

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



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# What is PulseNet USA?

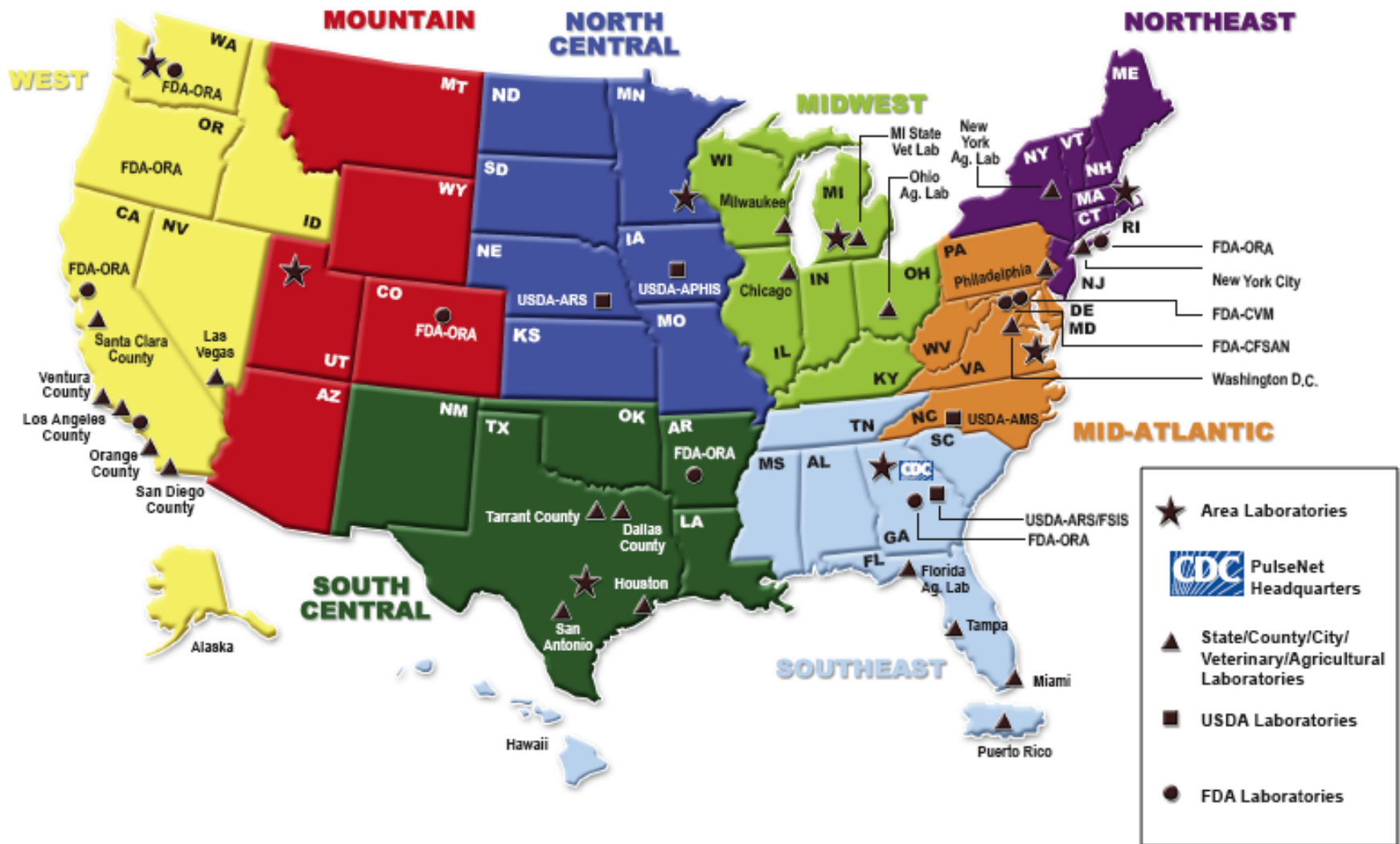
- Established in 1996, The Molecular Subtyping Network for Foodborne Disease Surveillance
- A national network of >75 state and local public health/food regulatory agency laboratories (USDA, FDA) coordinated by CDC and APHL
- Perform standardized molecular typing of foodborne disease-causing bacteria by Pulsed-field gel electrophoresis (PFGE)
- Dynamic databases of DNA “fingerprints” at CDC—available on-demand to participants



# PulseNet Objectives

- To detect foodborne disease case clusters that may be widespread outbreaks
- Provide real-time molecular surveillance of the most important bacterial foodborne diseases
- Assist epidemiologists in investigating outbreaks
  - Separate outbreak-associated cases from other sporadic cases (case definition)
  - 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

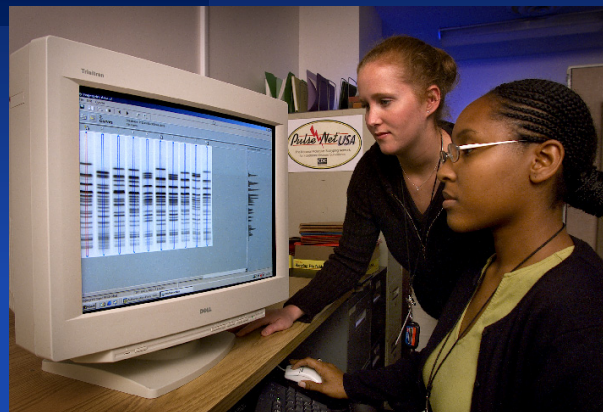




# The Three Basic Elements of PulseNet



## 1. Data acquisition



## 2. Data analysis

A screenshot of the PulseNet CDC Team workspace. The page title is "CDC Team" and the user is "Kelley Hise". The workspace contains a "Messages of the day" section with links to "CDC Team Sign up for a New User or Administrator Training Session!" and "CDC Team Training and Support Want to add new users to your workspace? Click Here!". There is also a link to "Click to jump to the PulseNet CDC Team Orientation Video!". A "PulseNet CDC Team Policy on Sharing Information" section is visible, stating that PulseNet CDC Team postings contain preliminary information on presumptive disease clusters and ongoing outbreak investigations. The policy requires prior approval for sharing information with persons outside public health and food regulatory agencies. A "Workspaces" sidebar on the left lists various PulseNet workspaces, including "PulseNet NDA", "CDC Team Training and Support", "OSH State Information Forum", "PulseNet", "CDC Team Migration from WebBoard", "2008 PulseNet Update Meeting", "2008 Update Meeting Agenda Committee", "Important PulseNet Documents", "QA/QC Manual", "Campylobacter", "E. coli", "Listeria", "Salmonella", "Shigella", "Vibrio", "Other Organisms", and "General PulseNet Information".

## 3. Data exchange





**Patient Specimen Collection**



**Specimen**



**Culture growth**



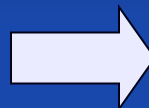
**Cell Suspension**

+

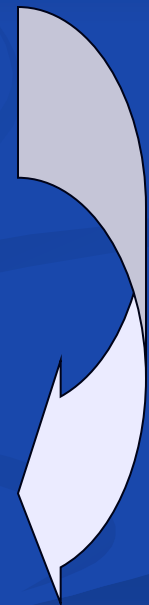


**Agarose**

=



**Cells Trapped in Plug  
-Lyse cells**



**Restriction**

↓  
...TCTAGA...  
...AGATCT...  
↑



**Finished Product!**

**DNA fingerprinting process**



# Intra-lab Comparison of PFGE Patterns

Fragment Sizes \*

(in kilobases)



- Online databases
- CDC Team postings
  - Cluster detection
  - Outbreak investigations
  - Active Cluster Reports/Bundles
  - Technical support
  - Reports
- “PulseNet News” Newsletter
- PulseNet Website  
([www.cdc.gov/pulsenet](http://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

DNA “fingerprints”

\*Global Reference Standard



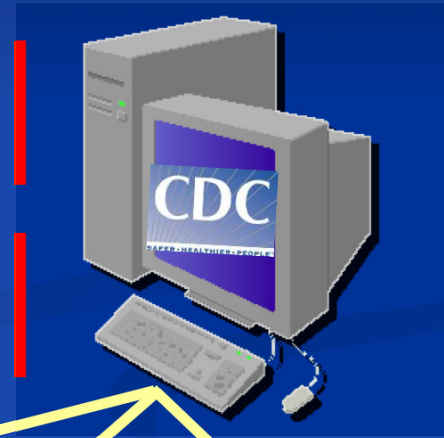
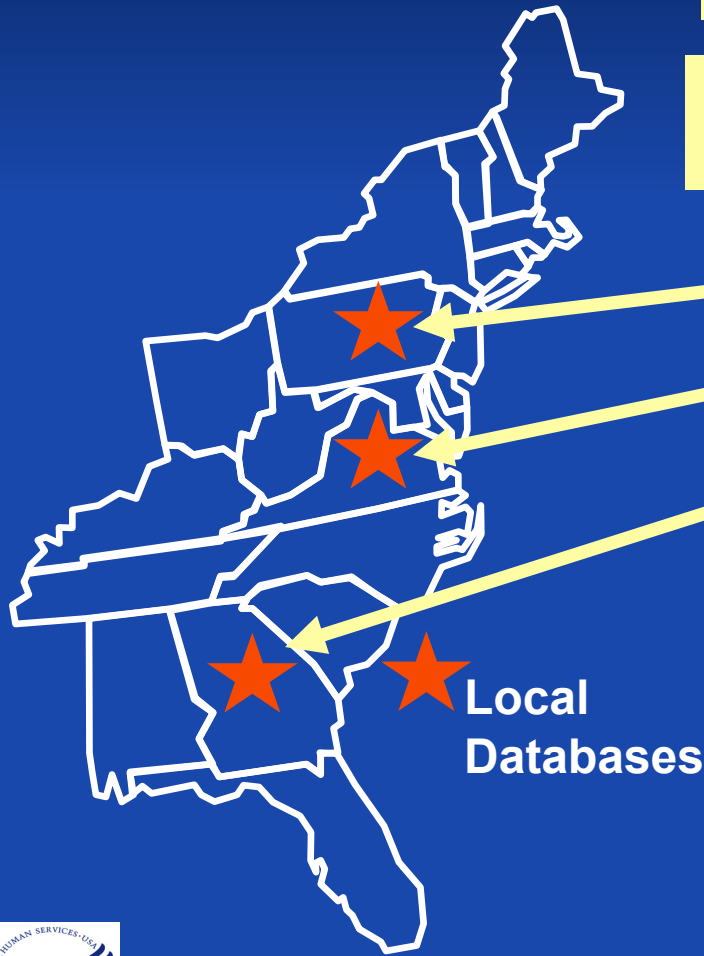
# PulseNet Laboratory Network

**Participating Labs**

**PFGE Patterns & Demographic Data**

**PulseNet National Databases (CDC)**

**TAT from receipt to upload: ~4 working days**

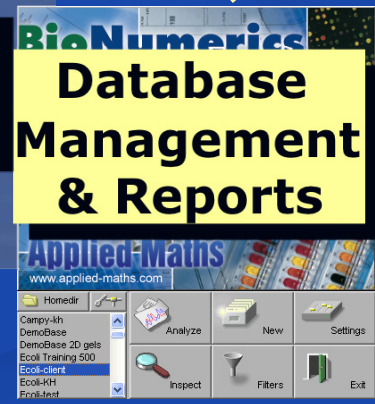


**Cluster Follow-Up/  
Communication w/  
Epis**

**Database  
Management  
& Reports**

**Cluster  
Detection**

**Local  
Databases**





# PulseNet Activity

\*as of January 1, 2009

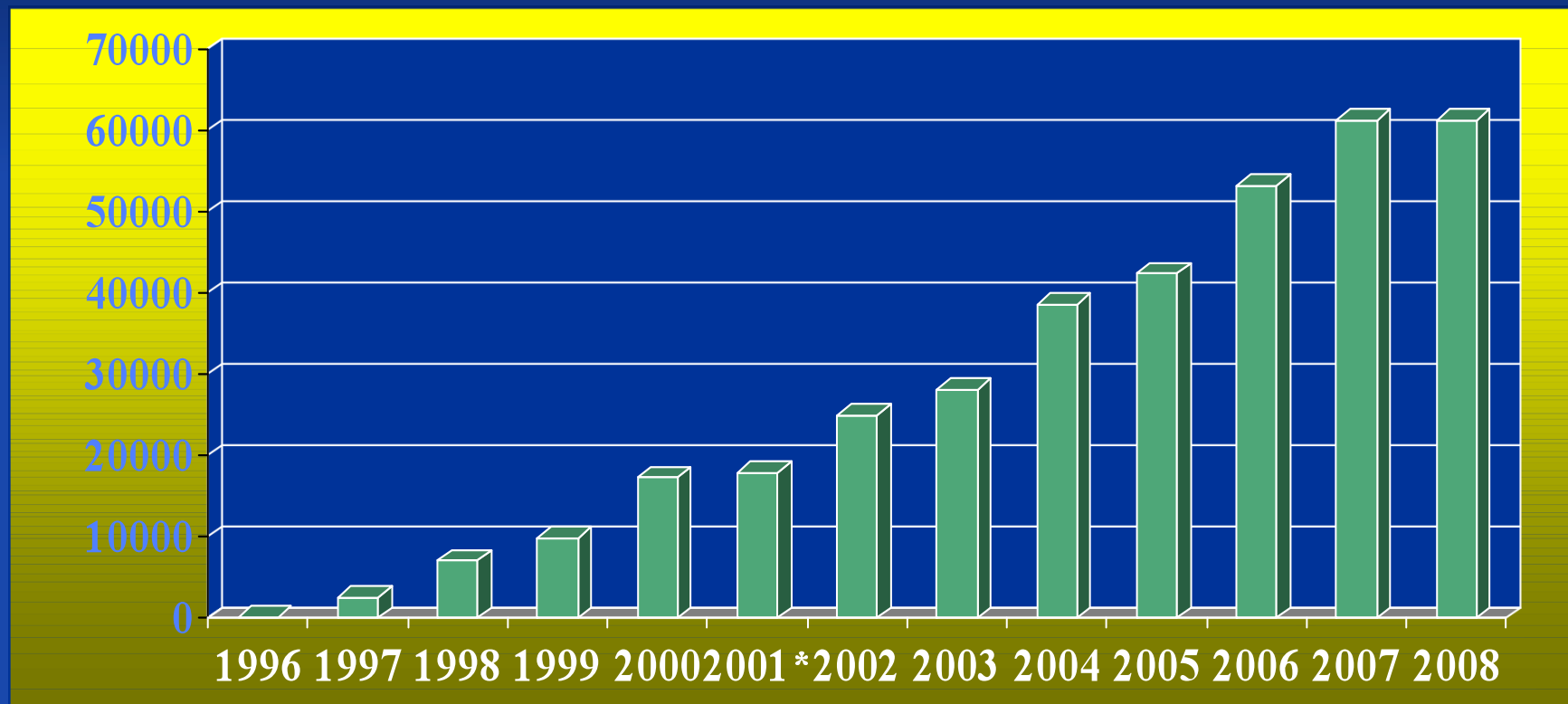
Over 375,000 PFGE patterns or DNA “fingerprints” submitted to PulseNet databases since 1996

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



# PulseNet Activity, 1996-2008

PFGE patterns submitted to PulseNet Databases

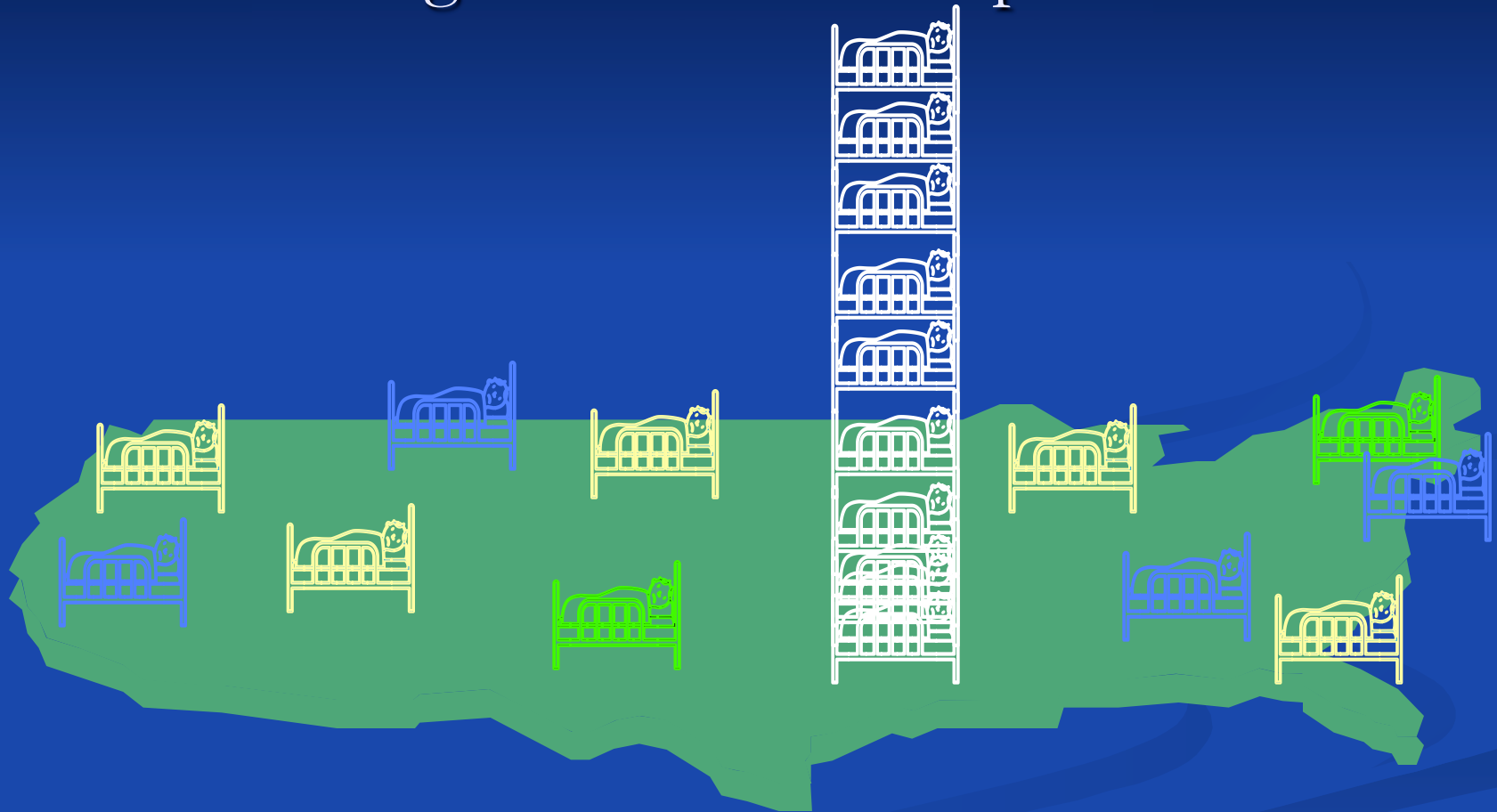


# Molecular Surveillance

- Subtyping is the differentiation of isolates of the same species.
- PulseNet is a network of public health laboratories that use subtyping to detect, investigate, and control outbreaks of foodborne infections.
- Monitoring the trends of specific subtypes of a specific pathogen by a *high discriminatory* molecular subtyping method
  - Plasmid profiling
  - Pulsed-Field Gel Electrophoresis (PFGE)
  - Multi-Locus VNTR Analysis (MLVA)
  - SNP-analysis

# Epidemiologic Investigations:

A large outbreak in one place

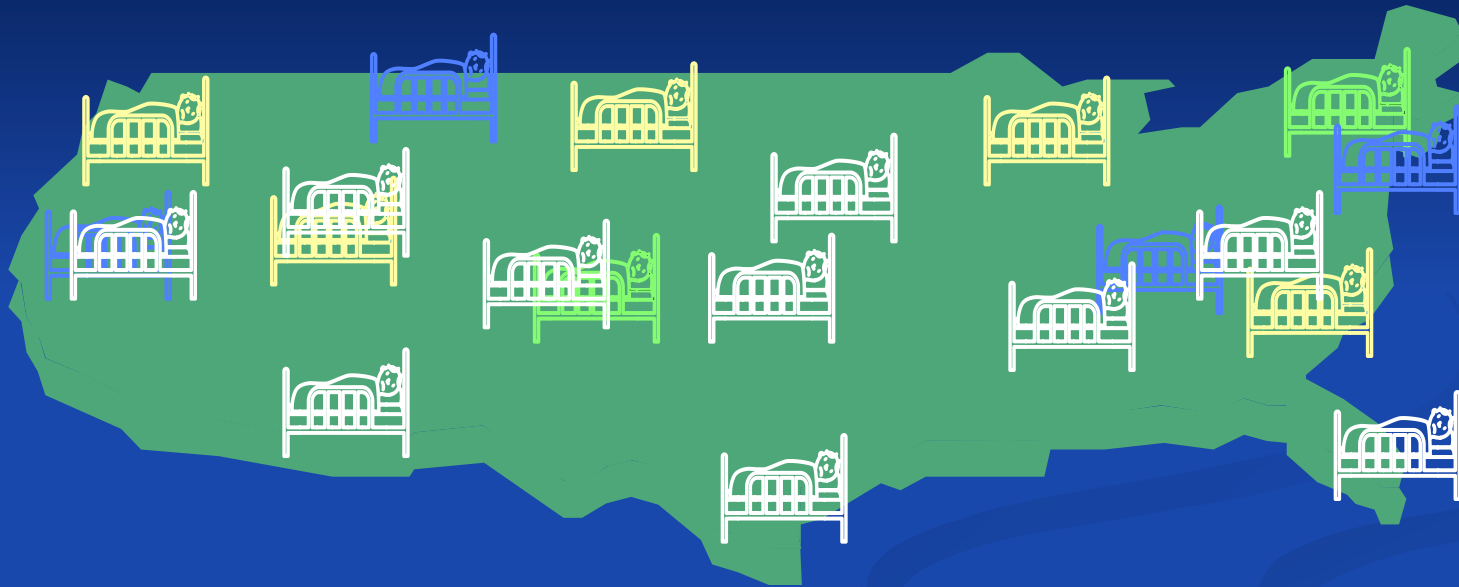


- Outbreak may be obvious
- Detected and investigated locally



# 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

# Local Cluster Detection and Follow-Up

## Initial Detection

- Perform cluster search within local database
  - Name local patterns and/or download CDC pattern names
  - Look for clusters of 3 or more within past 60 days (120 days for *Listeria*)
- Create a frequency graph for suspected pattern(s)
  - Look at pattern frequency over time
  - >2x increase over a month
  - May take into consideration seasonality of pattern
- Report local clusters internally using in-house procedures

# Local Cluster Detection and Follow-Up

## Initial Detection Continued...

- Compare pattern(s) to national database (only if necessary\*)
  - Use match against server and/or other query tools to see matches in other labs
  - It is not necessary to post matches from other states to CDC Team
- Post message to CDC Team IF at least one of the following:
  - Epidemiologic links
  - Above normal pattern baseline frequency in local database
  - DO NOT include CDC pattern names or line lists
- Monitor topic on CDC Team for CDC's and other laboratories' responses



\*If local epis, supervisors, etc. wish to see if there are matches at the national level/neighboring states



# Local Cluster Detection and Follow-Up

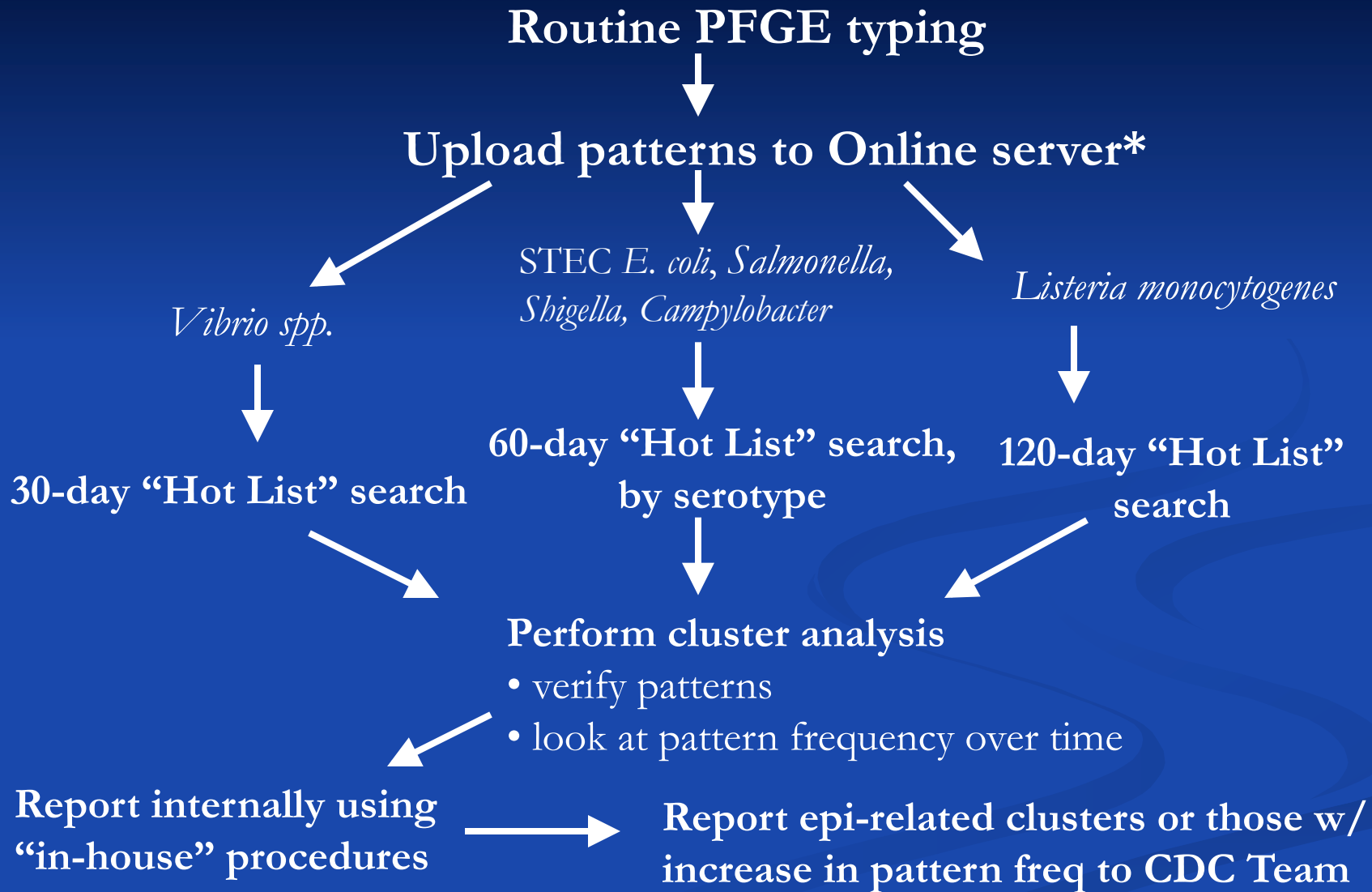
## Existing Clusters on CDC Team

- Once a week save cluster bundle files provided by CDC in “Important PulseNet Documents” in local databases
  - See if anything new matches a cluster
  - If matches exist, refer to the Active Cluster Report to see if the cluster is still active
- Sign up to receive email notifications for all organisms on CDC Team
- When you receive new notification, wait for CDC to rename posting and post line list
  - If your local patterns are in list, download outbreak code
  - Continue to compare new patterns to posted bundle





# Local Cluster Detection



\*If no laboratorian is certified for analysis, must email TIFFs and demographic information to CDC (pfge@cdc.gov)



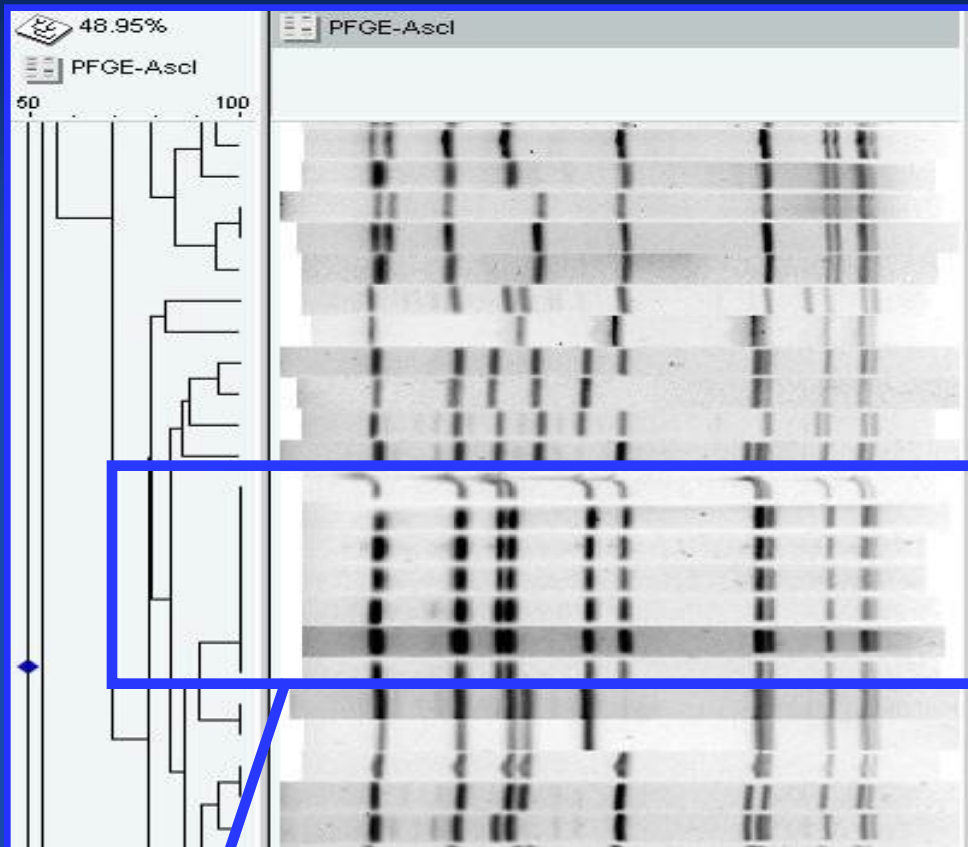
# PulseNet Cluster Detection System

- 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

# National Cluster Detection and Response

- Perform cluster search within national database *or* respond to local CDC Team posting or other request
- Assign cluster codes to clusters that indicate an increase in a particular PFGE pattern at the national level
- Create: line lists, frequency graphs, pie charts, cluster reports
- Report multi-state clusters that indicate an increase in a particular PFGE pattern
- Respond to epi requests

# What is a Cluster Search?



- Patterns submitted
- Cluster searches performed
- Visually compare indistinguishable patterns with 1<sup>st</sup> enzyme, then 2<sup>nd</sup> (always with *E. coli* and *Listeria*)
- Patterns/clusters named by CDC

Cluster of indistinguishable patterns by primary enzyme

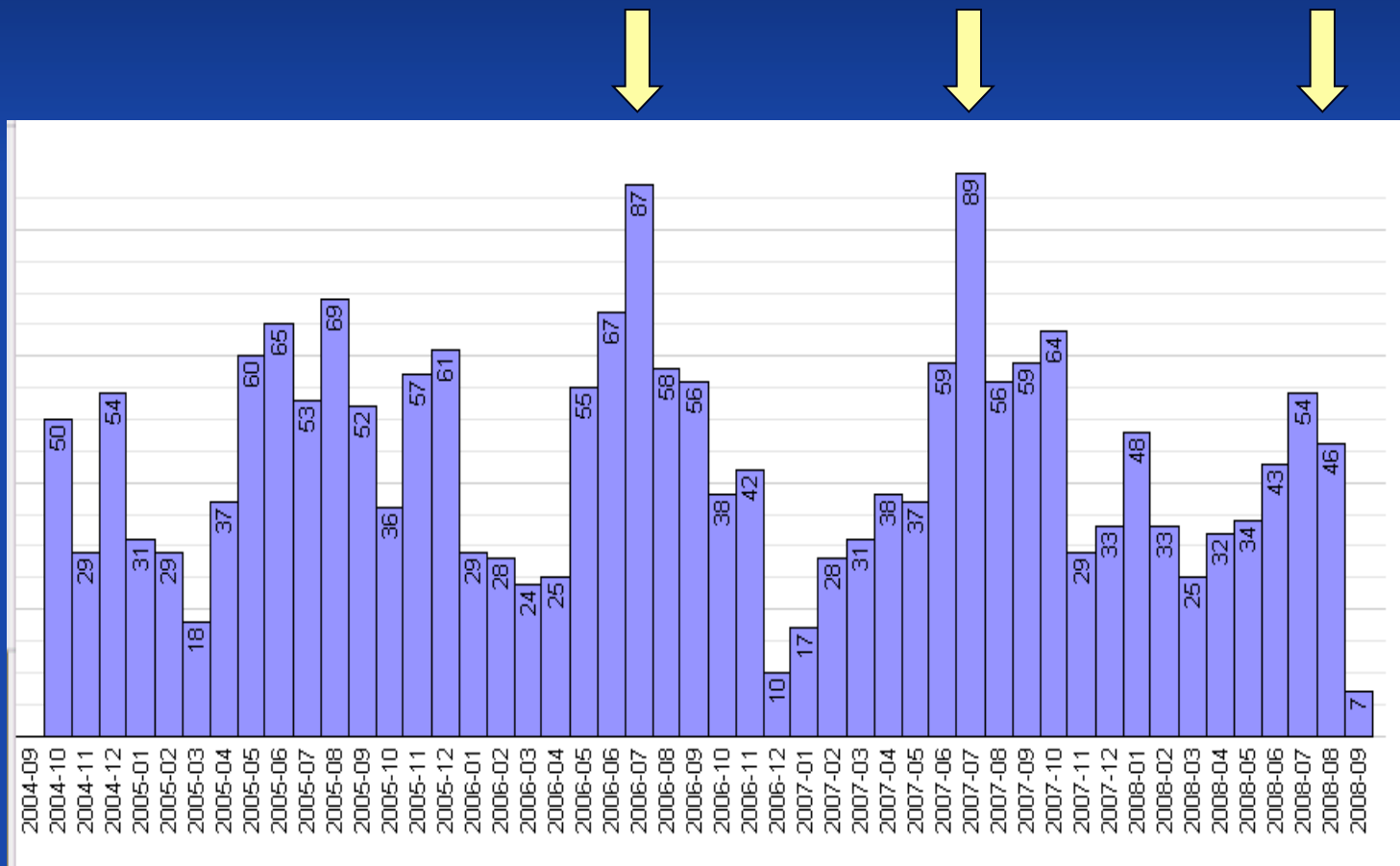


# Does the cluster warrant further investigation?

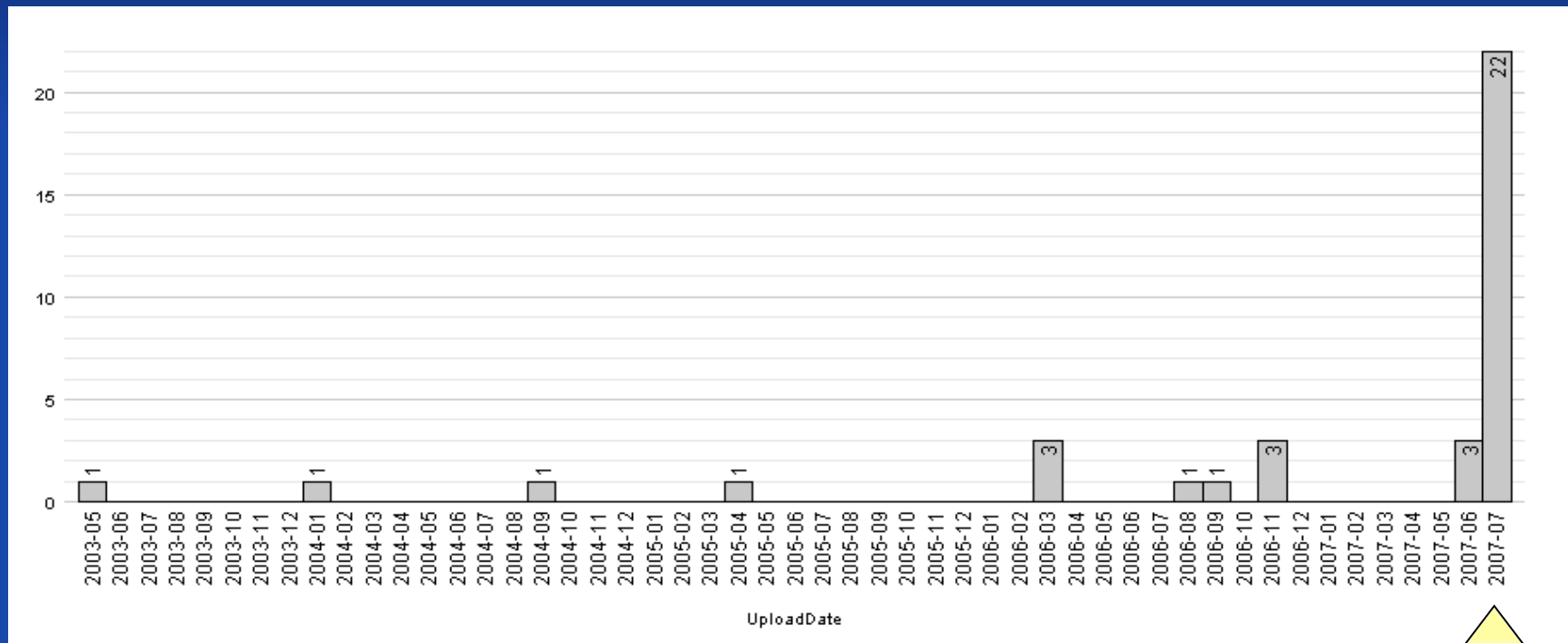
- Pattern Frequencies (national and regional)
  - If frequency is more than twice what is expected in a month's time
    - Take into consideration seasonal increases
- Non-human matches
  - If a pattern from a non-human isolate matches 3+ patterns from human isolates

# Pattern Frequencies

## Seasonal Peaks in *Salmonella* Berta



# Pattern Frequencies



An obvious cluster

# Pattern Frequencies

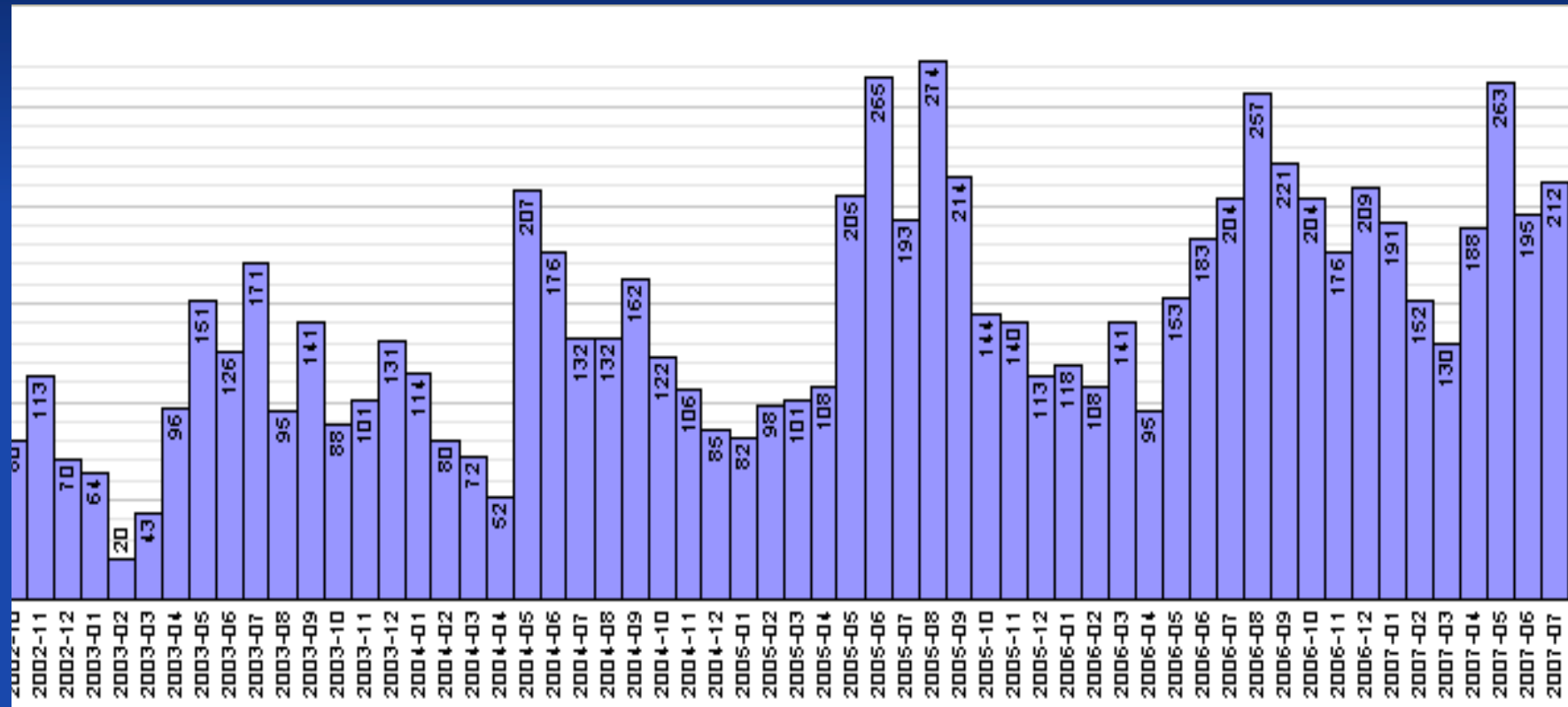
## Example

- A local PulseNet participant has detected a cluster of 8 *Salmonella* Enteritidis isolates that are pattern JEGX01.0004
  - JEGX01.0004 is the MOST common pattern for *Salmonella* Enteritidis, representing 45% (~14,120 patterns) of all *Salmonella* Enteritidis in the database
  - More common patterns tend to have less obvious clusters, and are often defined by an increase above baseline



# Pattern Frequencies

## *Salmonella* Enteritidis' Most Common Pattern



**Common Patterns: Detecting true increases can be difficult**

# Cluster Detection in PulseNet: Communication within the Network

**CDC Team** -- Search --

CDC Team: PulseNet

**Workspaces**

- CDC Team
  - + PulseNet NDA
  - + CDC Team Training and Support
  - + DGP/CD
  - PulseNet
    - CDC Team Migration from WebBoard
    - 2008 PulseNet Update Meeting
    - 2008 Update Meeting Agenda Committee
    - Important PulseNet Documents
    - QAQC Manual
    - Campylobacter
    - E. coli
    - Listeria
    - Salmonella
    - Shigella
    - Vibrio
    - Other Organisms
    - General PulseNet Information
    - Quarterly & Annual Reports
    - BioNumerics
    - Troubleshooting PFGE
    - Next Generation Subtyping
    - Proficiency Testing/Certification
    - Publications & Reports: Subtyping & Food Safety
    - PulseNet Workspace Comments
    - Comparisons

**Subject:0802LACJPX-1c (JPXX01.0088)\_LAC\_Typhimurium**

LAC has a cluster of 3 Typhimurium isolates that are indistinguishable by *Xba*I and *Bln*I. The collection dates are from 12/10/2007 to 1/10/2008. Epi under investigation. The isolate numbers are:

- LAC\_Z19766 2 year old boy, no travel
- LAC\_Z19999, 43 year old female, no travel
- LAC\_Z20072, 60 year old female, no travel

We have seen this pattern before in LAC. Our epi contact is (*name and phone*)

LAC08008PN.BDL  
Posted: 05 March 2008 09:52 AM



# Communication: Cluster Reports

Sample cluster report that is sent to the CDC epidemiologists

## Salmonella

JKA

0808OHJPX-1c. *S. Typhimurium* posted by OH

XbaI: JPXX01.1126 (0.13%)

BlnI: JPXA26.0029 (0.13%%)

60 Days: AK, ME, NM(2), NYC, OH(5), PA(4), VT

Source:

Notes no recent matches in PNC

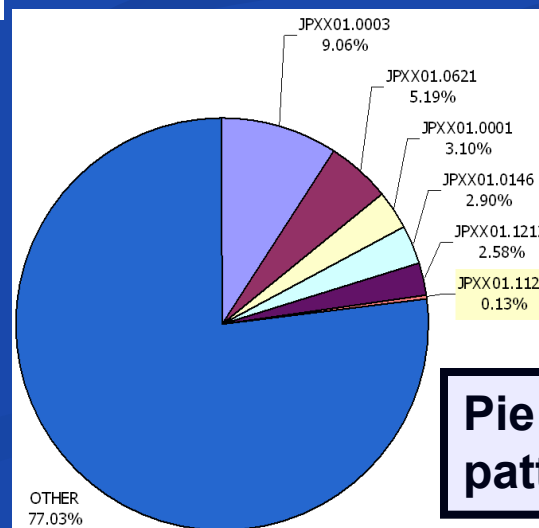
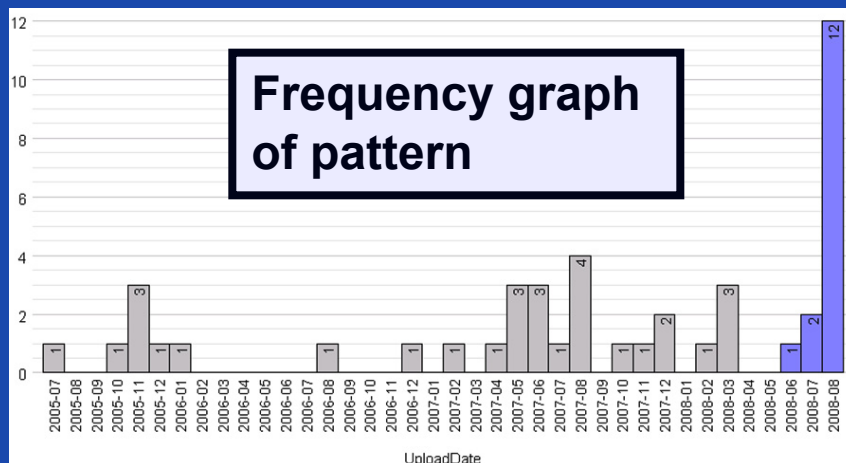
USDA/FDA Nothing recent in VetNet

MLVA Info:

Epi Update: Sent to

for follow-up

- Outbreak Code, serotype, posting lab
- Patterns involved and %
- States involved
- Notes, USDA/FDA/MLVA info
- Epi Update



# 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



# Collaboration with Other Agencies

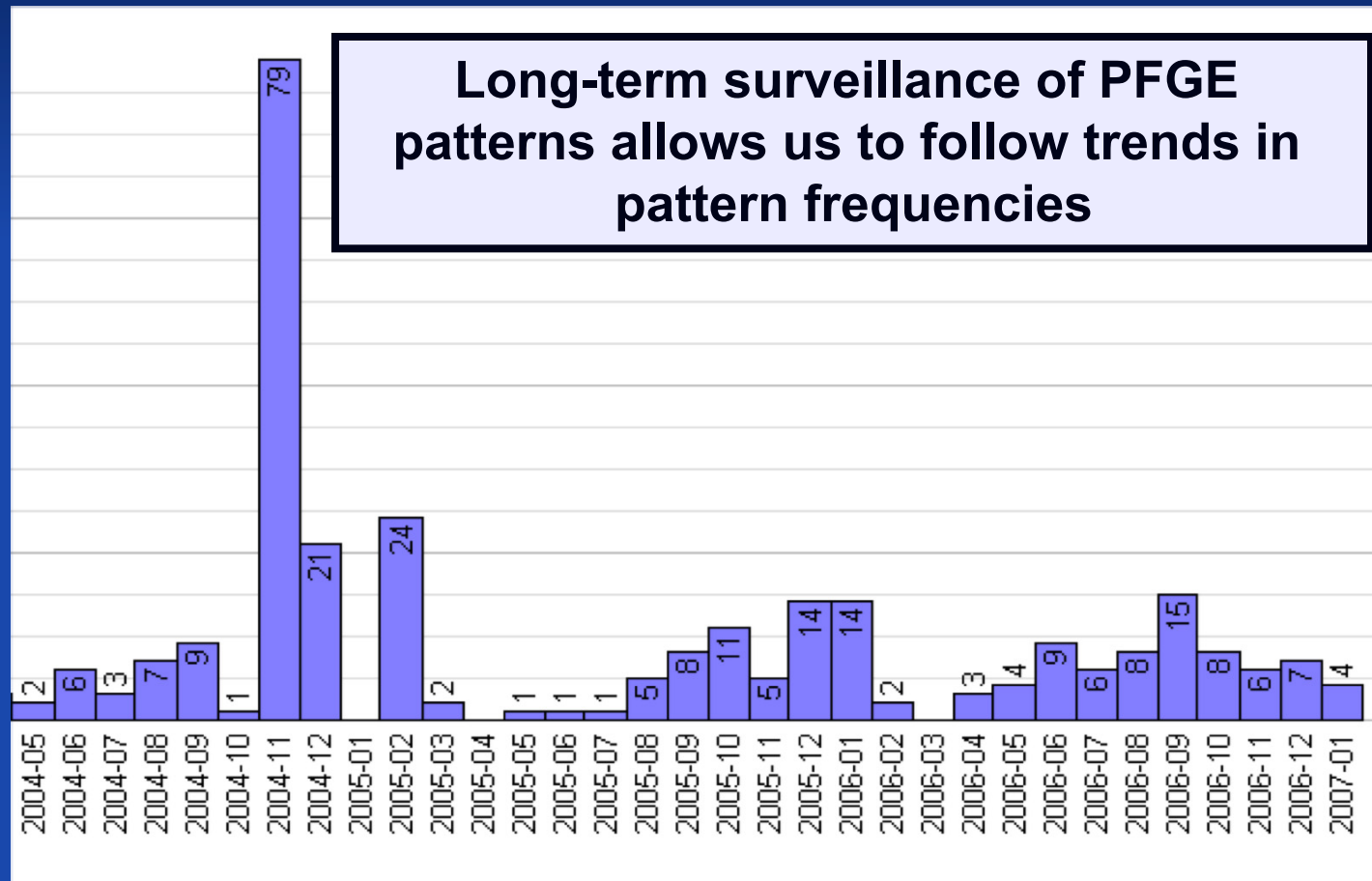
- Patterns submitted directly to PulseNet USA
  - United States Department of Agriculture (USDA)
    - Food Safety and Inspection Service (FSIS)
    - Agricultural Marketing Service (AMS)
    - Animal and Plant Health Inspection Service (APHIS)
  - U.S. Food and Drug Administration (FDA)
    - Center for Food Safety and Nutrition (CFSAN)
    - Center for Veterinary Medicine (CVM)
  - Agricultural Labs
    - Ohio, New York, Florida
  - Veterinary Labs
    - Michigan State University



# Collaboration with other Networks

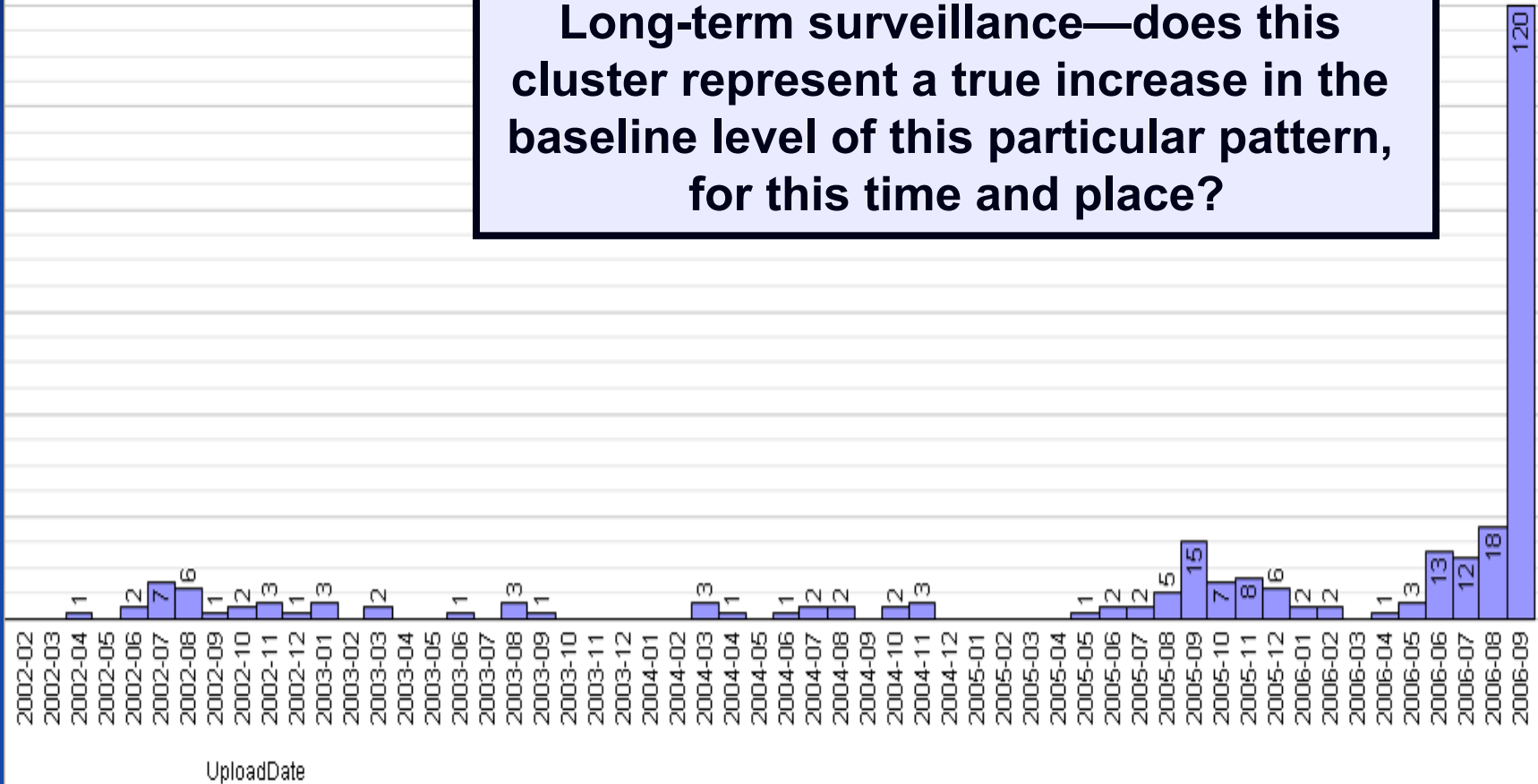
- Direct connection to other databases
  - USDA Agricultural Research Service (VetNet)
  - PulseNet Canada
  - Checked against all clusters reported to CDC epidemiologists
- Other International Networks
  - PulseNet Latin America, Europe, Asia Pacific, and Middle East
  - Postings on PulseNet International web-based discussion forum
  - Emails to contacts in each network during outbreak investigation

# Database Uses: Pattern Trends



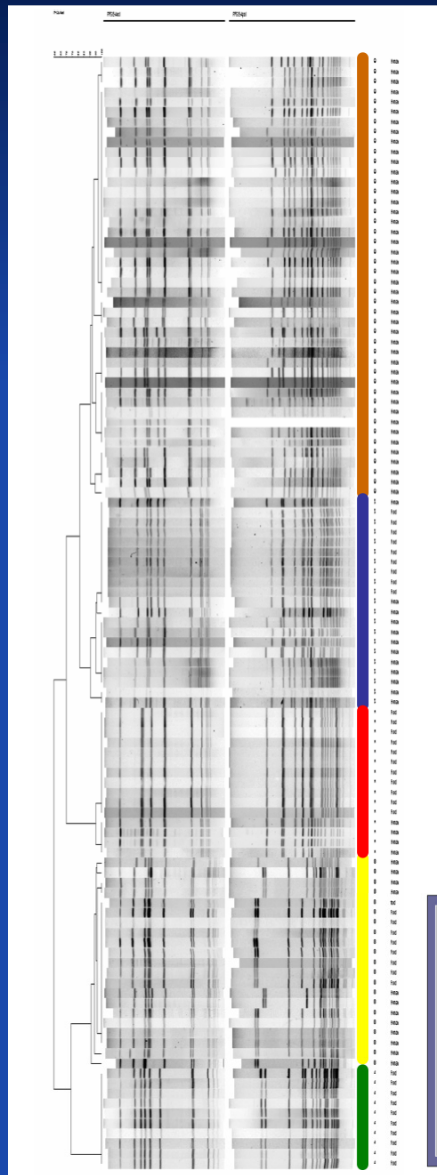
# Database Uses: Pattern Frequencies

Long-term surveillance—does this cluster represent a true increase in the baseline level of this particular pattern, for this time and place?





# Database Uses: Attribution Analysis

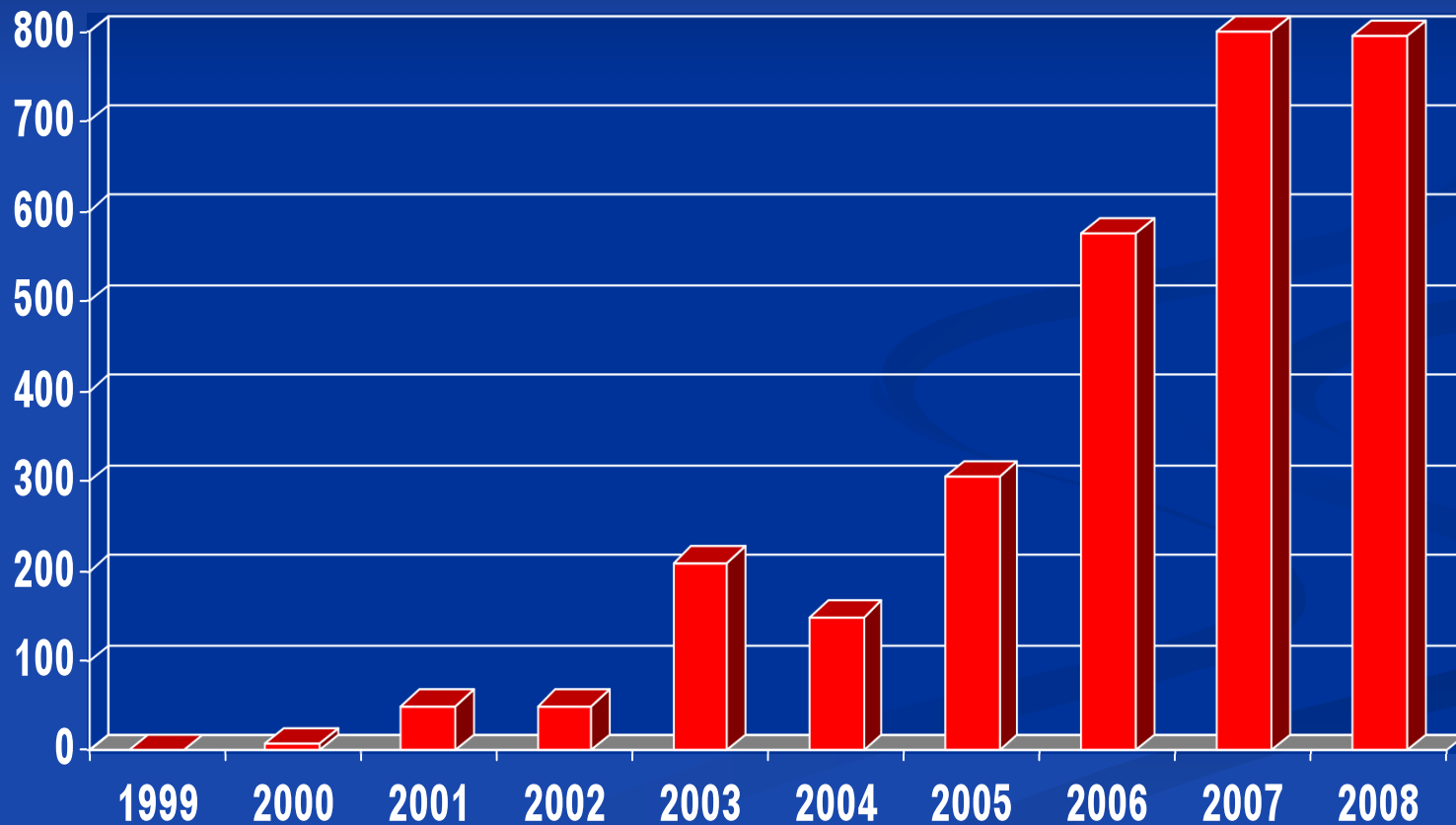


**What is the relative contribution of each food source to the burden of foodborne illness in humans?**



# Database Uses: Trends in Emerging Pathogens

Annual submissions of non-O157 STEC to PulseNet



# Questions?



# DFBMD

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

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