The Fight Against *Salmonella* in Agriculture – Research Perspective

(a view from the trenches)

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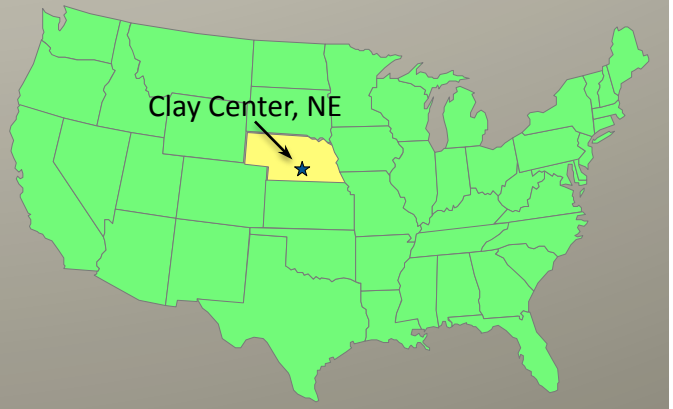
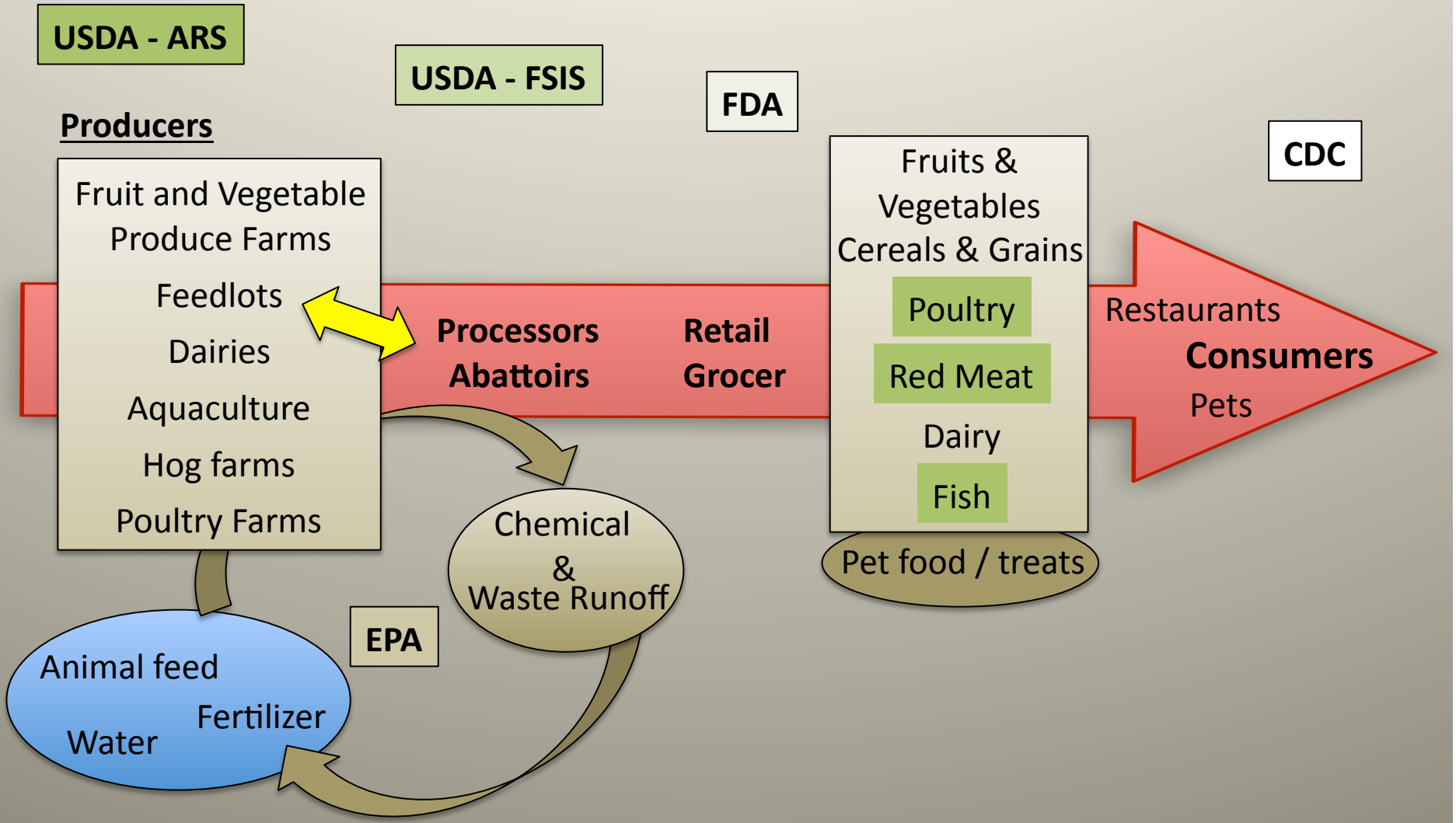


The Food Safety Continuum



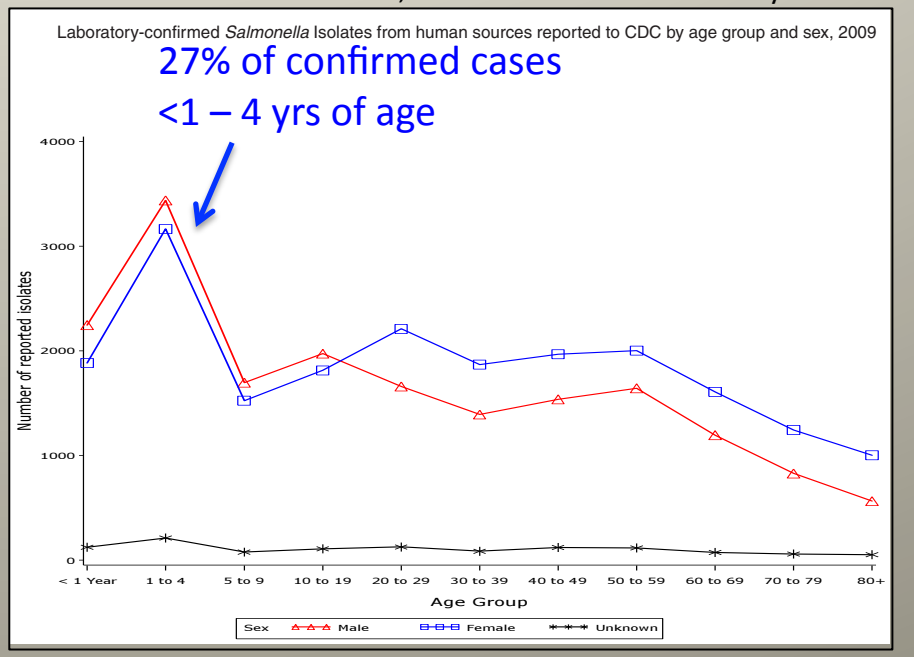
How do we **prevent** *Salmonella* foodborne illness…?

- ✓ 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



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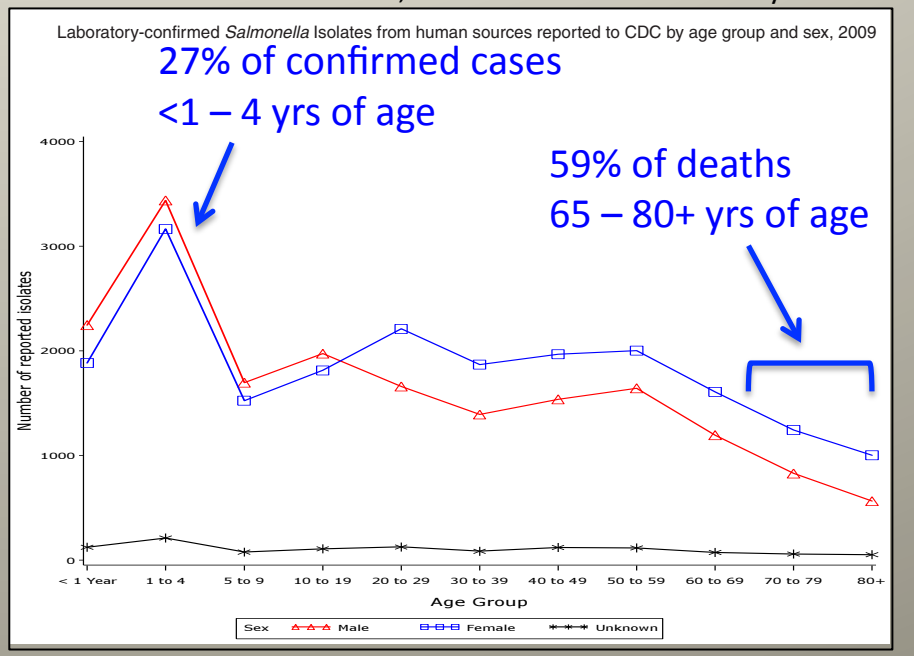
- Generally self limiting enteritis but can be invasive
  - Hospitalization ~15,000 per year
  - Death ~1% of confirmed cases/yr

- 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



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?

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

- Complex etiology

  ~10% cases attributed to outbreaks (OB)

  ~90% sporadic

Many potential sources of *Salmonella*!

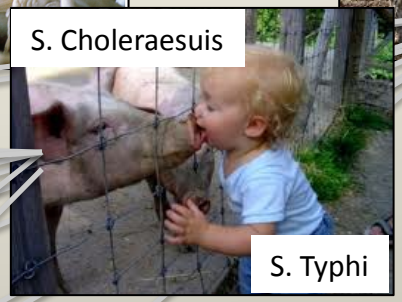
Reservoirs for *Salmonella* and Host Specificity

CDC Top Serotypes
Human salmonellosis

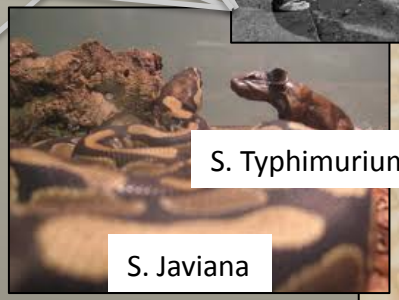
Enteriditis
Typhimurium
Newport
Javiana
Heidelberg
Montevideo
Saint Paul
Muenchen
Braenderup
Infantis
Thompson
Agona
Schwarzengrund
Bareilly
Hadar
Oranienburg

S. Gallinarum

S. Dublin


S. Choleraesuis

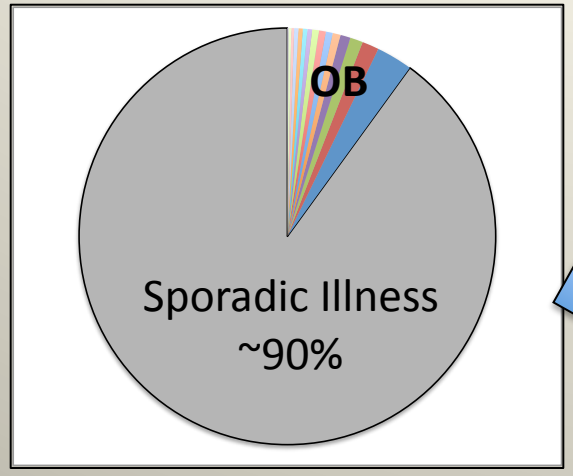
S. Typhi


S. Typhimurium

S. Javiana

Salmonella
Host specific
vs.
Host generalist

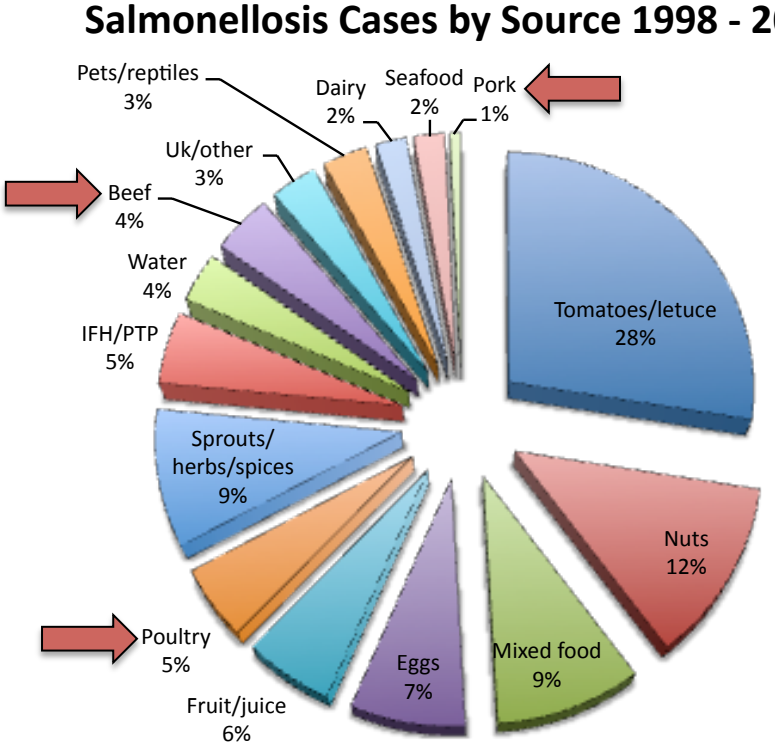
The Complexity of *Salmonella* Etiology



~40,000 confirmed cases / yr

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

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



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

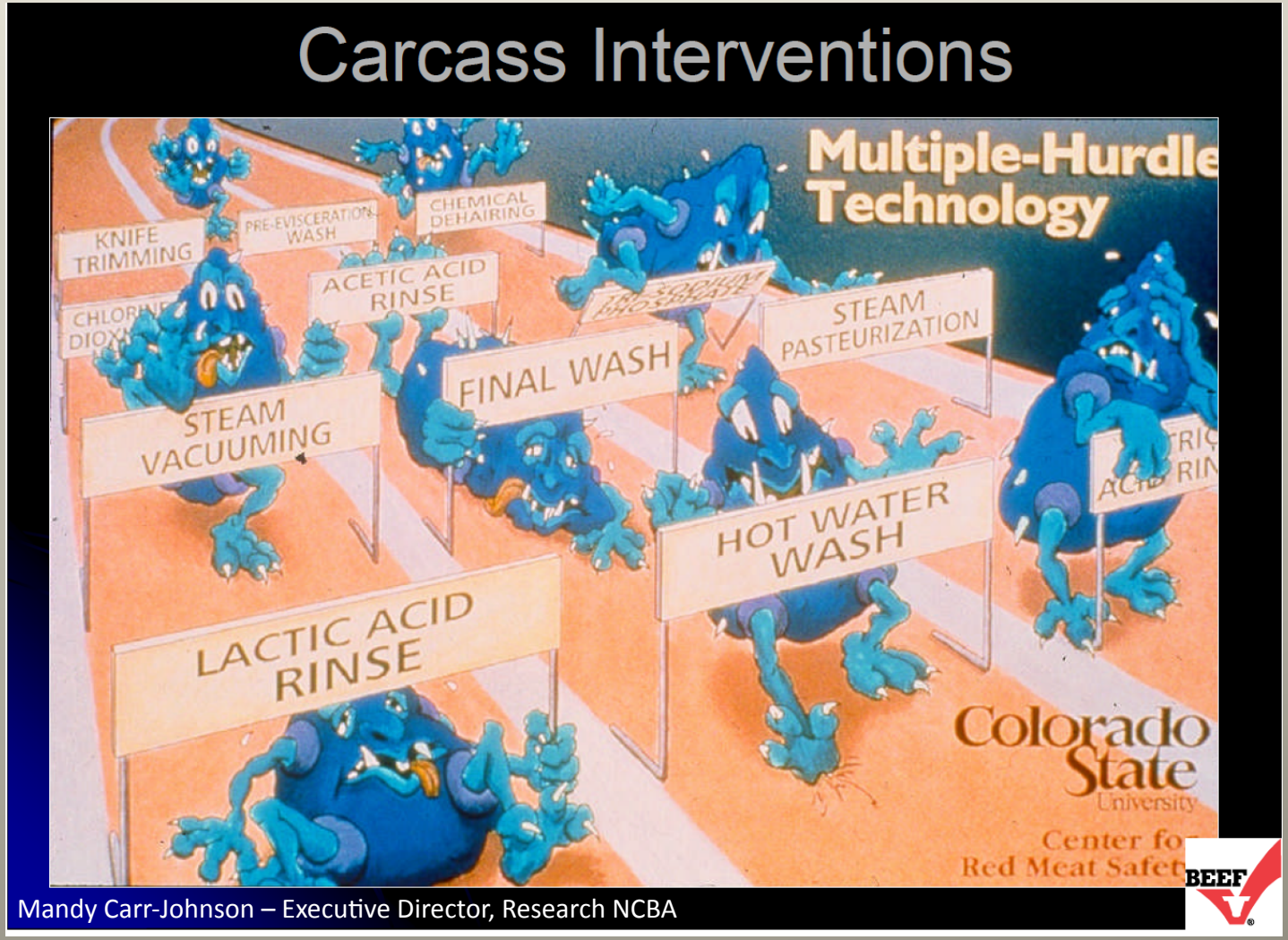
M. Ellin Doyle et. al., (2009) http://fri.wisc.edu/docs/pdf/FRI_Brief_Salmonella_Human_Illness_6_09.pdf

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



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

Beef Processing

~98% Reduction in *Salmonella* prevalence on carcasses

89.6%

Hide (*n*=3040)

50.2%

Pre-evisceration (*n*=3040)

0.8%

Post-intervention (*n*=3040)

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

BEEF

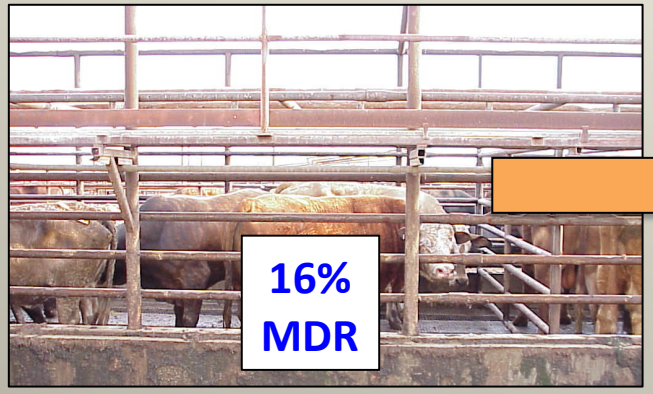
USDA ARS

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

Prevalence of Multidrug Resistant *Salmonella* at Harvest

Beef Processing

89.6%



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Pre-evisceration
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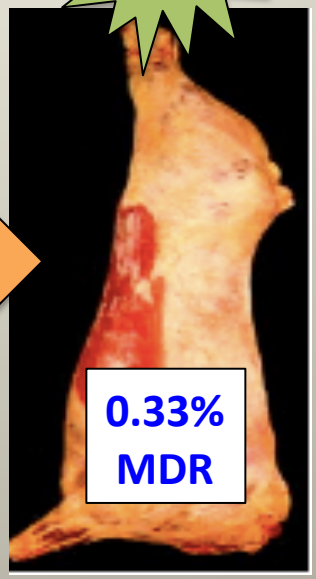


Post-intervention
(*n*=3040)

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

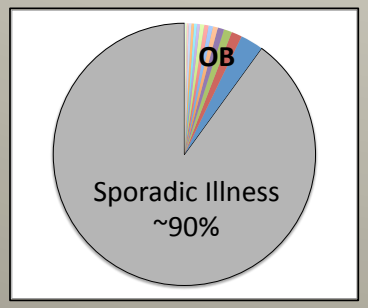
Newport MDR-AmpC	**MDR Typhimurium**
➢ 2002 47 ill	2003 58 ill
➢ 2007 43 ill	2009 14 ill
➢ 2009 42 ill	2011 19 ill

- 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*

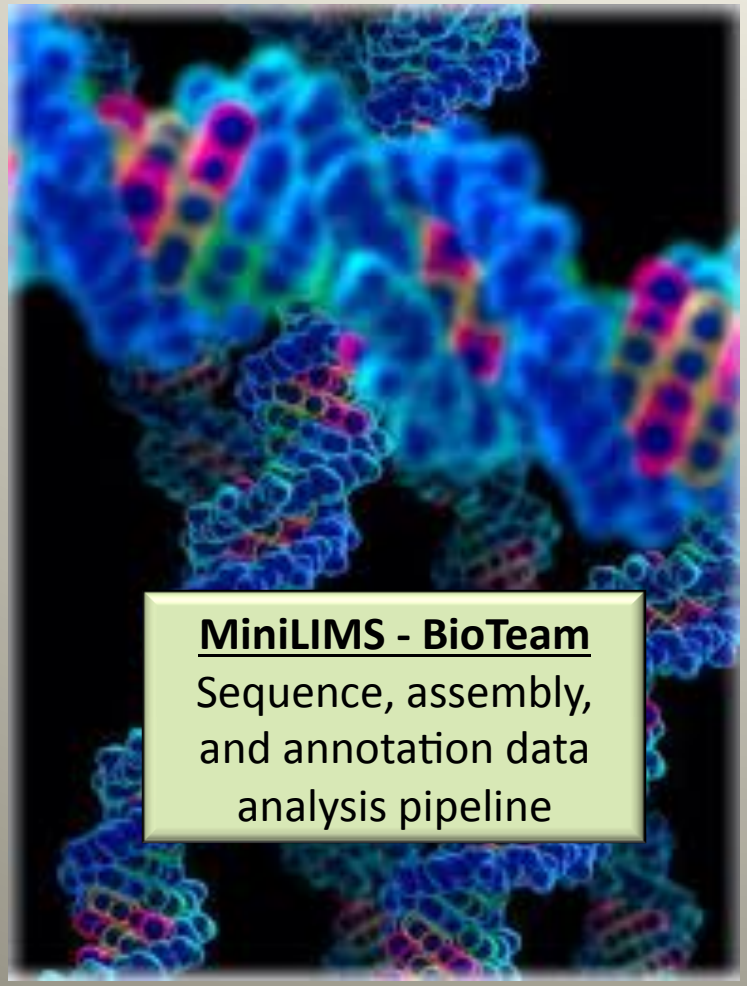
- Most commonly observed serotypes: **Montevideo (21%), Anatum (15%), Muenster (8%), Mbandaka(6%)**
- **MDR *Salmonella*** – 0.6%
  - MDR Typhimurium 0.09%
  - MDR Newport 0.07%



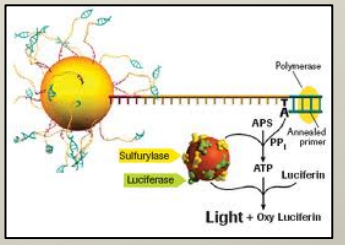
~0.4% of outbreak cases (1998-2011)

No Montevideo outbreaks linked to Ground Beef…

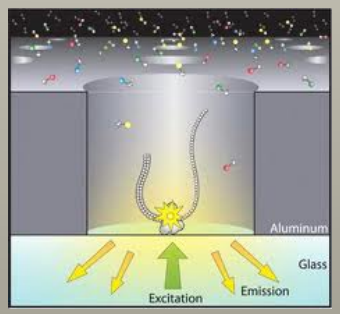
The Future Landscape of the Fight Against *Salmonella* in Agriculture…



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

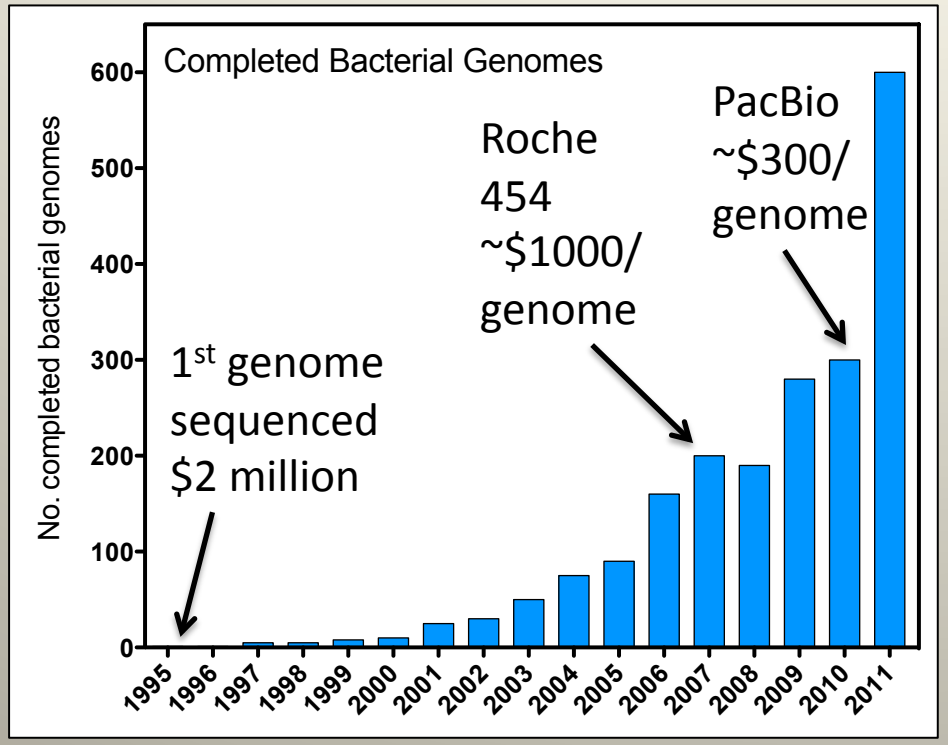


USMARC
acquired 2008



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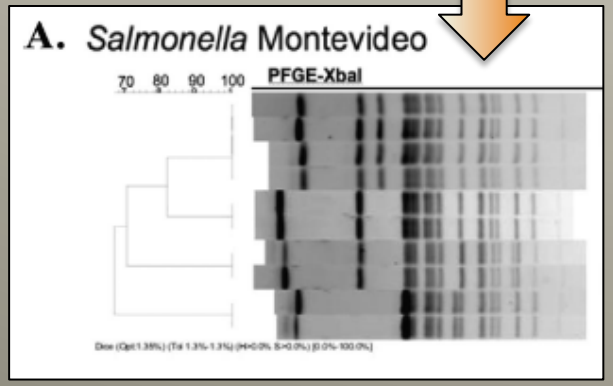
Outbreak occurred
July 2009 – April 2010
273 ill (44 states)
52 hospitalized
0 deaths

Causative agent: *S*. Montevideo

Highly clonal strains confound epidemiologic investigations



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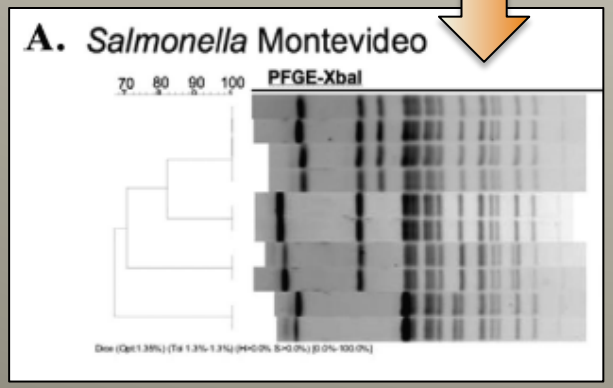

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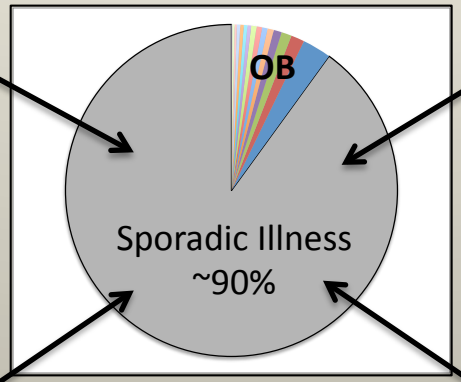
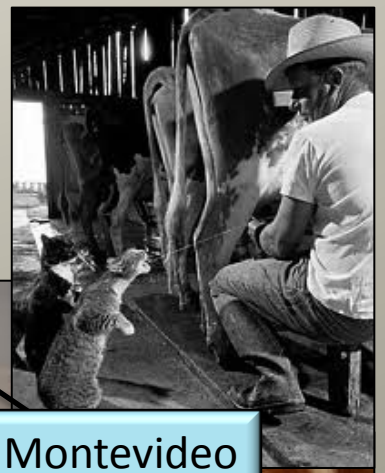
- Performed whole genome sequencing (WGS) of 40 Montevideo Isolates with PFGE patterns matching the OB strain (clinical, environmental, laboratory and food isolates)

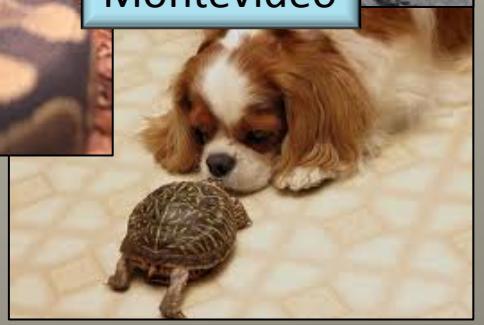
- Found a core set of SNPs that defined the outbreak strains – they were even able to determine that one of the "outbreak" cases in California was unrelated

Pathogen Source Tracking

Salmonellosis

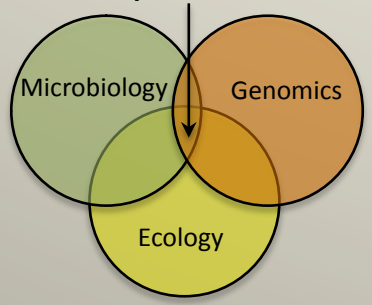


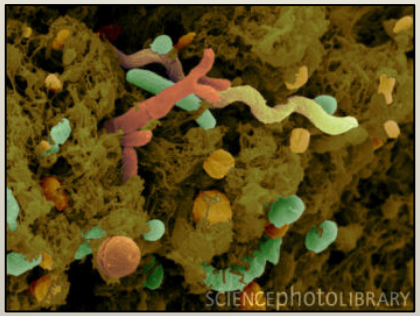




Other Future Trends: Metagenomics & Transcriptomics

Metagenomics: the study of complex mixtures/communities





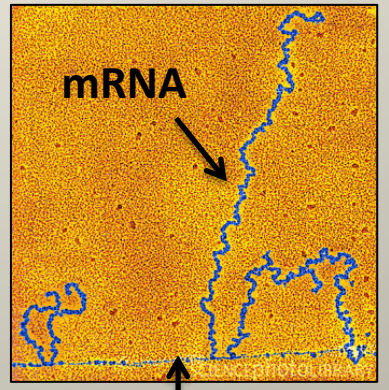
Scanning electron micrograph (SEM) of bacteria in cattle feces – magnification ~10,000x

Isolate total genomic DNA

Sequence

Characterize the "players" in a given microbial niche

Transcriptomics: Direct sequencing of total expressed RNA



Isolate total expressed RNA

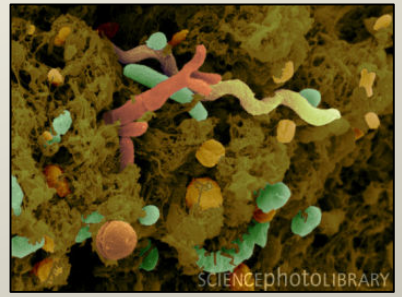
Convert to cDNA

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

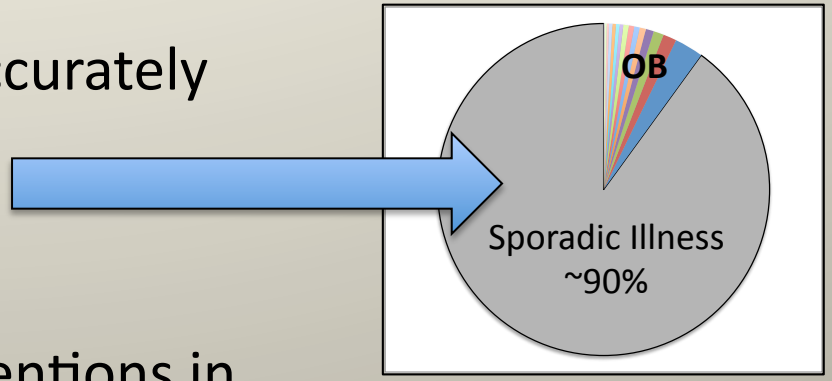
Transcriptional profiling of intervention survivors provides information on how to further target these *Salmonella*

Conclusions and Thoughts…

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

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

- SNP based

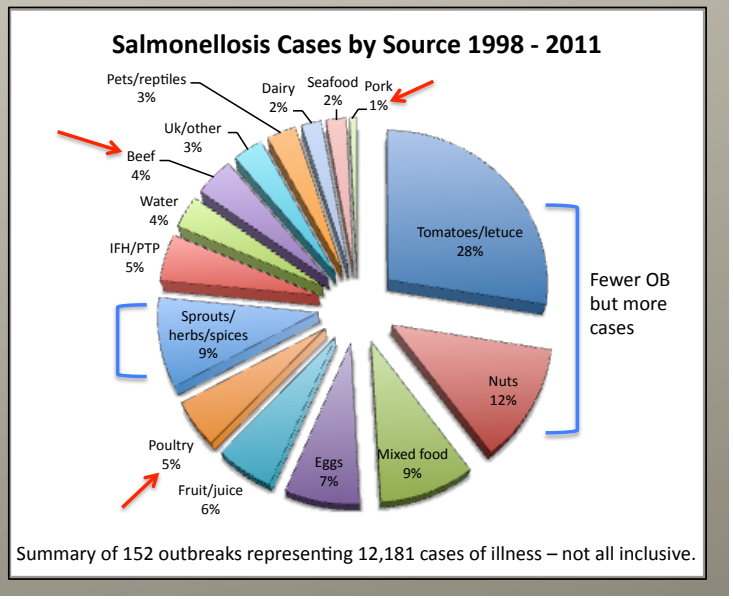


✓ Identify opportunities for interventions in Pre-harvest environments

- Metagenomics
- Probiotics (competition)

✓Increase our understanding of Intervention survivors – Transcriptomics

- Identify targets for elimination



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Partners in Industry – *Thank you!*