PulseNet USA: Overview of the Molecular Subtyping Network for Foodborne Disease Surveillance

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Overview

- PulseNet Network
  - What is PulseNet
  - Objectives
  - Basic Elements
- PulseNet Communication and QA/QC
  - QA/QC: PFGE Lab and Data Analysis/CDC Team
  - Certification and Proficiency Testing
- Active Surveillance
  - PulseNet Network and Activity
  - Cluster Searches
  - Epidemiologic Investigations
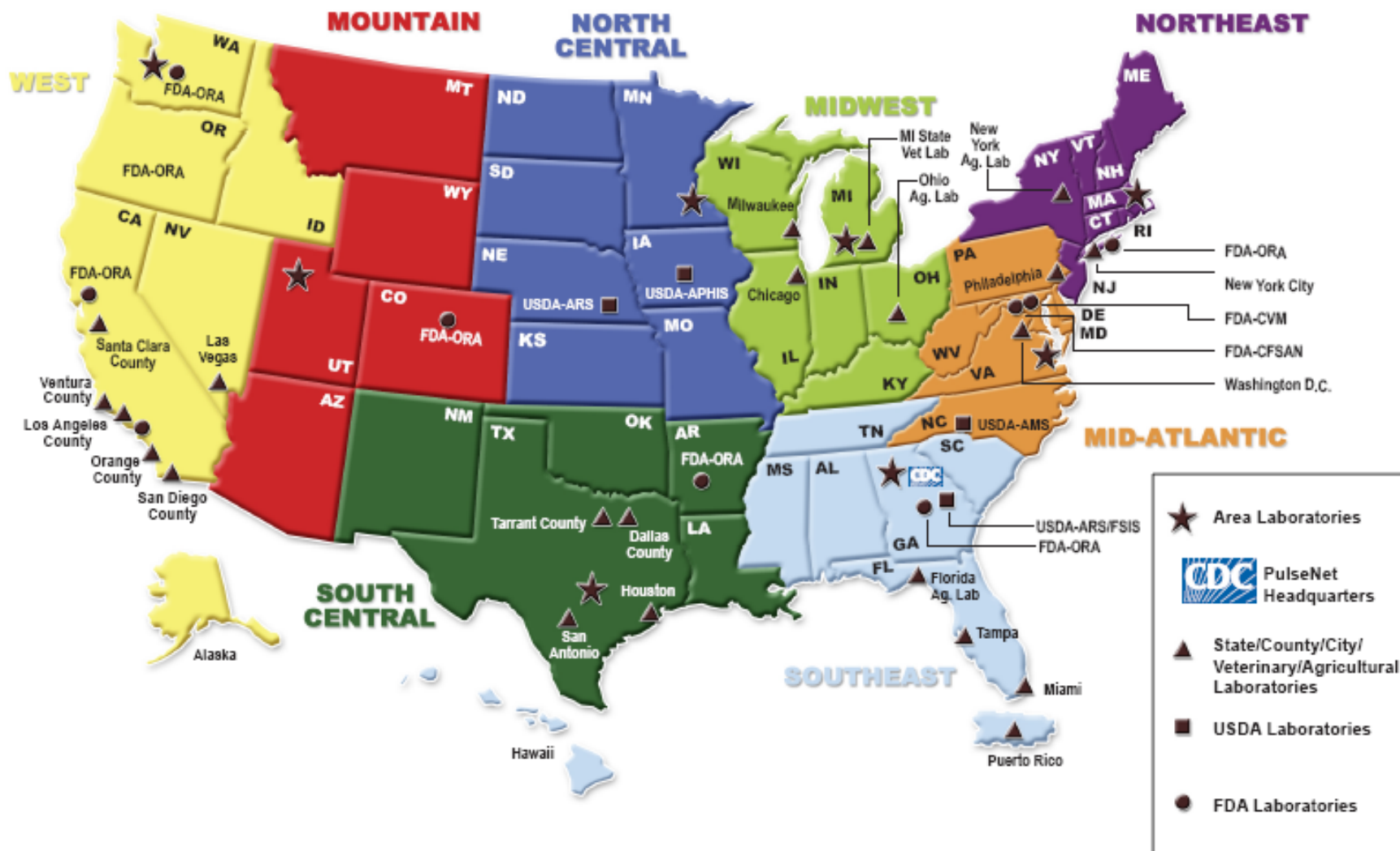- Database Uses
  - Pattern Frequencies
  - Trends

What is PulseNet USA?

- Established in 1996, The Molecular Subtyping Network for Foodborne Disease Surveillance

- A national network of >75 state and local public health/food regulatory agency laboratories (USDA, FDA) coordinated by CDC and APHL

- Perform standardized molecular typing of foodborne disease-causing bacteria by Pulsed-field gel electrophoresis (PFGE)

- Dynamic databases of DNA "fingerprints" at CDC—available on-demand to participants

PulseNet Objectives

- To <u>detect foodborne disease case clusters</u> that may be widespread outbreaks

- Provide real-time molecular surveillance of the most important bacterial foodborne diseases

- <u>Assist epidemiologists</u> in investigating outbreaks

  - Separate outbreak-associated cases from other sporadic cases (<u>case definition</u>)

  - Assist in rapidly identifying the source of outbreaks

- Act as a rapid and effective means of communication between public health laboratories

The National Molecular Subtyping Network for Foodborne Disease Surveillance

The Three Basic Elements of PulseNet

1. Data acquisition

2. Data analysis

3. Data exchange

Communication and QA/QC

- On-line databases
- CDC Team postings
  - Cluster detection
  - Outbreak investigations
  - Active Cluster Reports/Bundles
  - Technical support
- "PulseNet News" Newsletter
- PulseNet Website (www.cdc.gov/pulsenet)
- Annual update meetings

- Standardized protocols and molecular size standards
- QA/QC Manual
- Standardized software and nomenclature
- Training workshops (lab & software)
- Certification and proficiency testing

QA/QC: PFGE Laboratory

- Follow standardized protocols as closely as possible
- Be consistent
- Document
  - Any variations observed in an individual run
  - Temperature of water baths
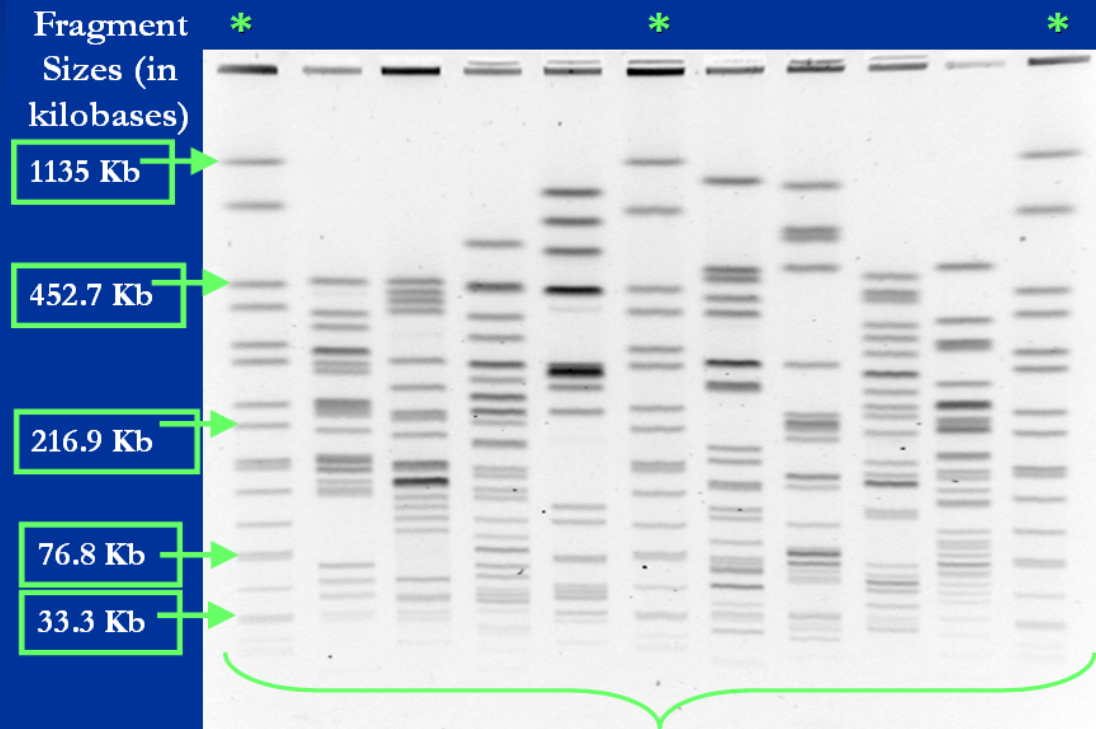 - Expiration date and lot numbers of reagents

QA/QC: PFGE Laboratory

- **Before Image Analysis:**
  - Critically review the appearance of the gel
  - Consider all points of the gel
  - Print gel image for reference
  - Make note of any obvious problems

PFGE Patterns of *E. coli* O157:H7

Fragment Sizes (in kilobases)

1135 Kb
452.7 Kb
216.9 Kb
76.8 Kb
33.3 Kb

DNA "fingerprints"

*Global Reference Standard

QA/QC: Data Analysis/CDC Team

- Follow standardized protocols for analyzing TIFFs and marking bands
  - Name TIFFs according to PulseNet protocols
  - Check all band markings before uploading
- Pay close attention to data format
  - Dates: YYYY-MM-DD
  - Age: use Entry Properties screen to enter (YY- MM-DD)
  - Pull downs: Patient Sex, Serotype, Source Type, etc.
- CDC Team
  - Name bundle files according to PulseNet protocols
  - Follow standardized protocols for posting initial and reply postings

Certification

- **Purpose:** to allow accurate comparisons within the PulseNet National databases by:
  - Ensuring TIFFs of all gel images are comparable and of satisfactory quality
  - Ensuring correct normalization and consistent band assignment
- **Process**
  - Certification sets include: 4 strains and instructions sent to requesting laboratory
  - Individual completes the subtyping and submits the TIFF and/or bundle file
  - TIFF and/or bundle file are evaluated to determine if they meet acceptable quality
  - Report is sent and individuals certified in analysis receive access to that national database (SecurID fob, username, and password)

Proficiency Testing (PT)

- Purpose: To ensure laboratories meet/exceed the PulseNet standards of PFGE gel quality and analysis
- Process:
  - Annually receive one PT isolate for each pathogen in which certified (every certified participant should run PT)
  - Perform subtyping; analyze in-house TIFF (currently only one TIFF is submitted per lab) and a TIFF sent by CDC
  - Results from analysis of both gels are submitted for evaluation
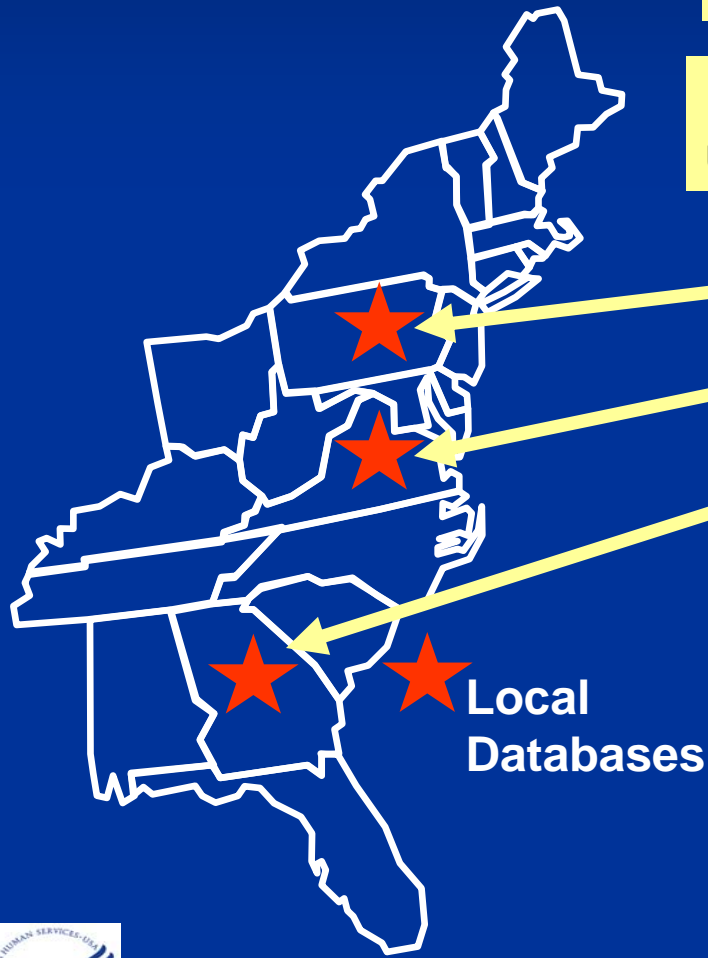
PulseNet Laboratory Network

Participating Labs

PFGE Patterns & Demographic Data

PulseNet National Databases (CDC)

TAT from receipt to upload: ~4 working days

Local Databases

Cluster Follow-Up/Communication w/Epis

Database Management & Reports

**Cluster Detection**

CDC

PulseNet Activity*

Over 375,000 PFGE patterns or DNA "fingerprints" submitted to PulseNet databases since 1996

Database	Entries Submitted	Patterns submitted	
		1st Enzyme	2nd Enzyme
Campylobacter	6,008	5,959	2,016
E. coli	35,414	34,070	20,310
Listeria	9,918	9,007	8,787
Salmonella	221,806	219,026	33,809
Shigella	37,638	37,423	2,545
V. cholerae	312	291	281
V. parahaemolyticus	37	37	37
Y. pestis	2,202	2,202	33

PulseNet Cluster Detection System

- PulseNet is a cluster detection tool, not an outbreak detection system
  - A PulseNet CLUSTER is a group of patterns that are found indistinguishable by PFGE
  - CLUSTERS of cases identified by PulseNet are investigated by epidemiologists
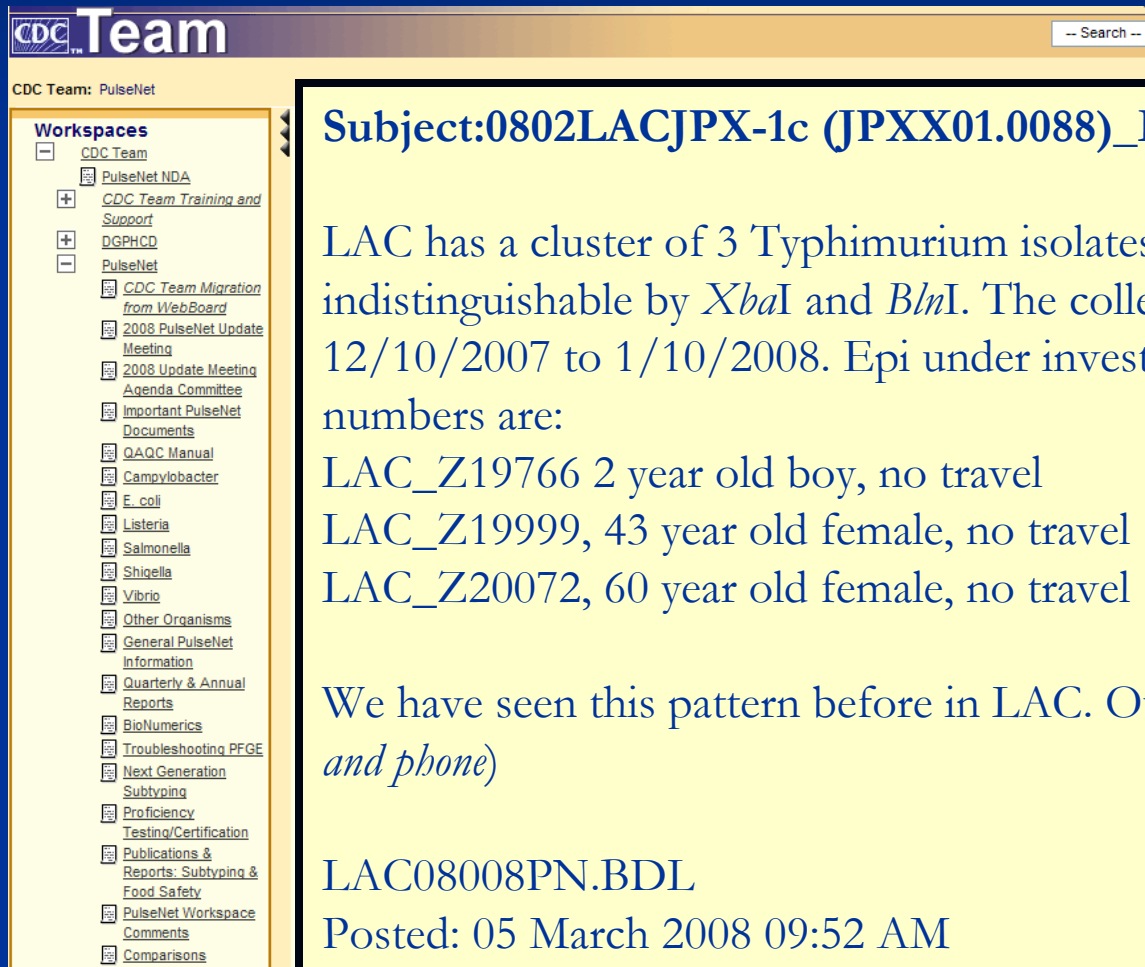 - If epidemiologic links are found between cases, the cluster is classified as an OUTBREAK

What is a Cluster Search?
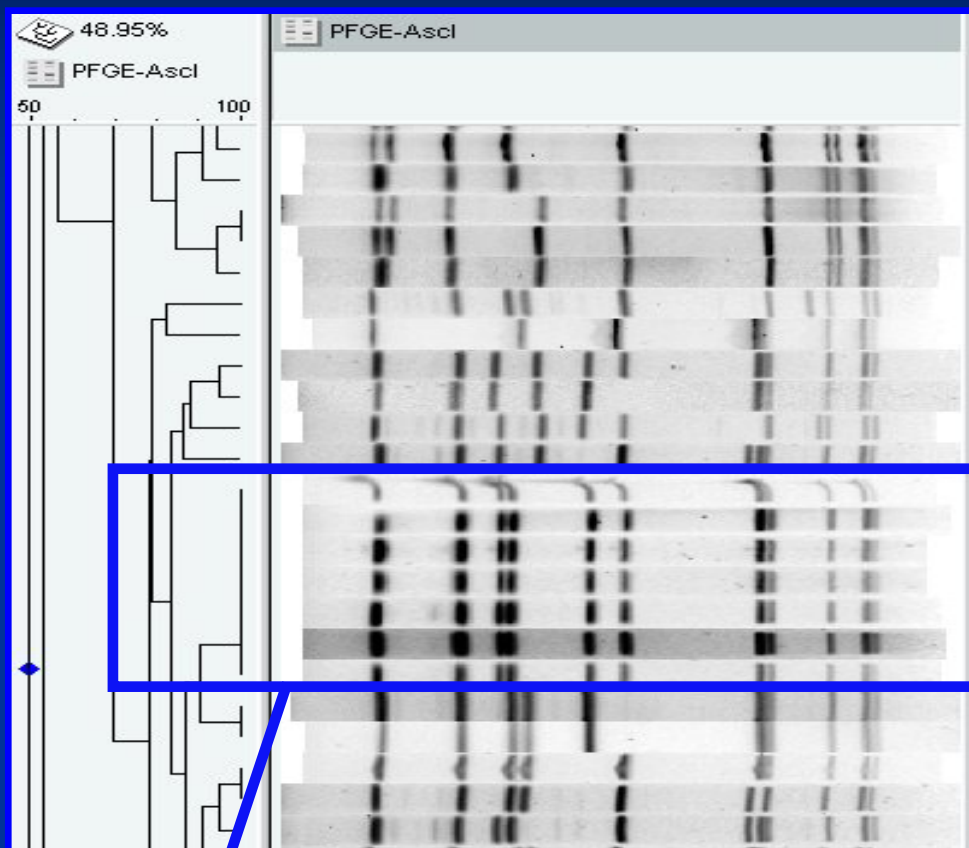
- **Patterns submitted electronically**
- **Cluster searches performed**
- **Visually compare indistinguishable patterns with 1st enzyme, then 2nd (if necessary)**
- **Patterns/clusters named by CDC**

Cluster of indistinguishable patterns by primary enzyme

Cluster Detection in PulseNet: PulseNet Workspace on CDC Team

MLVA Analysis

- Sequence-based subtyping
- Can further discriminate common PFGE patterns through highly variable target sequences
- Data may be epidemiologically more relevant than PFGE data
- Results more straightforward
- Currently MLVA results are housed in databases separate from PFGE; however, the ultimate goal is to have them in combined databases
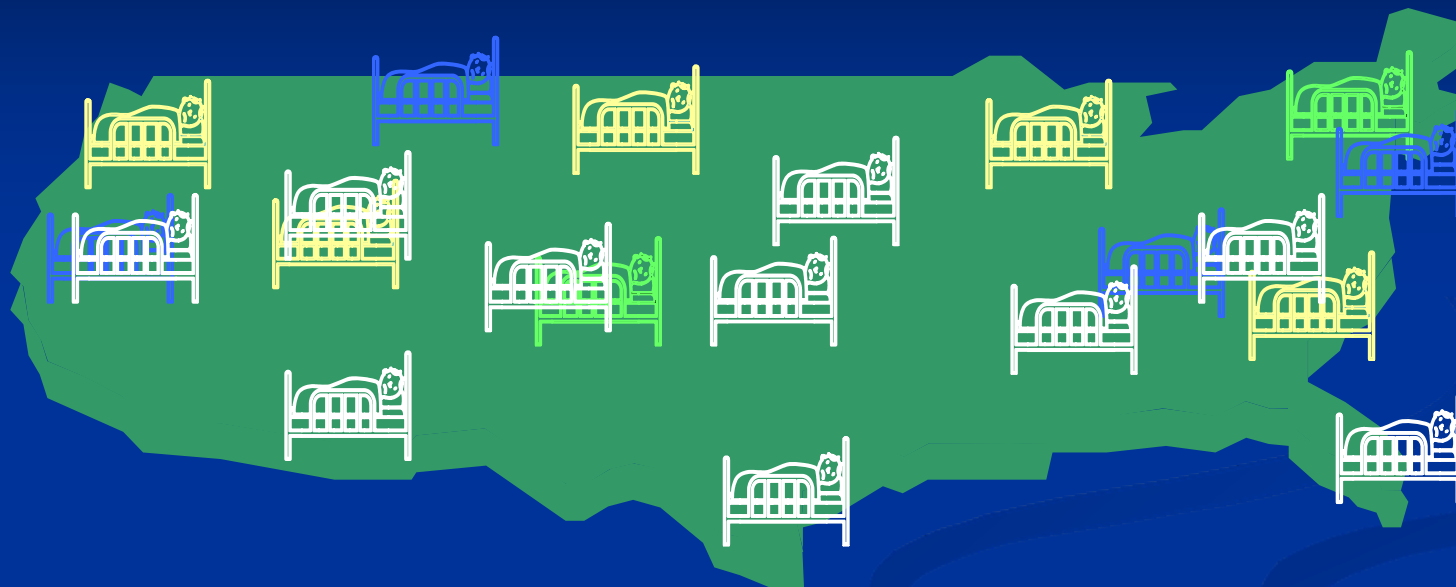
Epidemiologic Investigations:
A large outbreak in one place

■**Outbreak may be obvious**
■**Detected and investigated locally**

Epidemiologic Investigations:
A dispersed outbreak in many places

- Detect outbreaks centrally (or locally) through surveillance (widely dispersed, organism too common to notice small increase, identify related cases)
  - Investigation coordinated centrally
- Distinguish from concurrent sporadic cases
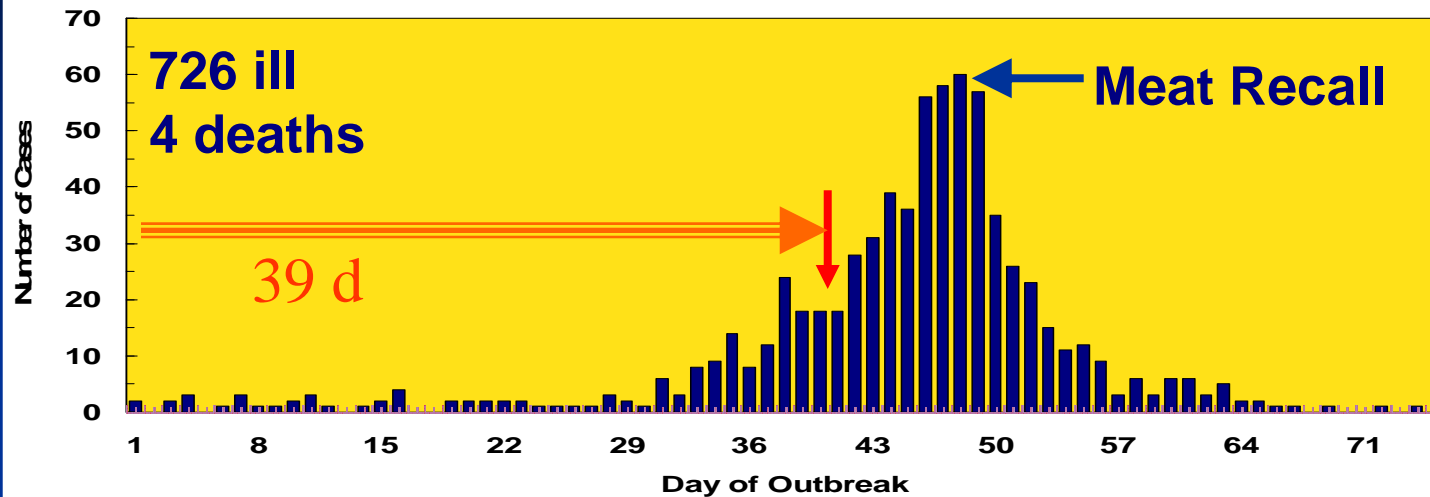 - Provide microbiological evidence of sources of outbreaks

Recent Foodborne Outbreaks With PulseNet Involvement

- *Salmonella* **Typhimurium – Peanut Butter products**
- *Salmonella* **Saintpaul – Raw Produce**
- *Salmonella* **Tennessee – Peanut butter**
- *Salmonella* **I 4,[5],12:i:- – Pot pies**
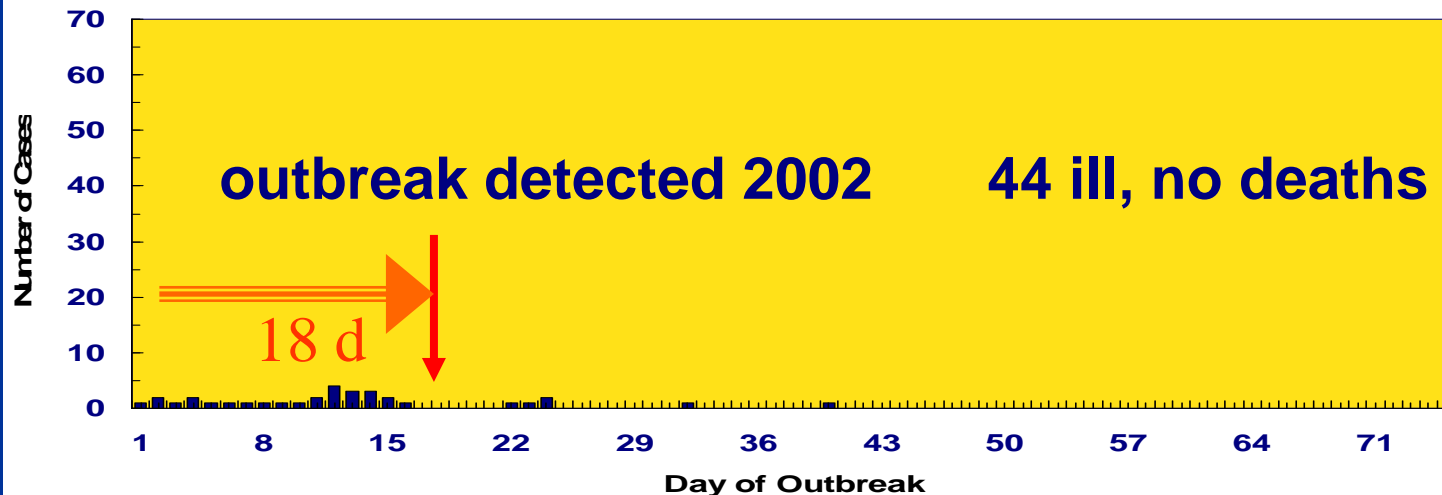- *E. coli* **O157 – spinach; ground beef**
- *Listeria monocytogenes* **– Milk**

Just to name a few.......

PulseNet as an Early Warning System

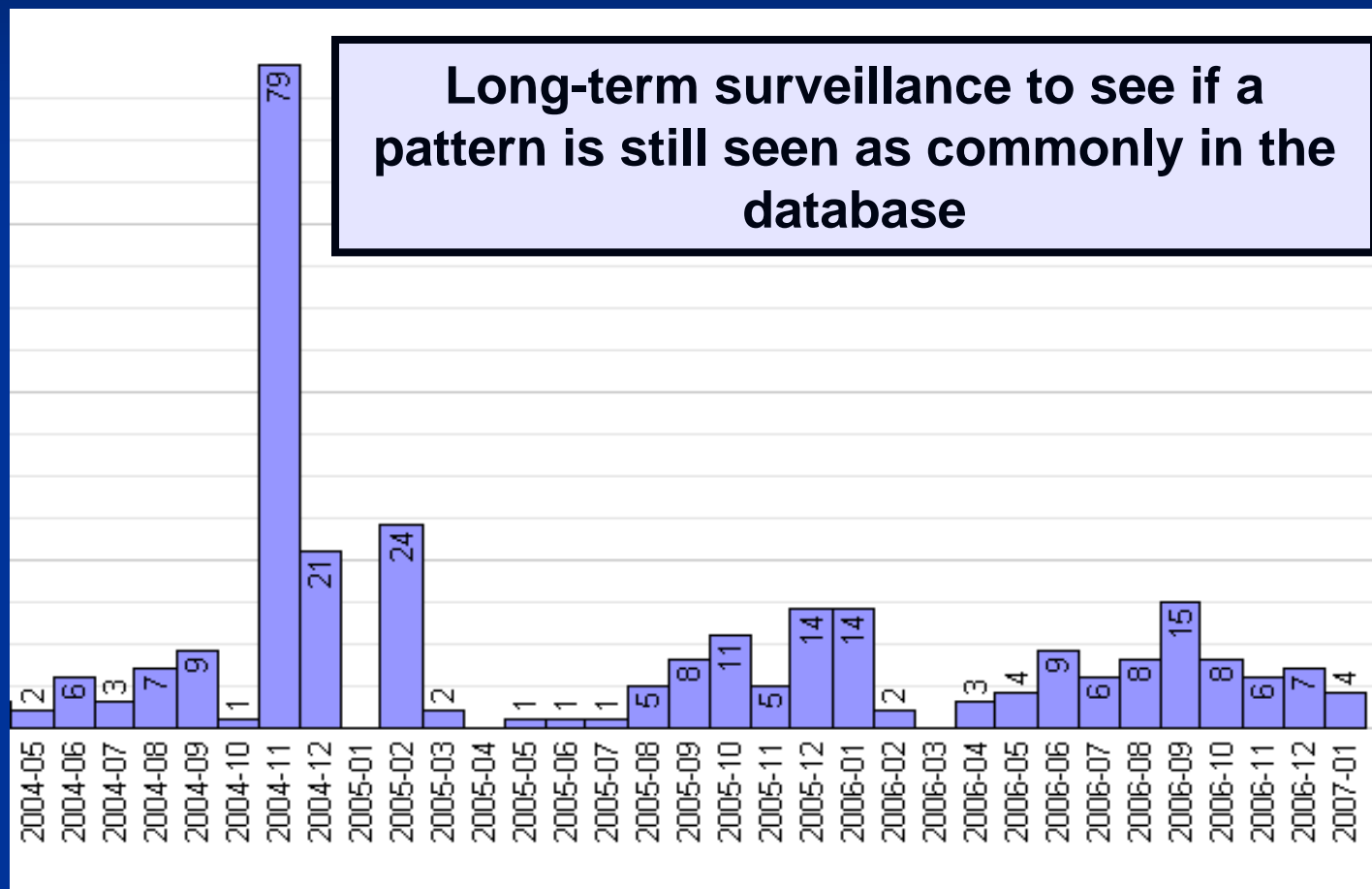## 1993 Western States *E. coli* O157 Outbreak

726 ill
4 deaths

Meat Recall

39 d

Number of Cases

Day of Outbreak

2002 Colorado *E. coli* O157 Outbreak

outbreak detected 2002   44 ill, no deaths

18 d

Number of Cases

Day of Outbreak

Database Uses: Pattern Trends

Long-term surveillance to see if a pattern is still seen as commonly in the database
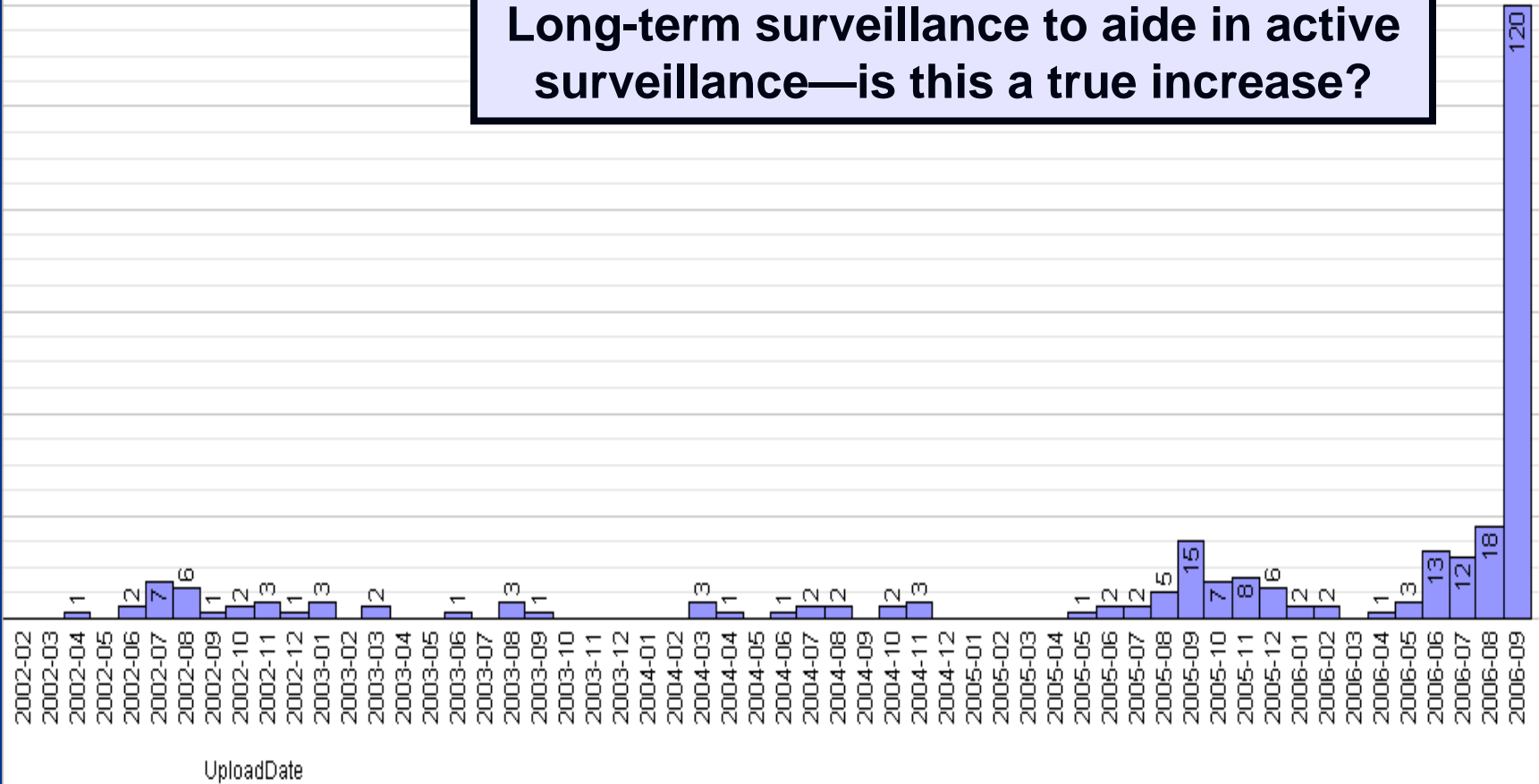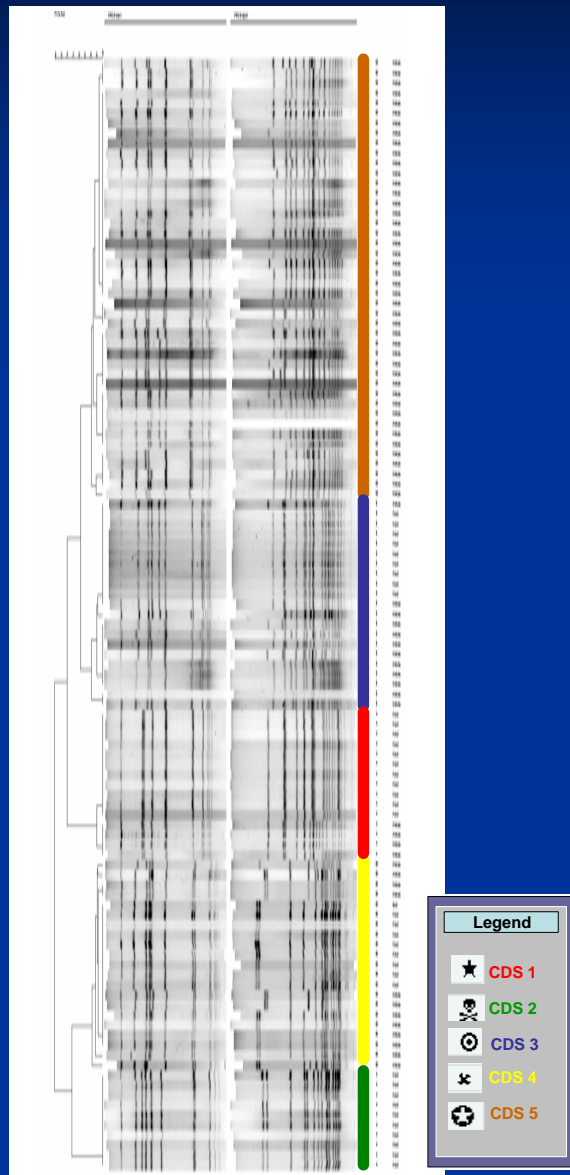
Database Uses: Pattern Frequencies

Long-term surveillance to aide in active surveillance—is this a true increase?

Database Uses: Attribution Analysis

What is the relative contribution of each food source to the burden of foodborne illness in humans?

Legend

★	CDS 1
☠	CDS 2
☉	CDS 3
✄	CDS 4
✪	CDS 5

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Questions?

Division of Foodborne, Bacterial, and Mycotic Diseases

Thank you for your attention
The findings and conclusions in this presentation are those of the author and do not necessarily represent the views of the Centers for Disease Control and Prevention