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## Leveraging Data to Mitigate Food Safety Risk in Live Production and Processing

Maurice Pitesky, DVM, MPVM, Dipl. ACVPM  
Assistant Specialist in Cooperative Extension, University of California  
Poultry Health and Food Safety Epidemiology  
Poultry Institute: 2014 Program, Puyallup, WA  
November 4<sup>th</sup> 2014

**Questions?**

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**Connecting the dots...**

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Decision Trees

Correlational Analyses

Logistic Regression

GIS mapping\*

Odds Ratios

**Using a Conditional Decision Tree to Understand Risk in the Processing Plant**

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**Risk factors at the processing level identified**

**Correlational Analysis**

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Decision Trees

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**Review of Backwards and Forwards Logistic Regression**

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Develop a model of multiple independent variables to predict Salmonella positivity and negativity

**Backwards**

**Forwards**

Decision Trees

Correlational Analyses

**Logistic Regression**

GIS mapping

Odds Ratios

### Using Logistic Regression to Identify Risky Farms

RANCH GROUP	Sig.	Odds Ratio	95% C.I. for Odds Ratio	
			Lower	Upper
Farm 1	.001	42.5	4.579	394.435
Farm 2	.001	42.5	4.579	394.435
Farm 3	.003	6.5	1.653	26.350
Farm 4	.560	.83	.129	3.035
Farm 5	.864	3.8	.924	15.228
Farm 6	1.000	1.0	2.284	4.468
Farm 7	.006	9.2	1.871	44.622
Farm 8	1.000	1.0	.262	3.815
Farm 9	.920	1.1	.279	4.115
Farm 10	.637	.68	.139	3.341
Farm 11	.148	2.9	.688	12.361
Farm 12	.995	1.04	.268	4.262
Farm 13	.244	2.2	.580	8.511
Farm 14	.169	2.04	.651	1.683
Farm 15	.099	3.5	.795	15.495
Farm 16	1.000	1.0	.007	1.402
Farm 17	.998	.000	0.000	
Farm 18	.448	.5	.084	2.992
Farm 19	.005	25.0	2.601	240.338
Farm 20	.995	.000	0.000	
Farm 21	.938	.94	.154	4.785
Farm 22	.101	3.8	.772	18.209
Farm 23	.784	1.3	.233	6.898
Farm 24	.550	1.8	.381	6.759
Farm 25	.402	2.0	.398	10.108
Farm 26	.999	.000	0.000	
Farm 27	.999	.000	0.000	
Farm 28	1.000	1.0	.151	6.643
Farm 29	.550	1.8	.381	6.759
Farm 30	.999	.000	0.000	

Decision Trees  
Correlational Analyses  
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### Overall Predictive Ability of Logistic Regression Models

#### Backwards Logistics Model

Observed	Salmonella	Predicted		Percentage Correct
		NEG	POS	
Step 1 Salmonella NEG		333	36	90.2
POS		52	134	72.0
Overall Percentage				84.1

#### Forwards Logistic Model

Observed	Salmonella	Predicted		Percentage Correct
		NEG	POS	
Step 1 Salmonella NEG		326	43	88.3
POS		69	117	62.9
Overall Percentage				79.8

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### Odds Ratios

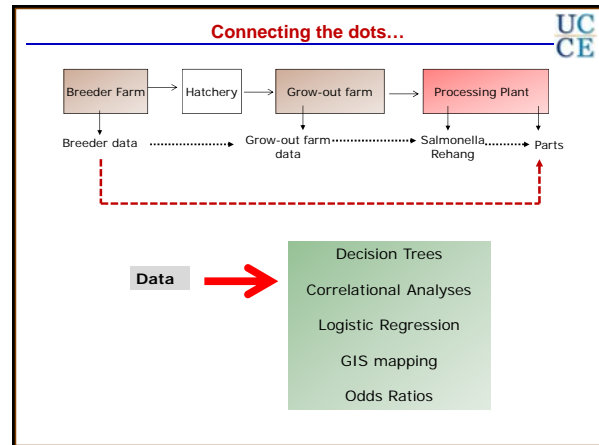
Variables in the Equation		B	S.E.	Wald	df	Sig.	Exp(B)	95% C.I. for EXP(B)	
								Lower	Upper
Step 1*	Farm(Selection)	2.550	.372	47.092	1	.000	12.812	6.184	26.543
	Constant	-.229	.188	1.517	1	.218	1.237		

a. Variable(s) entered on step 1: Farm(Selection).

Variables in the Equation		B	S.E.	Wald	df	Sig.	Exp(B)	95% C.I. for EXP(B)	
								Lower	Upper
Step 1*	Farm(Selection)	1.871	.261	55.477	1	.000	6.493	3.960	10.622
	Constant	-1.778	.128	191.803	1	.000	.169		

a. Variable(s) entered on step 1: Farm(Selection).

Decision Trees  
Correlational Analyses  
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### Next Generation Sequencing of Salmonella Heidelberg Under Different Processing Conditions

aka...  
Using the Genome of SH to Understand the 'Transcriptome' of SH to Make the Consumption of Poultry Products Safer?

Maurice Pitesky, DVM, MPVM, Dipl. ACVPM,  
Rodrigo Gallardo DMV, PhD, Dipl. ACVPV, and Huaijun Zhou PhD,  
University of California Davis  
Poultry Institute: 2014 Program, Puyallup, WA  
November 4<sup>th</sup> 2014

### Current Control Principles for Salmonella

- Biosecurity
- Cleaning & Disinfection
- On-farm surveillance
- Vaccination
- Competitive exclusion
- Pre and pro-biotics
- Feed and water hygiene
- HACCP
- Processing plant dips and sprays
- FSIS surveillance (performance standard)

How do we evaluate the efficacy of these approaches?

Is it that simple?

No, we need to think about the remaining Salmonella

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### What happens to *Salmonella* when you challenge them and don't kill them?

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Appl Environ Microbiol. 2013 Dec;79(23):7281-9. doi: 10.1128/AEM.12311-13. Epub 2013 Sep 20

**Preadaptation to cold stress in *Salmonella enterica* serovar Typhimurium increases survival during subsequent acid stress exposure.**

Shah, P. Cesar PT, Chen D, Stevens JB, Weiner BC

Cold and acid stress (as opposed to killing) 'rescues' virulent *Salmonella* from subsequent cold and acid challenge

Choice of Bacterial Growth Medium Alters the Transcriptome and Phenotype of *Salmonella enterica* Serovar Typhimurium

Jessica M. A. Blair, Grace E. Richmond, Andrew M. Bailey, Alireza, Laura J. V. Piddock

Published: May 21, 2013 - DOI: 10.1371/journal.pone.0063912

Differences in gene expression including transcripts associated with flagella and PI when growing *Salmonella* on different media (Minimal media vs LB)

To answer the question: they get creative and stubborn (i.e. they express different genes that allow them to survive and make it more difficult to kill them the next time you challenge them).

### New Tools to Mitigate Virulence in Food Systems?

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Condition 1: Genome, Transcriptome, Prot

Condition 2: Genome, Transcriptome, Prot

Same organism BUT different 'transcriptome' and 'proteome'. Therefore different virulence?

Can we use Next Gen techniques like RNA-seq to better understand the 'transcriptome' of pathogenic bacteria in a food system?

Can we alter the 'transcriptome' to make virulent bacteria less virulent in a food system?

### Candidate virulence genes for *Salmonella*

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*Salmonella* have several 'pathogenicity islands' (SPIs), 2 of which encode a type III secretion system for virulence proteins

SP1 required for invasion

SP2 required for intracellular accumulation

A large number of virulence genes are required for the successful pathogenesis of *Salmonella* infections (Forshell, 2006)

### Experimental Design

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Breeder Farm → Hatchery → Grow-out farm → Processing Plant

Processing in More Detail...  
Slaughter --- Evisceration --- Second Process

Chiller

#### Simulating the Chiller at benchtop

Control: SH      SH + PAA      SH + Ach      SH + Secure

Keep at 4 C for 90 minutes to 'simulate' chiller conditions

### Next Gen Sequencing Protocol

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1. RNA 'freeze' and extraction
2. DNase Treatment
3. rRNA depletion
4. RNA library preparation
  1. RNA fragmentation
  2. Synthesize ds cDNA
  3. Adapter ligation
  4. PCR enrichment
5. Bioanalyzer to determine fragment length
6. Clone and sequence to confirm presence of SH genome
7. Pool and sequence
8. Library analysis

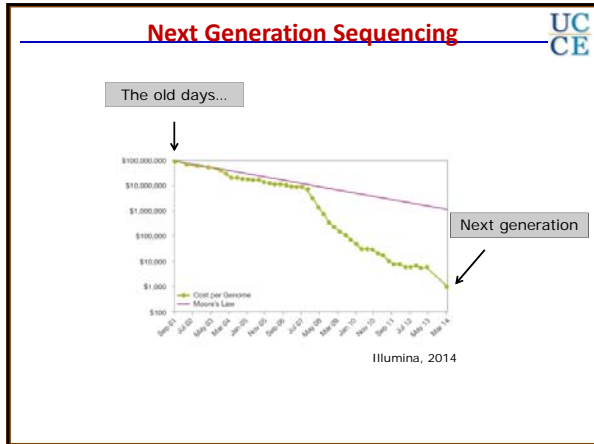
### Data Analysis

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Appl Environ Microbiol. 2014 Feb;80(2):896-906. doi: 10.1128/AEM.12314-13. Epub 2013 Nov 22

**RNA sequencing reveals differences between the global transcriptomes of *Salmonella enterica* serovar enteritidis strains with high and low pathogenicities.**

Genes that are down regulated in 'low pathogenic strains of SE'



### Acknowledgements

Bruce Charlton, CAHFS  
Tereza Chylkova, UC Davis  
Aura Ferreira, UC Davis  
Gabriel Senties-Cue, CAHFS  
Ying Wang, UC Davis

Funding: NIFA-CFAH

