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Whole Genome Sequencing from a State Regulatory Perspective: Impacts on Foodborne Disease Surveillance and Regulatory Testing

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Foodborne Illness in the US

- "One of the safest food supplies in the world"…

- CDC estimates 1 in 6 Americans (48 million people) are sick annually with foodborne illness
  - ✓128,000 hospitalizations
  - ✓3,000 deaths

✓Laboratory Confirmed Case Reports Annually in US

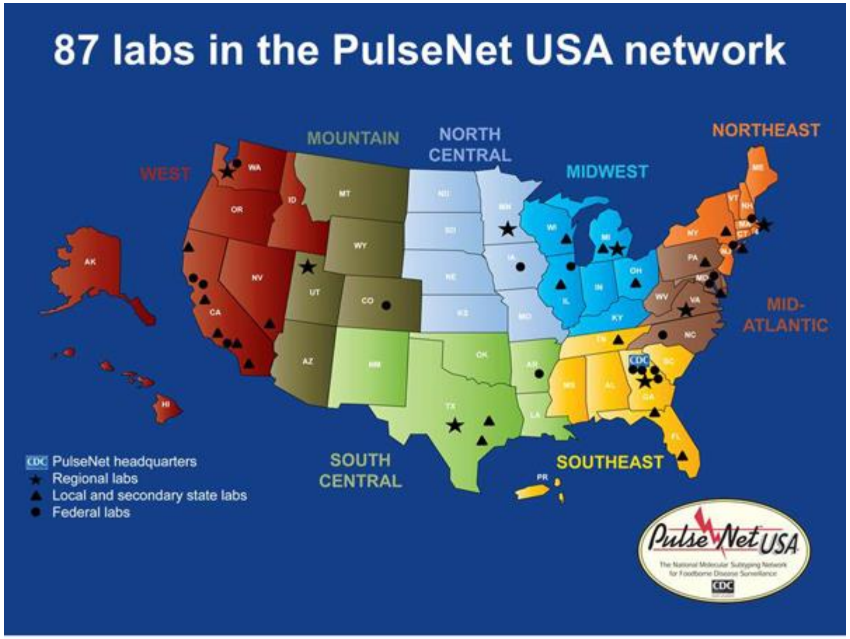
- ✓*Campylobacter* – 43,696
- ✓*Salmonella* – 41,930
- ✓*E. coli* O157 – 3,704
- ✓Shiga-toxin producing *E. coli* (STECs) – 1,579
- ✓*Listeria monocytogenes* – 808

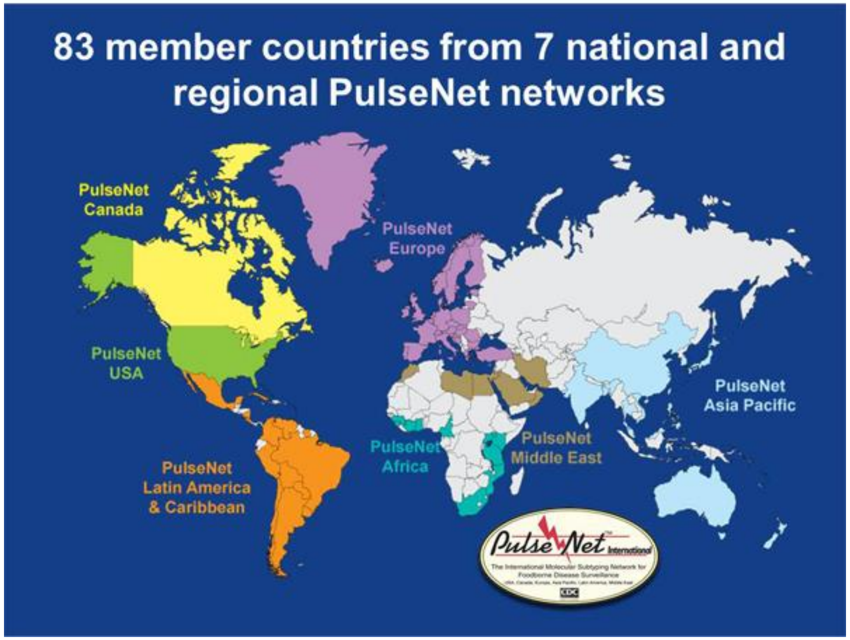
Tracking Foodborne Pathogens

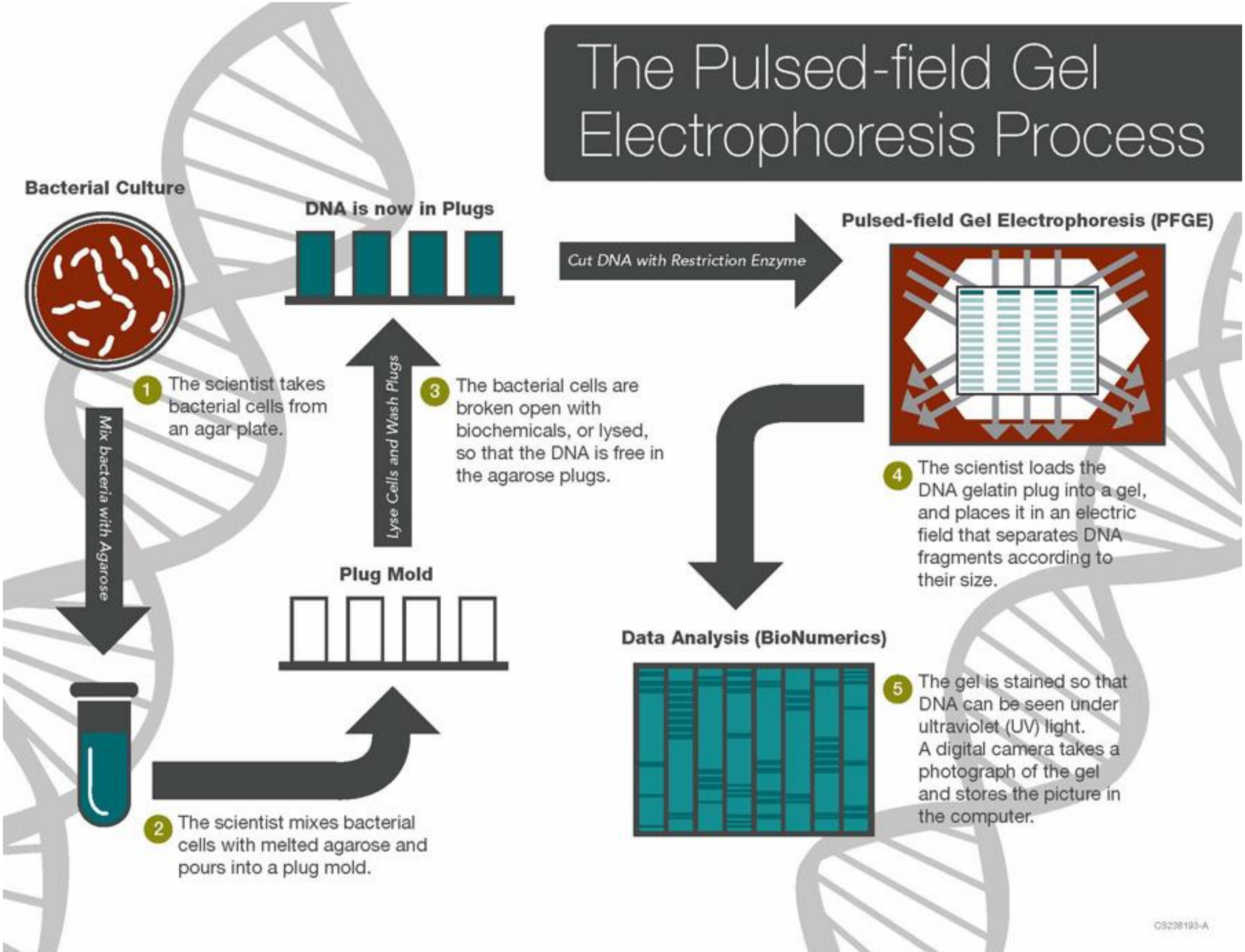
- Traditionally performed with by observation of biochemical reactions and often in combination with specific serotyping schema
  - Advantage: relatively low tech, low complexity
  - Disadvantage: expensive (materials and labor), requires growth of organism, slow to produce results
  - Example: *Salmonella* Typhimurium in milk from Hillfarm Dairy, IL
    - 16,284 cases from IL, IA, IN, MN, and WI
- Molecular subtyping brought methods that allowed visualization of genetic material or so-called "fingerprints"
  - Advantage: based on genetic information; more specific/discriminating; in some cases faster results; more widespread geographical recognition of "case clusters"
  - Disadvantage: in some cases less expensive (materials), requires growth of organism
  - Examples: *Ribotyping, pulsed-field gel electrophoresis* (PFGE), *PCR RFLP* ; many different methods, multiple examples of application to outbreak investigations

PulseNet

- Network of public health labs
- Perform standardized protocols of PFGE on:
    - ✓ *Salmonella*
    - ✓ *Campylobacter*
    - ✓ *E. coli* O157 and other Shiga-toxin producing *E. coli* (STECs)
    - ✓ *Listeria monocytogenes*
    - ✓ *Shigella*
- Data uploaded with 4 days of receipt of isolate
- Additional data required for some organisms (e.g. serotyping for *Salmonella*)
- Each analyst is certified for gel and data analysis
- Data sharing is performed securely in a private network

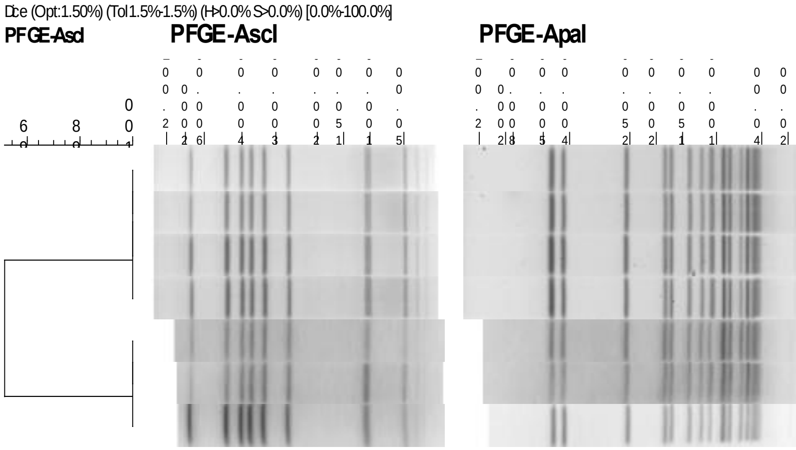





The Pulsed-field Gel Electrophoresis Process
Bacterial Culture
DNA is now in Plugs
Cut DNA with Restriction Enzyme
Pulsed-field Gel Electrophoresis (PFGE)
1 The scientist takes bacterial cells from an agar plate.
Mix bacteria with Agarose
Lyse Cells and Wash Plugs
3 The bacterial cells are broken open with biochemicals, or lysed, so that the DNA is free in the agarose plugs.
4 The scientist loads the DNA gelatin plug into a gel, and places it in an electric field that separates DNA fragments according to their size.
Plug Mold
Data Analysis (BioNumerics)
5 The gel is stained so that DNA can be seen under ultraviolet (UV) light.
A digital camera takes a photograph of the gel and stores the picture in the computer.
2 The scientist mixes bacterial cells with melted agarose and pours into a plug mold.
CS238193-A

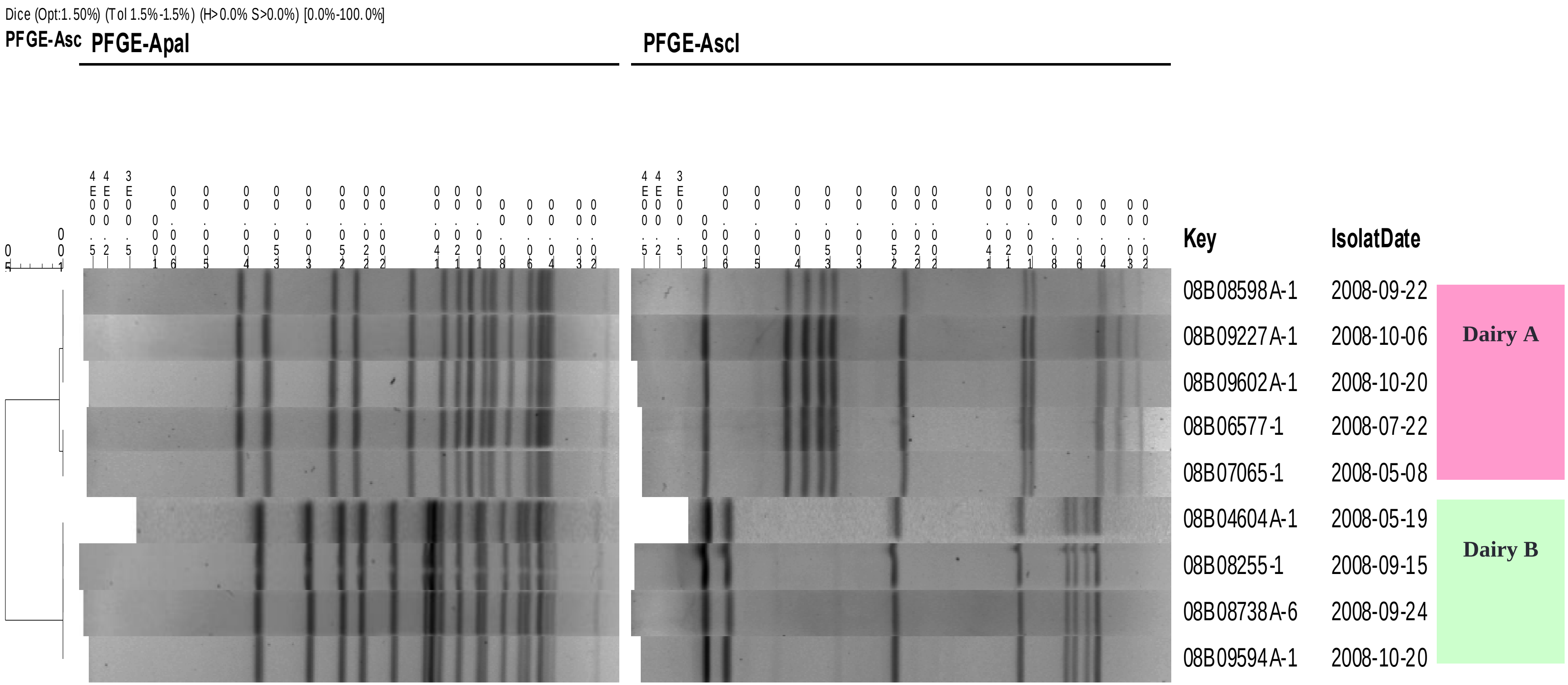
Comparison of PFGE patterns of *Listeria monocytogenes* isolated from salads produced by Food Processing Company A (2005-2011)

(7 entries)



Key	rceSSourceCounty	SourceCity	SourceSite	SourceTyp	TypeDetails	PPatIsolatDate
11B02457A-1	Albany	Slingerlands	Salad	Food	salad-potato	2011-03-23
11B02457A-2	Albany	Slingerlands	Salad	Food	salad-potato	2011-03-23
11B02457A-6	Albany	Slingerlands	Salad	Food	salad-potato	2011-03-23
11B02457A-7	Albany	Slingerlands	Salad	Food	salad-potato	2011-03-23
05B07990A-1	Oneida	New Hartford	Salad	Food	salad-tuna	2005-09-19
05B07991A-1	Oneida	New Hartford	Salad	Food	salad-tuna	2005-09-19
05B08708A-1	Oneida	New Hartford	Salad	Food	salad-tuna	2005-10-03

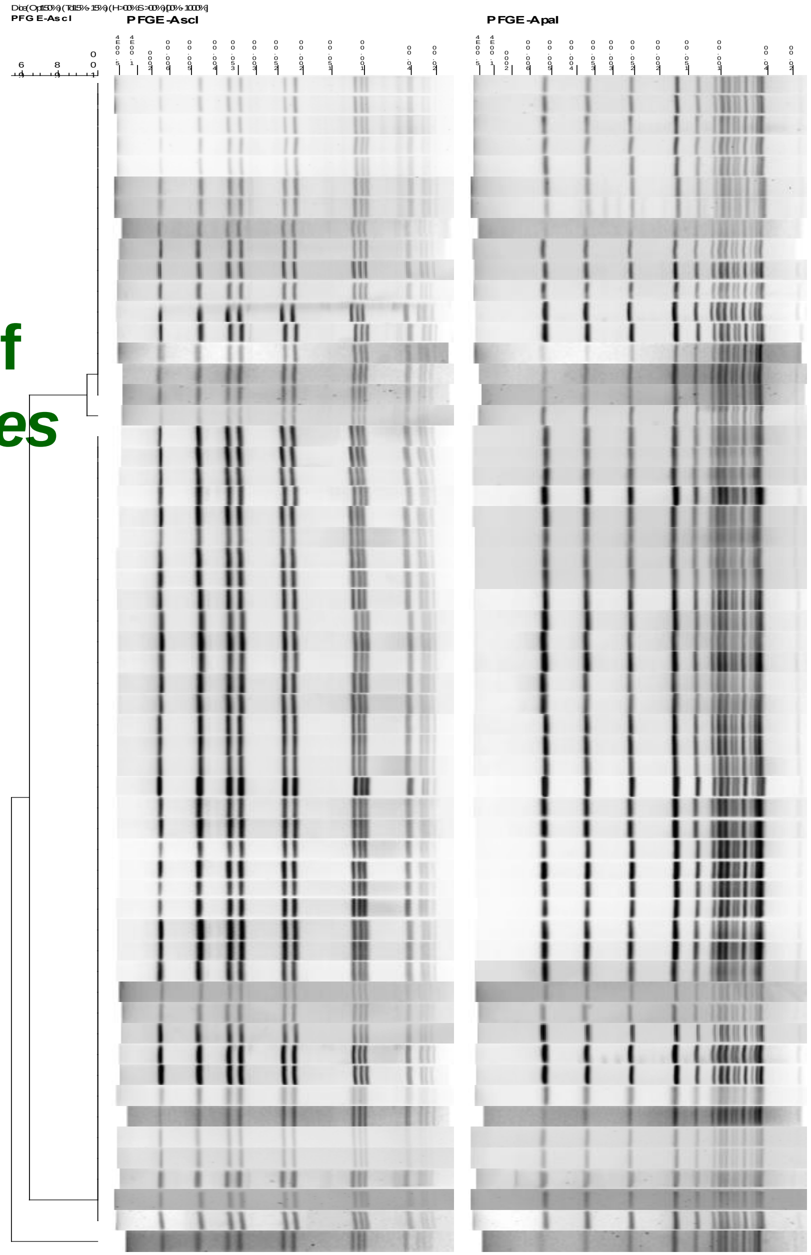
Persistence of *Listeria monocytogenes* in farms producing raw bovine milk for sale



Listeria monocytogenes in Sprouts

- **March 2008-March 2009**
  - 20 cases of listeriosis
  - Indistinguishable *Asc*I and *Apa*I PFGE patterns
  - CA (1), MA(6), NY(6); NJ(4); MD(1); ME(1); NH(1)
  - *No common* source identified through initial epidemiologic investigation
  - Source identified via routine random sampling of high risk foods (sprouts) by NYSDAM

PFGE Patterns of *L. monocytogenes* isolates associated with this sprout associated outbreak investigation



Key	SourceState	SourceSite	SourceType
CT___02033917	CT	Alfalfa Sprouts	Food
CT___02033918	CT	Alfalfa Sprouts	Food
CT___02033919	CT	Alfalfa Sprouts	Food
CT___02033920	CT	Alfalfa Sprouts	Food
CT___02033921	CT	Alfalfa Sprouts	Food
CT___02033931	CT	Factory swab	Environmental
CT___02033933	CT	Alfalfa Sprouts	Food
MA___08PF0425	MA	BLOOD	Human
MA___08PF1182	MA	BLOOD (SUBCULTURE)	Human
MA___08PF1521	NJ	BLOOD	Human
MA___08PF1749	CA	blood	Human
MA___08PF2122	NJ	BLOOD	Human
MA___09PF0297	NJ	Blood	Human
NH___2008027278	NH	blood	Human
NY___BAC0800002720	NY	Amniotic fluid	Human
NY___BAC0900001363	NY	Blood	Human
MA___08PF1952	MA		Human
FCF__507437-1	CT	Spent irriagation water	Environmental
FCF__507437-3	CT	Spent irriagation water	Environmental
FCF__507437-4	CT	Spent irriagation water	Environmental
FCF__507437-7	CT	Spent irriagation water	Environmental
FCF__507438-1	CT	Alfalfa sprouts	Food
FCF__507438-5	CT	Alfalfa sprouts	Food
FCF__507439-1	CT	Alfalfa sprouts	Food
FCF__507439-7	CT	Alfalfa sprouts	Food
FCF__507442-106	CT	Environmental Swab	Environmental
FCF__507442-11	CT	Environmental Swab	Environmental
FCF__507442-16	CT	Environmental Swab	Environmental
FCF__507442-2	CT	Environmental Swab	Environmental
FCF__507442-21	CT	Environmental Swab	Environmental
FCF__507442-57	CT	Environmental Swab	Environmental
FCF__507442-68	CT	Environmental Swab	Environmental
FCF__507442-93	CT	Environmental Swab	Environmental
FCF__507443	CT	alfalfa/clover sprouts in l.	Food
FCF__507443-1b	CT	alfalfa/clover sprouts in l.	Food
FCF__524837-1	CT	Spicy Sprout Blend	Food
FCF__524837-4	CT	Spicy Sprout Blend	Food
FCF__524838-3	CT	Clover Sprouts	Food
FCF__524838-7	CT	Clover Sprouts	Food
FCF__524839-1	CT	Broccosprouts	Food
FCF__524839-2	CT	Broccosprouts	Food
FCF__524840-10	CT	BroccoSprout Blend	Food
FCF__524840-2	CT	BroccoSprout Blend	Food
FCF__524844	CT	Crunchy Sprout Blend	Food
MA___08PF1125	MA	SPINAL FLUID	Human
MA___08PF1323	ME	BLOOD	Human
MA___09PF0062	MA	BLOOD	Human
MA___09PF0331	NJ	Blood	Human
MA___09PF0345	MA	Blood	Human
MD___MDA08223487	MD		Human
NY___BAC0900000221	NY	Blood	Human
NYAG_09B02865A-1	NY	Alfalfa Sprouts	Food
NYAG_09B02866A-6	NY	Clover Sprouts	Food
NYAG_NYAG09B0232.	CT	Alfalfa Sprouts	Food
NYC__nyc08-100647716	NY		Human
NYC__nyc09-100753729	NY		Human
NY___BAC0800004932	NY	Blood	Human


STEVEN SPIELBERG PRESENTS
BACK TO THE FUTURE
PG
A ROBERT ZEMECKIS FILM
OUTATIME

Changes in Technology (1983-2014)

1983 First Cell Phone: Martin Cooper who invented the first "Cell" phone; weighed 2.5lbs and could only be used for 20min before the battery died.

Use: phone calls; not widely adopted until late 1990's/early 2000's

Apple iPhone 6: Up to 24hr of phone talk time; up to 16 days of standby time; weighs 4.55 oz; 128GB on board storage;

Use: Phone calls, texts, web browsing, fitness tracking, photo/videos, GPS tracking, books, music, movies, games, and the list keeps growing….

Why whole genome sequencing?

- PFGE: served a practical public health function; but data are qualitative and requires difficult to support IT structure
- Technology is advancing at an exponential pace
- Whole genome sequencing (WGS) reveals the complete DNA make-up of an organism, enabling us to better understand variations both within and between species.
- Public health labs are now using this technology to perform basic foodborne pathogen identification during foodborne illness outbreaks

Why whole genome sequencing? (cont)

- Whole genome sequencing performs the same function as PFGE but has the power to differentiate virtually all strains of foodborne pathogens, no matter what the species

- May be used to extrapolate other important information on the organism such as;
  - ✓ Serotype
  - ✓ Virulence gene profiles
  - ✓ Antibiotic resistance patterns
  - ✓ Other novel markers

- This technology can be applied to all microorganisms which makes it ideal for public health laboratories.

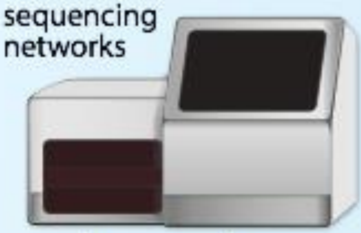
Basic Data Flow for Global WGS Public Access Databases

DATA ACQUISITION

Sequence and upload genomic and geographic data

GENOME TRAKR

Other distributed sequencing networks



DATA ASSEMBLY, ANALYSIS, AND STORAGE

International Nucleotide Sequence Database Collaboration (INSDC)
Shared Public Access Databases

- *NCBI – National Center for Biotechnology Information*
- *EMBL – European Molecular Biology Laboratory*
- *DDBJ – DNA Databank of Japan*

PUBLIC HEALTH APPLICATION AND INTERPRETATION OF DATA

- *Find clinical links*
- *Identify clusters*
- *Conduct traceback*
- *Develop rapid methods*
- *Develop culture independent tests*
- *Develop new analytical software*

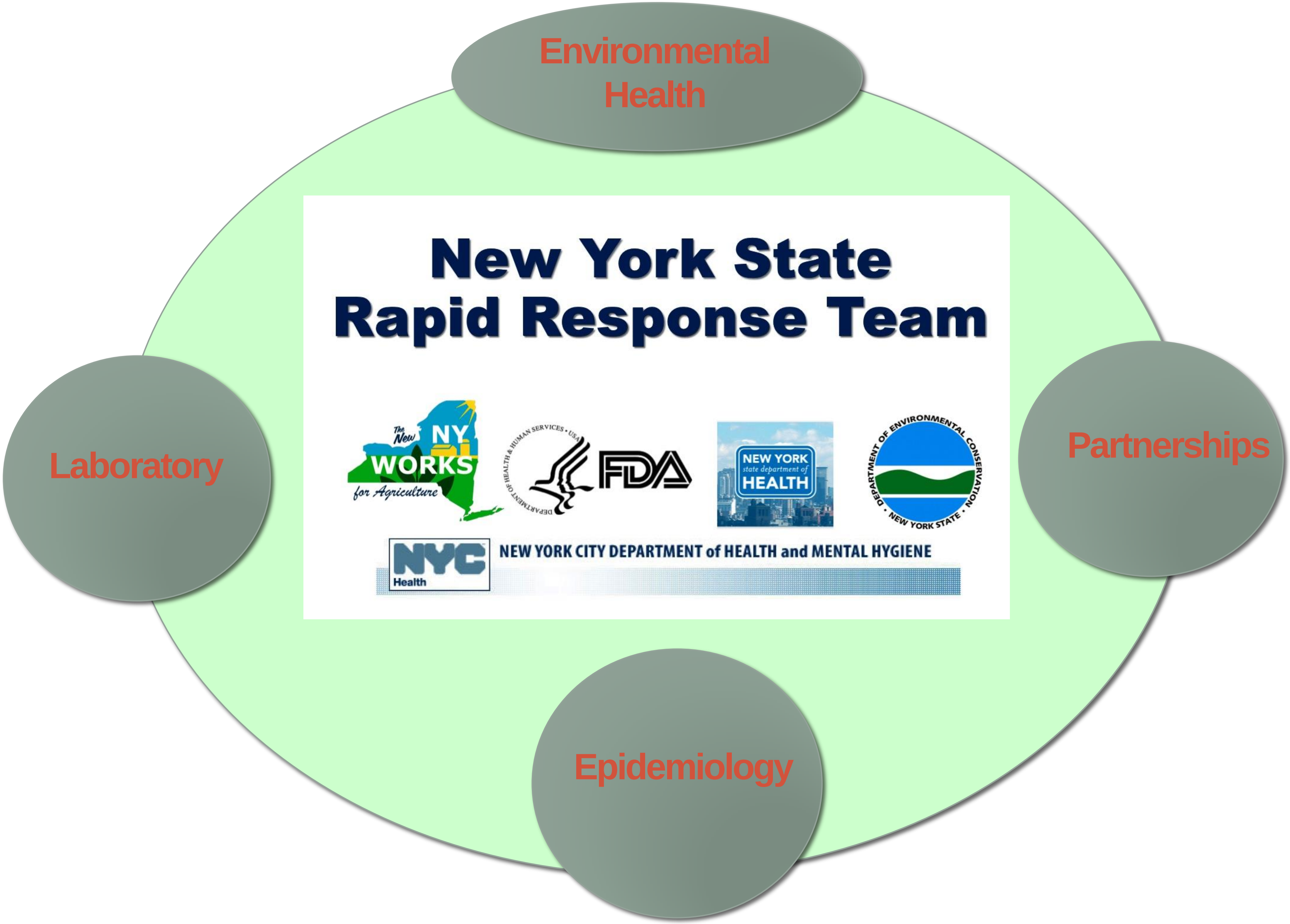
11/2014

State, Local, Federal, and Foreign Public Health Agencies

Academia/Industry

Why do this at the State Level?

- Power in a distributed network of laboratories with a common capability; this model has worked well for PulseNet for the past 20 years
- State and local public health laboratory involvement was crucial to the success of the network
- Foodborne outbreak tracking still relies on coordination and collaboration between the laboratory, epidemiology, and environmental health
- Partnerships are key to the success of an *Integrated Food Safety System (IFSS)*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NEW YORK CITY DEPARTMENT of HEALTH and MENTAL HYGIENE
Laboratory
Partnerships
Epidemiology

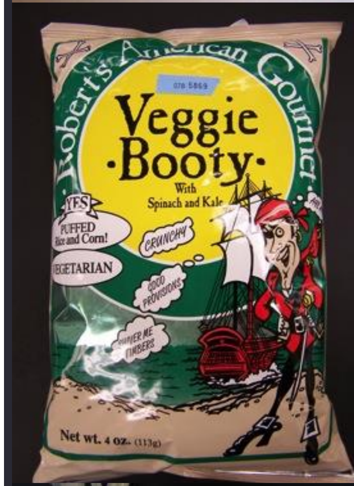
Linking Food & Environmental Isolates to Human Disease (2008-2012)

Pathogen	No. of Isolates	No. (%) Overlap with Human Cases*	No. (%) Associated with Cluster or Outbreak**
Campylobacter spp.	8	4 (50%)	2 (25%)
E. coli O157:H7	5	2 (4%)	0 (0%)
Non-O157 STECs	4	1 (25%)	0 (0%)
Listeria monocytogenes	56	44 (79%)	5 (9%)
Salmonella enterica	31	30 (97%)	6 (19%)

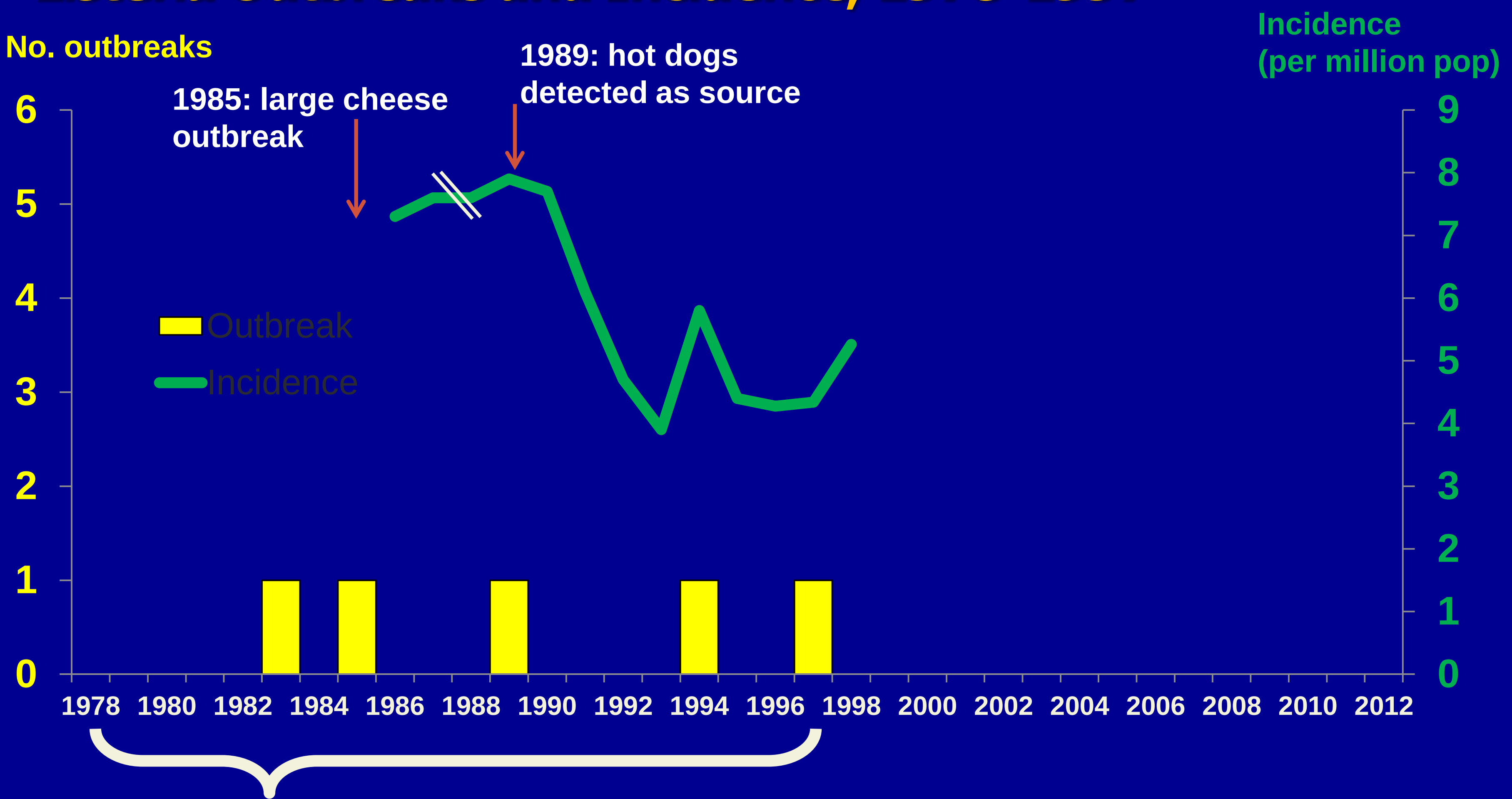
*=PFGE pattern with at least one human case in PulseNet database
**=Isolates assigned PulseNet outbreak code or linked to New York State only cluster

Historical Examples of Major Outbreak & Recall Investigations

- *E. coli* O157:H7 in fresh spinach (2006)
- *E. coli* O157:H7 in ground beef (2007)
- *Salmonella* associated with peanut butter (2007)
- Salmonella associated with "Veggie Booty" (2007)
- *Salmonella* associated with fresh produce (2008)
- *Listeria monocytogenes* associated with sprouts (2008-09)
- *Listeria monocytogenes* in a hospital cafeteria (2008)
- *Salmonella* associated with peanut butter (2009)
- *Listeria monocytogenes* in Spanish-style soft cheese (2009)
- *Salmonella* associated with deli meats/spices (2010)
- Shiga-toxin producing *E. coli* O145 in lettuce (2010)
- *Listeria monocytogenes* in potato salad (2011)
- *Salmonella* in chicken livers (2011)
- *E. coli* O157:H7 associated with produce (2013)
- *Listeria monocytogenes* in imported seafood (2012-13)
- *Salmonella* in pet foods/treats (2013)



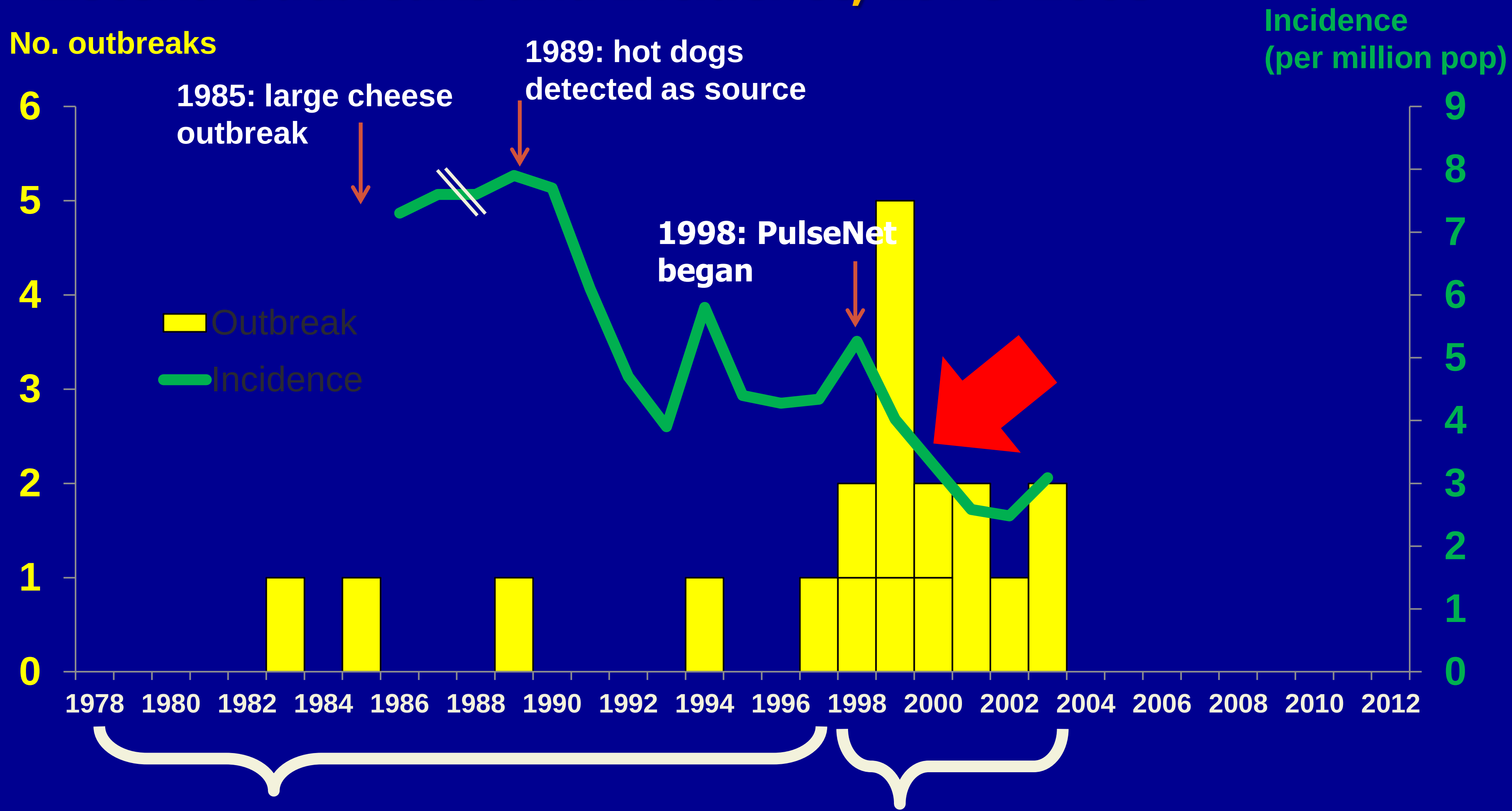
Listeria Outbreaks and Incidence, 1978-1997



<u>Before PulseNet</u>
(20 years)
1978-1997
5 outbreaks
Median **69** cases/outbreak

SOURCE: John Besser (CDC)

Listeria Outbreaks and Incidence, 1978-2003

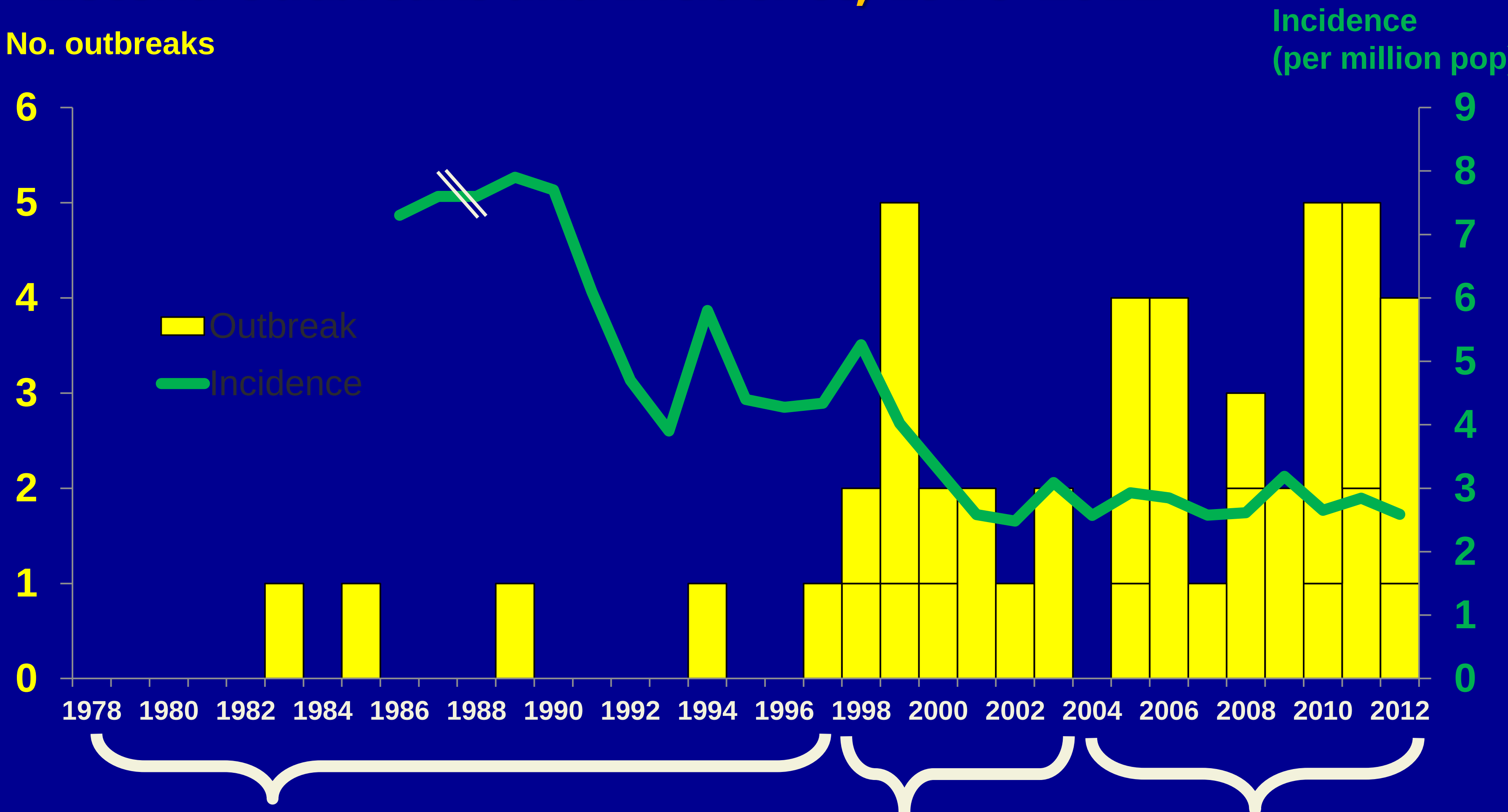


Before PulseNet
(20 years)
1978-1997
5 outbreaks
Median **69** cases/outbreak

PulseNet's first years
(6 years)
1998-2003
14 outbreaks
Median **11** cases/outbreak

SOURCE: John Besser (CDC)

Listeria Outbreaks and Incidence, 1978-2012



Before PulseNet
(20 years)
1978-1997
5 outbreaks
Median **69** cases/outbreak

PulseNet's first years
(6 years)
1998-2003
14 outbreaks
Median **11** cases/outbreak

***Listeria* Initiative & PulseNet** (9 years)
2004-2012
28 outbreaks
Median **5.5** cases/outbreak

SOURCE: John Besser (CD

Listeria monocytogenes Project with Cornell University

- Phase 1 – 60 Large chains and 60 large/medium independents (27, 000ft^2)
- Phase 2 – 60 Small independents (2, 200ft^2)
- Phase 3 – 60 establishments with poor sanitation history (5, 500ft^2) (3 consecutive – food equip./x contam.)

Retail Study Overall Summary

Environmental Samples	Phase 1	Phase 2	Phase 3
Stores Sampled	121	60	60
Store positive for Lm	73 (60%)	33 (55%)	39 (65%)
Multiple Positive sites	44 (36%)	19 (32%)	24 (40%)
Prevalent Ribotypes	27 (22%)	11 (18%)	16 (27%)
Multiple Prevalent Ribotypes	0	1 (2%)	5 (8%)

Retail Study Environmental Summary

Sponge Description	# Tested	# Present	% Present	Phase 2	Phase 1
Slicer/Utensils	131	6	4.6%	3.6%	2.6%
Deli Case	63	5	7.9%	8.5%	3.3%
Deli Sink	60	10	16.7%	11.7%	12.2%
Deli Floor Drain	11	3	27.3%	0%	19.7%
Deli Floor	13	1	7.7%	14.3%	
Dairy Case	53	8	15.1%	18.5%	10.9%
Raw Meat Floor Drain	15	10	66.7%	61.5%	34.9%
Dry Aisle	55	4	7.3%	10%	7.3%
Walk-in Cooler Floor	50	16	32%	27.1%	
Entrance floor mat	60	8	13.3%	15.8%	

Summary

- NY State Department of Agriculture and Markets Food Laboratory is joining this network and will begin using this technology in the Summer of 2015
- Well characterized environmental (food, water, facility, etc.) isolates are critical to the success of GenomeTRACKR and PulseNet
- Whole genome sequencing technology is transforming public health microbiology in nearly real-time

Food Safety News

Breaking news for everyone's consumption

How Culture-Independent Diagostics Threaten Public Health Surveillance

BY LYDIA ZURAW | NOVEMBER 12, 2014

Traditional methods for diagnosing foodborne illness infections such as Salmonella, Campylobacter and E. coli involve cultivating patient samples in an artificial nutrient medium. But tests that don't require isolates from pure culture are becoming increasingly popular.

There are different kinds of culture-independent diagnostic tests (CIDTs), but they all take a broad look at the DNA in samples, screening for the general types of pathogens that are present. The type of CIDT public health folks think will really overtake culture tests are syndrome-based panels that can test for multiple agents at once. There are five such tests currently licensed for gastrointestinal illnesses, with more expected to follow in coming years.

These CIDTs are particularly attractive to clinicians because, in addition to testing for many different pathogens, they can be faster than traditional methods and can detect bugs that would otherwise be difficult to find. They also don't need as much equipment or highly trained technicians, so they can save labs money.

http://FoodSafetyNews.com November 12, 2014