Agricultural Outlook Forum
U.S. Department of Agriculture

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The Fight Against Salmonella in Agriculture – Research Perspective
(a view from the trenches)

Dayna M. Harhay

The Fight Against *Salmonella* in Agriculture – Research Perspective

(a view from the trenches)

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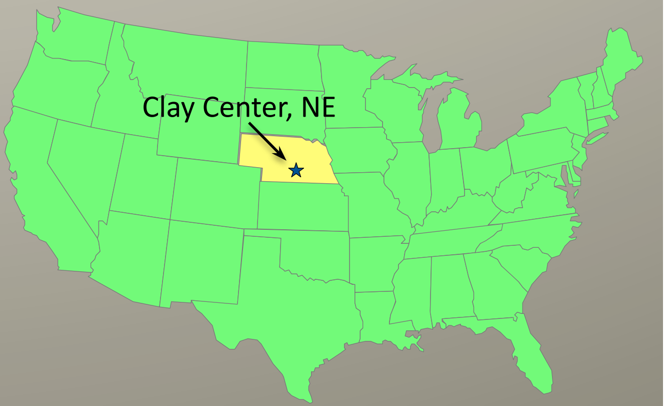
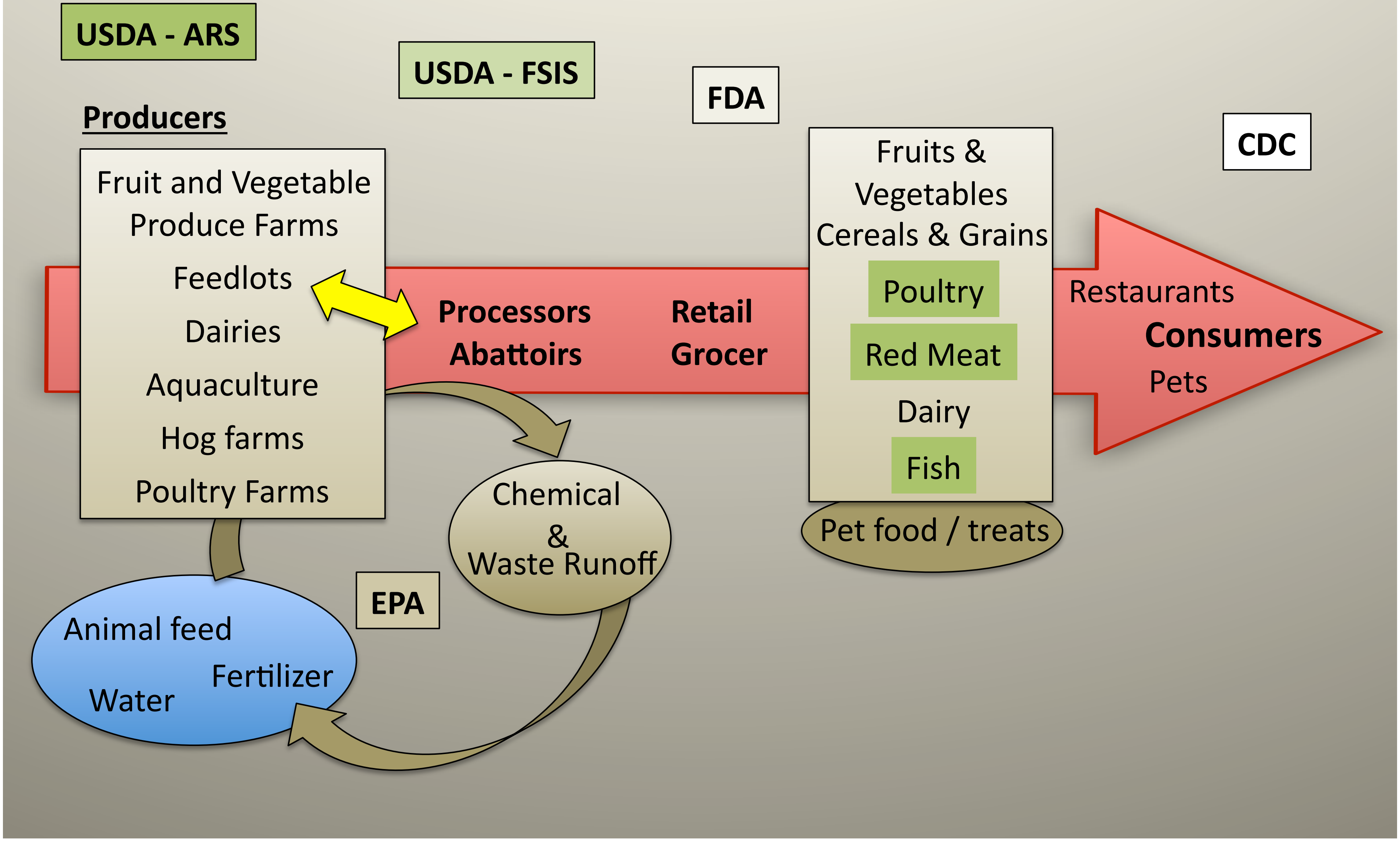


The Food Safety Continuum



USDA - ARS

USDA - FSIS

FDA

CDC

Producers

Fruit and Vegetable Produce Farms

Feedlots

Dairies

Aquaculture

Hog farms

Poultry Farms

Processors

Abattoirs

Retail

Grocer

Fruits & Vegetables

Cereals & Grains

Poultry

Red Meat

Dairy

Fish

Pet food / treats

Restaurants

Consumers

Pets

Chemical & Waste Runoff

EPA

Animal feed

Fertilizer

Water

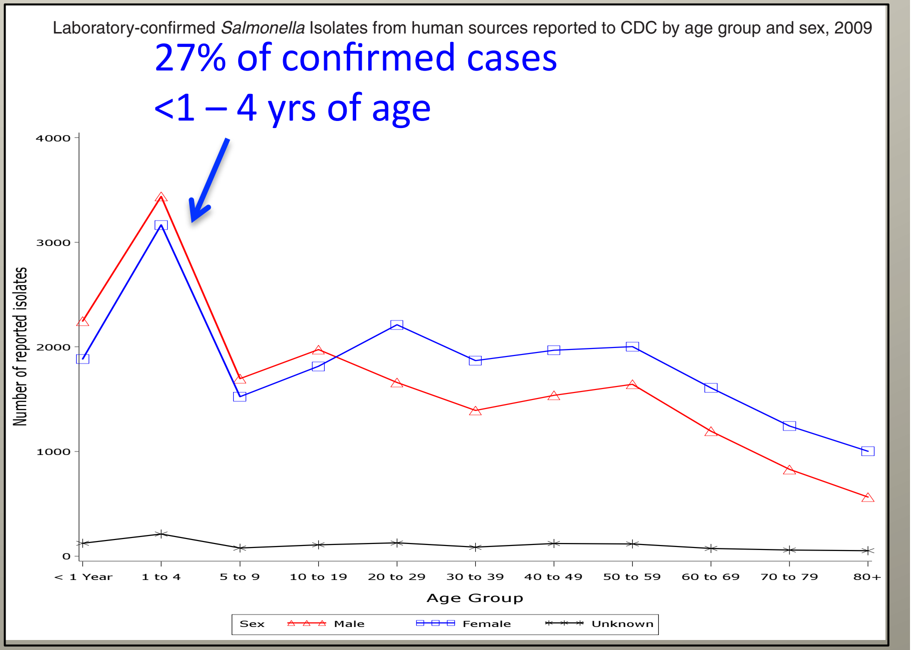
How do we **prevent** *Salmonella* foodborne illness…?

- ✓ Know the enemy
- ✓ Understand the scope of the problem
- ✓ What is being done about the problem?
- ✓ What more can be done?

Salmonella and Foodborne Disease

- Salmonellosis – Non-typhoidal *Salmonella enterica*
- > 2,500 serotypes (~1,700 noted for making animals sick)
- U.S. cases per year:
  - Confirmed ~40,000
  - **Estimated ~ 1.4 million**

CDC, *Salmonella* Annual Summary - 2009


Laboratory-confirmed *Salmonella* Isolates from human sources reported to CDC by age group and sex, 2009

27% of confirmed cases

<1 – 4 yrs of age

Salmonella and Foodborne Disease

- Salmonellosis – Non-typhoidal *Salmonella enterica*
- > 2,500 serotypes (~1,700 noted for making animals sick)
- U.S. cases per year:
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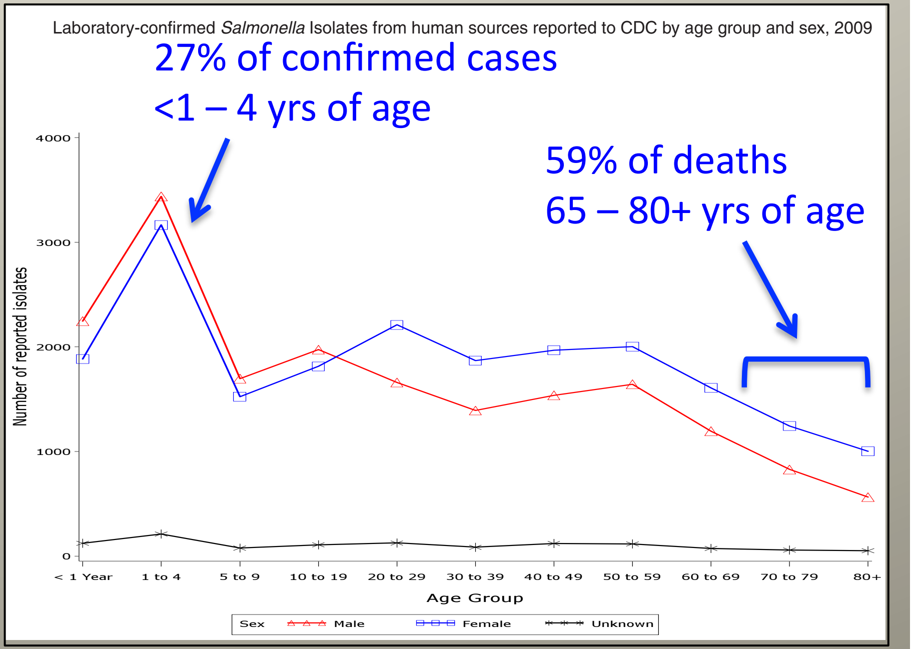
  - **Estimated ~ 1.4 million**

- Generally self limiting enteritis but can be invasive
  - Hospitalization ~15,000 per year
  - Death ~1% of confirmed cases/yr
- Human health cost ~ $330 million/yr
  - Decreased productivity
  - Medical expense

http://www.ers.usda.gov/Data/FoodborneIllness/

- Industry cost ~$100s of millions
  - Product recall
  - Plant closures and clean up
  - Liability costs

CDC, *Salmonella* Annual Summary - 2009



Laboratory-confirmed *Salmonella* Isolates from human sources reported to CDC by age group and sex, 2009

27% of confirmed cases
<1 – 4 yrs of age

59% of deaths
65 – 80+ yrs of age

Salmonella and Foodborne Disease

- Primary habitat – animal large intestine / feces
  - "Hearty bug" also survives well in the environment

- 6 serotypes account for >50% human cases

  20 serotypes account for >70% cases

  Are some serotypes more virulent than others?

- Complex etiology

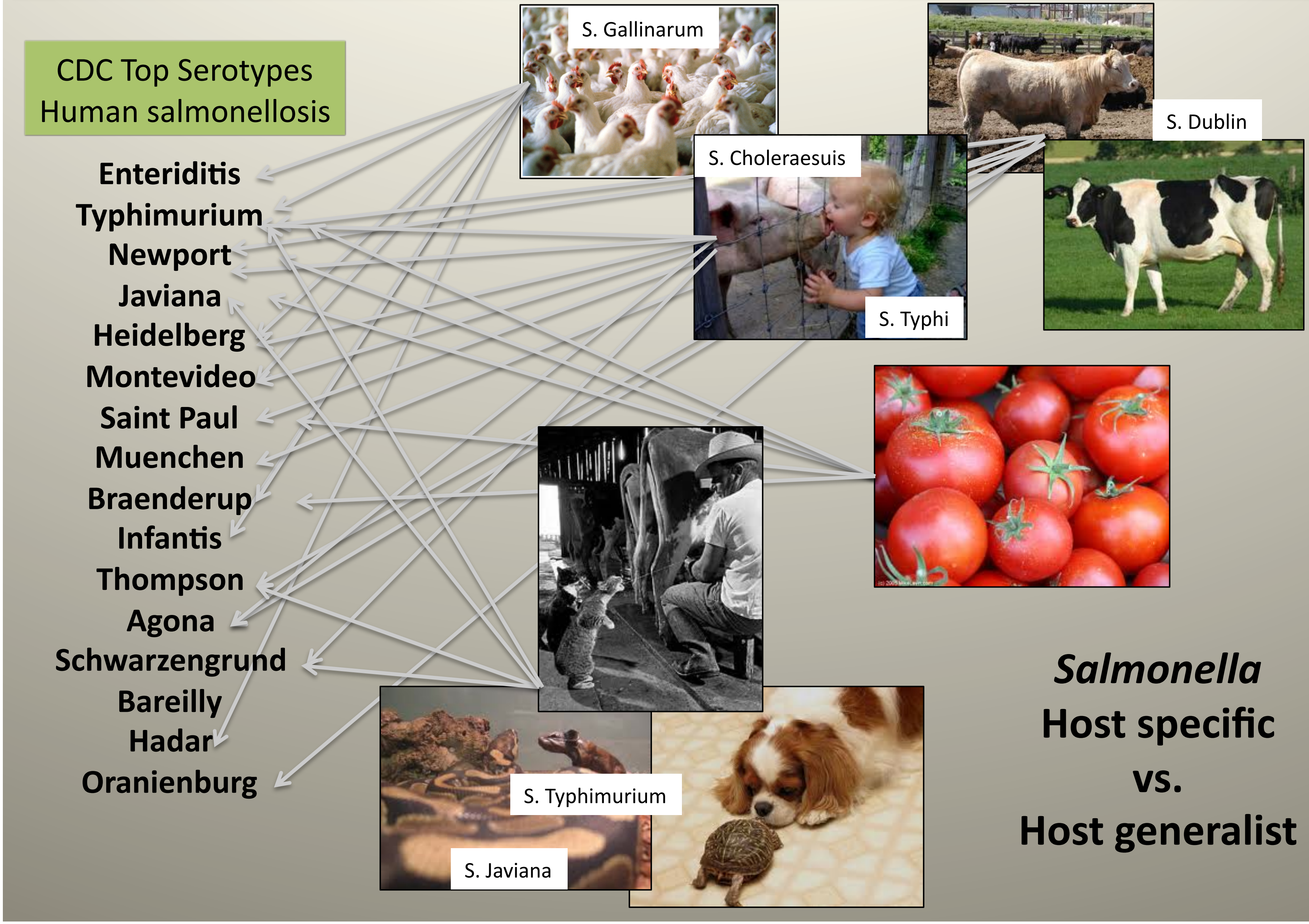
  ~10% cases attributed to outbreaks (OB)

  ~90% sporadic

Fecal Bacteria
~10^{11} CFU/g feces
100 billion/g!

Many potential sources of *Salmonella*!

Reservoirs for *Salmonella* and Host Specificity

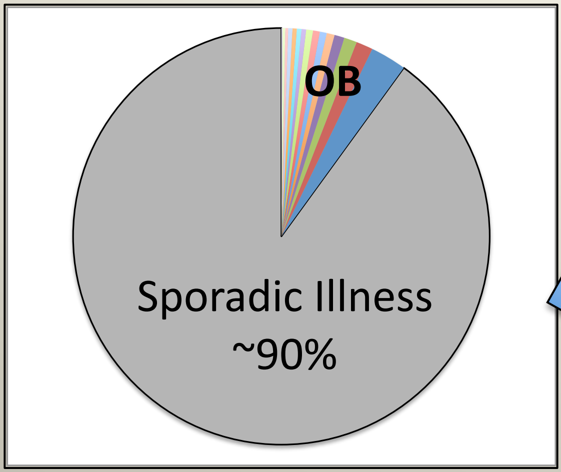


CDC Top Serotypes
Human salmonellosis

Enteriditis
Typhimurium
Newport
Javiana
Heidelberg
Montevideo
Saint Paul
Muenchen
Braenderup
Infantis
Thompson
Agona
Schwarzengrund
Bareilly
Hadar
Oranienburg

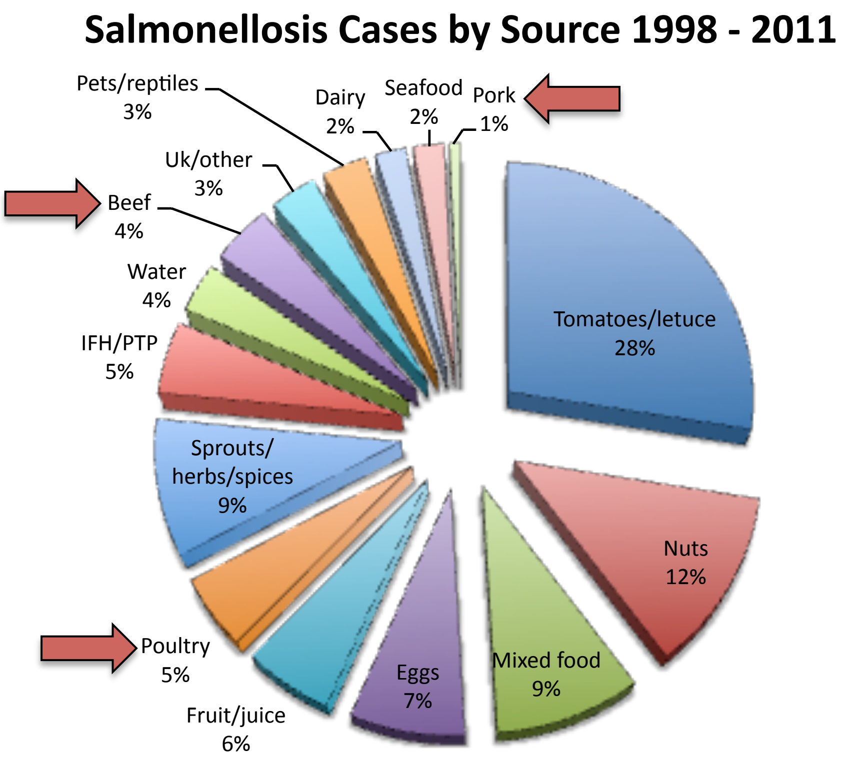
Salmonella
Host specific
vs.
Host generalist

The Complexity of *Salmonella* Etiology



~40,000 confirmed cases / yr

~10-15% of confirmed cases in U.S. attributed to OB linked with contaminated meat consumption



Summary of 152 outbreaks representing 12,181 cases of illness – not all inclusive.

HHS Healthy People Initiative 2020
Salmonellosis Target:
6.8 cases / 100K people
Presently ~14 cases / 100K

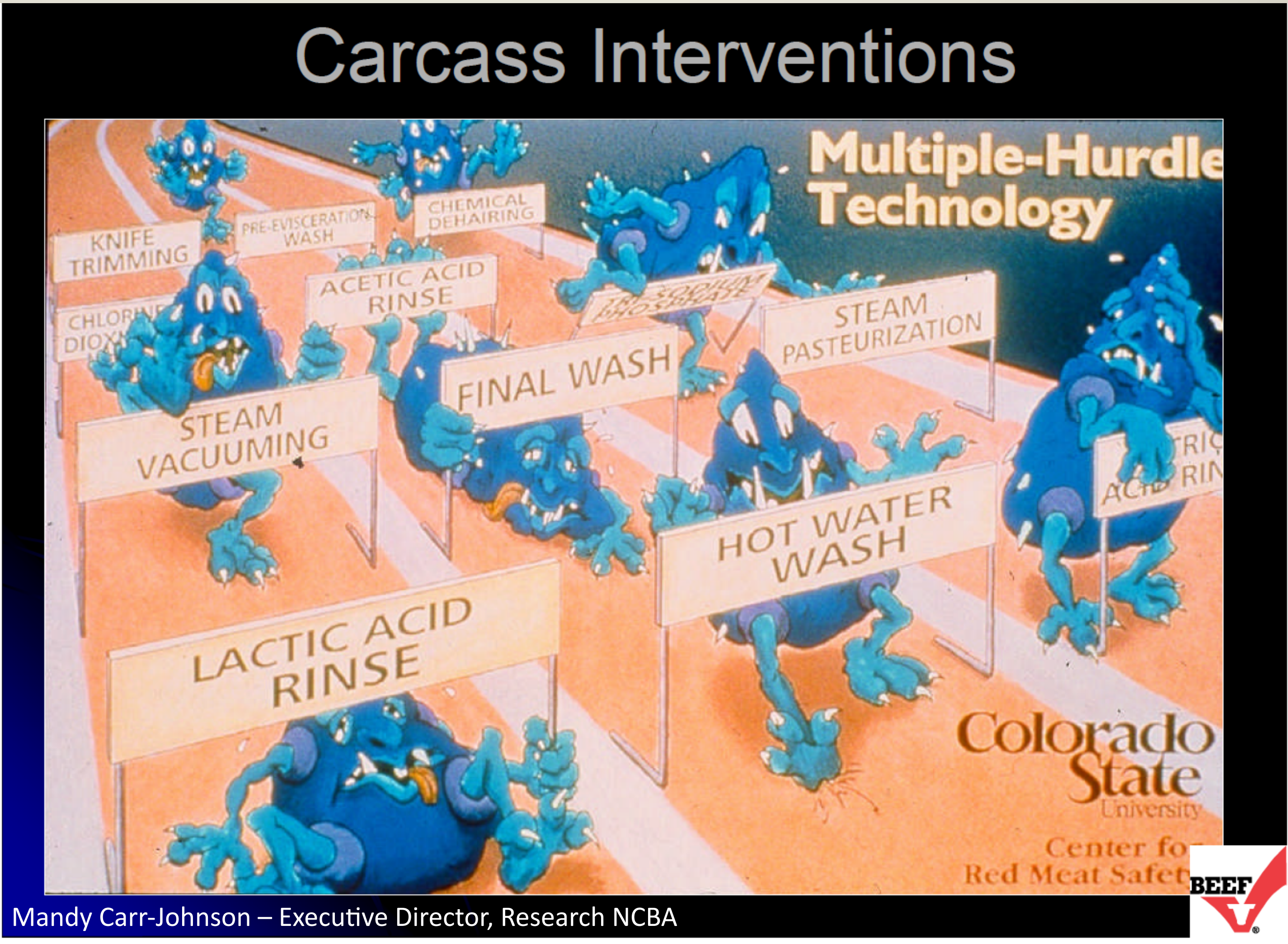
M. Ellin Doyle et. al., (2009) http://fri.wisc.edu/docs/pdf/FRI_Brief_Salmonella_Human_Illness_6_09.pdf

http://wwwn.cdc.gov/foodborneoutbreaks/

Post-Harvest Multiple Hurdle Carcass Interventions

FSIS – 1996
Issued Final Rule:
Pathogen
Reduction;
Hazard
Analysis and
Critical
Control
Point
(**PR/HACCP**)
Systems

2006 – FSIS
Increased those
performance
standards



Carcass Interventions

Mandy Carr-Johnson – Executive Director, Research NCBA

Efficacy of Multiple Hurdle Carcass Processing Interventions at Reducing *Salmonella* Prevalence

Beef Processing

~98% Reduction in
***Salmonella* prevalence**
on carcasses

89.6% **50.2%** **0.8%**

Hide (*n*=3040)

Pre-evisceration
(*n*=3040)

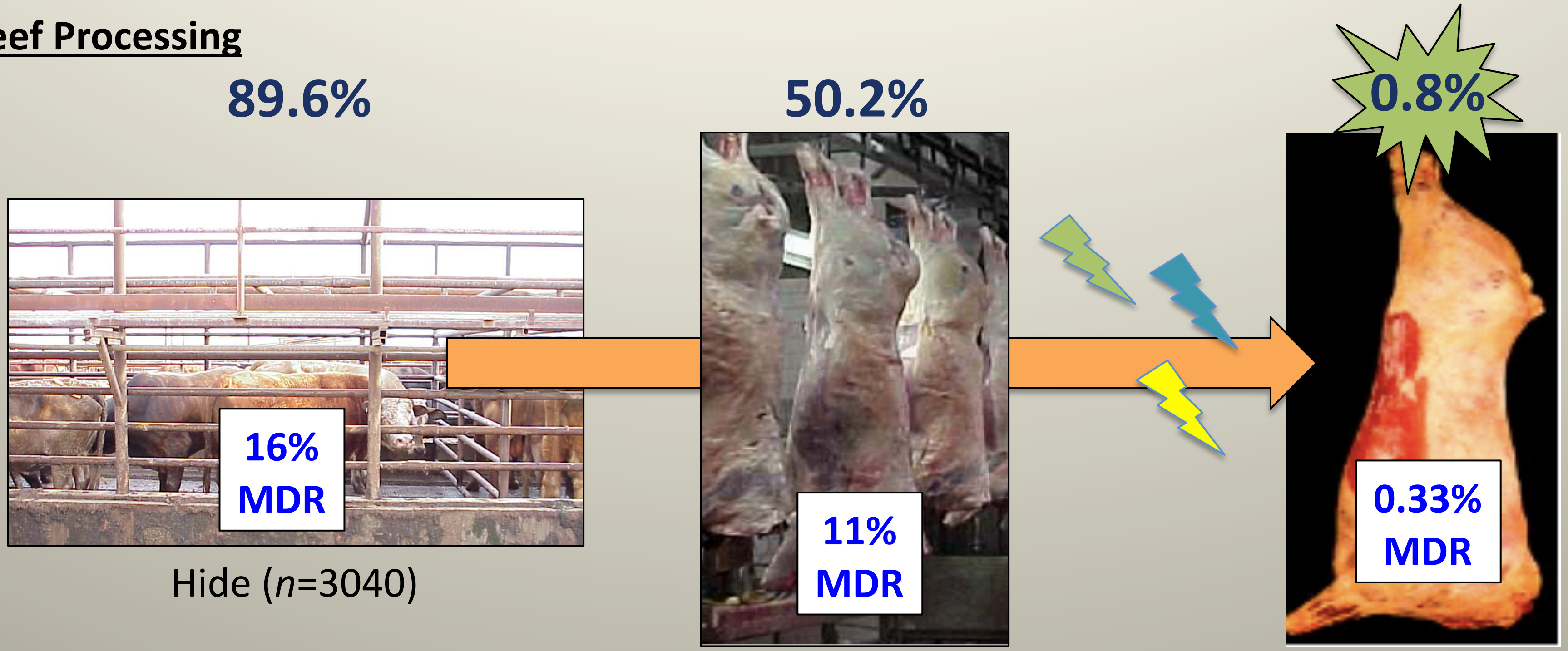
Post-intervention
(*n*=3040)

Multiple hurdle interventions are very effective for reducing pathogen levels on final carcasses

Brichta-Harhay et al., (2008) 74:6289-97

Prevalence of Multidrug Resistant *Salmonella* at Harvest

Beef Processing



89.6%

16% MDR

Hide (*n*=3040)

50.2%

11% MDR

Pre-evisceration (*n*=3040)

0.8%

0.33% MDR

Post-intervention (*n*=3040)

6% of all *Salmonella* isolated in this study found to be MDR

- ***Consistent subpopulation of any niche***

~1.8% Ceftriaxone resistance

~0.1% Nalidixic acid resistance – uncommon

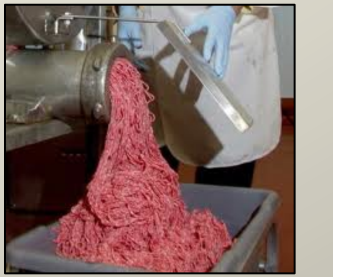
Predominant Serotypes

Newport
Typhimurium
Uganda

Brichta-Harhay et al., (2011) AEM 77:1783-96

Concerns about Drug Resistant *Salmonella* in Ground Beef

- Relatively few outbreaks and recalls, but when they occur they can involve MDR *Salmonella* Newport or Typhimurium



Newport MDR-AmpC	**MDR Typhimurium**
➢ 2002 47 ill	2003 58 ill
➢ 2007 43 ill	2009 14 ill
➢ 2009 42 ill	2011 19 ill

- Concern that multidrug resistant (MDR) *Salmonella* are more invasive than susceptible *Salmonella* - ***are they?***

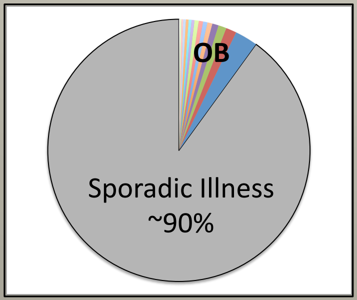
Salmonella Prevalence in Ground Beef

USMARC Nationwide survey 2007-2008 of *Salmonella* prevalence in ground beef (n=4,136 samples) found **mean prevalence to be 4.2%** *Bosilevac et. al., (2009) AEM 75:1892-1900*

- Most commonly observed serotypes: **Montevideo (21%), Anatum (15%), Muenster (8%), Mbandaka(6%)**

- **MDR *Salmonella*** – 0.6%

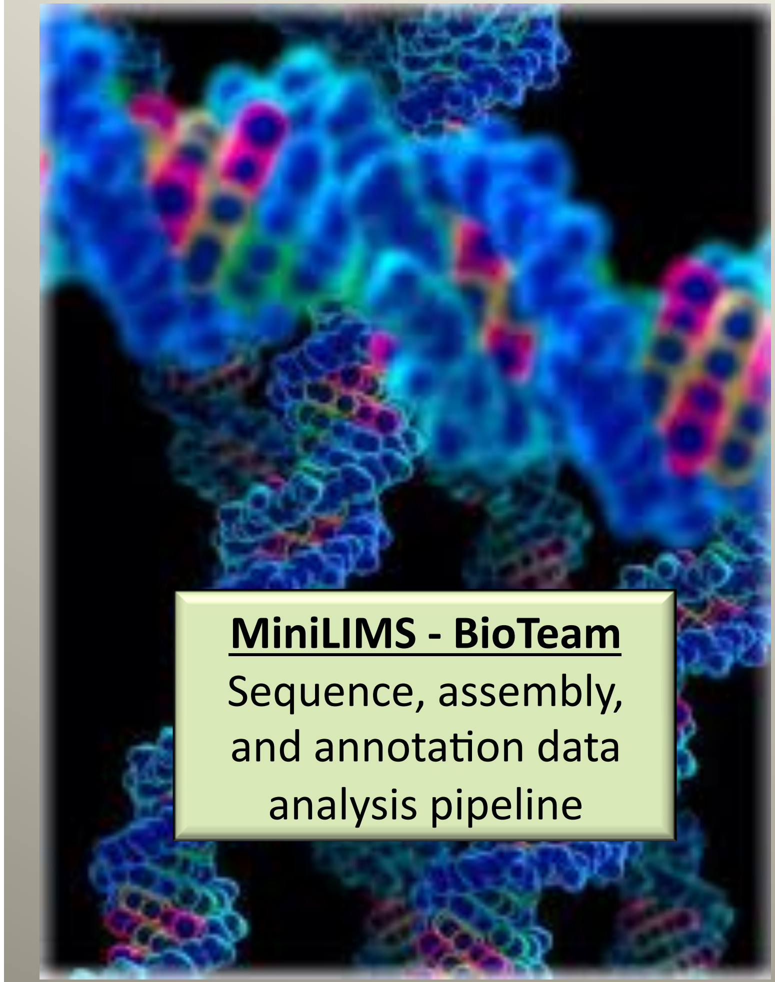
➢ MDR Typhimurium	0.09%
➢ MDR Newport	0.07%



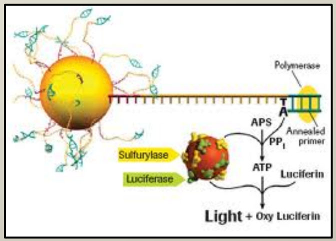
~0.4% of outbreak cases (1998-2011)

No Montevideo outbreaks linked to Ground Beef…

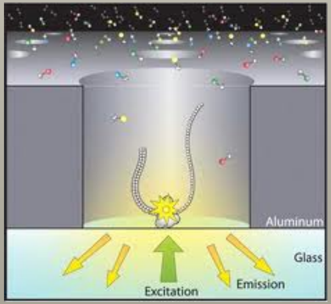
The Future Landscape of the Fight Against *Salmonella* in Agriculture…



MiniLIMS - BioTeam
Sequence, assembly,
and annotation data
analysis pipeline

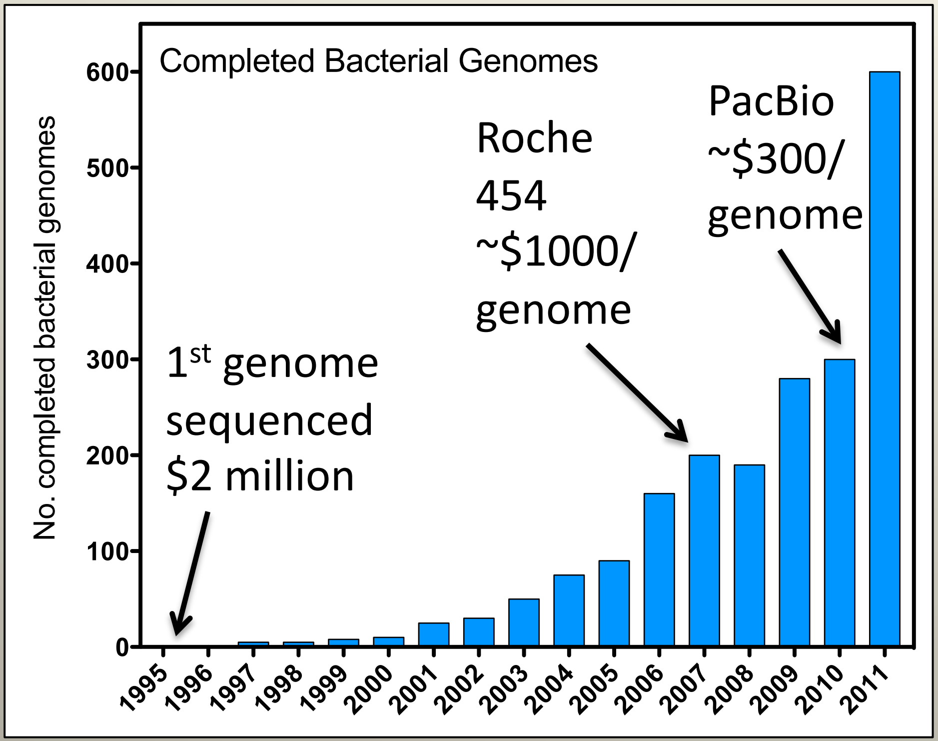


USMARC
acquired 2008



USMARC
acquired 2011

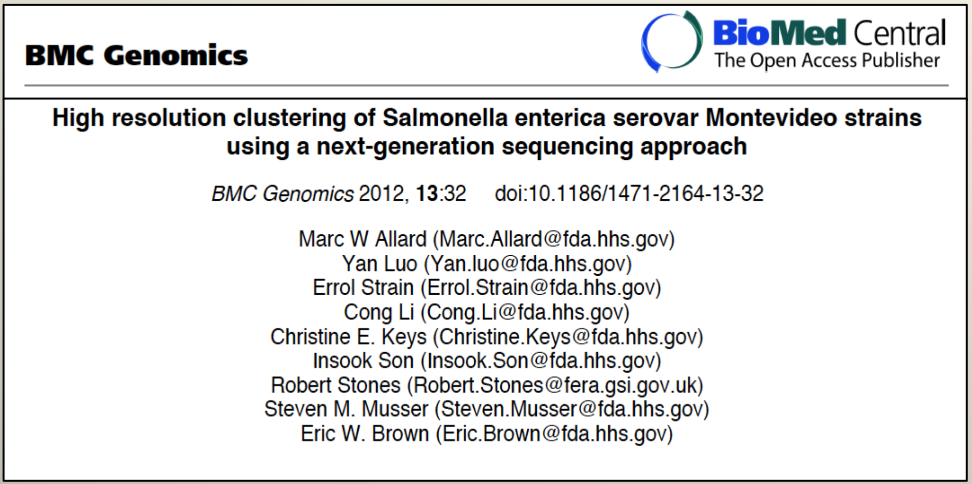
Bacterial Pathogen Genome Sequencing



"It is now readily possibly to determine the draft genome sequence of a bacterial pathogen within a two day time span"

***Source Tracking* - Molecular Epidemiology based on specific patterns of Single Nucleotide Polymorphisms (SNPs) within a pathogens genome sequence**

Pathogen Detection and Source Tracking



BMC Genomics

BioMed Central
The Open Access Publisher

High resolution clustering of Salmonella enterica serovar Montevideo strains using a next-generation sequencing approach

BMC Genomics 2012, **13**:32 doi:10.1186/1471-2164-13-32

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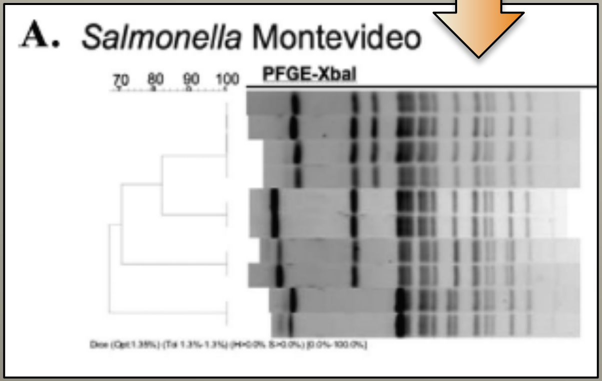
Outbreak occurred
July 2009 – April 2010
273 ill (44 states)
52 hospitalized
0 deaths

Causative agent: *S.* Montevideo

Highly clonal strains confound epidemiologic investigations



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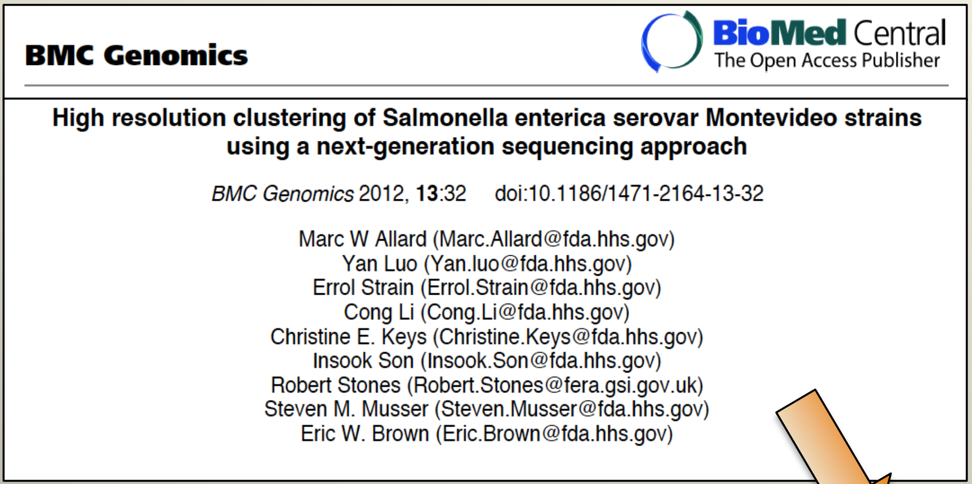

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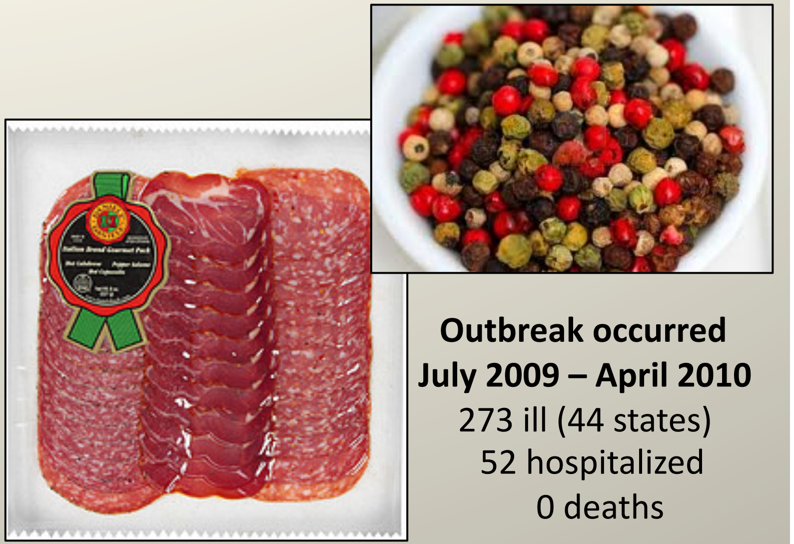

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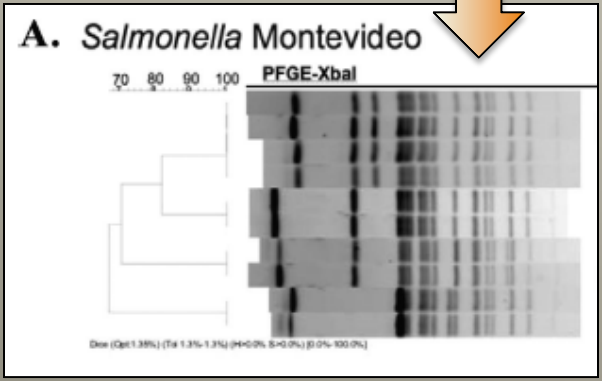
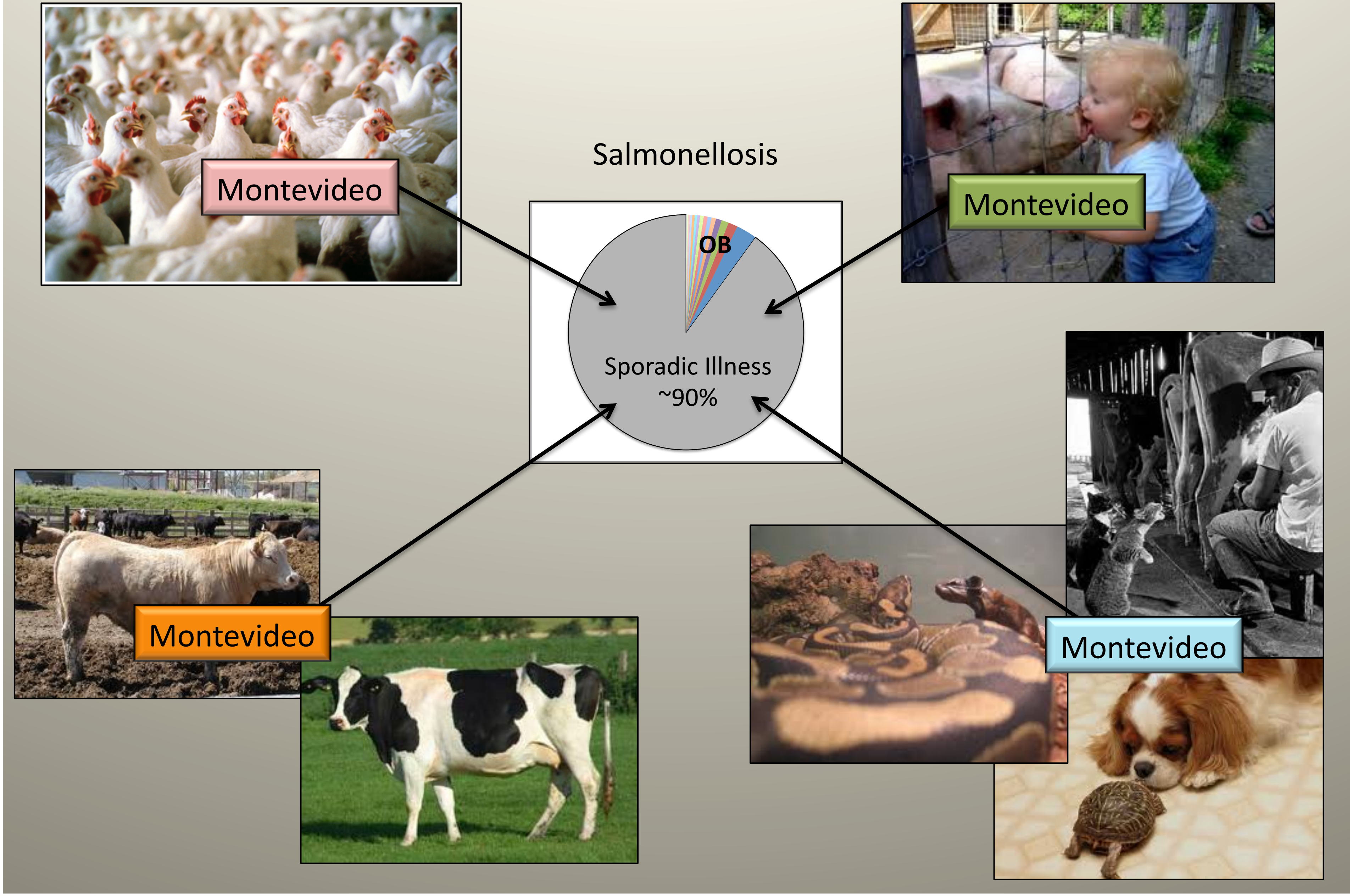


Bosilevac et. al., (2009) AEM 75:1892-1900

- Performed whole genome sequencing (WGS) of 40 Montevideo Isolates with PFGE patterns matching the OB strain (clinical, environmental, laboratory and food isolates)

- Found a core set of SNPs that defined the outbreak strains – they were even able to determine that one of the “outbreak” cases in California was unrelated

Pathogen Source Tracking



Salmonellosis

Montevideo

Montevideo

OB

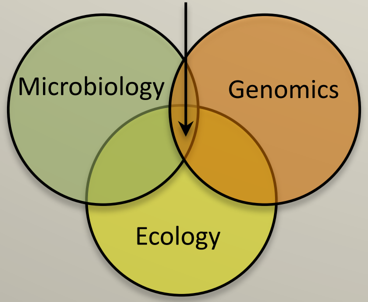
Sporadic Illness
~90%

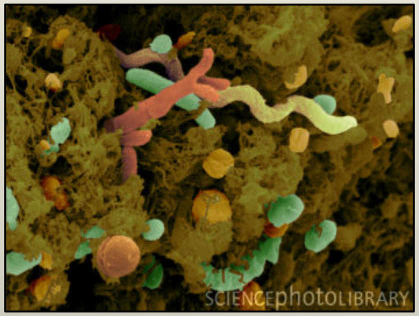
Montevideo

Montevideo

Other Future Trends: Metagenomics & Transcriptomics

Metagenomics: the study of complex mixtures/communities





Scanning electron micrograph (SEM) of bacteria in cattle feces – magnification ~10,000x

Isolate total genomic DNA

Sequence

Characterize the "players" in a given microbial niche

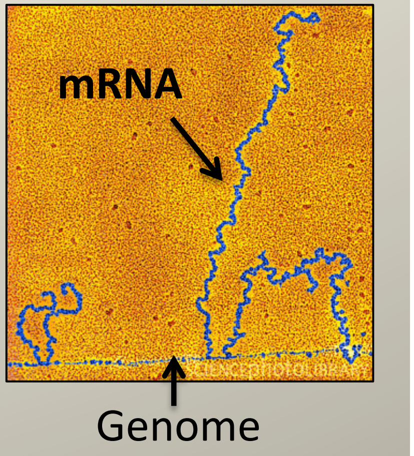
Transcriptomics: Direct sequencing of total expressed RNA

Isolate total expressed RNA

Convert to cDNA

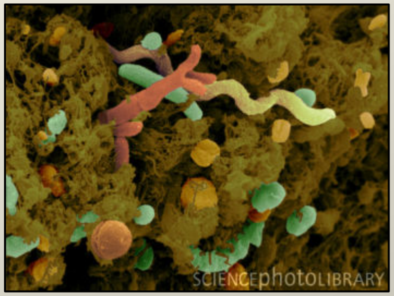
Sequence

Characterize gene expression in a given niche



Questions that can be asked using genomics technology:

Metagenomics –



How do fecal community profiles change with perturbation?

- Antibiotic exposure
- Pathogen colonization

Provides opportunity to discover new probiotics

- Non-pathogens that can displace *Salmonella* in fecal or environmental niche

Questions that can be asked using genomics technology:

Transcriptomics –

How do *Salmonella* respond to carcass processing interventions?

- ➢ Characterize the MDR Newport transcriptional profile in response to simulated carcass processing interventions

Expose *Salmonella* to simulated multiple hurdle carcass interventions

16% genes showed ↑ expression:

- ➢ Heat shock
- ➢ Acid stress
- ➢ DNA repair
- ➢ Virulence response

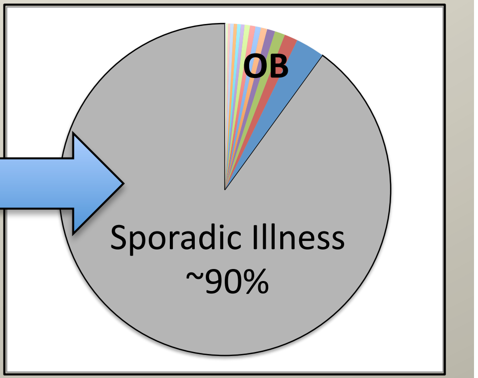
Transcriptional profiling of intervention survivors provides information on how to further target these *Salmonella*

Conclusions and Thoughts…

So how do we prevent *Salmonella* foodborne illness…?

✓ Better source tracking – more accurately identify sources of *Salmonella*

- SNP based



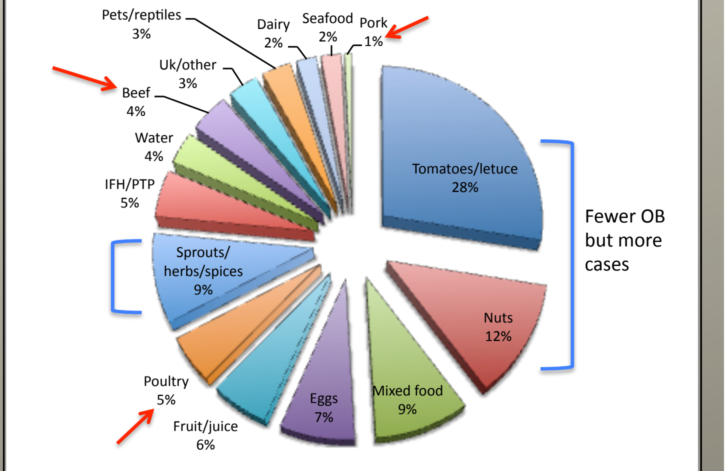
✓ Identify opportunities for interventions in Pre-harvest environments

- Metagenomics
- Probiotics (competition)

✓Increase our understanding of Intervention survivors – Transcriptomics

- Identify targets for elimination

Salmonellosis Cases by Source 1998 - 2011



Summary of 152 outbreaks representing 12,181 cases of illness – not all inclusive.

Acknowledgments

US MARC Scientists and Technicians

Terry Arthur
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Bruce Jasch
Greg Smith

Renee Godtel
Bob Lee

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International Center for Food Industry Excellence

Guy Loneragan
Sara Gragg
Mindy Brashears
Chance Brooks
Tyson Brown

National Cattlemen's Beef Association
Beef Industry Food Safety Consortium (BIFSCo)

Mandy Carr-Johnson
Bo Reagan

Partners in Industry – *Thank you!*