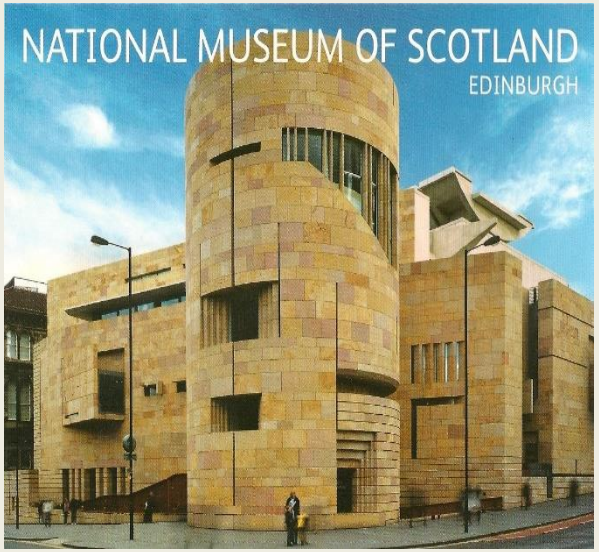
National Center for Emerging and Zoonotic Infectious Diseases

Whole Genome Sequencing & Food Safety

Peter Gerner-Smidt, MD, DSc
Branch Chief



Food Standards Scotland's first food Conference
Edinburgh, March 28, 2018

Food Standards Scotland
For safe food and healthy eating

A foodborne infection

is an infection you get by ingesting food, which contains pathogenic microorganisms

But….
Infections caused by foodborne pathogens are not always foodborne !

May be transmitted:

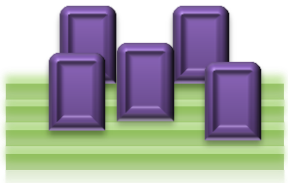
- **From water/the environment**
- **From person to person**
- **From contact to animals**

Impact of Foodborne Diseases

Disease Burden in the US

1 in **6** Americans

128,000 Hospitalizations



3,000 Deaths

Economic Impact

15+ Billion each year

Pathogens

Salmonella

Campylobacter

Vibrio

9 Billion $ each year

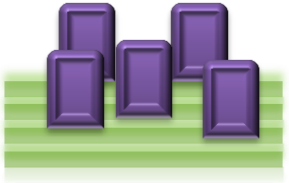
E coli

Listeria

Shigella

Flynn D 2014

Disease Burden Worldwide



230,000 Deaths

www.who.int/foodsafety

Foodborne illnesses are preventable

Food safety touches everyone

Agriculture

Research

Industry

Consumers

Media

Local, State, Federal Government

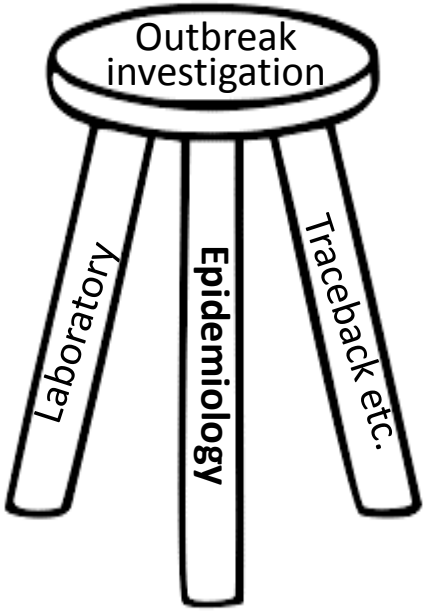
"You Are Never Alone"

Outbreak surveillance is a collaboration

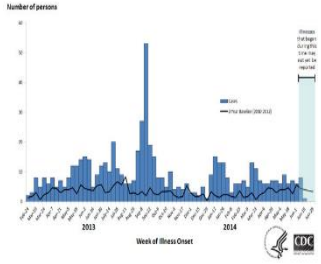
- Outbreaks detected, defined and confirmed by the laboratory

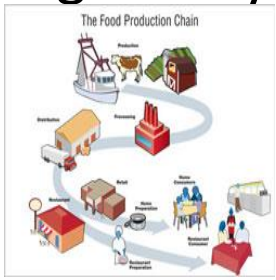
- Outbreaks investigated and solved by epidemiologists

- Inspection, tracebacks and regulatory action



Integrated Surveillan





Walmart Poultry *Salmonella* Control Initiative
a successful private - public partnership

SUPPLIERS MUST MEET NEW GUIDELINES BY MID-2016

Walmart leads the charge toward improved poultry safety (Part 1)

By Heidi Parsons

19-Dec-2014 - Last updated on 19-Dec-2014 at 08:52 GMT

FoodSafetyNews.com

Walmart, the world's largest retailer, is implementing stricter standards for poultry safety.

To better protect its customers against foodborne illnesses, Walmart announced it is beefing up its poultry safety guidelines for US suppliers.

Walmart
Save money. Live better.

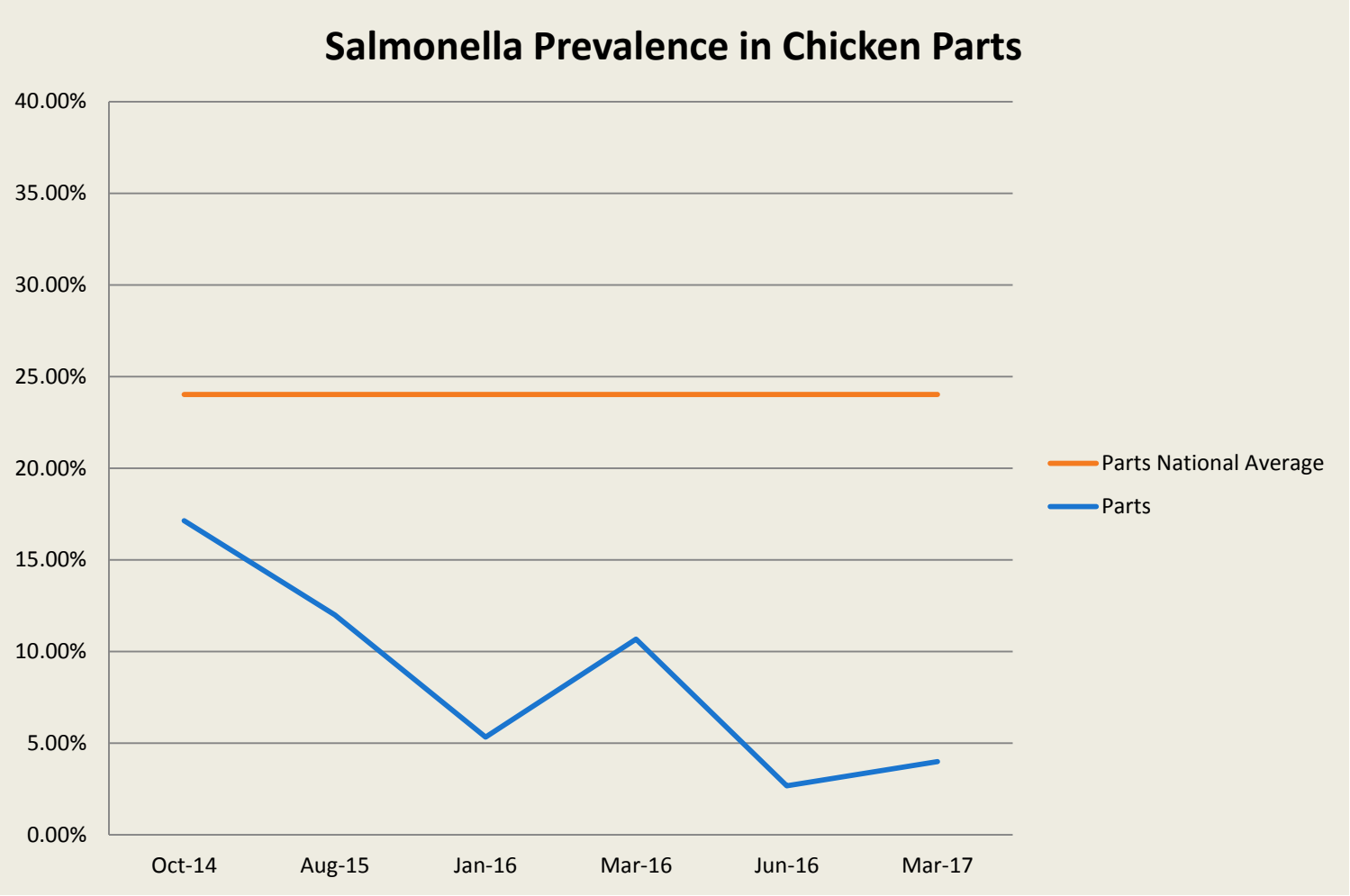
DEPARTMENT OF HEALTH & HUMAN SERVICES USA
CDC

Walmart Poultry *Salmonella* Control Initiative
a successful private - public partnership

Four Intervention Areas:

- **Primary Breeder Stock**
  - source from primary breeders who participate in USDA's National Poultry Improvement Plan (NPIP) for Breeding Poultry (9 CFR 145.83)
  - *Salmonella* data on a regular basis report on progress to Walmart
  - support changes targeted towards continuous reduction of prevalent *Salmonella* serotypes of human health concern
- **Bio-control Measures**
  - Directed at major human *Salmonella* serotypes in housing complex
  - vaccination of broiler-breeder (parental) flocks
  - adhere to disease prevention best practices associated with bio-security and vector control
- **Whole Chicken - Process Control**
  - regulatory approved intervention or a combination of interventions between pre-scald to post-chill
  - cumulative 4-log10 reduction of *Salmonella*
  - by December 31, 2015
- **Chicken Parts Intervention**
  - regulatory approved intervention or a combination of interventions post-chill, after cut-up of whole chickens prior to packaging
  - 1-log10 reduction

The Walmart Poultry *Salmonella* Control Initiative Works:

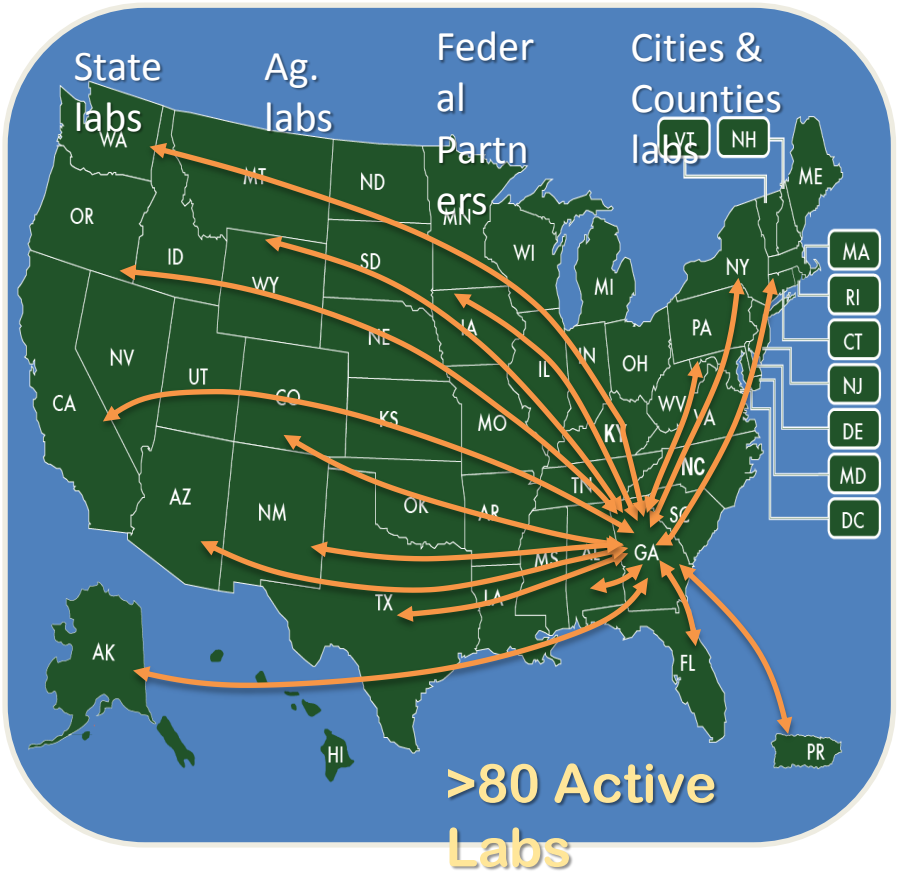


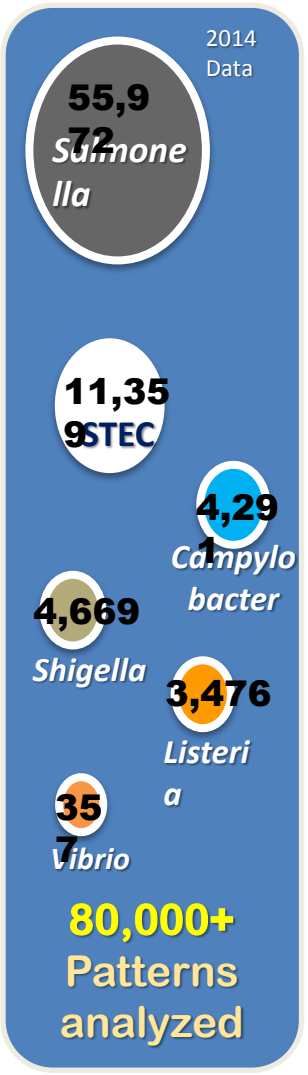
Courtesy Walmart- data are preliminary and not for public distribution

PulseNet

the National Molecular Subtyping Network for Foodborne Disease Surveillance

Easier and faster to exchange data than to exchange strains



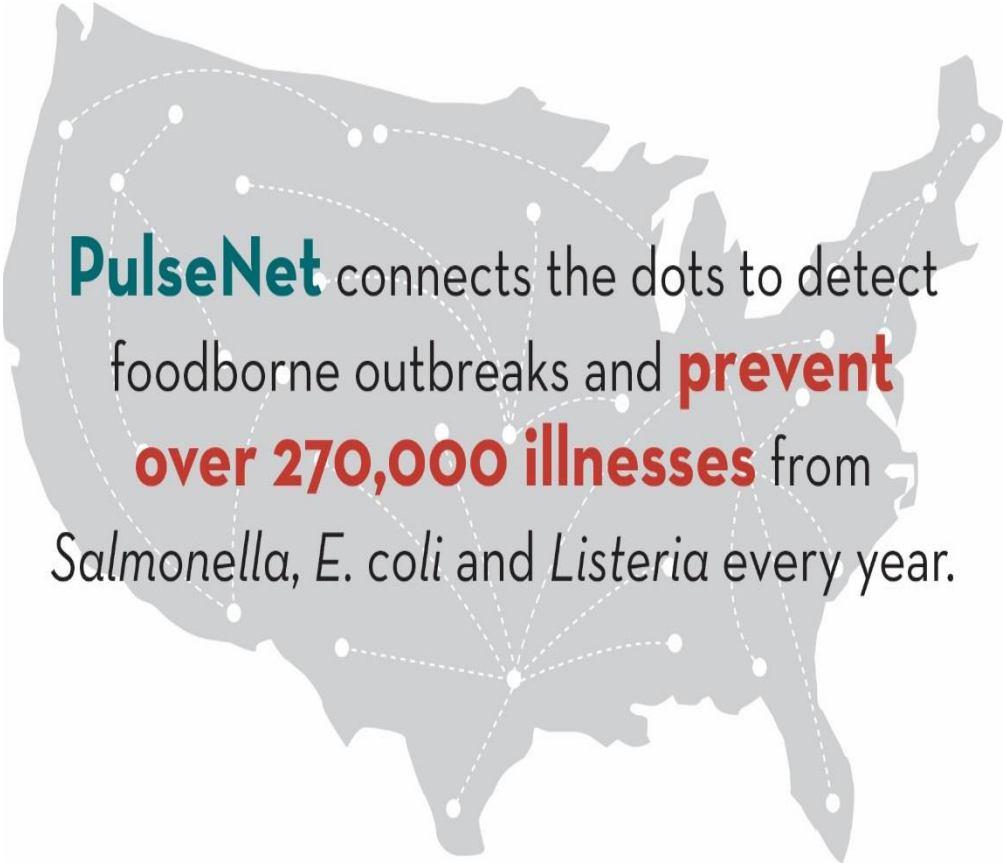


20 years of PulseNet USA 1996 CDC 2016

Molecular Surveillance (by PFGE) Saves Lives and Money

Every year **PulseNet** saves at least **half a billion dollars** in medical costs and lost productivity.

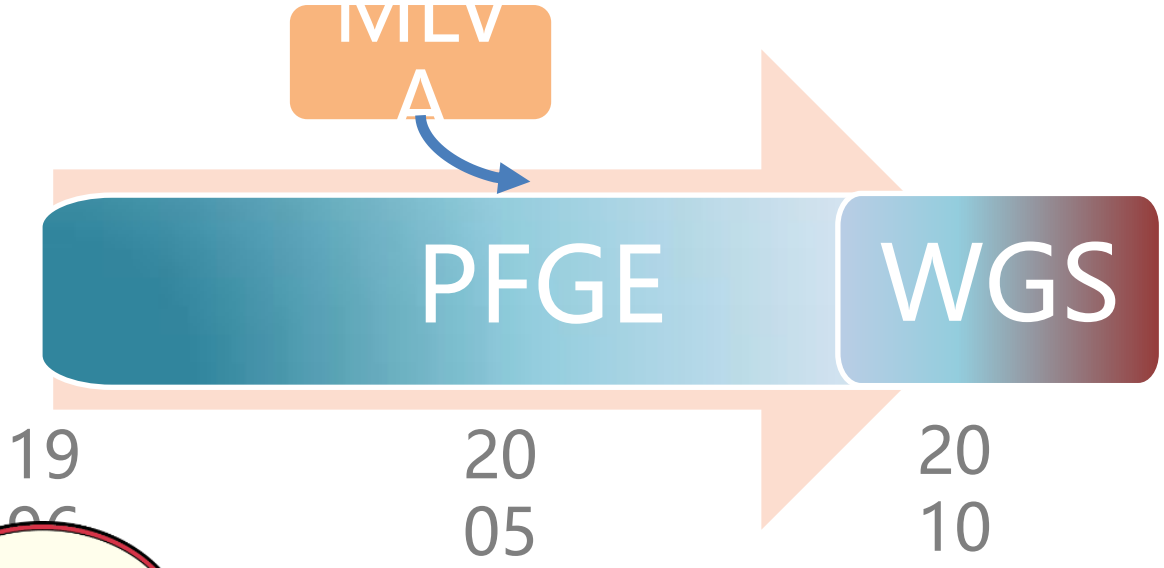
$1 spent = $70 saved!

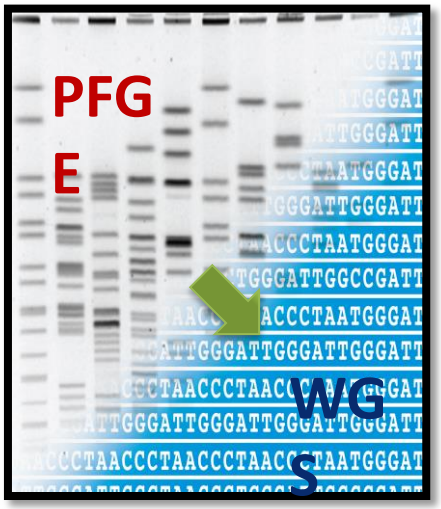


Scharff RL *et al.* 2016. An Economic Evaluation of PulseNet: A Network for Foodborne Disease Surveillance. Am J Prev Med 50:S66-73.

PulseNet moving to Whole Genome Sequencing

Standardized, automated methods to ensure comparability of data generated in different laboratories, save time and resources



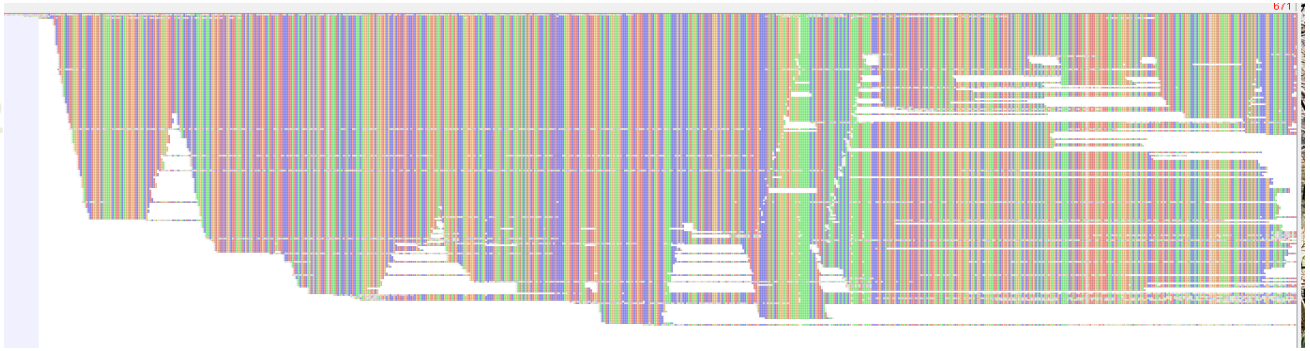


Modified from Carleton and Gerner-Smidt (ASM Microbe July 2016)

PulseNet International
USA, Canada, Europe, Asia Pacific, Africa, Middle East, Latin America and the Caribbean

What Is Whole Genome Sequencing ?

- A process to determine the full genetic code (sequence of DNA bases) of an organism
- "Massive parallel sequencing"
  - The whole genome sequenced in small random pie
    ('shotgun sequencing', 25- >1000 bp) multiple tim
    CCCGGCCCTTTGGCCCTTTGGGAAATCGCCCCAATGGAAATTTGTAGAAGT
  - Following sequencing the DNA base sequence is d
    by piecing the overlapping short sequencing toget
    electronically using specialized software

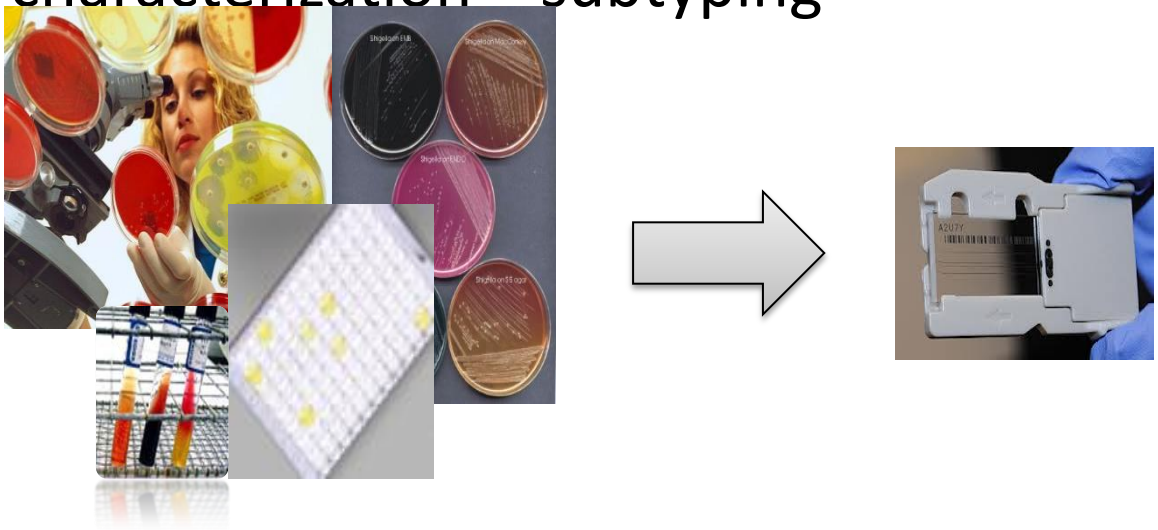


PFGE Is Amazing!
How Can WGS Possibly Be Better?

WGS Is More Than Subtyping

Replacing Traditional Microbiology with WGS

- Cost-efficient consolidation of multiple workflows into one: Identification – serotyping – virulence profiling – antimicrobial resistance characterization – subtyping



- Cost: (100-) 150- 200 US $/ bacterial strain

Reference Characterization by WGS:

'One Shot' Characterization Of STEC

Genus/Species: *Escherichia coli*

Serotype: O104:H4

Pathotype: Shiga toxin-producing and enteroaggregative *E. coli* (STEC/EAEC)

Virulence profile: *stx2a, aggR, aggA, sigA, sepA, pic, aatA, aaiC, aap*

Sequence Type: ST678

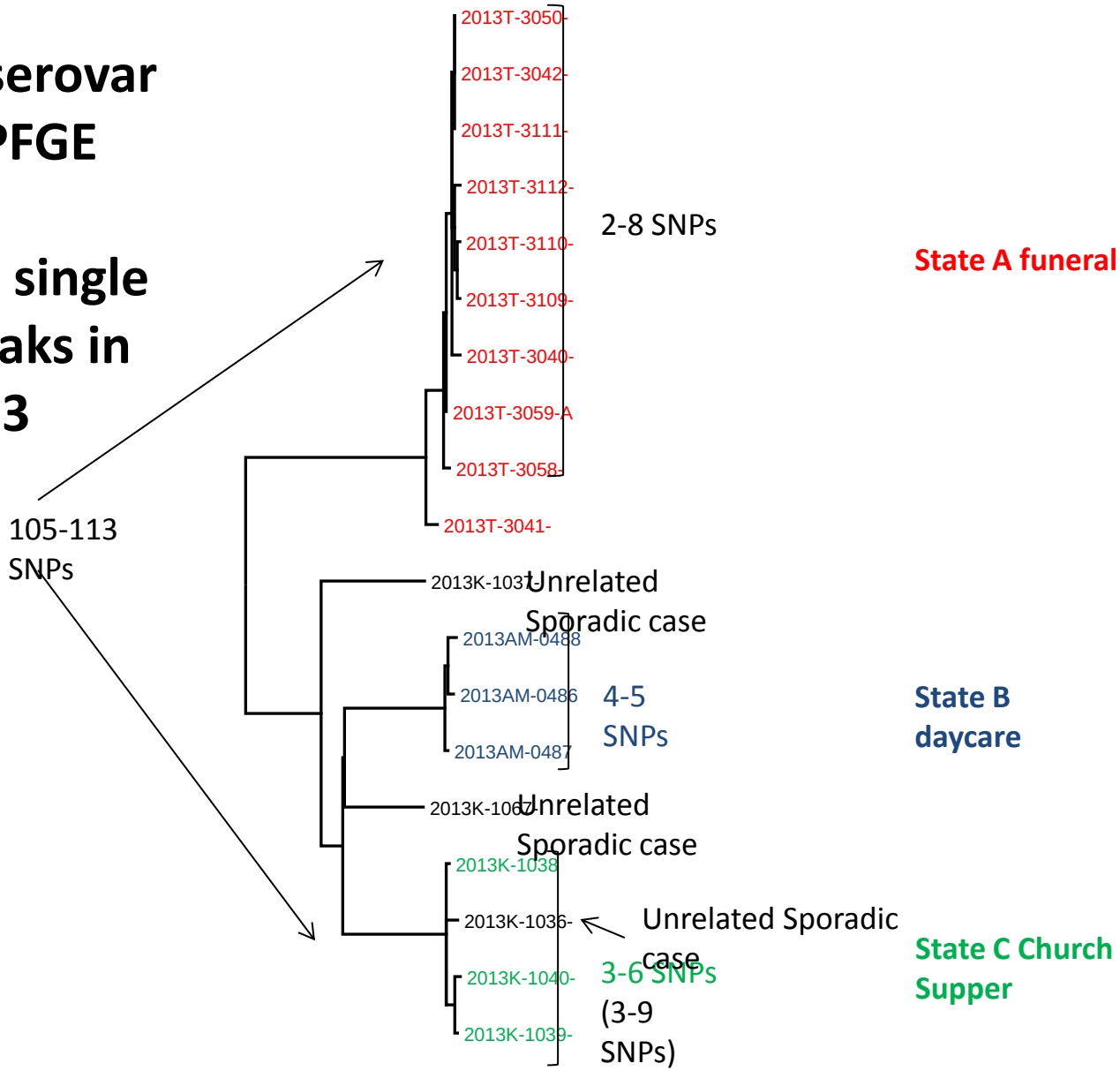
Allele code: 102.45.26.35.3

Antimicrobial resistance genes: bla_{TEM-1}, $bla_{CTX-M-15}$, *strAB, sul2, tet(A)A, dfrA7*

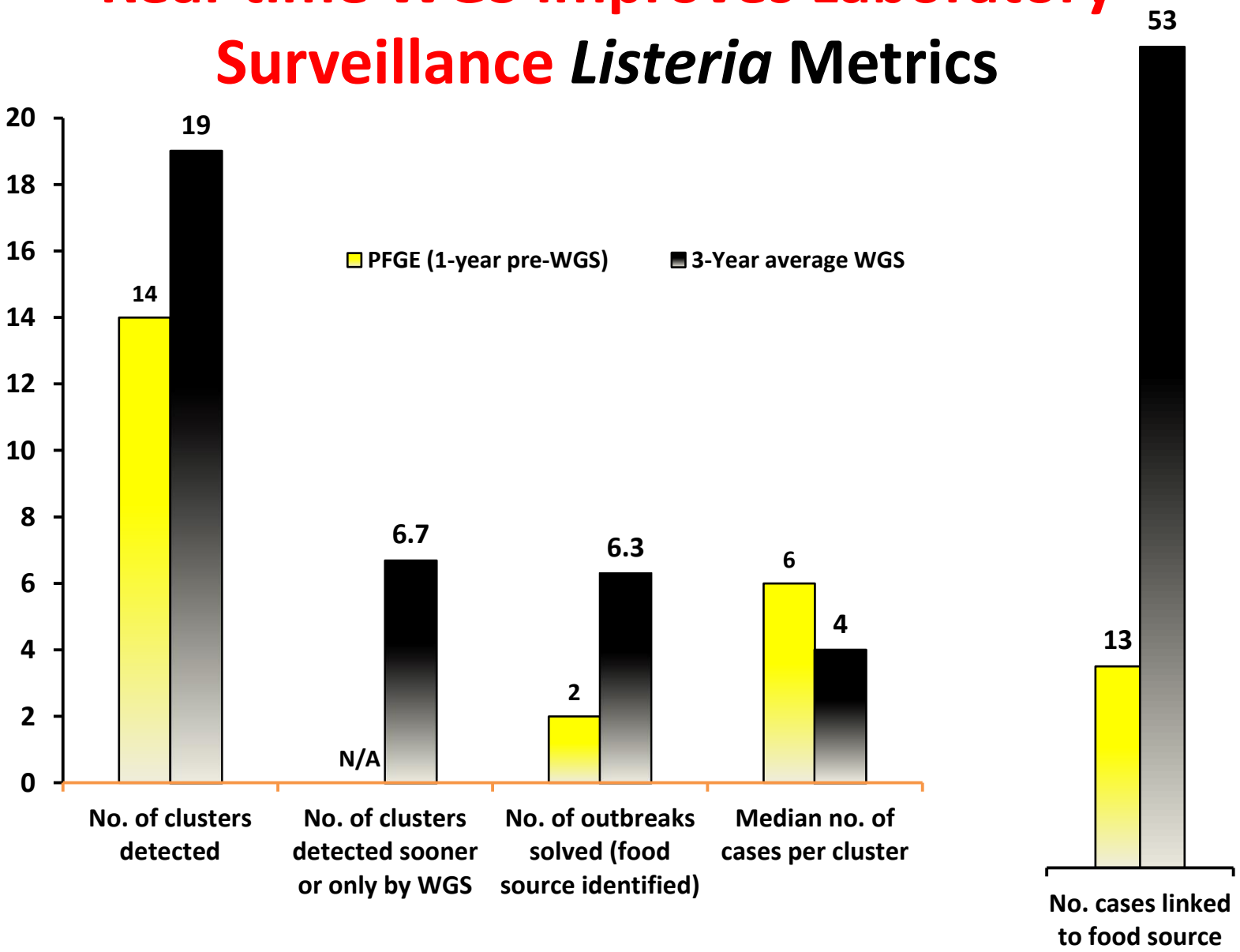
WGS Higher Resolution Than PFGE

Typical tight clustering in point-source outbreaks

***Salmonella* serovar Heidelberg PFGE pattern JF6X01.0022 single state outbreaks in summer 2013**



Real-time WGS Improves Laboratory Surveillance *Listeria* Metrics



Courtesy Amanda Conrad, CDC Outbreak Response & Prepare

Salmonella ser. I 4,[5],12:b:- 1712MLJKX-1 (JKXX01.1478)

Monoclonal: Typical point souce

- Salmonella outbreak 2017- 18
- ~ 90 cases as of March 23
- 4 serotypes
  - I 4,5,12:b:-
  - Thompson
  - Okatie
  - Javiana
- New vehicle: herbal supplement: **kratom**

Kratomguides.com

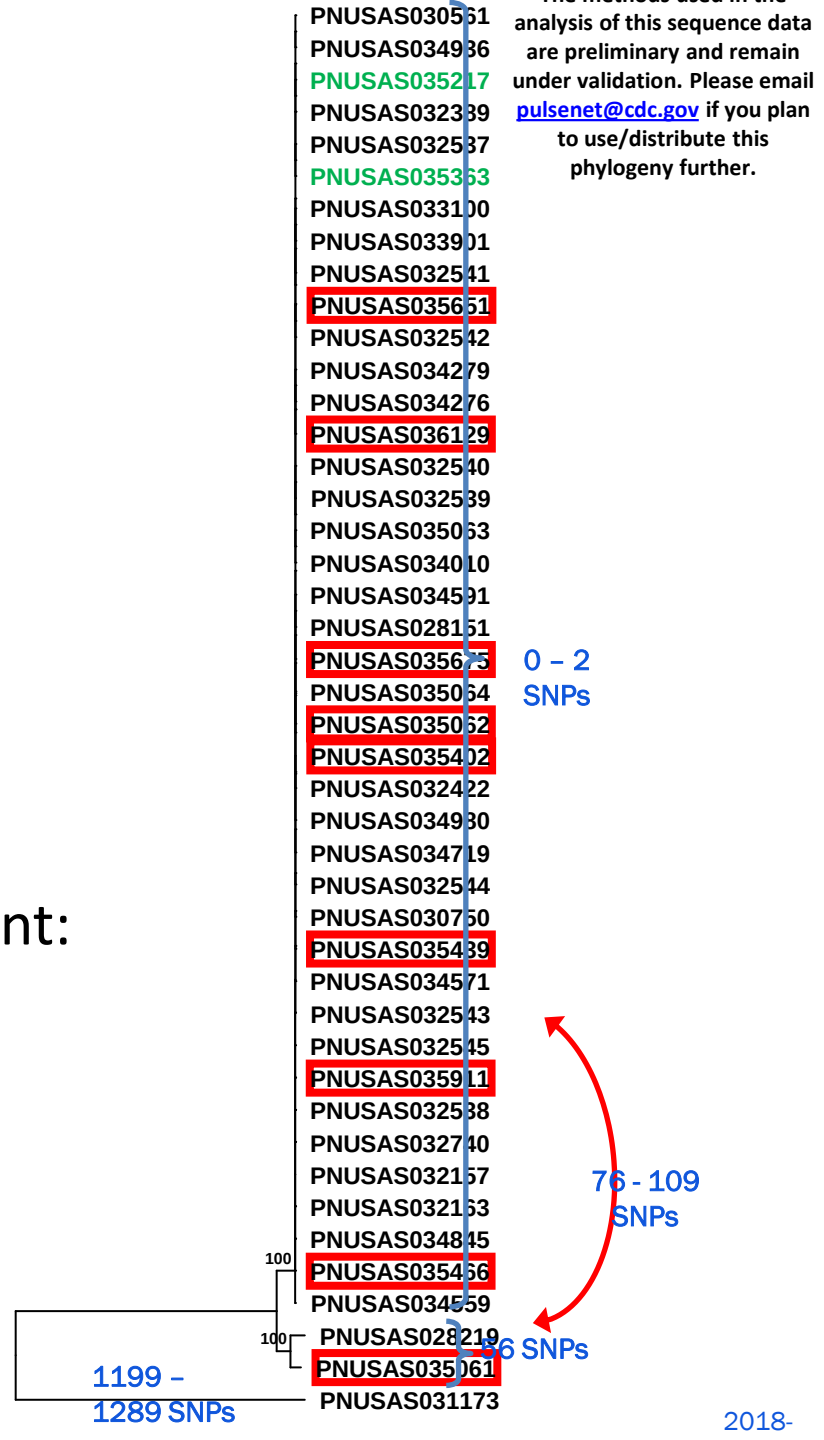
Kratompowders.org

Kratomonline.org

Settings:

LyveSET 1.1.4f used with reads trimmed using fastx_trimmer 5 bases from 3' ends before mapping by SMALT. R2 reads of PNUSAS032540 and PNUSAS032740 trimmed 15 bases from 3' ends. SNPs were called using Varscan at > 20x coverage, > 95% read support, and < 5 bp apart.

Reference:



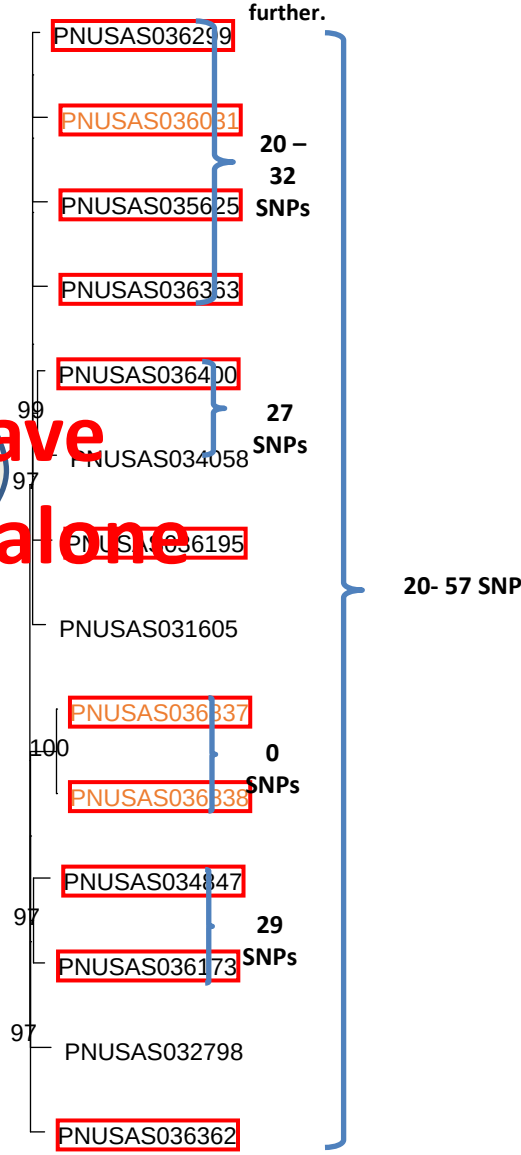
1712MLJKX-1 *Salmonella* ser. Thompson – (JP6X01.0684)

The methods used in the analysis of this sequence data are preliminary and remain under validation. Please email pulsenet@cdc.gov if you plan to use/distribute this phylogeny further.

	PNUSAS031605	PNUSAS032798	PNUSAS034058	PNUSAS034847	PNUSAS035625	PNUSAS036031	PNUSAS036173	PNUSAS036195	PNUSAS036299	PNUSAS036362	PNUSAS036363	PNUSAS036400	PNUSAS036837	PNUSAS036838
PNUSAS031605	0	38	39	38	30	30	32	33	21	32	30	26	45	45
PNUSAS032798	38	0	51	41	41	41	37	44	30	37	41	38		
PNUSAS034058	39	51	0	47	41	42	42	43	32	43	42	27		
PNUSAS034847	38	41	47	0	40	37	29	40	29	34	39	35		
PNUSAS035625	30	41	41	40	0	30	33	37	21	34	31	28		
PNUSAS036031	30	41	42	37	30	0	3		20	34	32	2		
PNUSAS036173	32	37	42	29	33	33	0			34				
PNUSAS036195	33	44	43	40	37	33	37							
PNUSAS036299	21	30	32	29	21	20	22	2						
PNUSAS036362	32	37	43	34	34	34	29	37						
PNUSAS036363	30	41	42	39	31	32	34	3						
PNUSAS036400	26	38	27	35	28	27	30							
PNUSAS036837	45	50	57	46	46	47								
PNUSAS036838	45	50	57	47	46	47								

This cluster could not have been detected by WGS alone

WGS_id	Key				te
PNUSAS031605		an			vn
PNUSAS032798		man	018	1712MLJ	
PNUSAS034058		uman	6/2018	1712MLJKX-1	S
PNUSAS034847		Human	1/30/2018	1712MLJKX-1	Stool
PNUSAS035625		Human	2/11/2018	1712MLJKX-1	Stool
PNUSAS036031		Food	2/15/2018	1712MLJKX-1	Kratom
PNUSAS036173		Human	2/20/2018	1712MLJKX-1	Stool
PNUSAS036195		Human	1/28/2018	1712MLJKX-1	Stool
PNUSAS036299		Human	1/29/2018	1712MLJKX-1	stool
PNUSAS036362		Human	2/6/2018	1712MLJKX-1	Stool
PNUSAS036363		Human	2/12/2018	1712MLJKX-1	Stool
PNUSAS036400		Human	2/13/2018	1712MLJKX-1	Stool
PNUSAS036837		Food	2/22/2018	1712MLJKX-1	Kratom Powder
PNUSAS036838		Food	2/22/2018	1712MLJKX-1	Kratom Powder

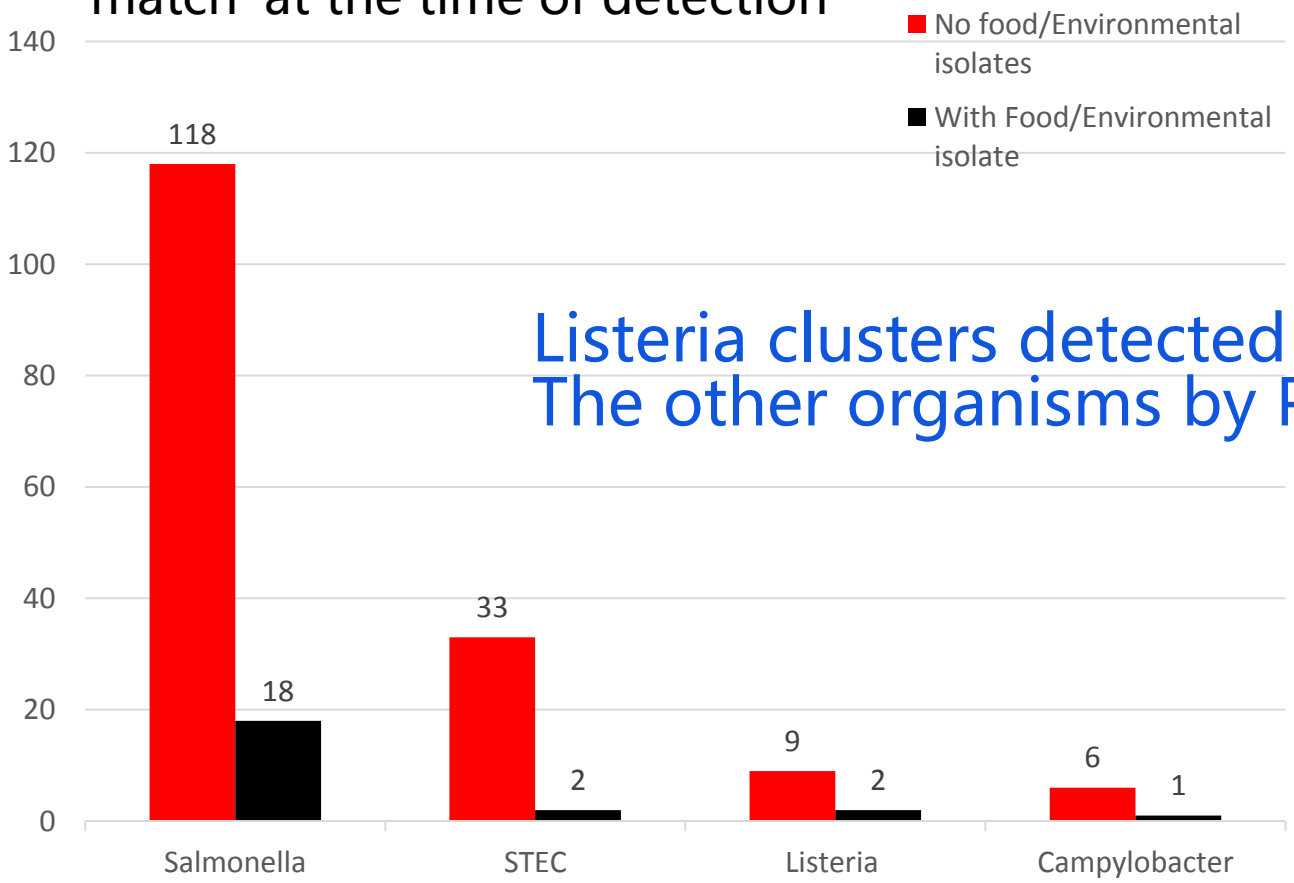


LyveSET 1.1.4f used with reads trimmed using fastx_trimmer 5 bases from 3' ends before mapping by SMALT. SNPs were called using Varscan at > 20x coverage, > 95% read support, and < 5 bp apart. PacBio assembly of str. 2013K-0463 with prophage regions masked at 87735-908183, 1963137-1971305, 2386740-2436933, 3249575-3263912, and 4084799-4101913.

PRODUCT TESTING-DRIVEN ("RETROSPECTIVE") OUTBREAK INVESTIGATIONS

PulseNet Clusters In 2016* With Or Without Isolates From Potential Sources Available At The Time Of Detection

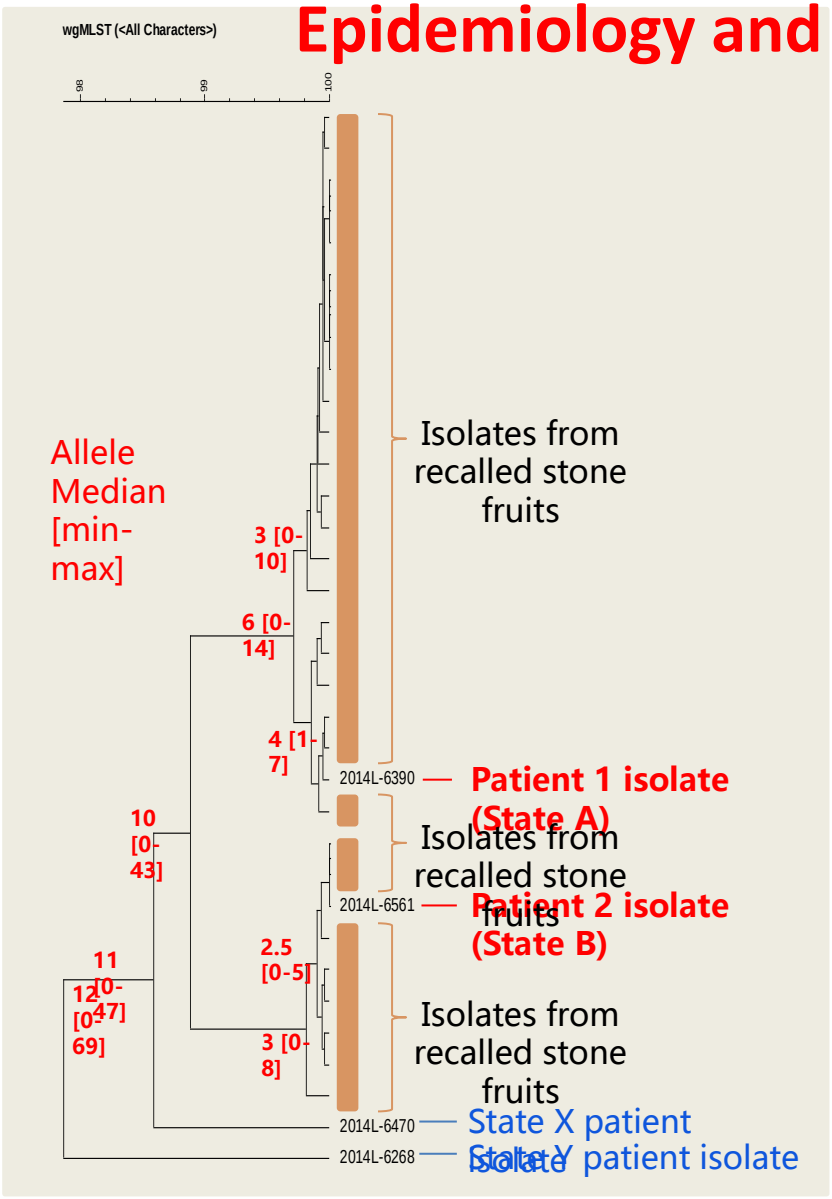
14% of PulseNet clusters has a non-clinical 'match' at the time of detection



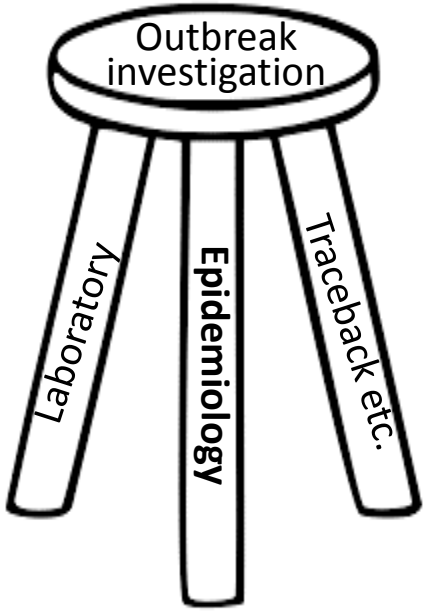
Listeria clusters detected by WGS
The other organisms by PFGE

2016*: Until 9/19/2016

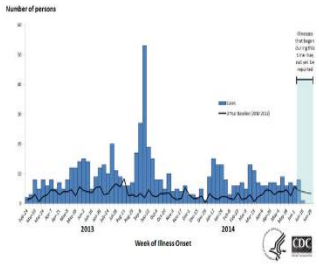
2014 Stone Fruit Outbreak: Concordance Between Epidemiology and WGS



We Still Need The Three Legged Stool !

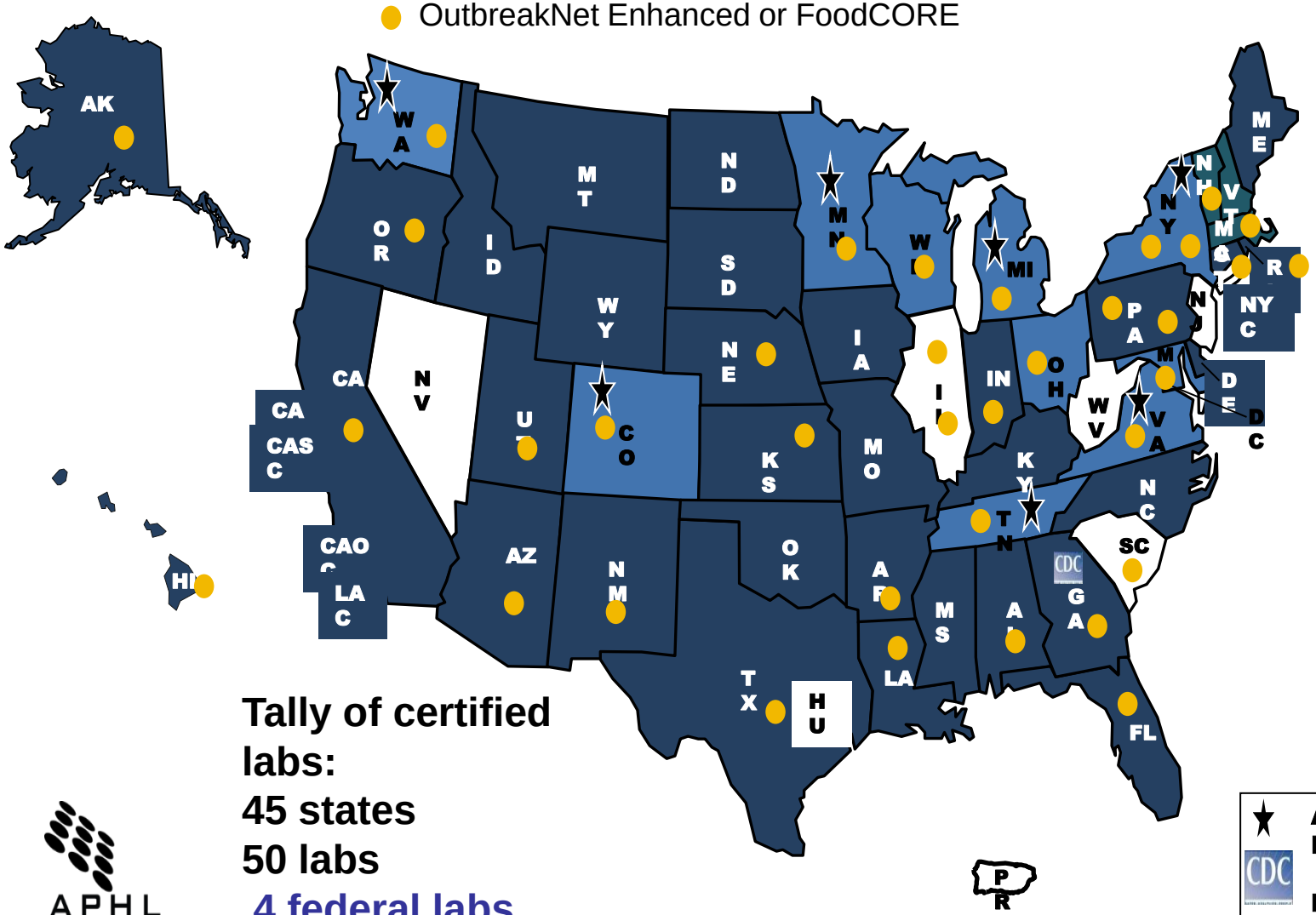


Integrated Surveillance



PulseNet, WGS and Enhanced Epidemiological Capacity

- WGS certified Bioinformatics 7.16 platform
- WGS certified
- OutbreakNet Enhanced or FoodCORE



Tally of certified labs:
45 states
50 labs
4 federal labs
3 international labs

APHL

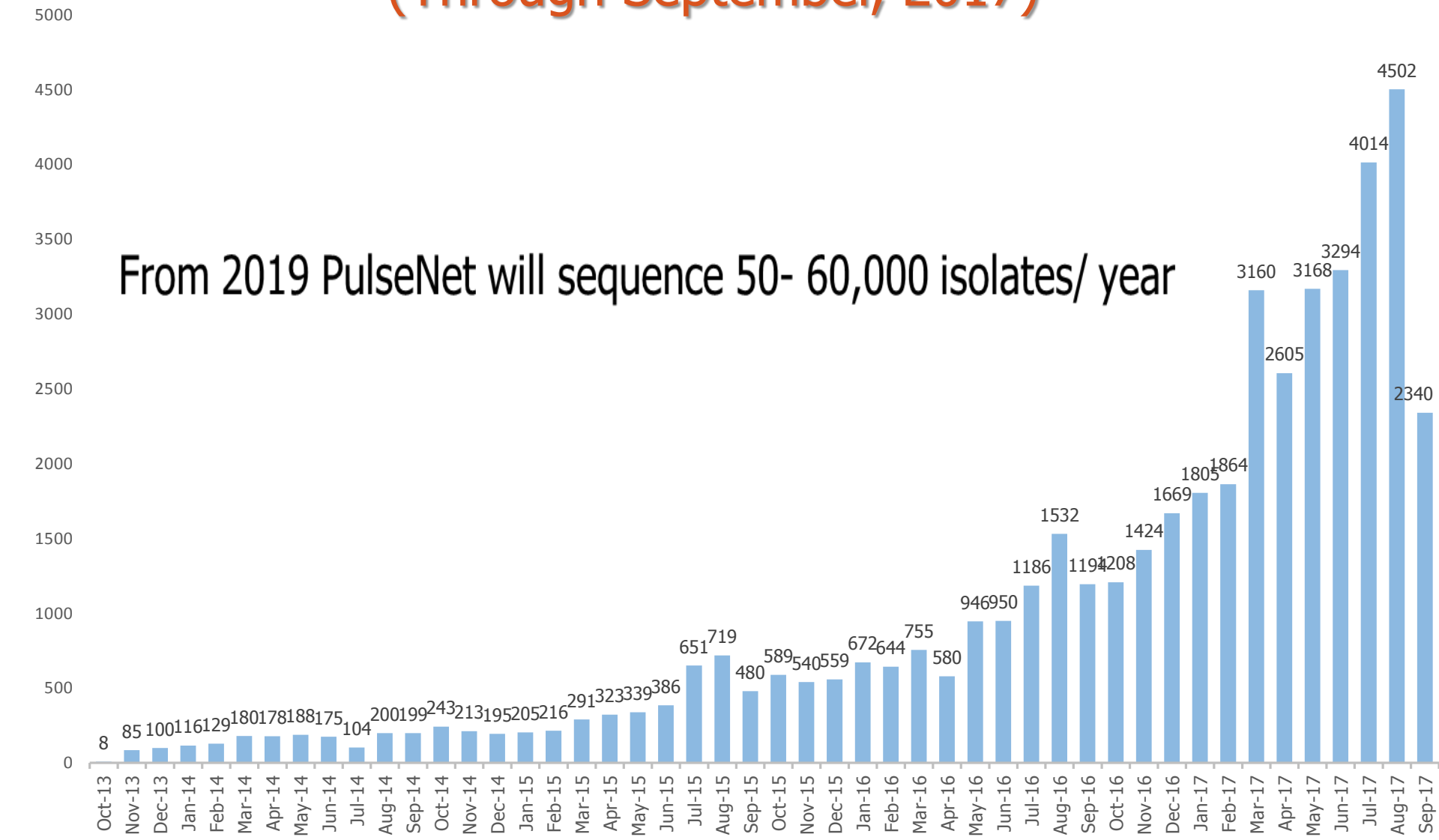
★ **Area Laboratories**

CDC **PulseNet Central**

Modified: March 16, 2018

Genomes Sequenced and Submitted to PulseNet & SRA, NCBI

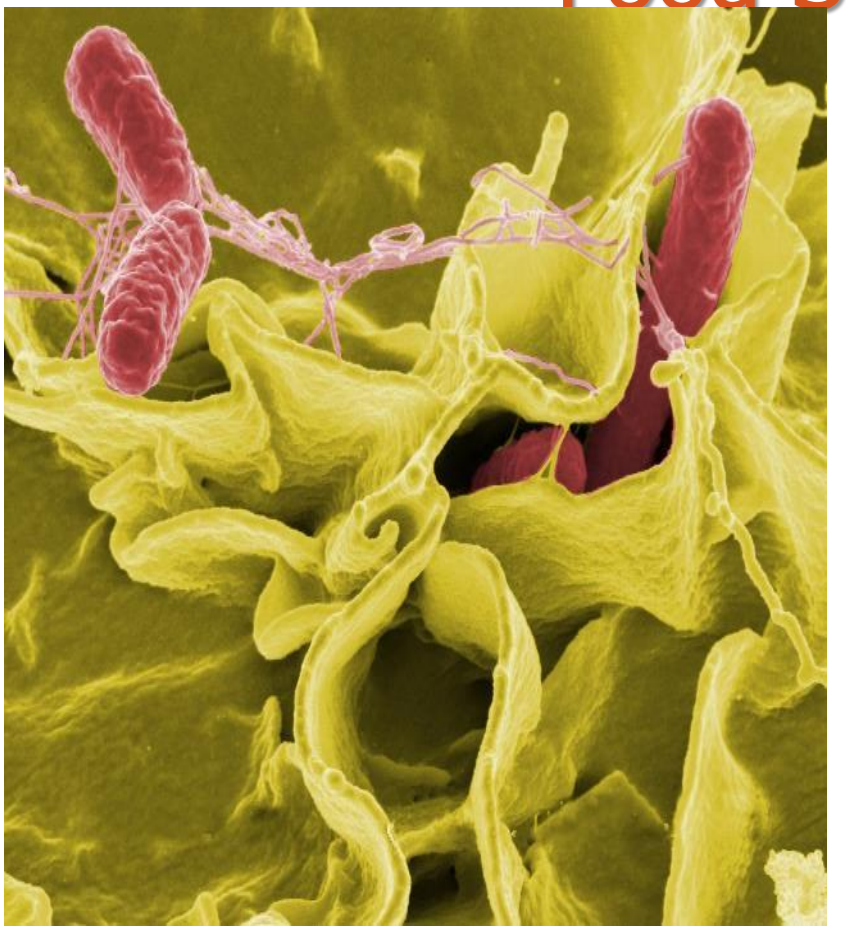
(Through September, 2017)



WGS: New Concerns

- WGS turnaround time issues
- Interpretation concerns
  - Confusion about the meaning of a "match" or "mis-match"
  - No "absolute" cutoff values (SNPs or allele)
- Food industry concerns
  - No "statute-of-limitations" on liability
  - No precise definition of "outbreak"
  - Data sharing

Coming Soon: Using Big Data to Improve Food Safety



- Pathogen characterization direct-from-specimen (faster)
- Compatible with CIDTs
- More information to inform policy

But

- Privacy issues
- Regulatory hurdles


DON'T EAT YOUR SPINACH!!
THERE IS A GOD!
NEWS
DEADLY E COLI FOUND IN SPINACH
9/2006 CARTOONCOX.ORG
CAGLECARTOONS.COM

Acknowledgements

Public Health Agency of Canada

Disclaimers:

"The findings and conclusions in this presentation are those of the author and do not necessarily represent the official position of the Centers for Disease Control and Prevention"

"Use of trade names is for identification only and does not imply endorsement by the Centers for Disease Control and Prevention or by the U.S. Department of Health and Human Services."