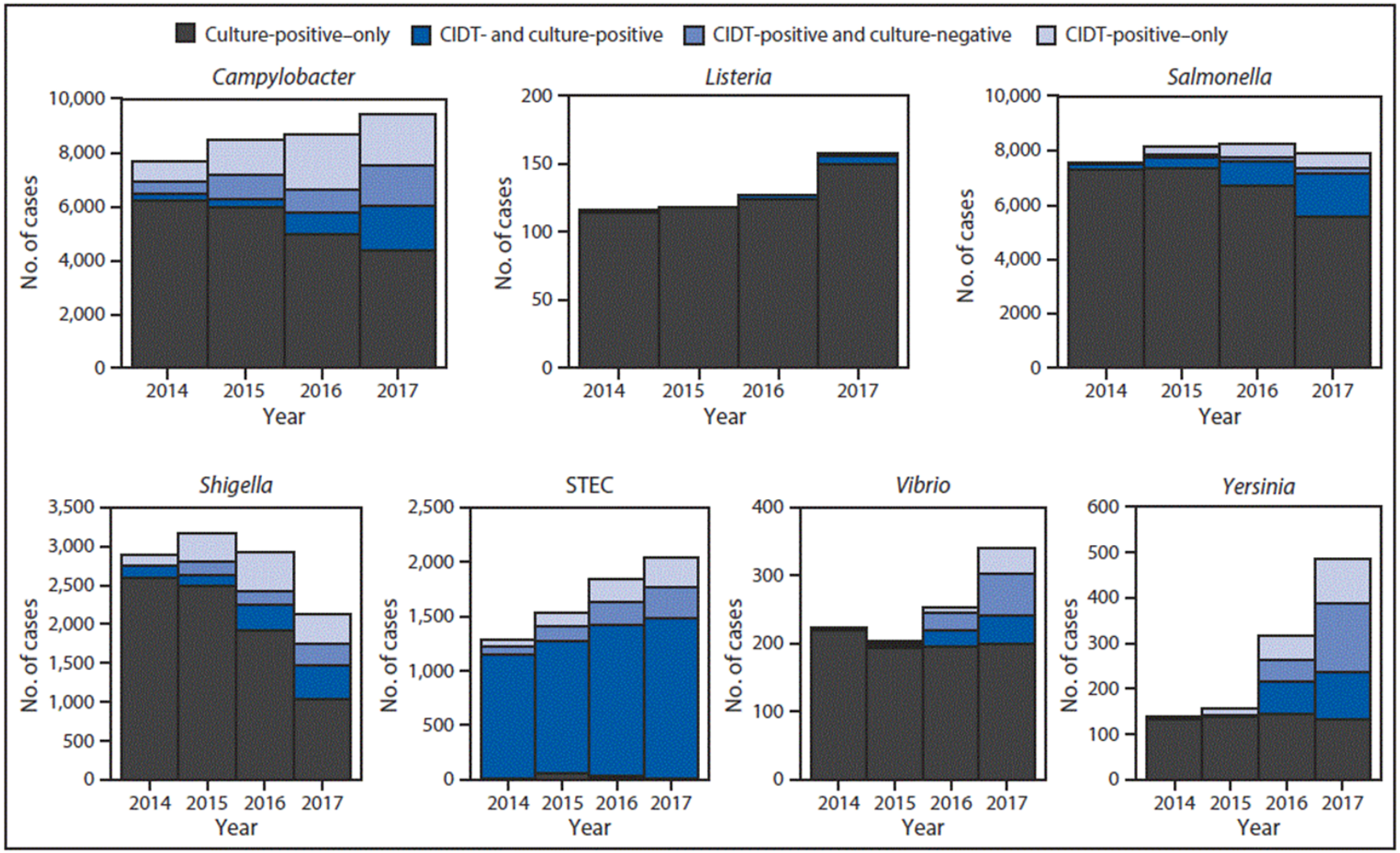
ISSUES/CHALLENGES

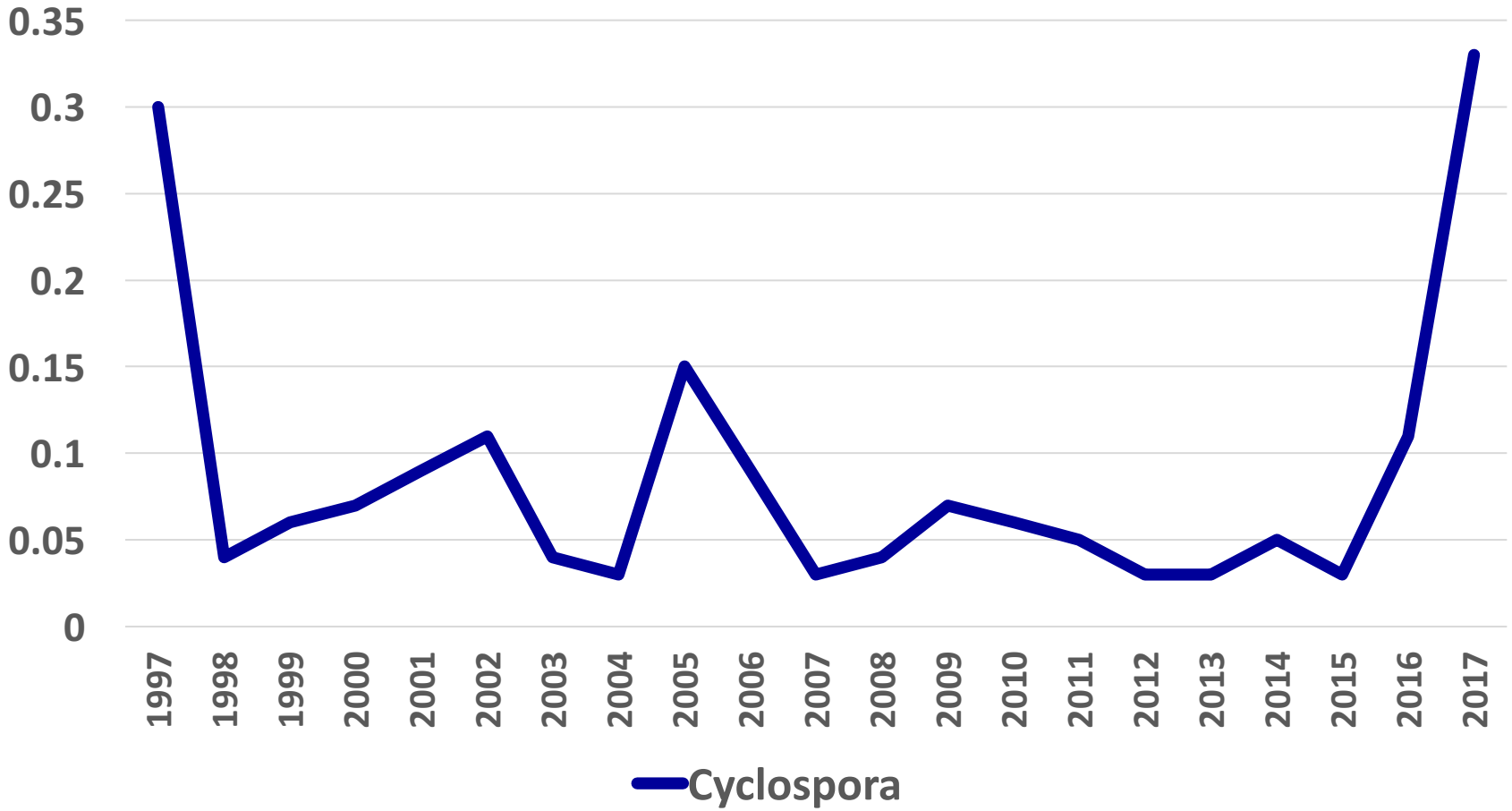
Potential Impact of Culture Independent Diagnostic Tests (CIDT)

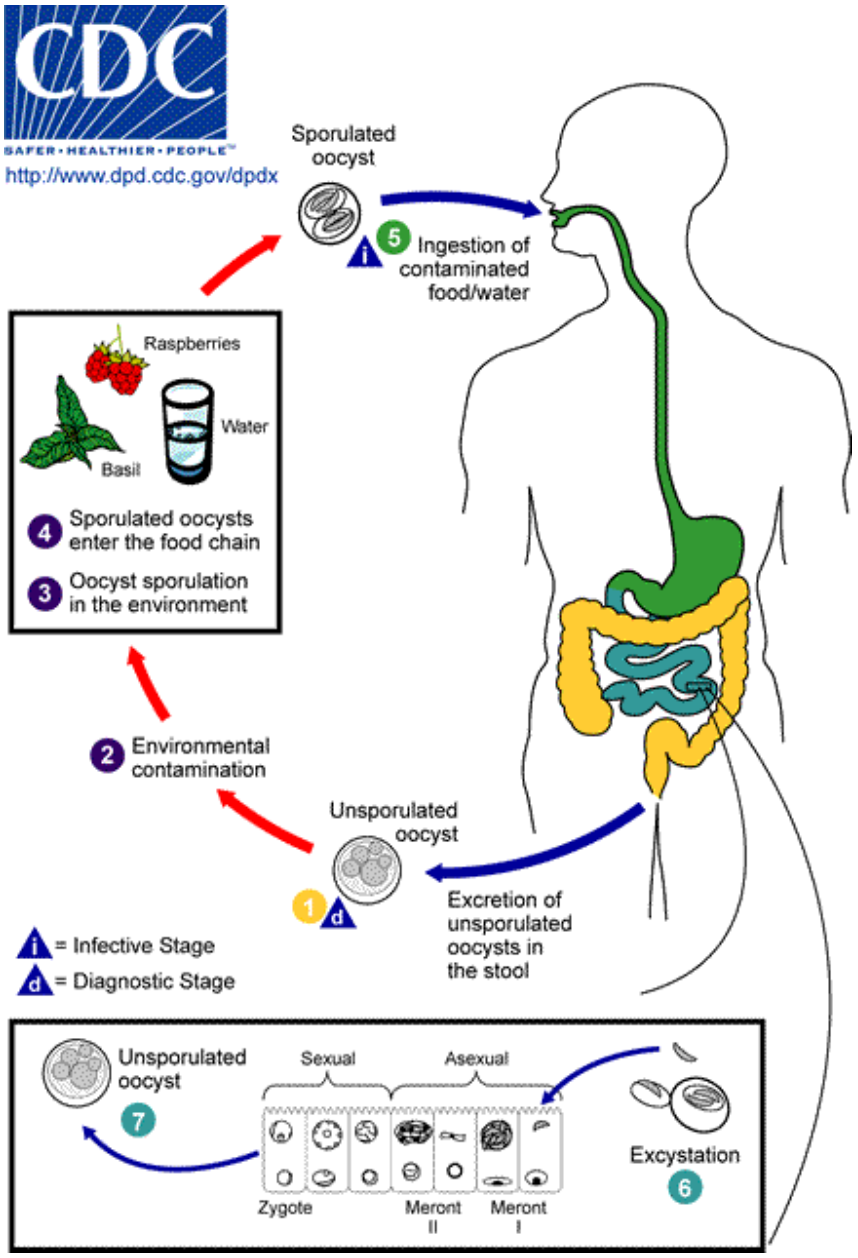
- More cases reported - *faster*
- Less laboratory information to include or exclude cases as disease clusters (early on)
- More demand to collect detailed exposure information
- Reporting of agents not readily detected by culture
- Multiple pathogens or spurious pathogens?

Number of infections diagnosed by culture or culture-independent diagnostic tests, by pathogen, year, and culture status — FoodNet sites, 2014–2017



Cyclospora Cases per 100,000 Population, FoodNET, 1997-2017





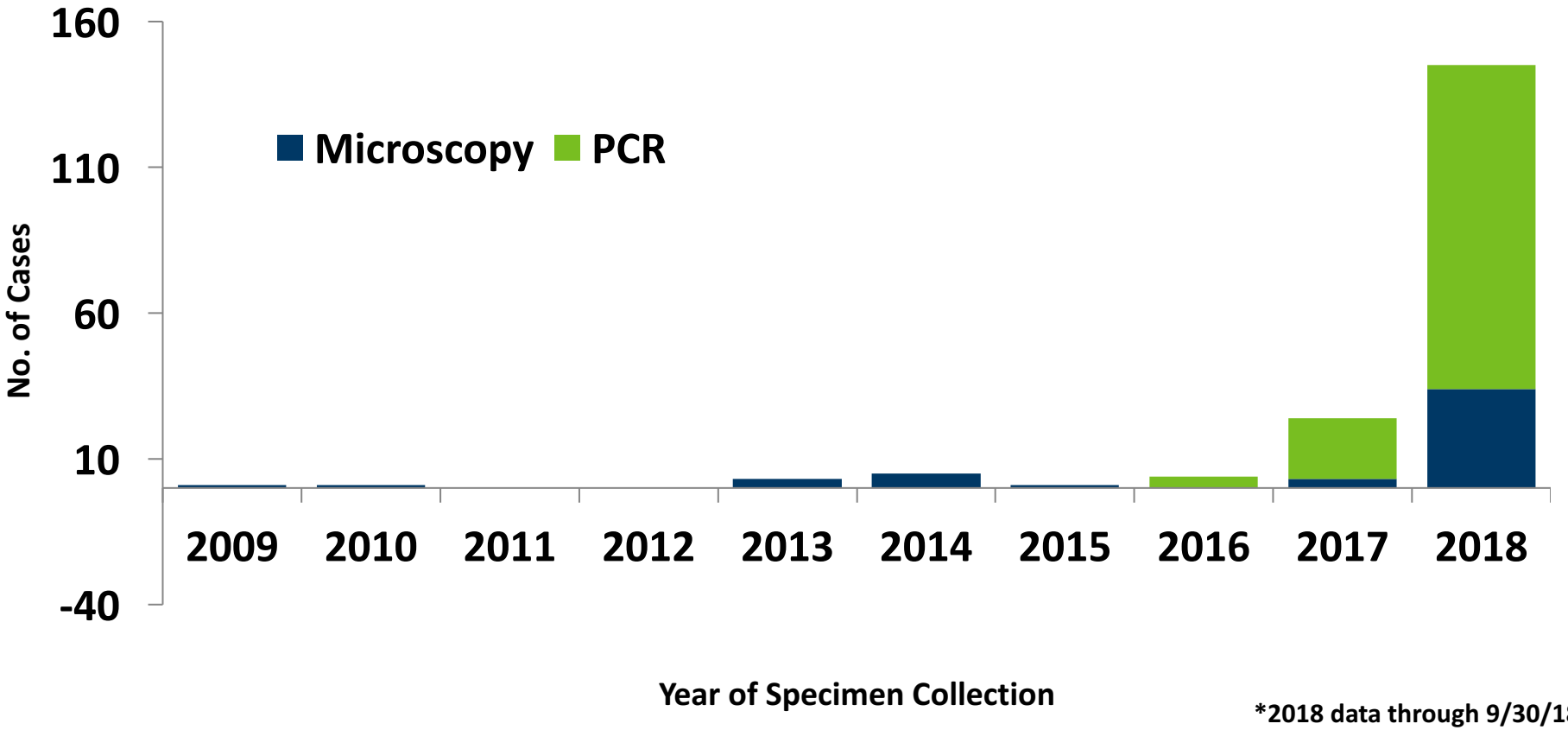
Expert opinion, Spring 2018:

There is no endemic transmission of *Cyclospora* recognized in the United States and there is no amplification of contamination possible at the point of service.

Thus, outbreaks of cyclopsoriasis in the United States **likely** represent primary contamination events of imported fresh produce items.

This was not *True*…this year

Cases of Cyclosporiasis by Method of Detection at Clinical Laboratory, Minnesota, 2009-2018*



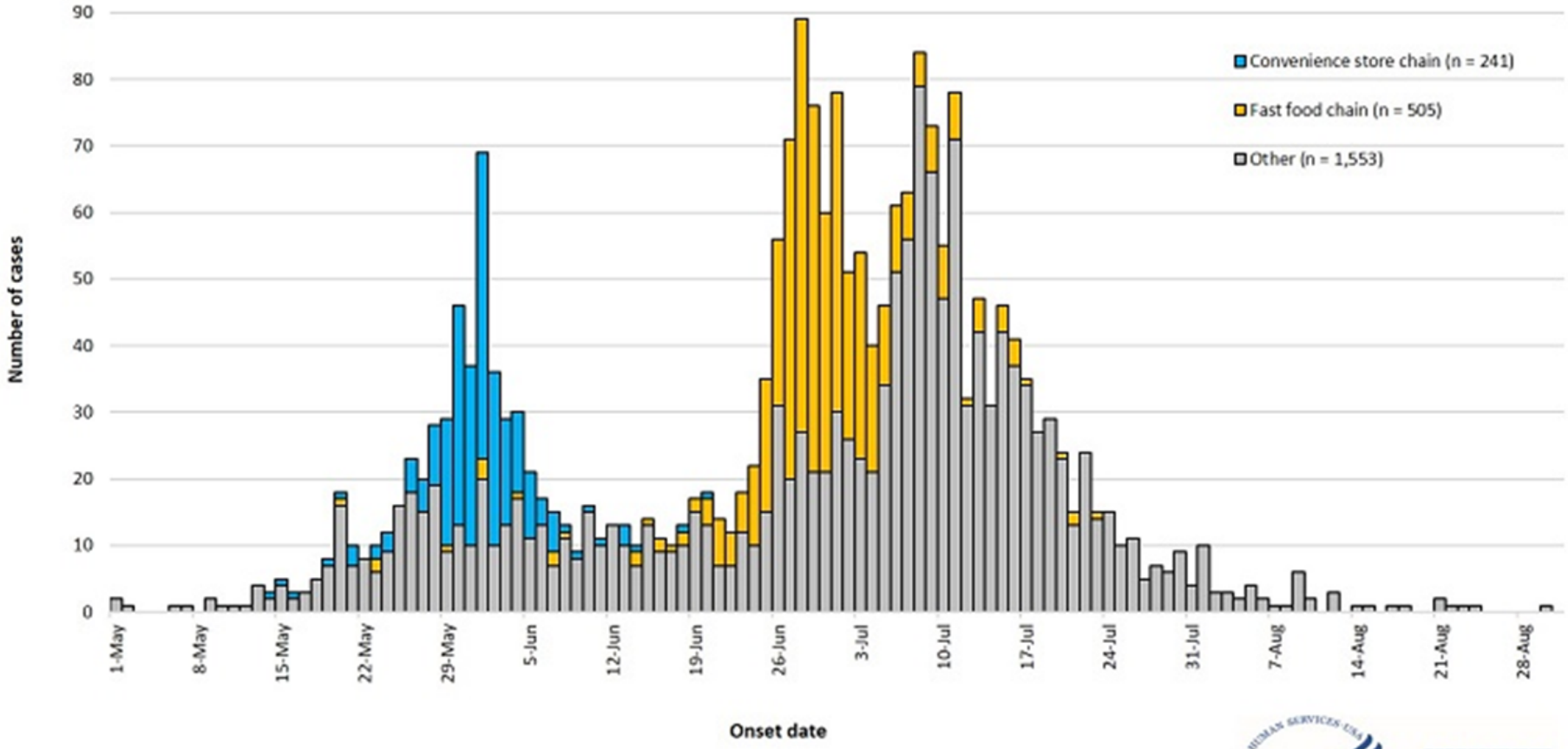
*2018 data through 9/30/18

Surveillance & Outbreak Response

- Cyclosporiasis is a nationally notifiable disease in most states
- CDC, in collaboration with public health authorities, analyzes each reported case for epidemiologic evidence of linkage to other cases, to facilitate rapid identification and investigation of outbreaks
- Yet, there are **no validated molecular tools** available yet for linking *C. cayetanensis* cases

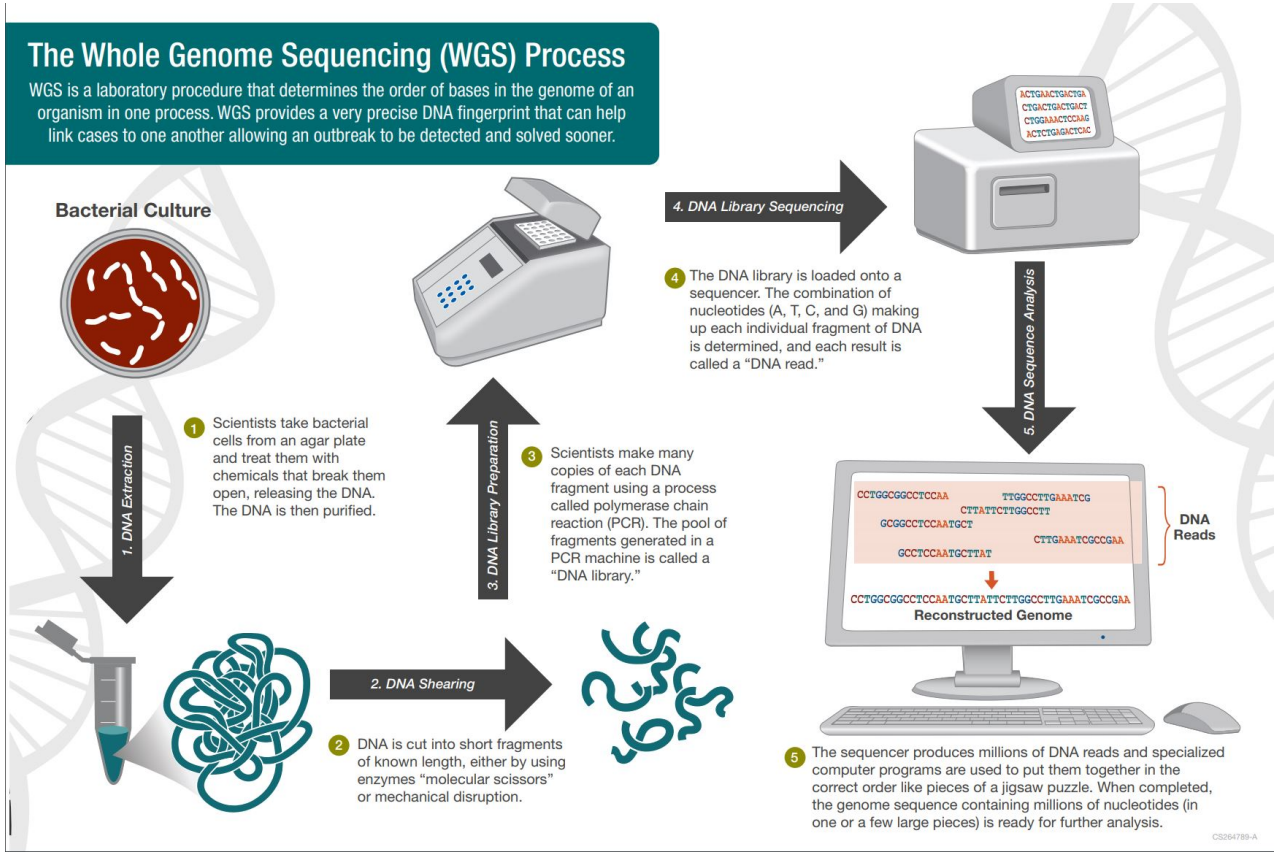
- Now what?

Domestically Acquired Cases of Cyclosporiasis — United States, May–August 2018

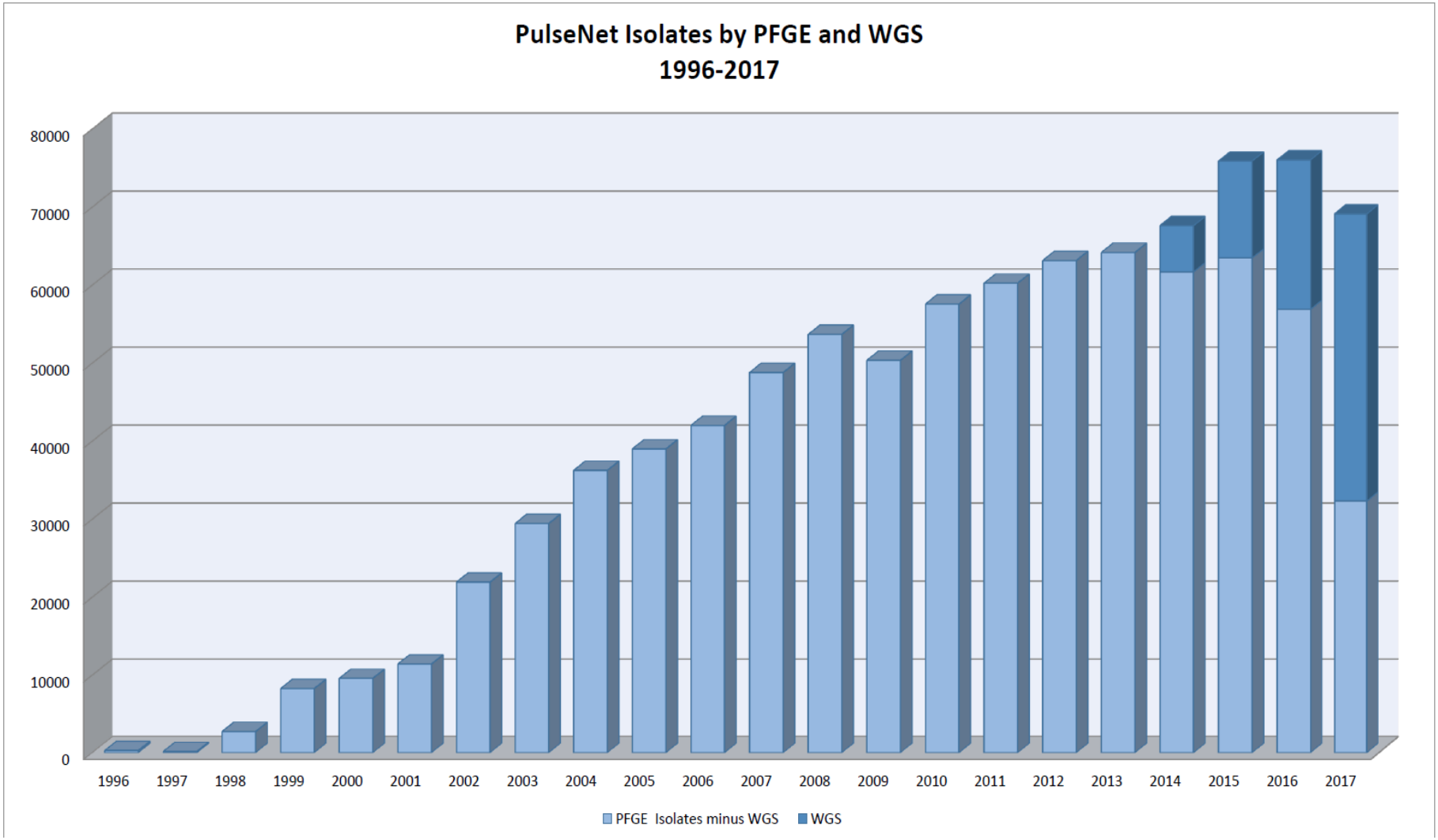


Enteric Pathogens Reported in 2016 by Clinical Laboratories that Started Using BioFire Prior to 2016		
Pathogen	# Reported	% of Total
Campylobacter	231	27.4
EAEC	175	20.7
Salmonella	108	12.8
STEC (O157 and non-O157)	99	11.7
ETEC	73	8.7
EPEC	61	7.2
Shigella	59	7.0
Yersinia	25	3.0
Vibrio	13	1.5
Total	844	100

Medus C, Minnesota Dept of Health 2018



IMPACT OF WHOLE GENOME SEQUENCING


PulseNet Isolates by PFGE and WGS
1996-2017
80000
70000
60000
50000
40000
30000
20000
10000
0
1996
1997
1998
1999
2000
2001
2002
2003
2004
2005
2006
2007
2008
2009
2010
2011
2012
2013
2014
2015
2016
2017
PFGE Isolates minus WGS
WGS

Potential Impact of Whole Genome Sequencing (WGS)

- More laboratory information to include or exclude cases from cluster investigations
- More demand for health authorities to investigate small clusters
- More focus on linking localized restaurant/retail outbreak investigations (sub-clusters) to common food chain source
- **Longer turn-around-times, delayed epidemiology work flow**

EDLB Vision

REPLACE all of these enteric workflows:

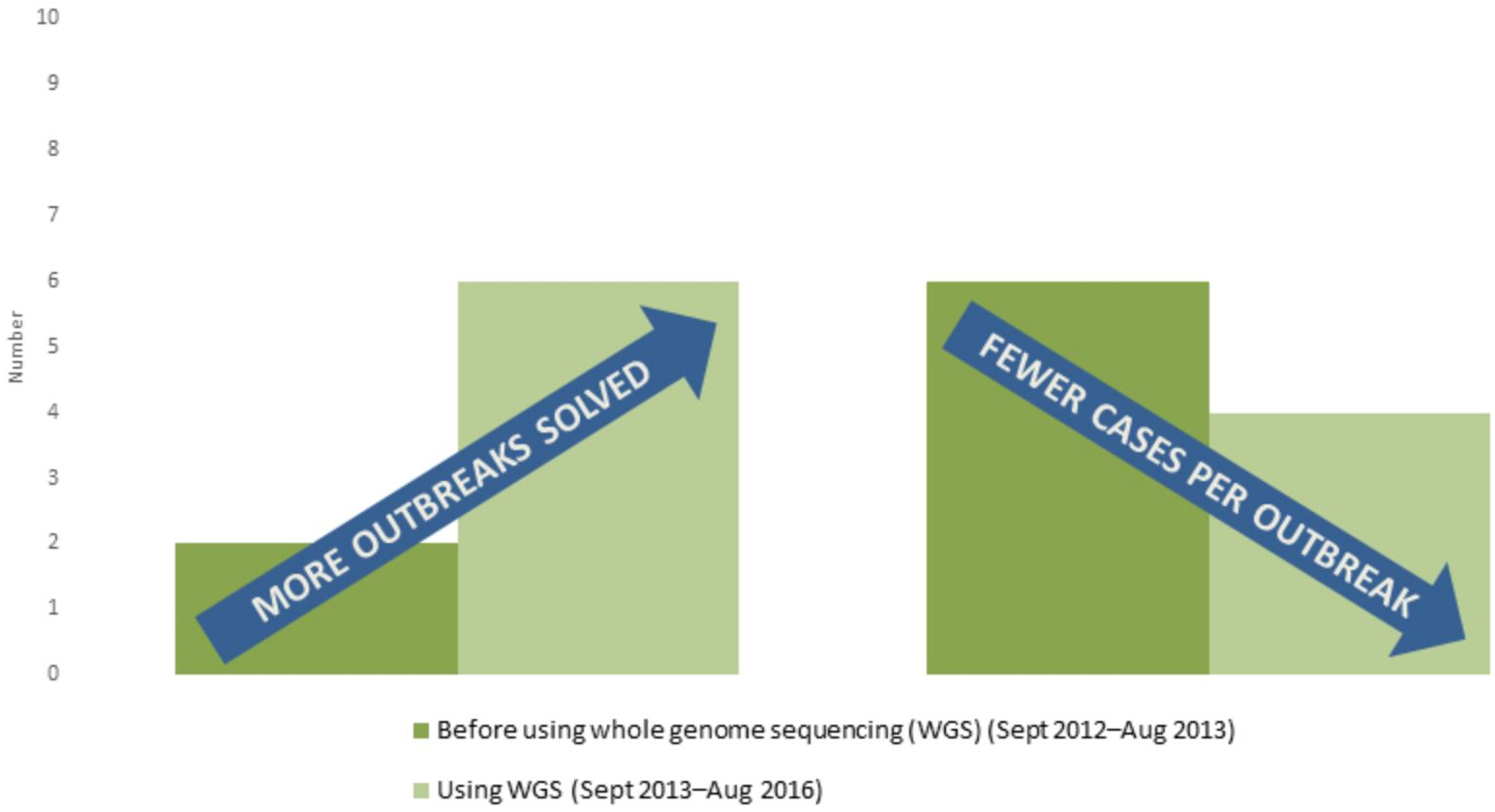
- Identification
- Serotyping
- Virulence profiling
- Antimicrobial susceptibility
- Subtyping for surveillance and outbreak investigations

With ONE cost-efficient and precise method: All of this information can be derived from the genome sequence

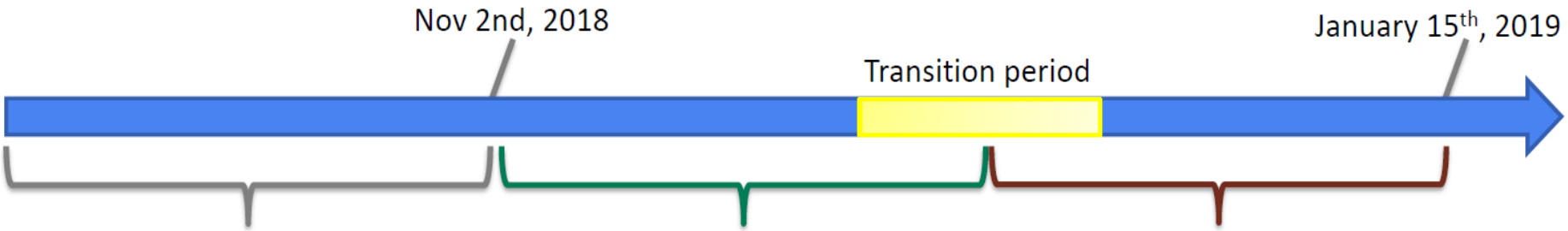
WGS

Listeria WGS Project at CDC

Whole genome sequencing prevents *Listeria* illness



PulseNet Transition Timeline to WGS Surveillance at States



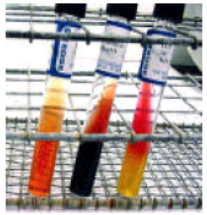
- Stopped PFGE for Listeria and Campylobacter
- Cleaned PFGE databases in preparation for upgrade to BN 7
- Talked and made preparations with IT for PulseNet database conversion and upgrade

- Convert databases to BN 7 and begin submitting PFGE data in BN 7
- Receive analysis certification in WGS from PulseNet
- Develop and implement validation plan for identification, serotyping, virulence and resistance gene detection if necessary under CLIA or CAP

- Submit state generated sequence data through BN for analysis; upload analyzed sequence data to PN national database; upload sequence reads to NCBI with limited metadata
- Perform local cluster detection using cgMLST and allele codes; compare to national database

Workflows and Turn-Around-Times

Day 0

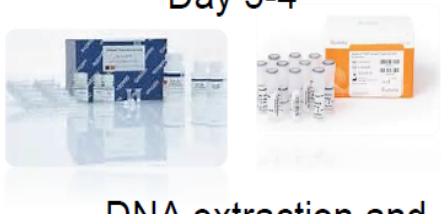


Isolate received or recovered at PHL

Day 2

Pure culture growth

Day 3-4

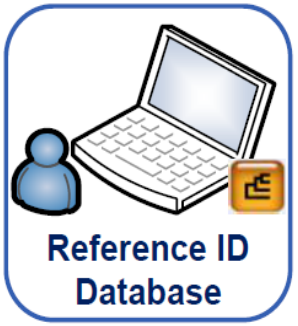


DNA extraction and Library preparation

Day 5-6

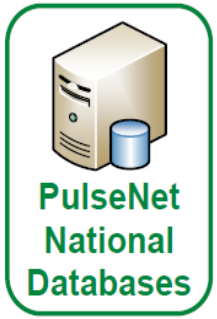
DNA sequencing

Day 6-7



Sequence data QC and identification

Genotyping and surveillance analysis



*TAT is measured at time of upload to National Database

PulseNet Prioritization for WGS

- **Conduct WGS on as many isolates as funds permit using the following priority schedule: (1) *Listeria monocytogenes*, (2) STEC, (3) *Salmonella*, (4) Other species**
  - If 100% of *Salmonella* cannot be sequenced, utilize a random sequencing approach (i.e. 1 of every 3 Salmonella received in laboratory)
  - Other organisms may be further prioritized if funded to do so (i.e. *Campylobacter* in FoodNet sites)

Potential Combined Impacts of CIDT and WGS

- More cases reported faster
- Reporting of agents not readily detected by culture
- Initially, less laboratory information to include or exclude cases from cluster
- Eventually, more laboratory information to include or exclude cases from cluster-for isolates that are submitted and subtyped
- Longer turn-around-times
- Disrupted epidemiology work flows

Likely

- More outbreaks associated with novel agents not previously detectable on a routine basis
- Epidemiologists will be unclear of epidemiologic linkages with delayed WGS data
- Impact on public health detection/response until tools improve

HOW TO HANDLE THIS TRANSITION?

Consider

- Focused training
- Capacity building – personnel with molecular techniques, data management, and investigation skills
- New investigative tools
- Big data tools to help with data mining

Team Diarrhea

Increasing the specificity of food exposure information provided by case-patients is as important as increasing the specificity of the case definition.

Collection of Laboratory Information

- More detailed information regarding the diagnostic methods
- Access to initial samples?

v 3.0 (May 2018)

Cyclosporiasis National Hypothesis Generating Questionnaire

State/NNDSS ID#: _____

Reset Form

Form Approved
OMB No. 0920-1198
Exp. Date 09/30/2020

General information (Questions to be completed by interviewer before the questionnaire is administered)

1. Classify case based on CDC case definition: ☐ Confirmed ☐ Probable

Laboratory information:

2. Date(s) stool collected for *Cyclospora* testing: _____
3. Test results: ☐ Positive ☐ Negative ☐ Indeterminate ☐ Pending
4. Specify type of testing laboratories (Check all that apply including confirmatory lab):
   ☐ Clinical lab (e.g., at a hospital/clinic) ☐ Commercial lab ☐ State lab ☐ CDC lab
5. Specify testing method(s) (Check all that apply including confirmatory test):
   ☐ O&P (e.g., light microscopy, UV fluorescence microscopy, stained smears)
   ☐ GI PCR Panel (e.g., BioFire FilmArray®) ☐ PCR (Not part of a panel) ☐ Lab-developed test
   ☐ Other, specify: _____
6. Specify name(s) of lab-confirmed coinfection:
   _____ ☐ Not applicable
7. Additional information (e.g., patient has appointment to submit stool, lab accession number, etc):
   _____

Detailed Case Investigation Forms

Section 8: Leafy greens (e.g., iceberg, romaine, mesclun, cabbage, spinach)

Now I have some questions about leafy greens (<u>not</u> canned, cooked, or frozen) that you (your child) may have eaten during the 14 days before your illness began. You could have eaten these leafy greens either in your home or away from home. I am only interested in leafy greens that were <u>not</u> grown at home. Please remember to include greens you might have eaten on sandwiches or burgers or as a garnish.

Yes	Maybe	No	Don't know	Did you (your child) eat:
☐	☐	☐	☐	58. Pre-made, single serving salads (e.g., ready to eat salads with toppings, meats, dressing)?
				a. What were the: Ingredients (lettuce, cabbage, carrots, etc.): _____ Brand(s): _____ Place(s) purchased (names, locations): _____
☐	☐	☐	☐	59. Iceberg lettuce?
				a. If eaten <u>at home</u>, what was the: Type(s): ☐ Prepackaged ☐ Head/Loose ☐ Topping/Garnish ☐ Unknown Brand(s): _____ Place(s) purchased (names, locations): _____ ☐ Not applicable (did not eat at home)
				b. If eaten <u>outside the home</u>: List name(s) of establishment(s) and location(s): _____ ☐ Not applicable (did not eat outside the home)

Summary

- New tools should help clinicians provide better patient care and support antibiotic stewardship activities
- Outbreak investigations may be take longer until they have better subtyping or grouping capabilities
- We may need patience as epidemiologic skills catch up with molecular tools (i.e. tools need to be more “investigator” friendly

Acknowledgements

- Special thanks to Dr. Craig Hedberg for sharing slides and perspectives

Questions?