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Salmonella in Poultry

Sources, Colonization & Mitigation

Harshavardhan Thippareddi, Ph.D.
Interim Associate Dean for Research
John Bekkers Professor in Poultry Science

Poultry & *Salmonella*

- Poultry meat has consistently been linked to salmonellosis, with over 23% of foodborne illnesses attributable to poultry consumption
- Of those attributed to poultry, 17% were from chicken (broilers and parts) and 6% from turkey
- Poultry industry has made significant progress by reducing the incidence in poultry meat through control at processing, using antimicrobial interventions
- An understanding of the sources and potential control at production followed by incorporation of control strategies at production may be necessary to achieve further reductions

Salmonella – Sources in Poultry Production

A systematic review and meta-analysis of the sources of *Salmonella* in poultry production (pre-harvest) and their relative contributions to the microbial risk of poultry meat

J. Wang,[*] S. Vaddu,[*] S. Bhumanapalli,[*] A. Mishra,[†] T. Applegate,[*] M. Singh,[†] and H. Thippareddi[*,1]

[*]*Department of Poultry Science, University of Georgia, Athens, GA 30602, USA; and* [†]*Department of Food Science and Technology, University of Georgia, Athens, GA 30602, USA*

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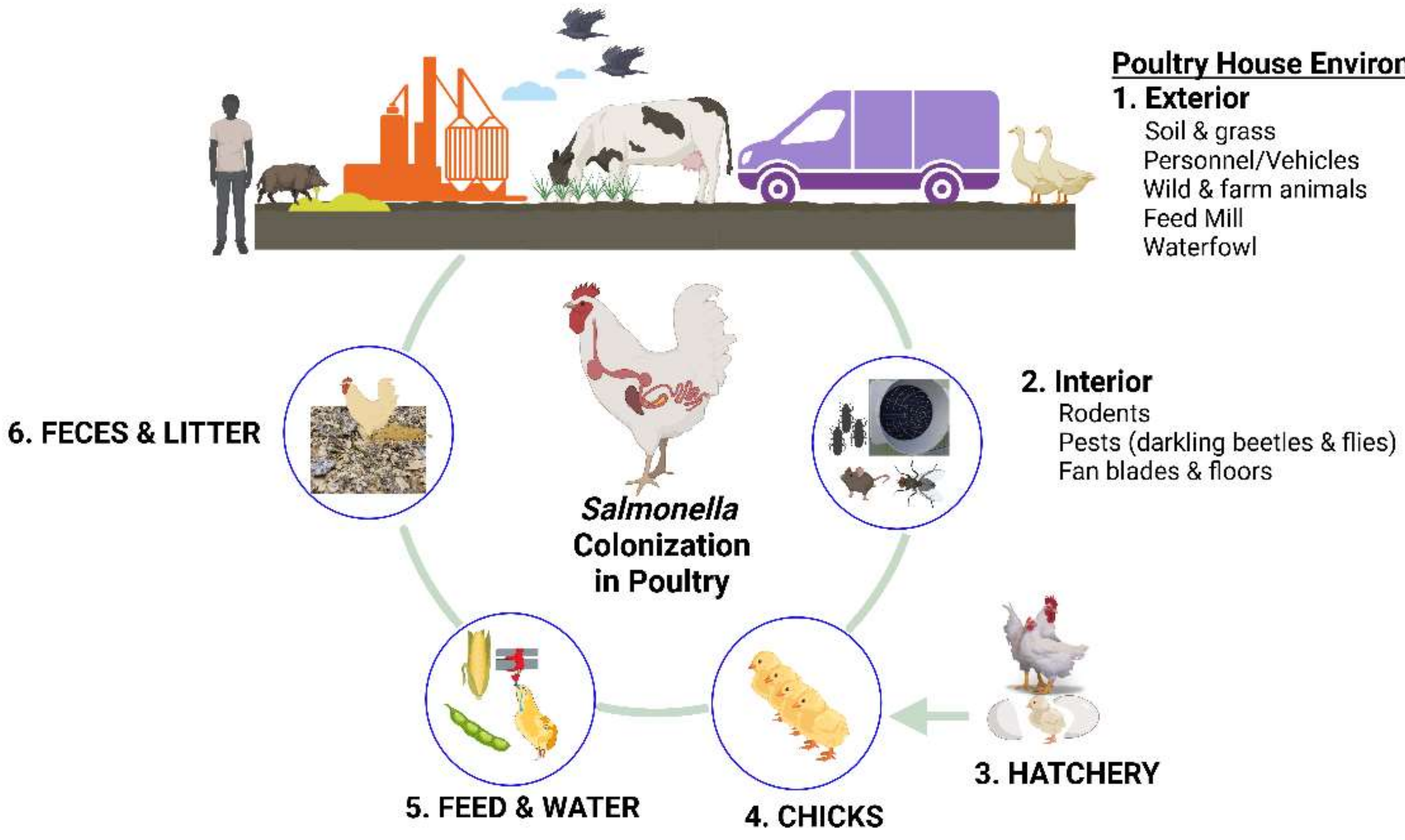
Salmonella – Sources in Poultry Production



Salmonella Sources – Exterior Environment

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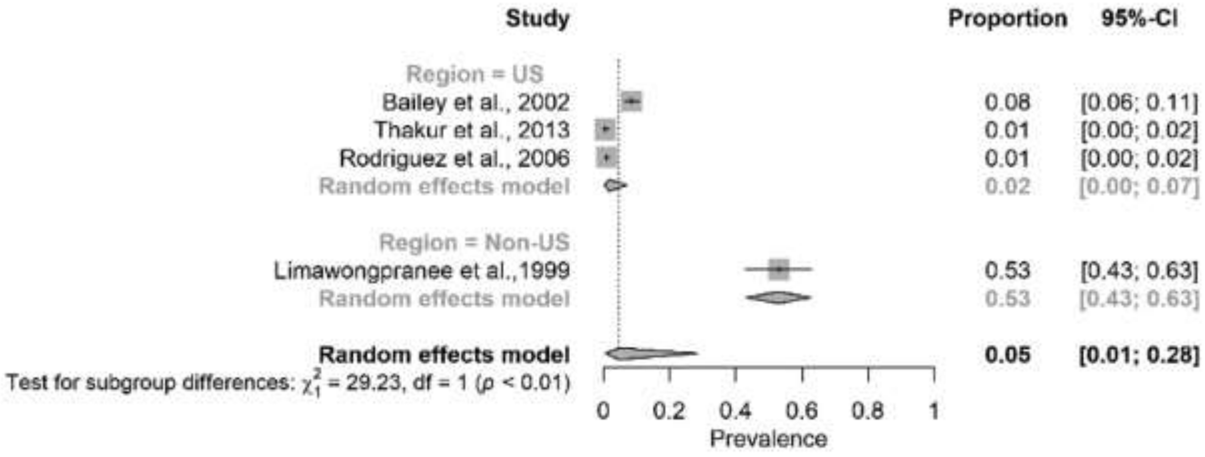
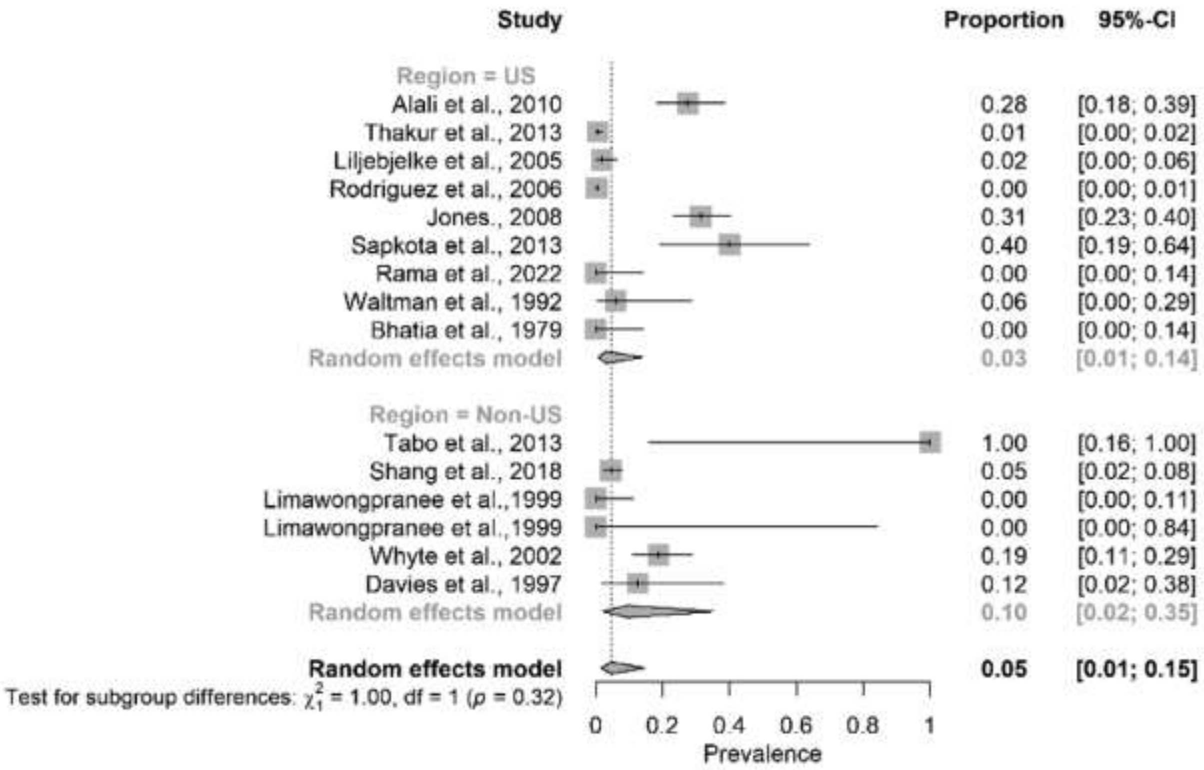
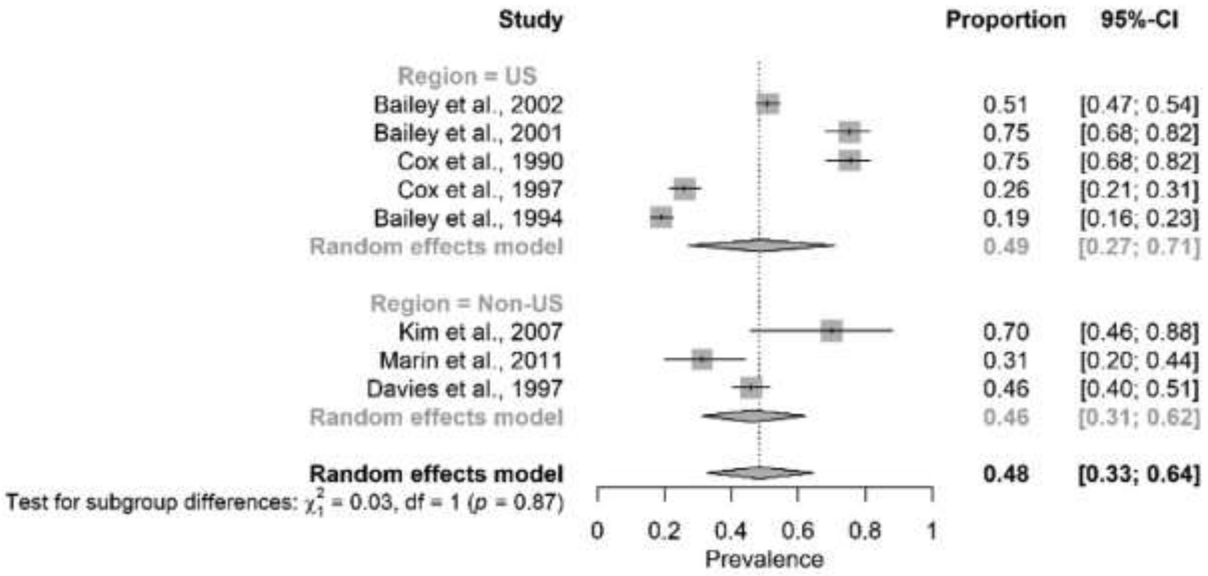
Salmonella Sources – Exterior Environment



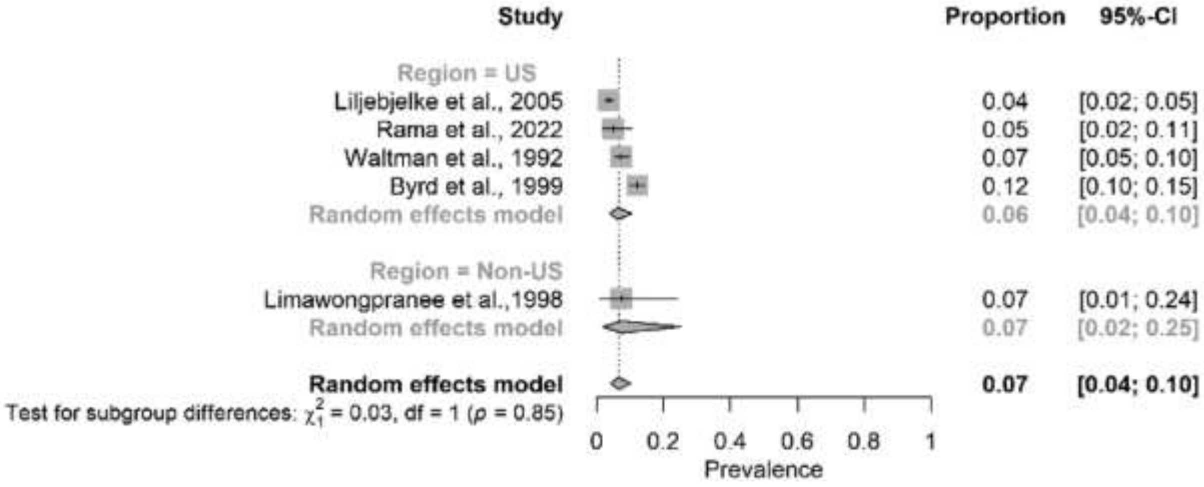
Salmonella Sources – Feed



Salmonella Sources – Hatchery



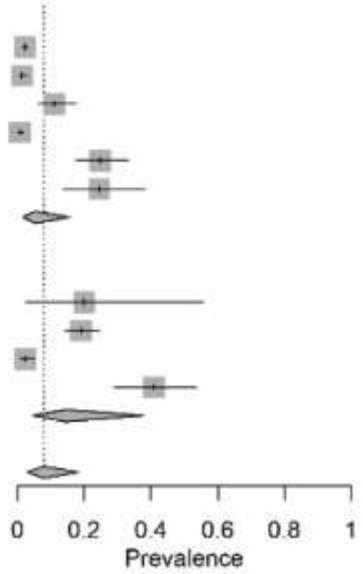
Salmonella Sources – Chicks



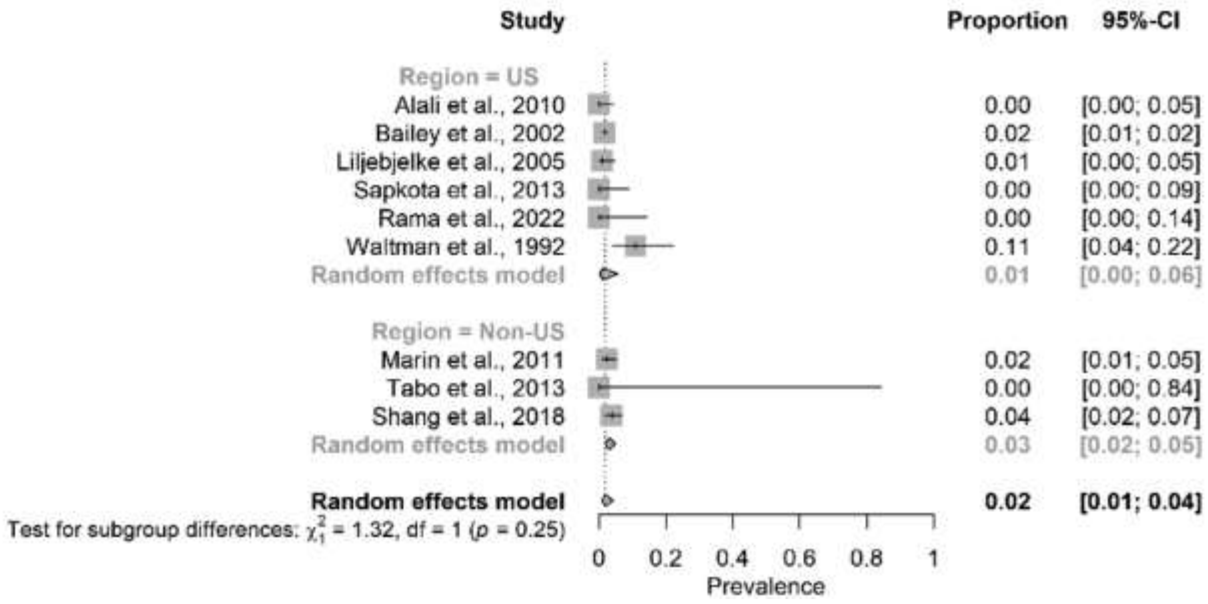
Salmonella Sources – Environment, Interior

Study	Proportion	95%-CI
Region = US		
Bailey et al., 2002	0.02	[0.02; 0.03]
Thakur et al., 2013	0.01	[0.01; 0.03]
Liljebjelke et al., 2005	0.11	[0.06; 0.18]
Rodriguez et al., 2006	0.01	[0.00; 0.02]
Waltman et al., 1992	0.25	[0.18; 0.33]
Bhatia et al., 1979	0.25	[0.14; 0.38]
Random effects model	0.05	[0.02; 0.16]
Region = Non-US		
Kim et al., 2007	0.20	[0.03; 0.56]
Marin et al., 2011	0.19	[0.14; 0.25]
Skov wt al., 2000	0.02	[0.01; 0.05]
Limawongpranee et al.,1999	0.41	[0.29; 0.54]
Random effects model	0.15	[0.05; 0.38]
Random effects model	**0.08**	**[0.03; 0.18]**

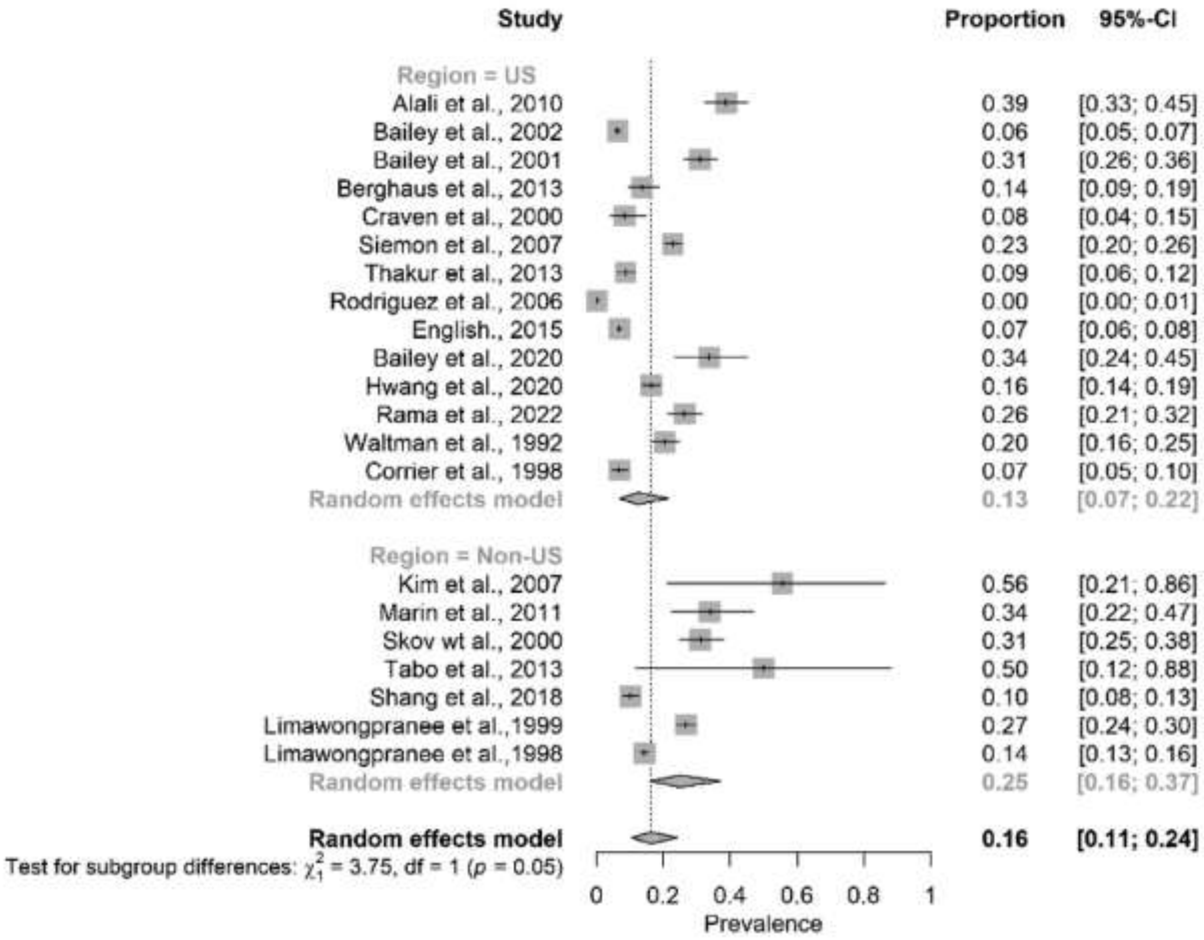
Test for subgroup differences: $\chi^2_1 = 1.56$, df = 1 ($p = 0.21$)



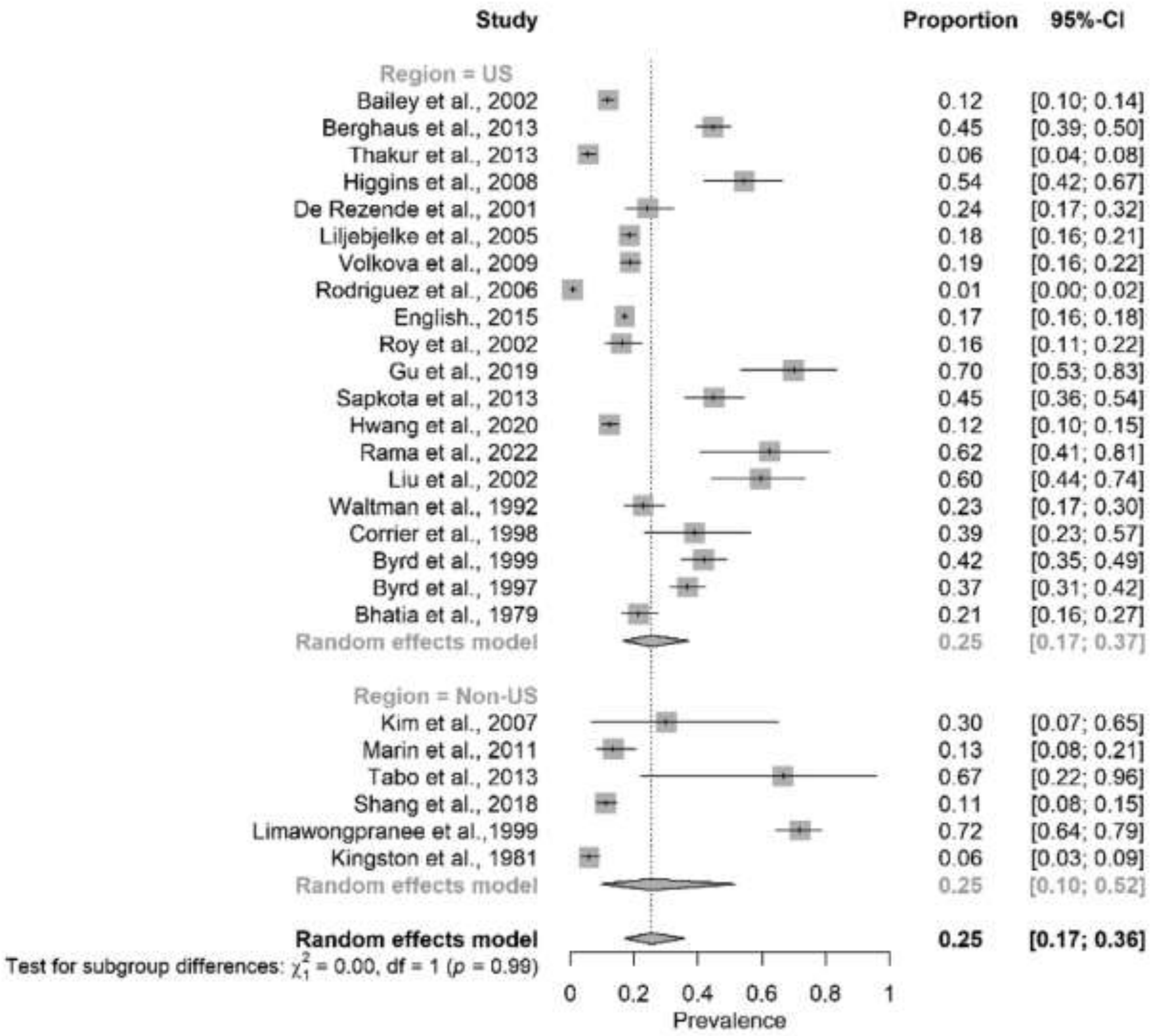
Salmonella Sources – Water

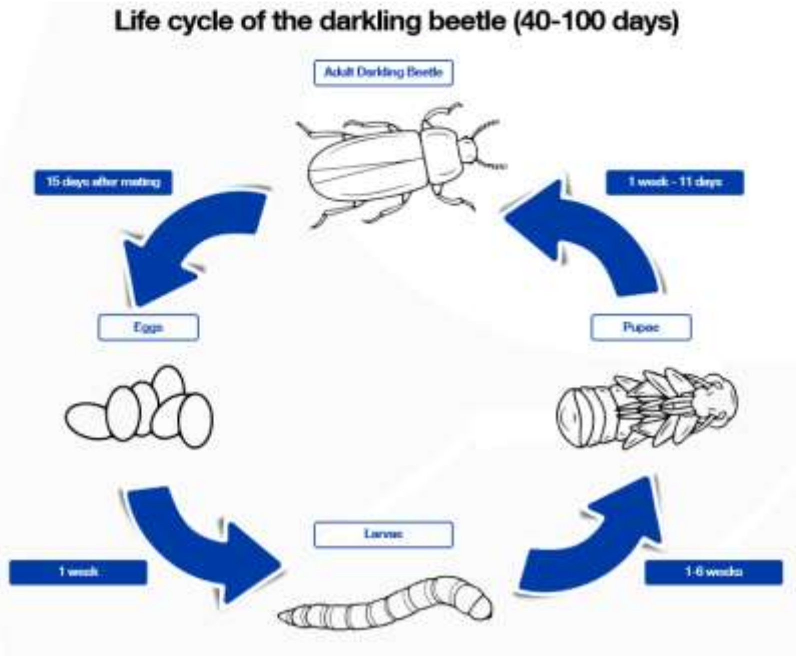


Salmonella Sources – Excreta

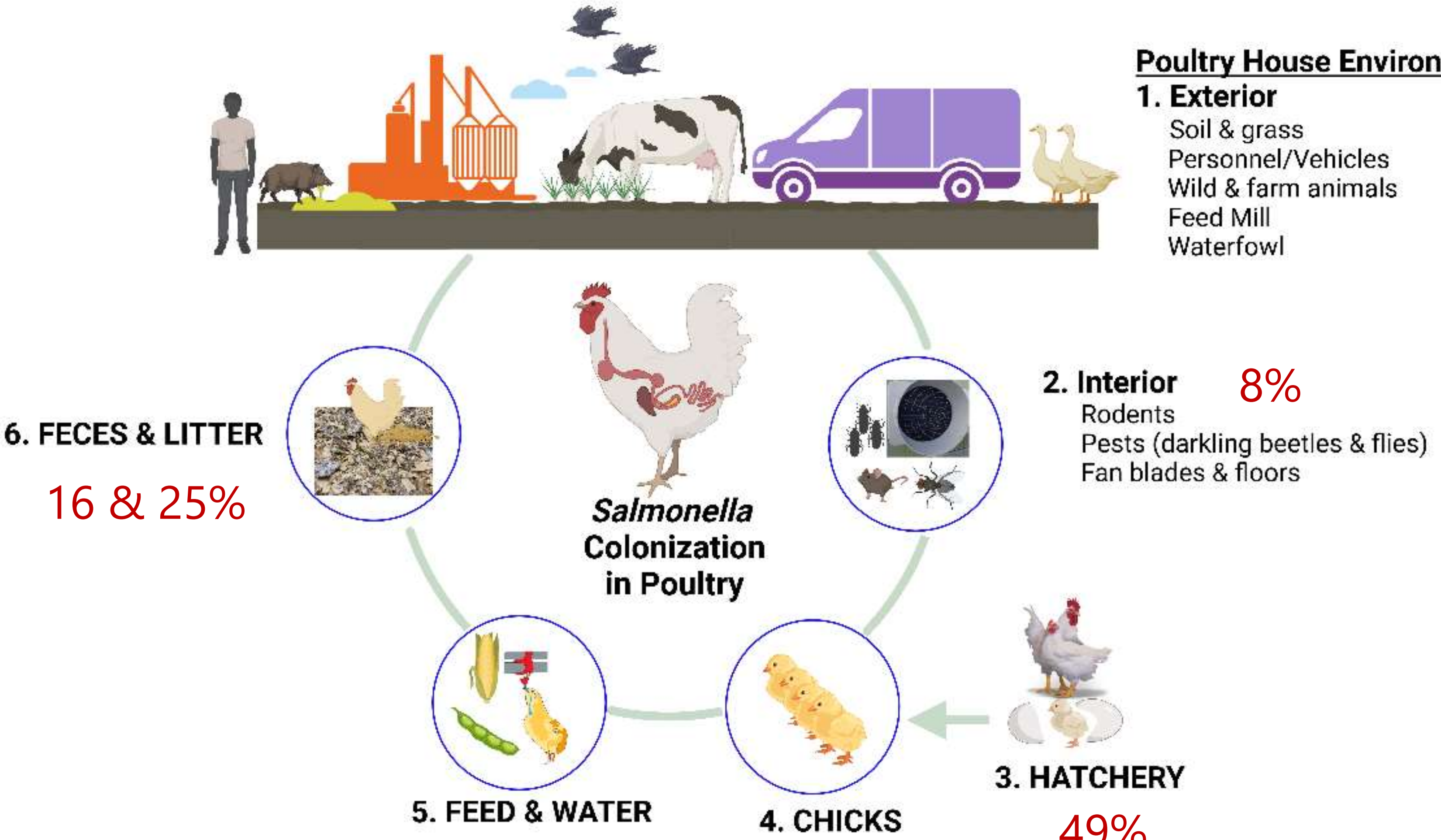


Salmonella Sources – Litter





Salmonella Sources - Summary



5%

8%

49%

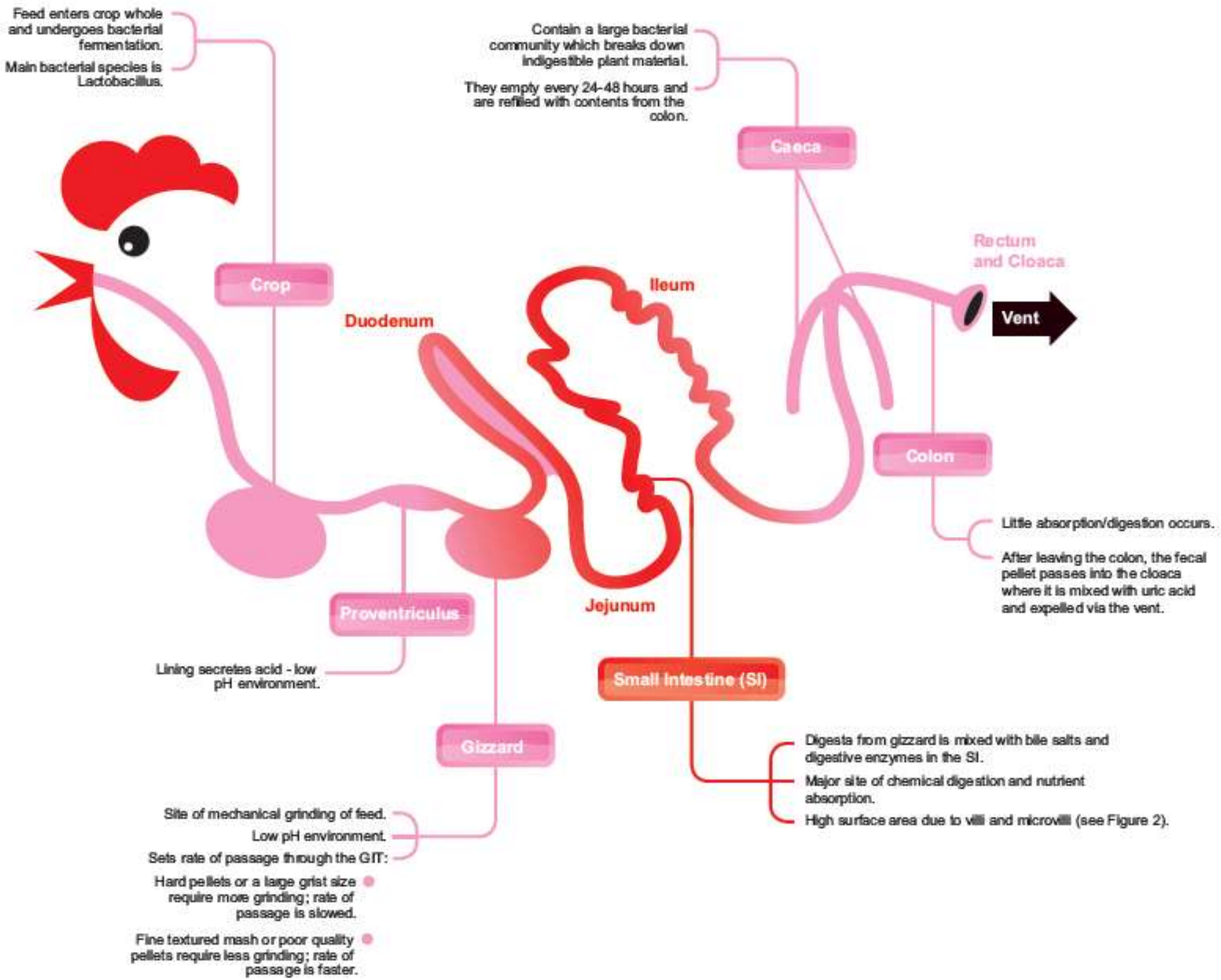
5%

5%

16 & 25%

Poultry Feeding Habits -

Poultry Gut & Importance



Salmonella Colonization in Broilers

Salmonella Species and _Campylobacter jejuni_ Cecal Colonization Model in Broilers

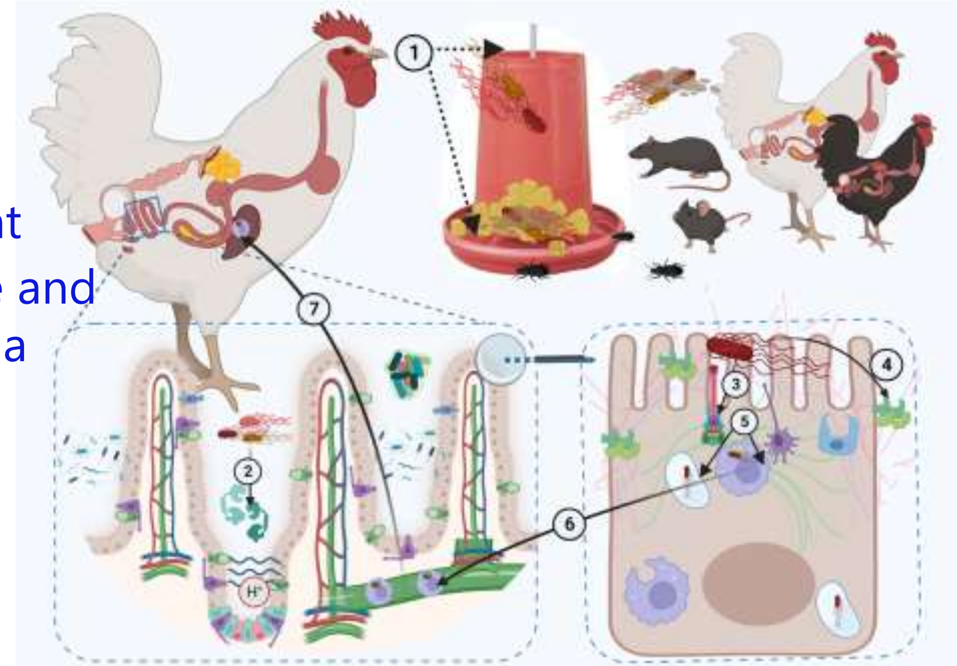
N. J. Stern[1]

Poultry Microbiological Safety Research Unit, Russell Research Center Agricultural Research Service, USDA, Athens, GA 30604

2008 Poultry Science 87:2399–2403
doi:10.3382/ps.2008-00140

Salmonella Transmission and Infection

Salmonella Colonization of the Poultry Gut



1. Infection through fecal-oral route
2. Production of acid shock proteins against acidic environment
3. Attachment to enterocyte facilitated by flagella and fimbriae and expression of injectisome, a type III secretion system (T3SS), a protein complex for uptake and invasion
4. Secretion of effector proteins to interact with extended cytoskeleton for facilitation of engulfment
5. Internalization of *Salmonella* by vacuole and macrophage
6. Transportation of *Salmonella* to mesenteric lymph nodes
7. Septicemia and translocation in various organs

Salmonella Colonization in Broilers

Table 1. Colonization of chicks challenged at 2 d posthatch with a composite of 3 serotypes of *Salmonella* spp.[1]

Challenge level	d 4	wk 1	wk 2	wk 3	wk 4
Trial 1					
Negative control	ND[2]	ND	ND	ND	ND
10^5 cfu	5.46 ± 1.42	4.64 ± 0.91	2.80 ± 0.79	0.61 ± 0.91	ND
10^6 cfu	6.03 ± 1.06	6.02 ± 0.20	4.95 ± 1.24	3.01 ± 1.88	0.50 ± 0.70
10^7 cfu	5.34 ± 0.69	5.50 ± 0.71	1.79 ± 0.98	1.09 ± 1.00	ND
10^8 cfu	5.56 ± 1.41	5.14 ± 0.27	3.03 ± 1.39	1.47 ± 1.36	1.47 ± 1.68
Trial 2					
Negative control	ND	ND	ND	ND	ND
10^4 cfu	4.92 ± 1.21	4.97 ± 0.78	4.80 ± 1.67	5.34 ± 0.68	2.09 ± 1.93
10^5 cfu	6.68 ± 0.58	6.62 ± 0.40	5.89 ± 0.26	4.97 ± 0.51	2.47 ± 1.42
10^6 cfu	5.35 ± 0.84	5.85 ± 1.14	4.35 ± 1.57	3.56 ± 0.54	1.90 ± 2.18
10^7 cfu	6.02 ± 0.36	5.34 ± 1.02	4.46 ± 1.35	5.02 ± 1.15	2.17 ± 1.92

[1]Birds were grown for 4 wk. Colonization quotient expressed as $\log_{10}$ cfu g^{-1} of cecal materials of 8 individuals.

[2]ND = not detected.

2008 Poultry Science 87:2399–2403
doi:10.3382/ps.2008-00140

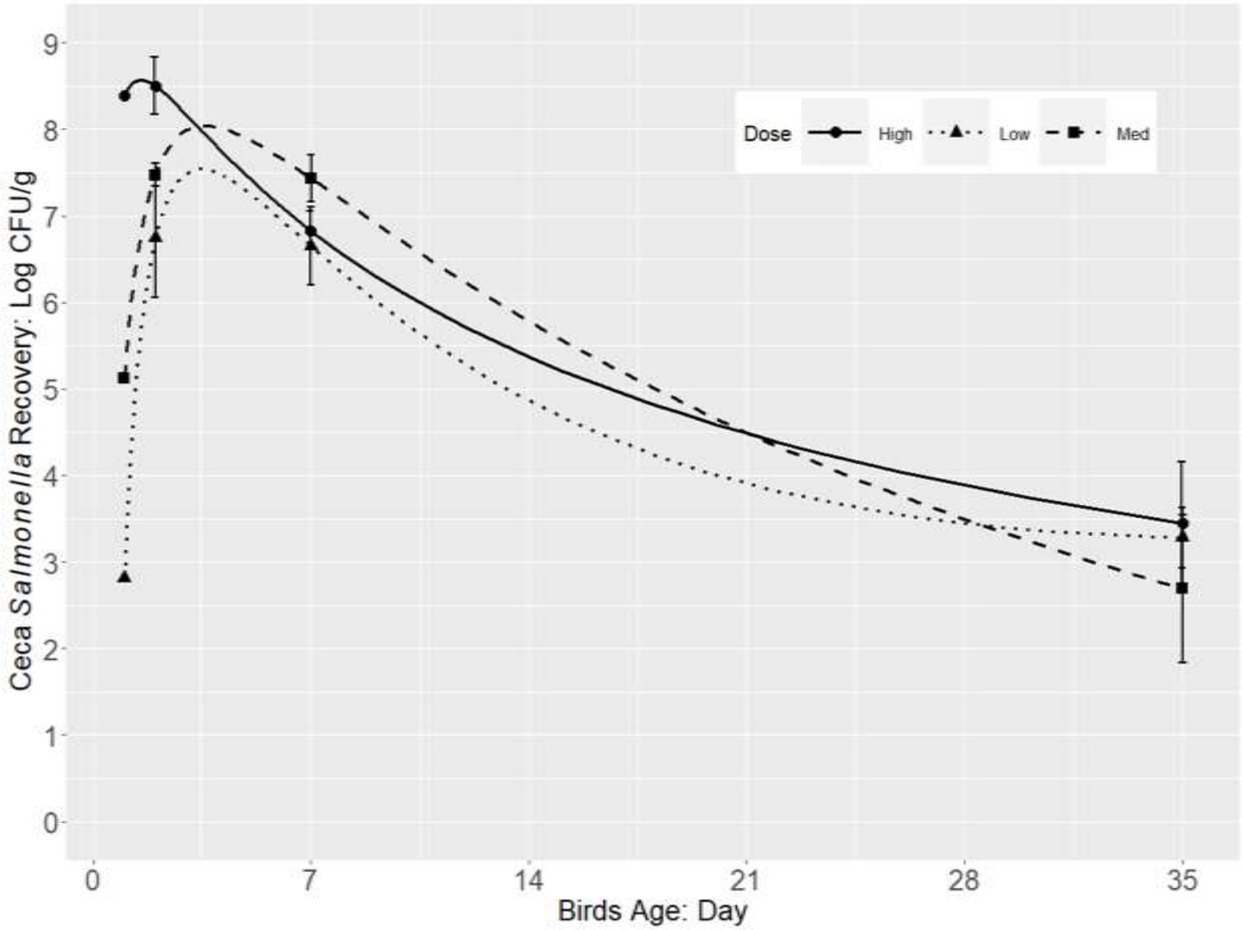
Salmonella Colonization Dynamics & Translocation

Translocation of *Salmonella* from the Gastro-intestinal Tract to Internal Organs in Broilers

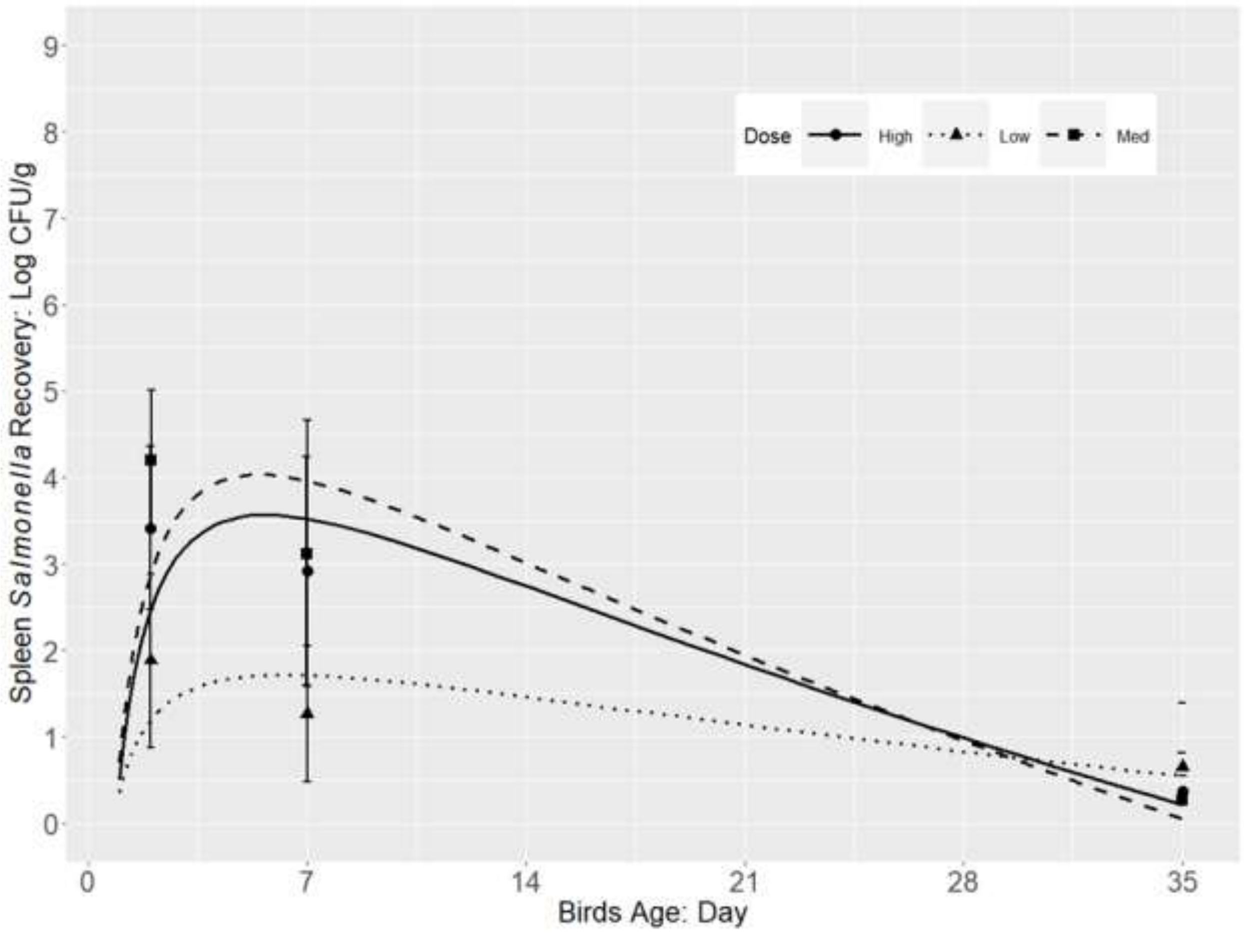
Jinquan Wang[1], Davis Fenster[1], Sasikala Vaddu[1], Sujitha Bhumanapalli[1], Rami Dalloul[1], Cortney Leone[2], Manpreet Singh[2], Harshavardhan Thippareddi[1]

[1]Department of Poultry Science, University of Georgia, Athens, GA, USA
[2]Department of Food Science and Technology, University of Georgia, Athens, GA, USA

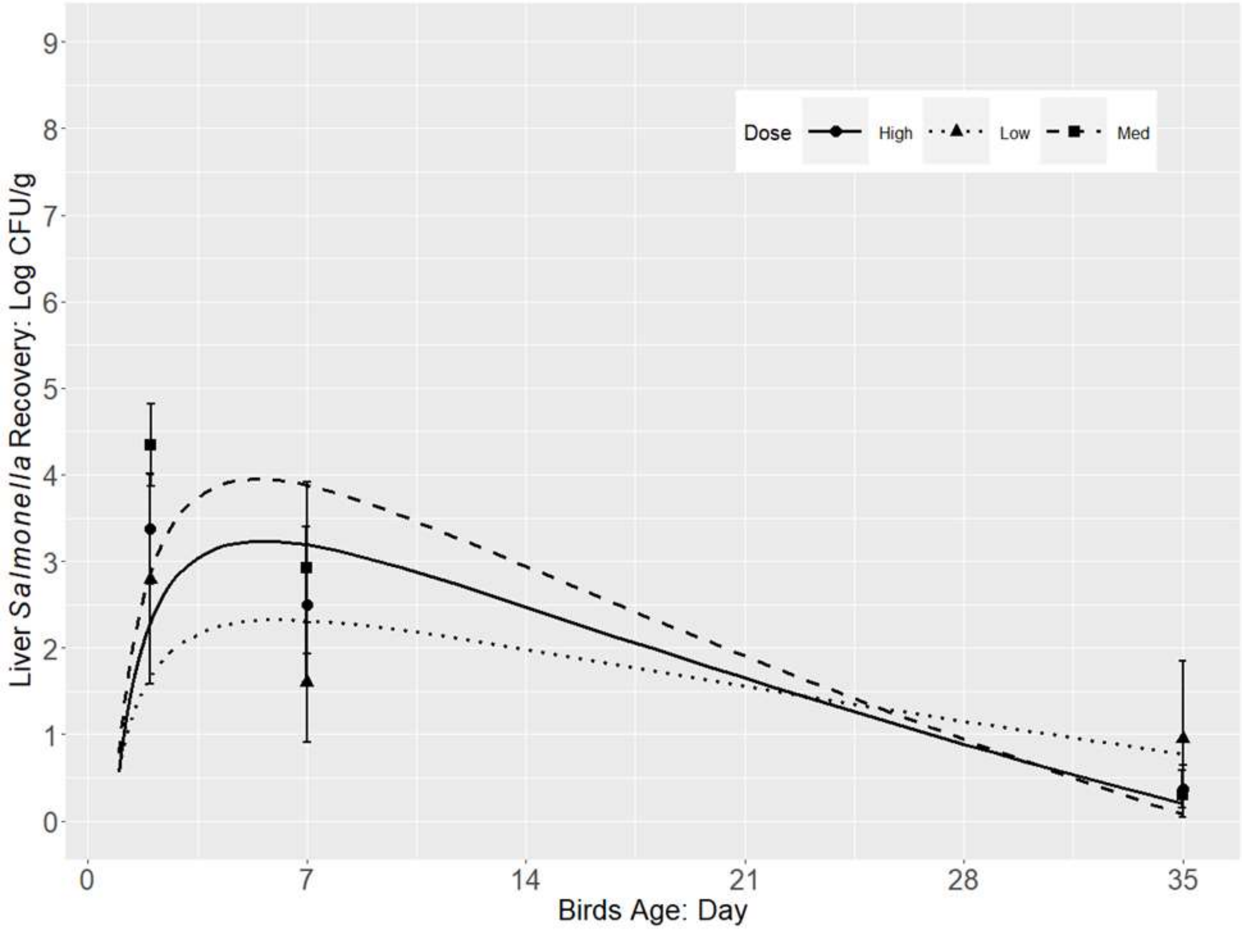
Salmonella Colonization Dynamics - Ceca



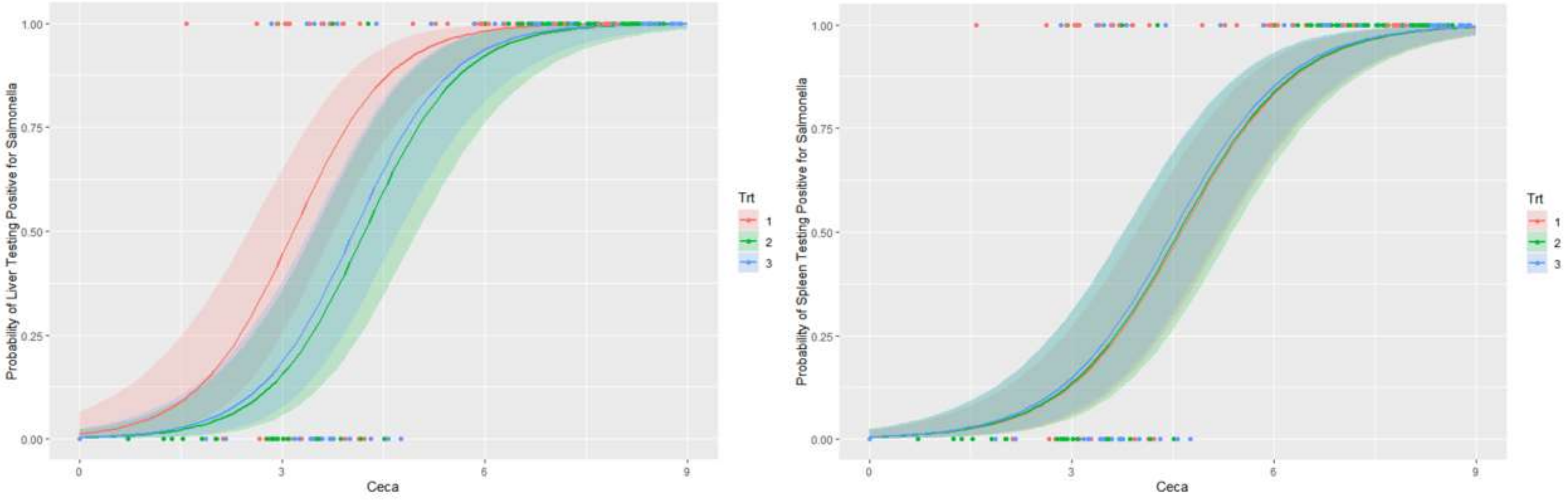
Salmonella Colonization Dynamics - Spleen



Salmonella Colonization Dynamics - Liver



Salmonella Colonization Dynamics - Probabilities



Salmonella Colonization Dynamics – Ceca, 35d

	S. Typhimurium	*S.* Infantis	*S.* Reading	Pooled Data
Dose				
Low	1.67 ± 0.49^{bx}	1.27 ± 0.7^{bx}	3.02 ± 0.36^{y}	3.28 ± 0.35
Med	1.10 ± 1.10^{abx}	0.19 ± 0.19^{ax}	2.48 ± 2.48^{y}	2.69 ± 0.85
High	0.82 ± 0.82^{bx}	0.25 ± 0.25^{ax}	3.41 ± 3.41^{y}	3.45 ± 0.71
p-values				
Linear	<0.001	<0.001	0.003	0.673
Quad	0.797	0.507	0.154	0.063

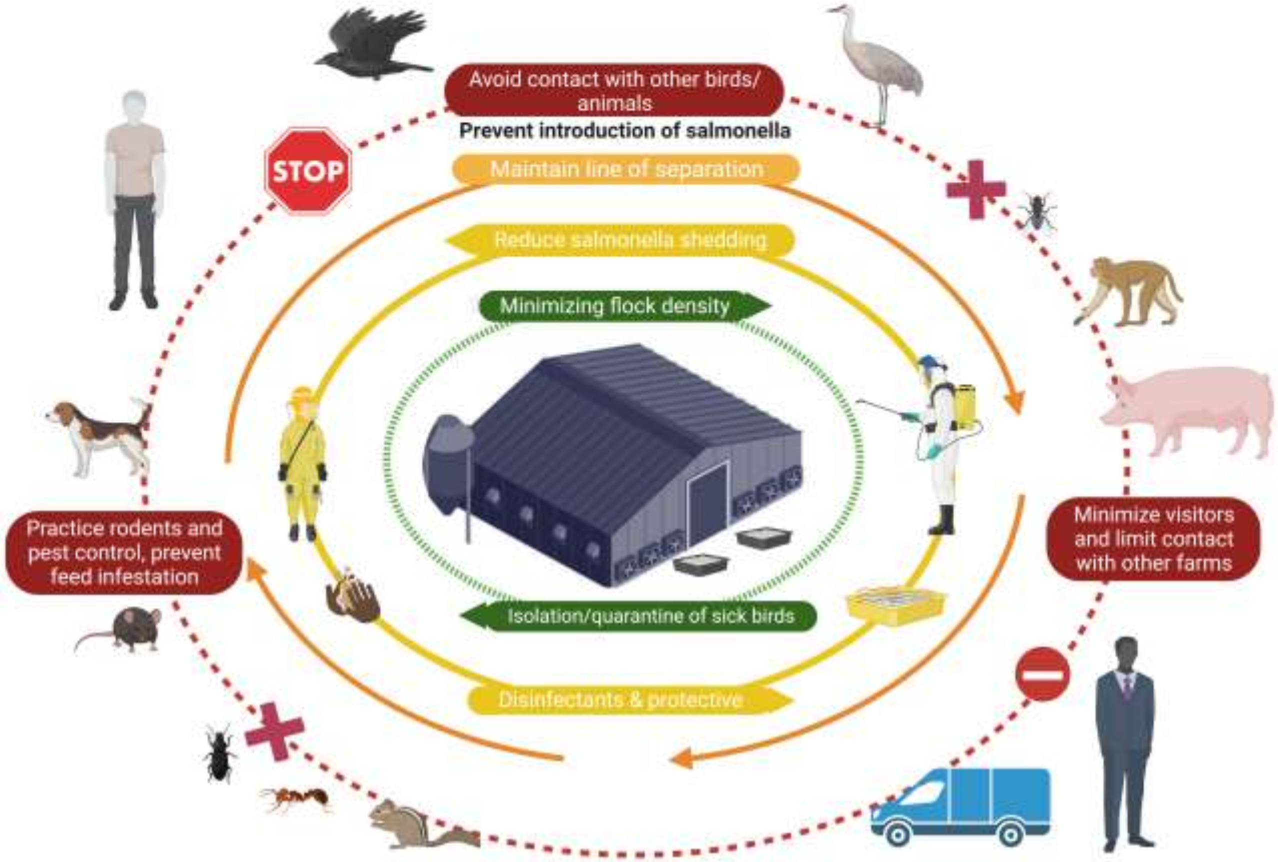
Salmonella risk reduction at production

Strategies to mitigate *Salmonella* risk at production

- Management strategies
  - Biosecurity and hygienic measures
  - Cleaning and disinfection of poultry houses between flocks
  - Litter management

Salmonella Control - Biosecurity

- Biosecurity
  - Preventive measures undertaken to stop or minimize the introduction and spread of disease



Strategies to mitigate *Salmonella* risk at production

- Nutritional strategies
  - Organic acids
  - Botanicals/Essential oils
  - Bacteriocins
  - Bacteriophages
  - Novel compounds and feed additive combinations
  - Probiotics, competitive exclusion and prebiotics

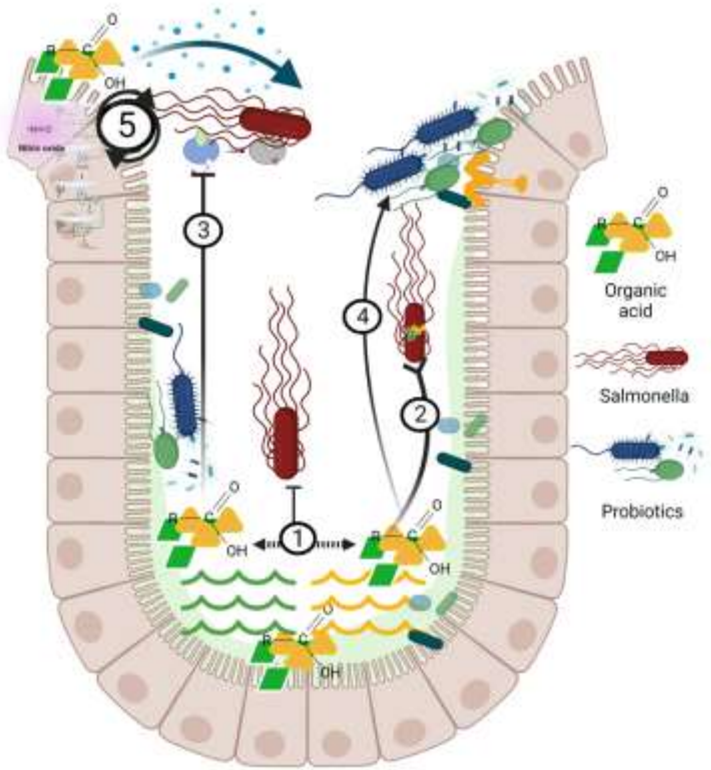
Salmonella – Organic Acids

- Organic acids
  - Naturally occurring compounds with carboxyl (-COOH) functional groups.

    Acetic acid, lactic acid, citric acid, propionic acid, butyric acid etc.
  - Can enter bacterium in undissociated form and dissociate within cell environment causing disruption of proton pump

Mode of Action

1. pH reduction causing unfavorable environment for *Salmonella* colonization ar multiplication
2. Internalization and dissociation causing disruption of bacterial cell
3. Inhibition of bacterial enzymes
4. Modulation of gut microbiome
5. Immunomodulation such as regulation of cytokines, activation of complement system and the production of nitric oxide to defend against *Salmonella*



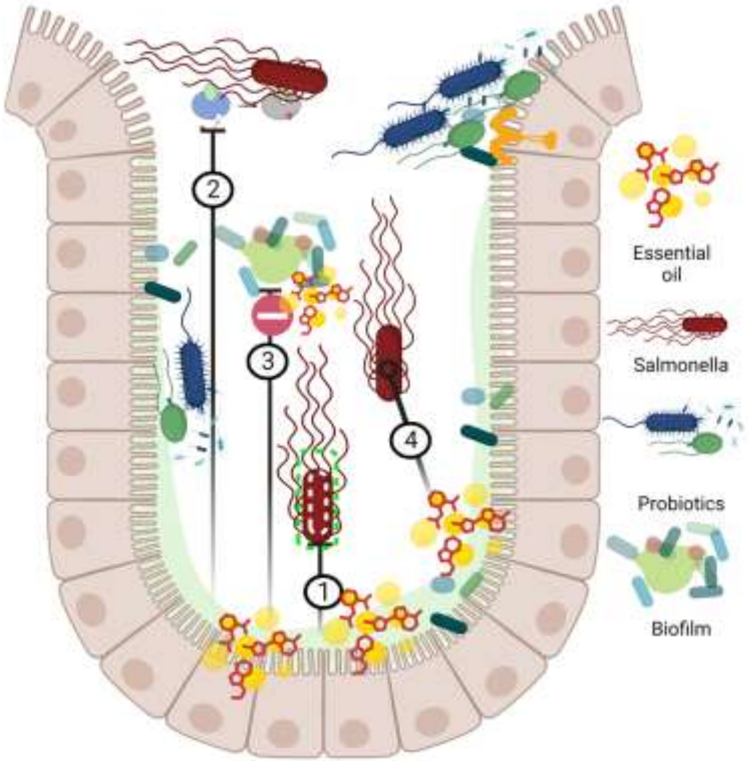
Salmonella Control – Botanicals/Essential Oils

- Essential oils
  - Complex mixture of volatile form of plant extract with antimicrobial effect

    e.g., Oregano, Eucalyptus, Rosemary, Thyme, etc.
  - Contains terpenes, phenolics, aldehydes, ketones, alcohols, etc.

Mode of Action

1. Interaction with lipid bilayer of *Salmonella* and causing disruption and leakage of cellular components
2. Inhibition of bacterial ATPase enzyme
3. Disrupt quorum sensing and biofilms
4. Alteration in bacterial physiology and metabolism by up-regulating stress response genes and downregulating virulence genes



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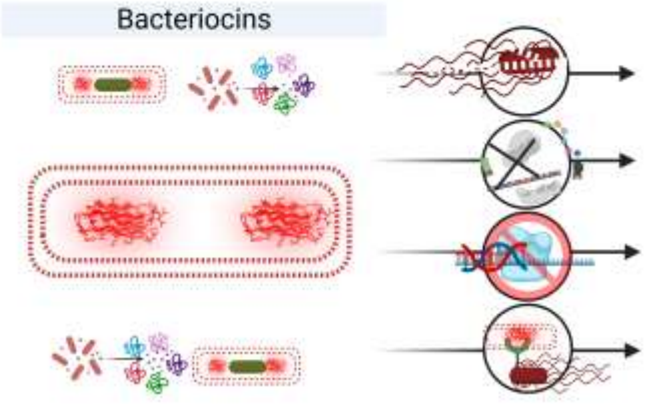
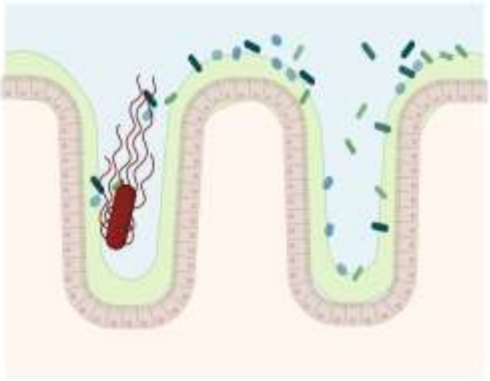
Salmonella Control – Bacteriocins

- Bacteriocins
  - Antimicrobial peptides produced by some bacteria to inhibit or kill other competing bacteria



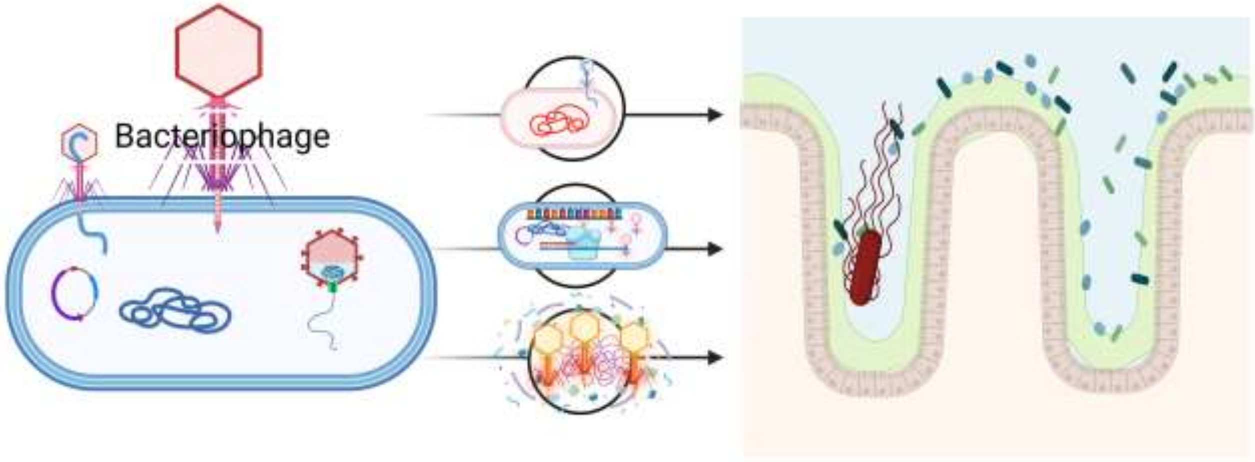


Mode of Action

1) Increased permeability of *Salmonella* cell membra
2) Inhibition of bacterial protein synthesis causing arrest of growth,
3) Bacterial DNA binding and replication arrest
4) Bind to receptors in *Salmonella* and disrupt cell function

Salmonella Control – Bacteriophages

- Bacteriophages
  - Virus that can infect bacteria, replicate using their cell machinery and cause their death while liberating

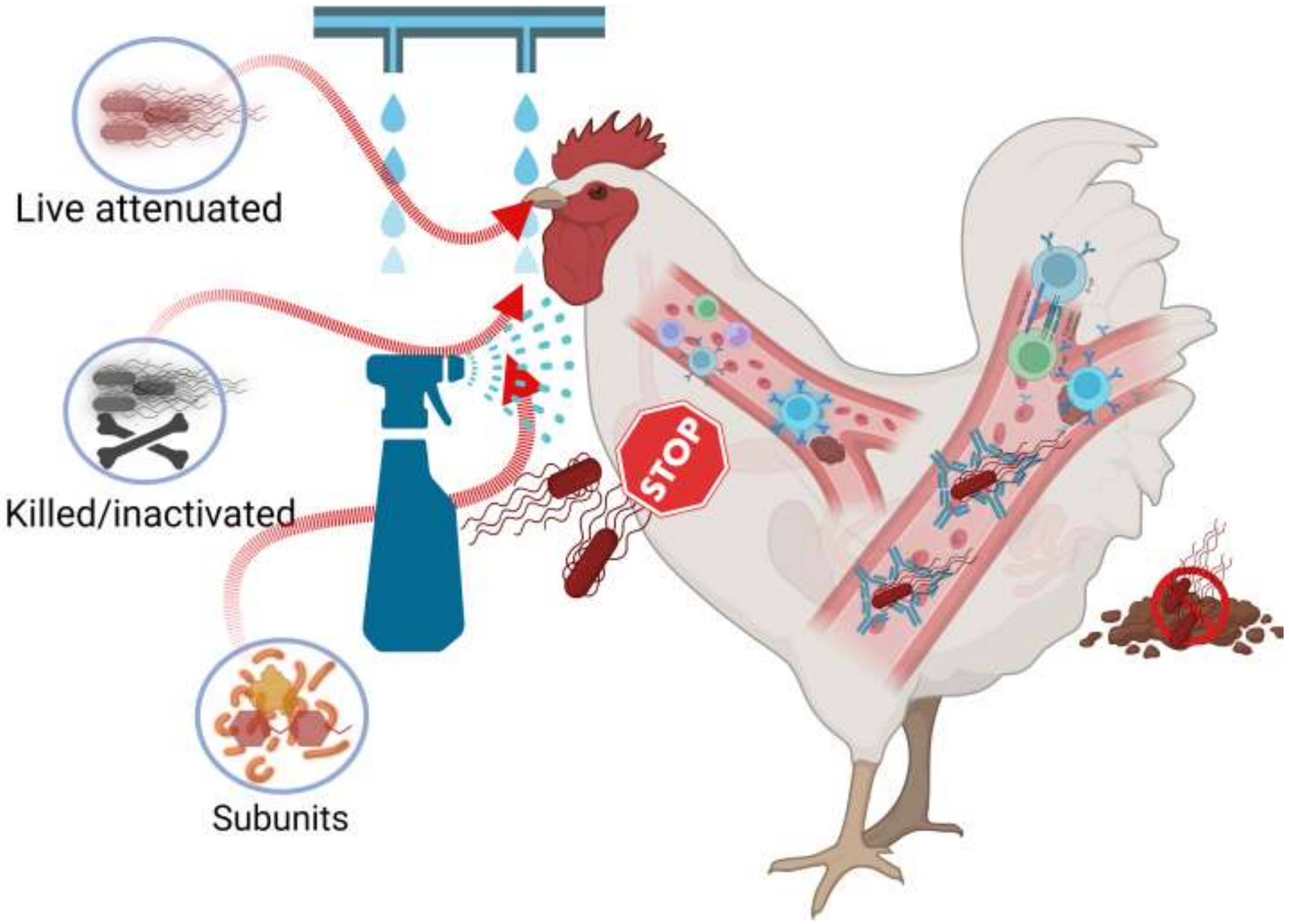


Mode of Action

1) Adsorption and penetration in bacterium,
2) Use bacterium machinery to replicate,
3) Cause lysis of bacterium (Salmocins against *Salmonella*)

Strategies to mitigate *Salmonella* risk at production

- Immunization
  - Oral vaccination
    - Breeder
    - Broiler

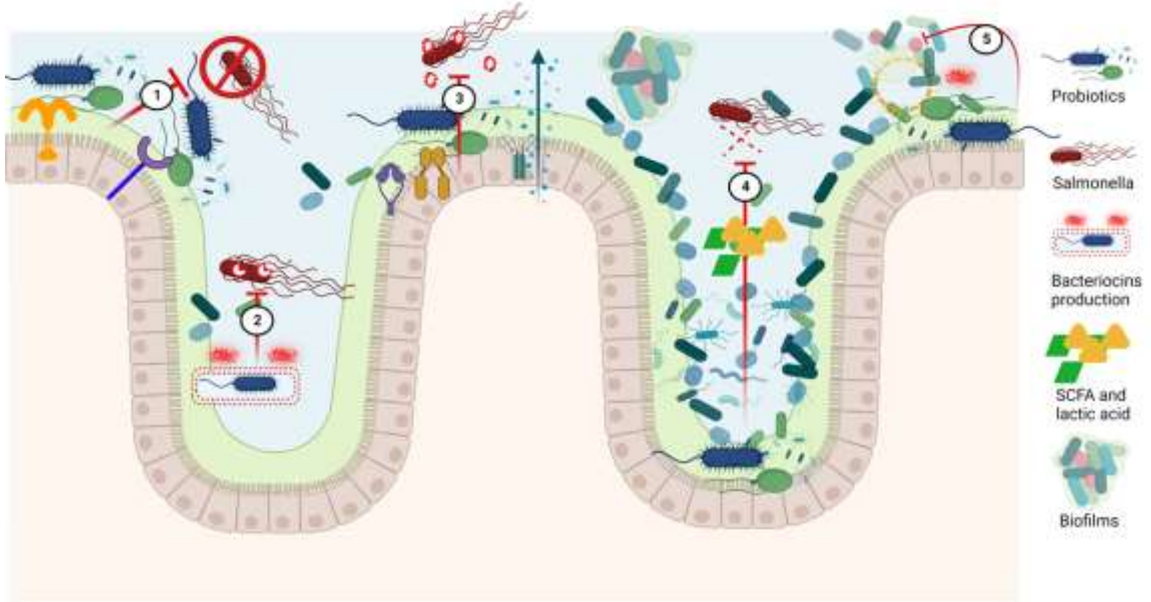


Salmonella Control - Probiotics

- Probiotics
  - Microorganisms, especially of bacterial and fungal origin that competes with pathogenic organism and provides health benefits to the host
  - They can be the part of normal gut microbiome or could be introduced from *in vitro* cultures

Mode of Action

1. Competitive exclusion by Probiotics for receptor binding and nutrients with *Salmonella*
2. Immunomodulation and change in gene expression of immunoglobulins, cytokines and antioxidants
3. Acidification of gut by increased fermentation metabolites such as lactic acid and other SCFA
4. Disruption/eradication of extracellular polymeric substances (biofilms) of *Salmonella* through surfactants, bacteriocins and other metabolites

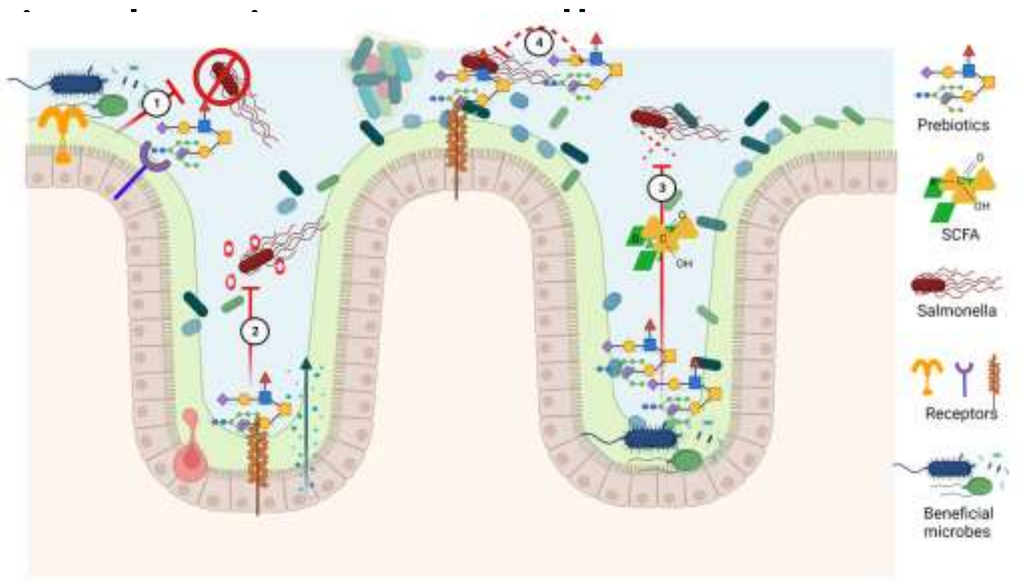


Salmonella Control - Prebiotics

- Prebiotics
  - Can be carbohydrates or peptides that are non-digestible by the host but utilized by beneficial microbes as the source of fermentation substrate
  - Can interact with some cell receptors and s

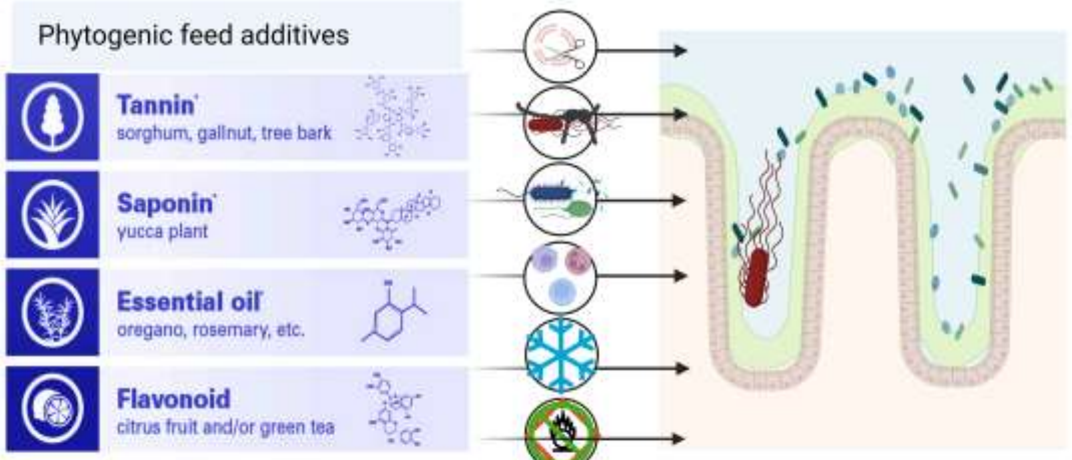
Mode of Action

1. Promote beneficial bacteria for competitive exclusion of *Salmonella*
2. Immunomodulation and change in gene expression of adaptive immune cells and cytokines
3. Acidification of gut by increased fermentation metabolites such as lactic acid and SCFA
4. Prebiotics like mannan-oligosaccharides can bind to *Salmonella* and prevent their adhesion to the gut wall



Salmonella Control – Phytogenic Feed Additives

- Phytogenic additives
  - Different active ingredients from plants with antimicrobial effects

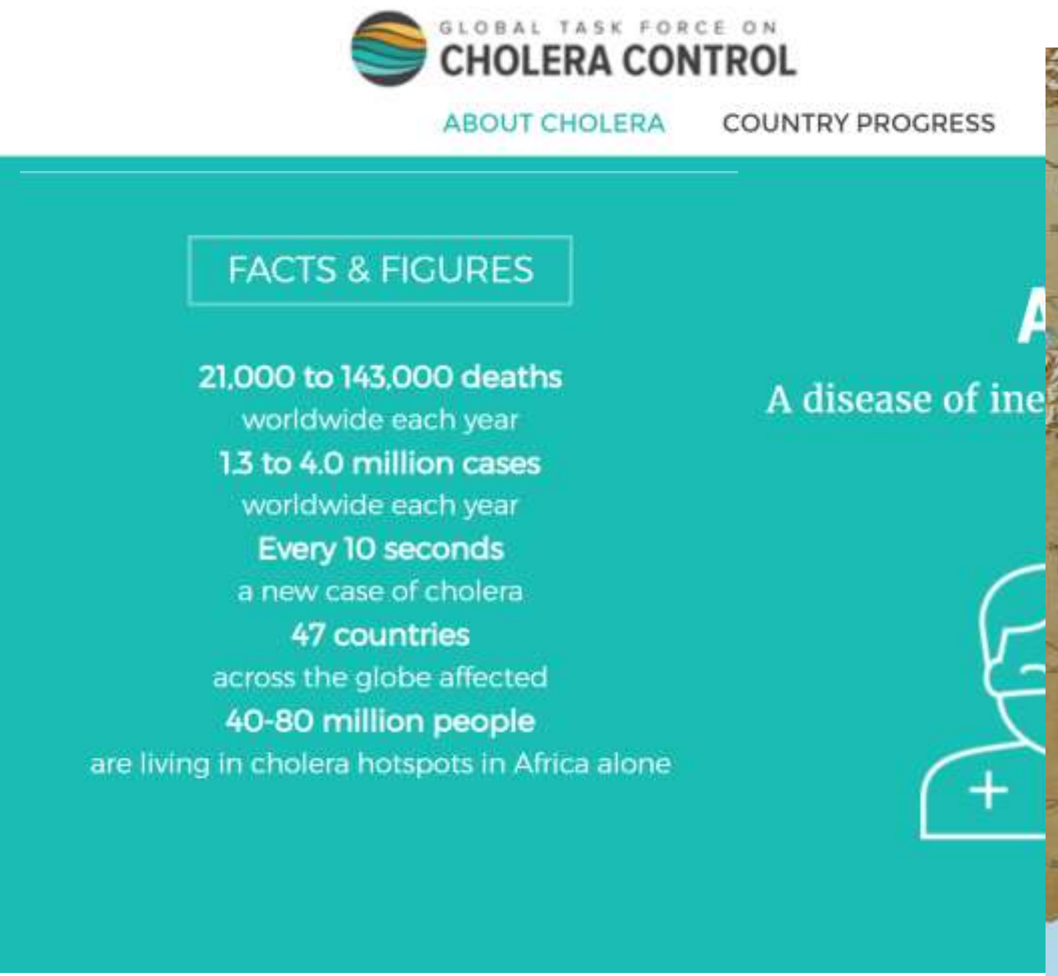


Source: Kemin products

Mode of Action

1. Disruption of bacterial cell membrane
2. Inhibition of bacterial motility
3. Modulation of beneficial microbiota
4. Stimulation of immune system against *Salmonella*
5. Antioxidation and anti-inflammatory response to reduce the severity of infection

Is *Salmonella* Control Necessary at Pre-



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Salmonella in Poultry & Risk Reduction

- Several sources of *Salmonella* in the poultry production environment
- The microorganism can colonize the gastro-intestinal tract of the poultry and persist in the gut through out the production timeframe
- Several strategies are available for poultry producers to reduce the risk
  - However, no silver bullets to eliminate the microorganism from the poultry <u>under current production system</u>
- *Salmonella* control at production is necessary to reduce the prevalence and concentrations at or subsequent to processing

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Thank You

Harshavardhan Thippareddi, Ph.D.
Interim Associate Dean for Research
John Bekkers Professor of Poultry Science

Email: harsha.thippareddi@uga.edu

Salmonella in Poultry—Proposed Regulatory Changes and Intervention Strategy Advancements

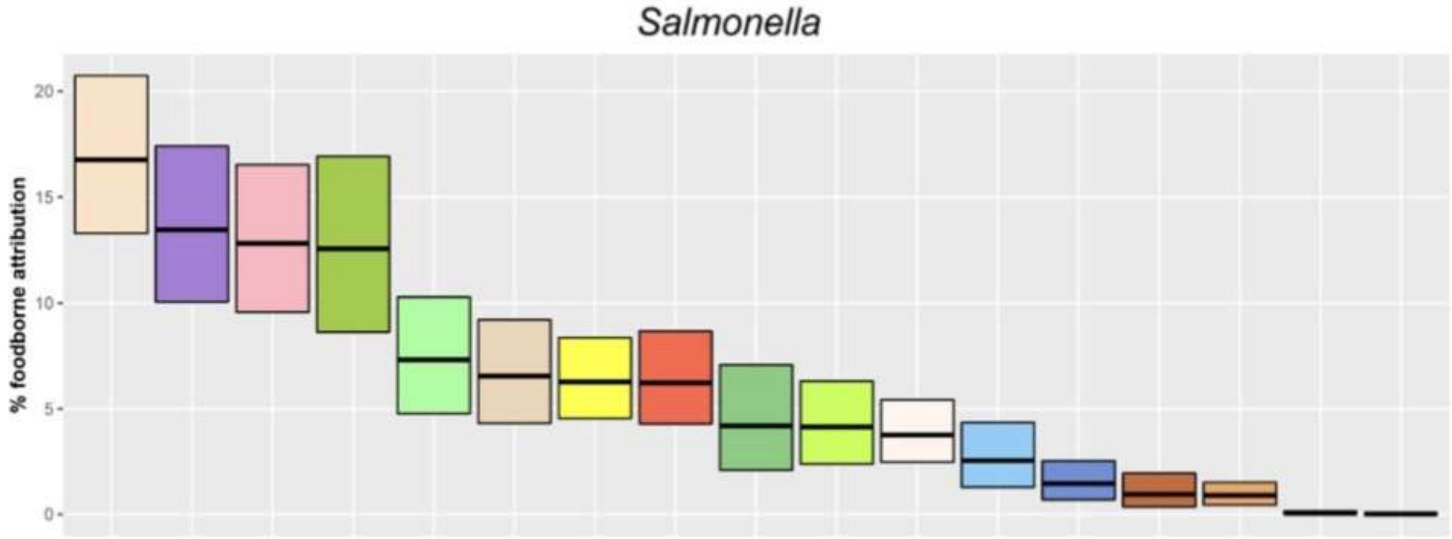
Mindy Brashears, PhD
Paul Whitfield Horn Distinguished Professor
Associate Vice President for Research
Director-International Center for Food Industry Excellence
Roth and Letch Family Endowed Chair in Food Safety
Texas Tech University

Salmonella What Drives Decisions?

- Attribution Data?
- Regulations?
- Science and Data?

Attribution Data

Figure 2: Estimated percentage of foodborne *Salmonella* illnesses (with 90% credibility intervals) for 2019, in descending order, attributed to each of 17 food categories, based on multi-year outbreak data,* United States. Click here to download relevant data.



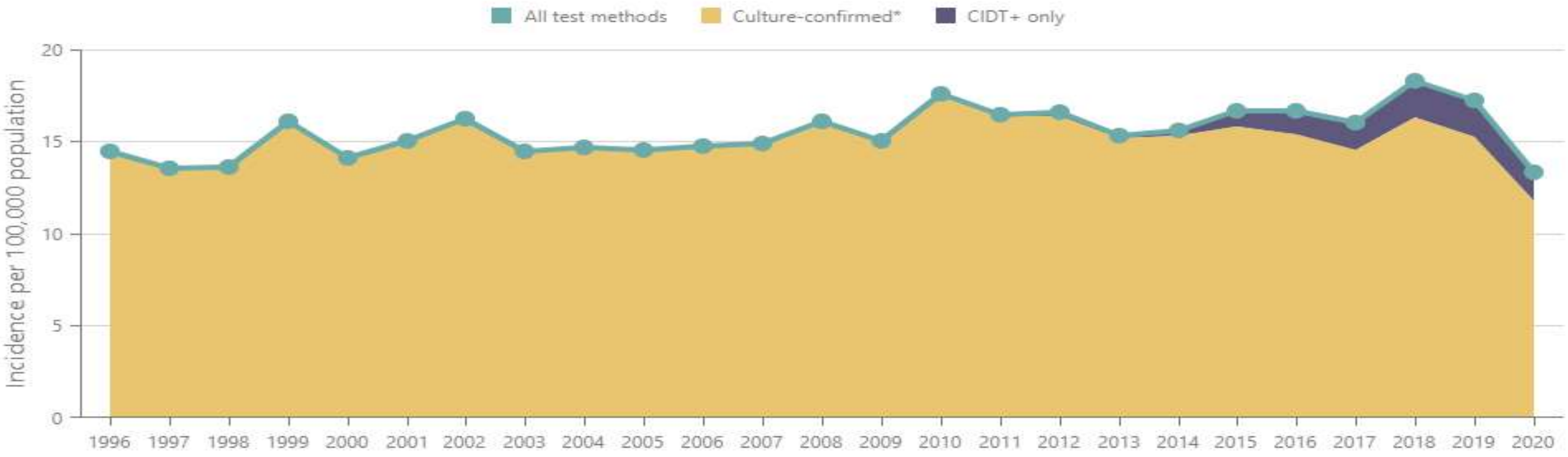
	Chicken	Fruits	Pork	Seeded Vegetables	Other Produce	Turkey	Eggs	Beef	Vegetable Row Crops	Sprouts	Dairy	Fish	Other Seafood	Grains-Beans	Other Meat/ Poultry	Oils-Sugars	Game
% attribution	16.8	13.5	12.8	12.6	7.3	6.6	6.3	6.2	4.2	4.2	3.8	2.6	1.4	0.9	0.9	< 0.1	< 0.1
cumulative attribution	75.9%							24.2%									

*Based on a model using outbreak data that gives equal weight to each of the most recent five years of data (2015-2019) and exponentially less weight to each earlier year (1998-2014).

Confirmed Infections

Salmonella infections by year; 1996-2020

Incidence per 100,000 population – FoodNet sites; all test methods
* Culture-confirmed includes those infections confirmed by culture only or by culture following a positive CIDT.
Source: FoodNet, Centers for Disease Control and Prevention



Current Regulatory Drivers

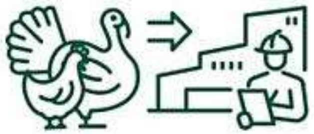
Food Safety and Inspection Service

Salmonella Framework

"Adulterant" Status for Raw Poultry

Performance Standards

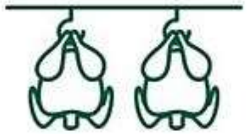
Proposed Regulatory Framework to Reduce *Salmonella* Illnesses Attributable to Poultry



Component 1

Requiring incoming flocks be tested for Salmonella before entering an establishment

Read More →



Component 2

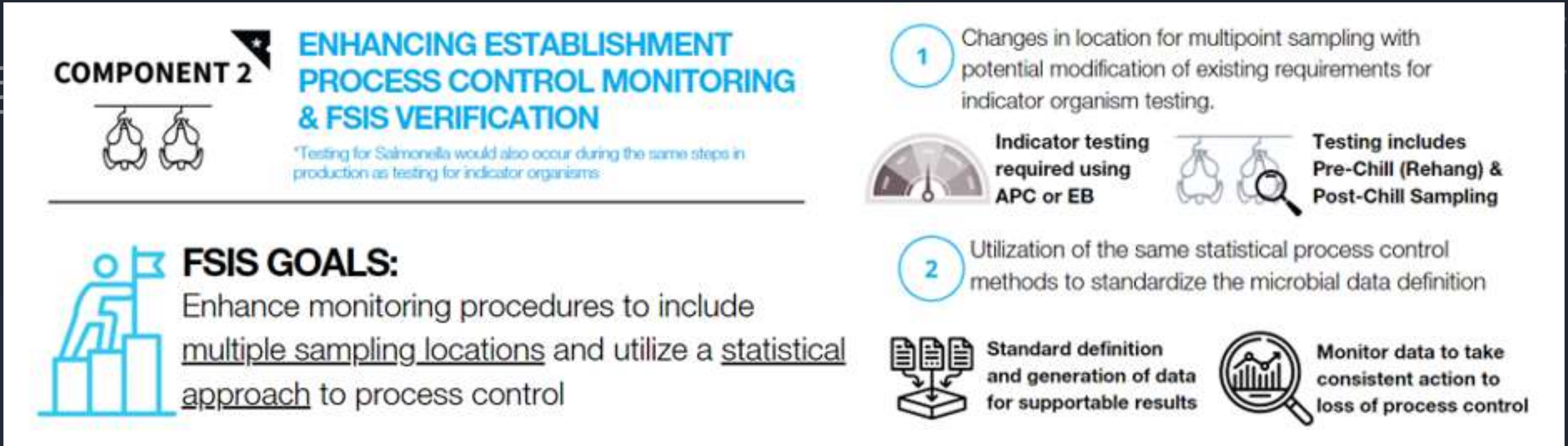
Enhancing establishment process control monitoring and FSIS verification

Read More →

Component 3

Implementing an enforceable final product standard

Read More →



- Component 2
  - Statistical Process Control of EB or APC and *Salmonella* presence
  - Pre-Chill and Post-Chill
  - Actionable Data

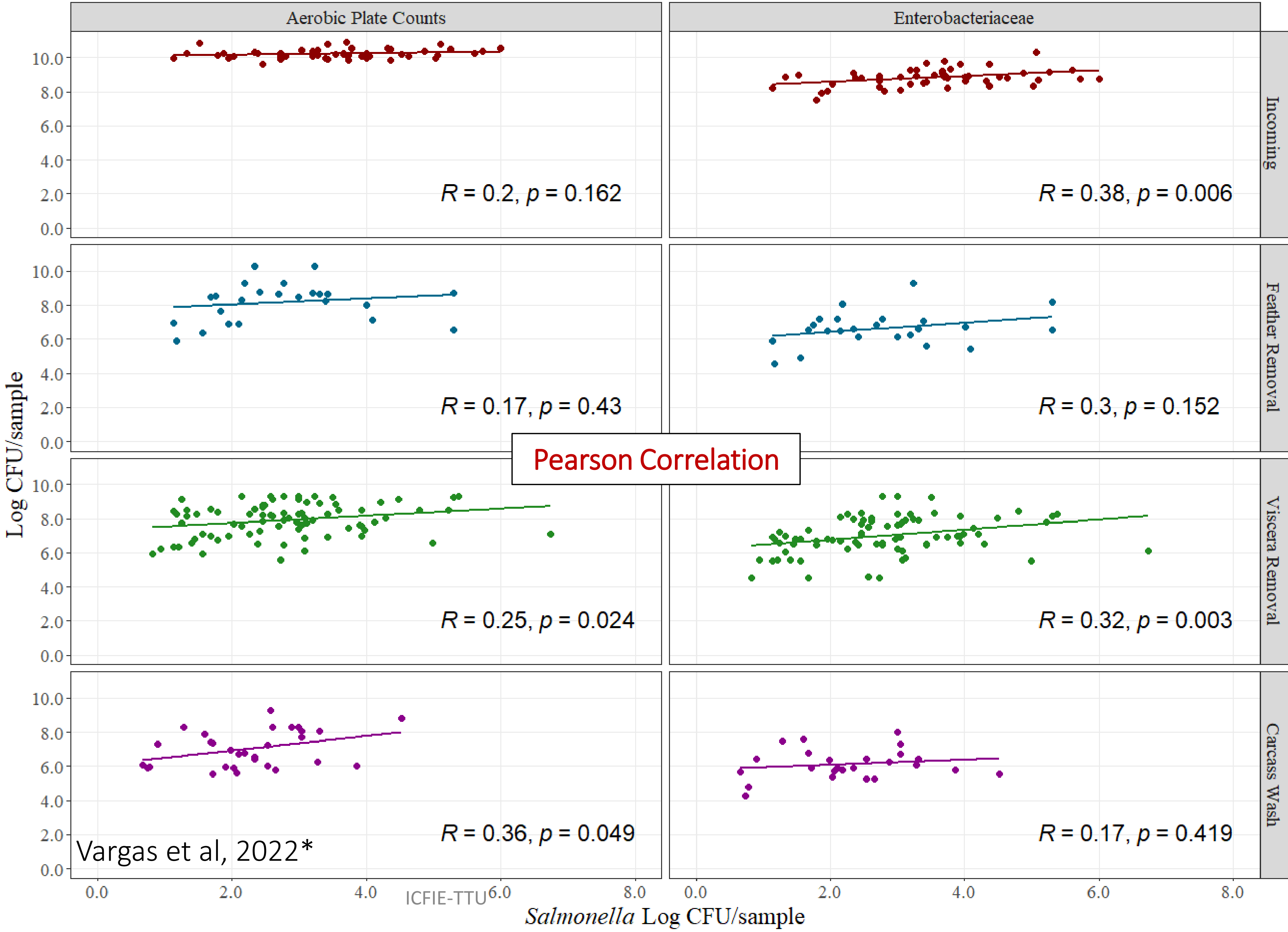
Why Biomap *Salmonella?*

Correlation of Indicators to Salmonella Behavior

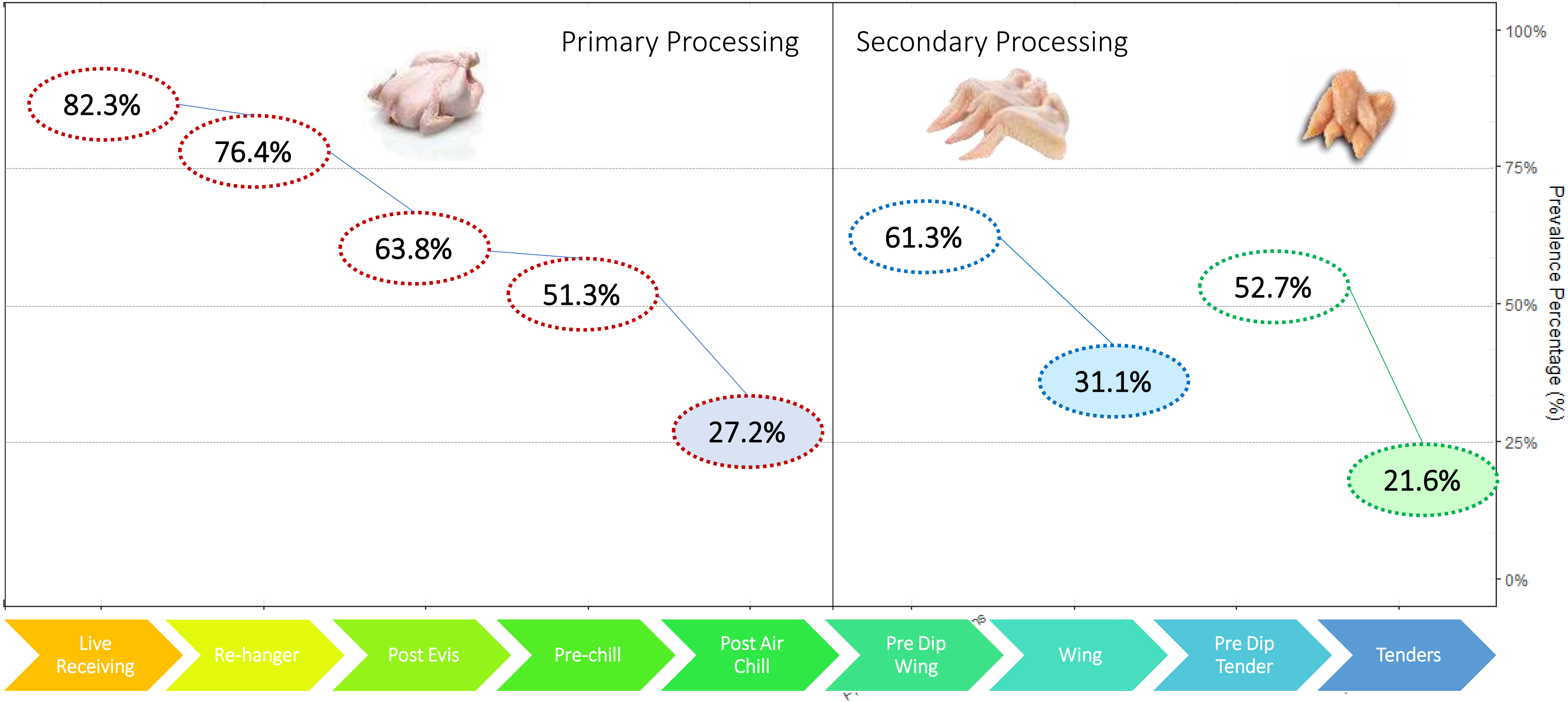
Vargas et al 2023 (Foods)

-Indicators should be used for Process control

-Not useful for targeting *Salmonella*

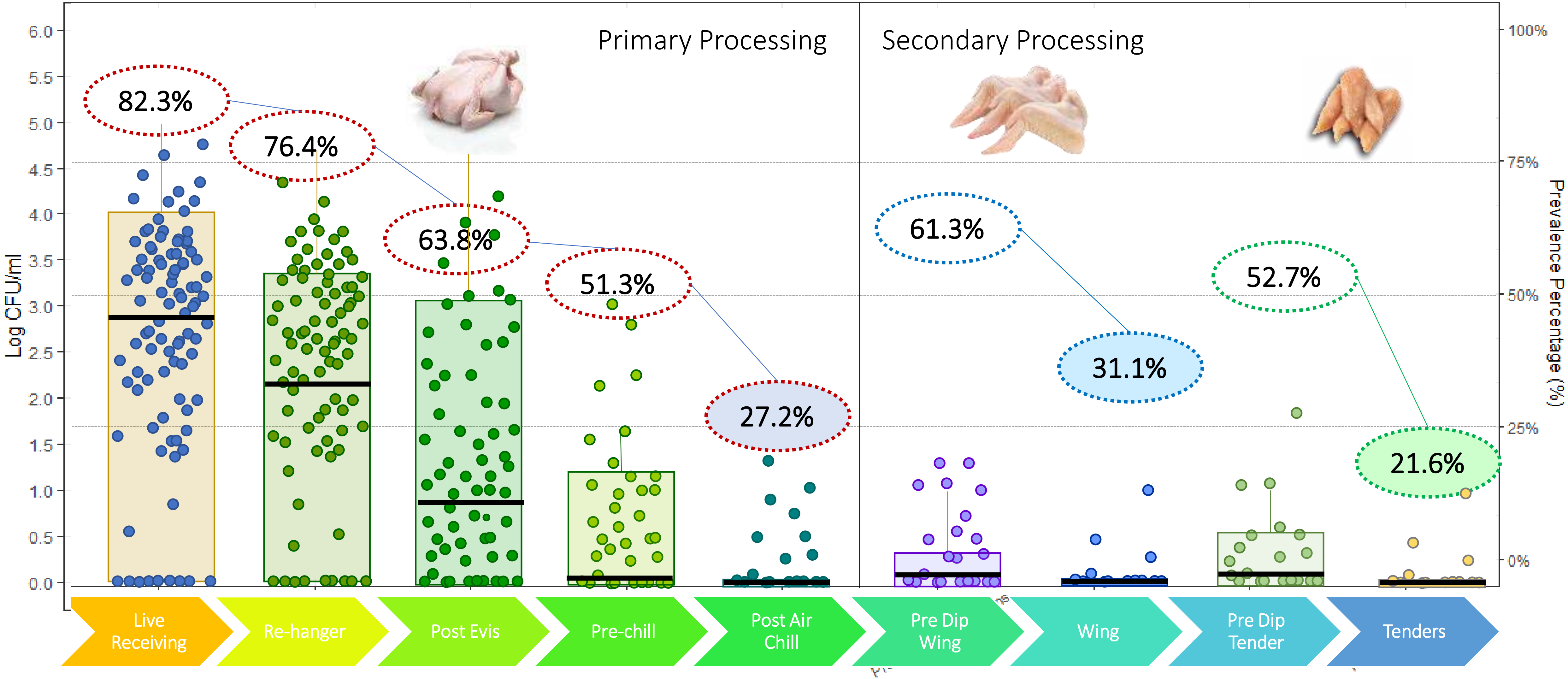


Salmonella Enumeration vs Prevalence



Sanchez-Plata et al, 2021

Salmonella Enumeration vs Prevalence



Sanchez-Plata et al, 2021

Salmonella Biomapping for Decision Making

Little correlation of *Salmonella* quantification and Indicators

Indicators are still a good measurement of process control

Quantification Data Gives more insight

- Wing and Tender prevalence is similar to Post-Evisceration Carcasses
- Quantification is much different
  - 3 logs post-evisc vs <0.5 log on parts – Lower Risk

Drive allocation of Resources

Performance Standards do not allow for Strategic Decision-Making

- Component 3
  - Actionable and Enforceable Final Product Standards

Salmonella as an "Adulterant" in Raw Poultry

USDA Announces Action to Declare Salmonella an Adulterant in Breaded Stuffed Raw Chicken Products

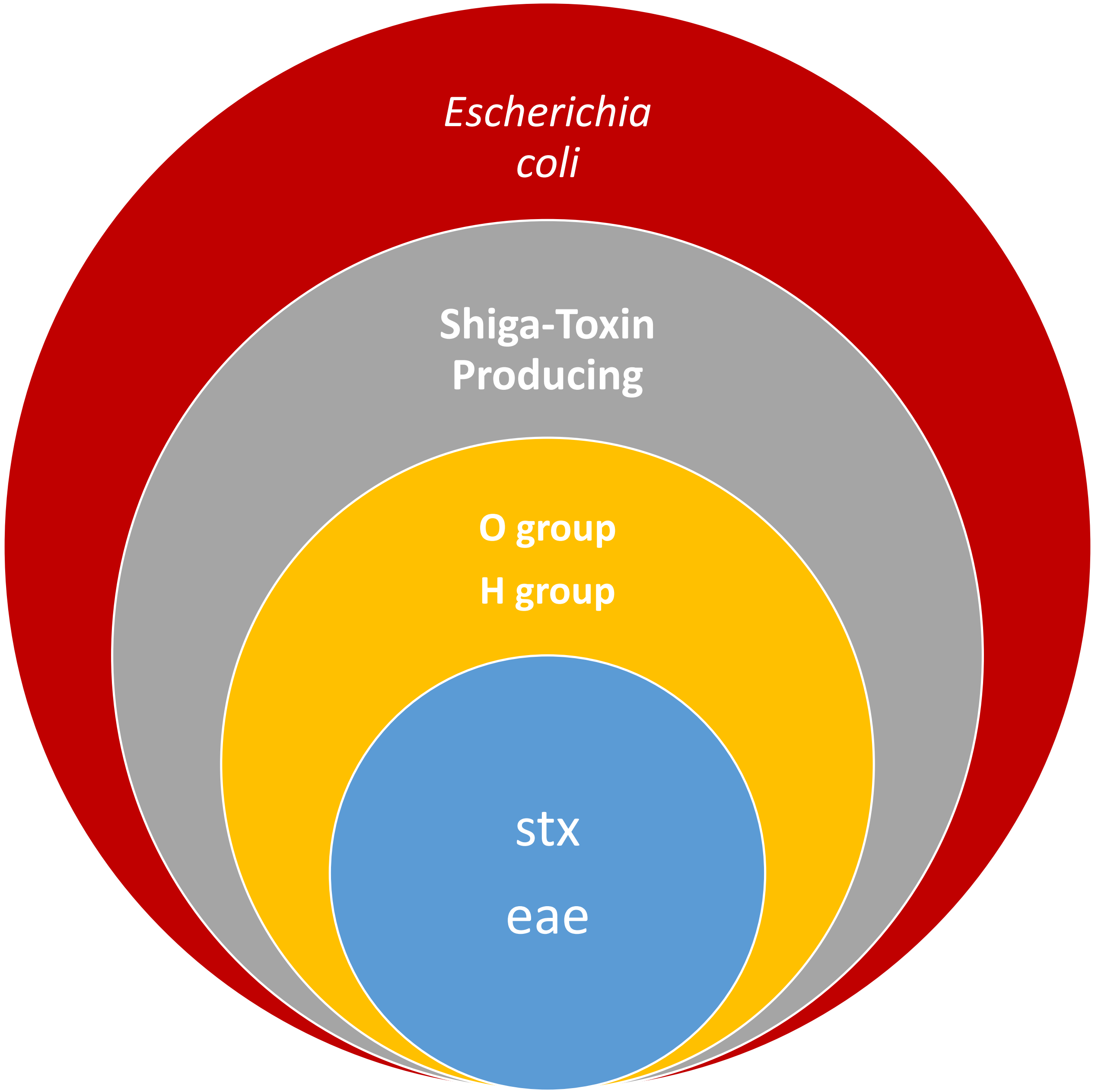
Press Release
Release No. 0167.22

Contact: USDA Press
Email: press@usda.gov

WASHINGTON, August 1, 2022 – The U.S. Department of Agriculture's (USDA) Food Safety and Inspection Service (FSIS) is announcing that it will be declaring *Salmonella* an adulterant in breaded and stuffed raw chicken products.

“Big 7”
ADULTERANTS
in
Raw Ground Beef
and RGB Components

Very Specific to a Pathogen in a Product that has a high consumption



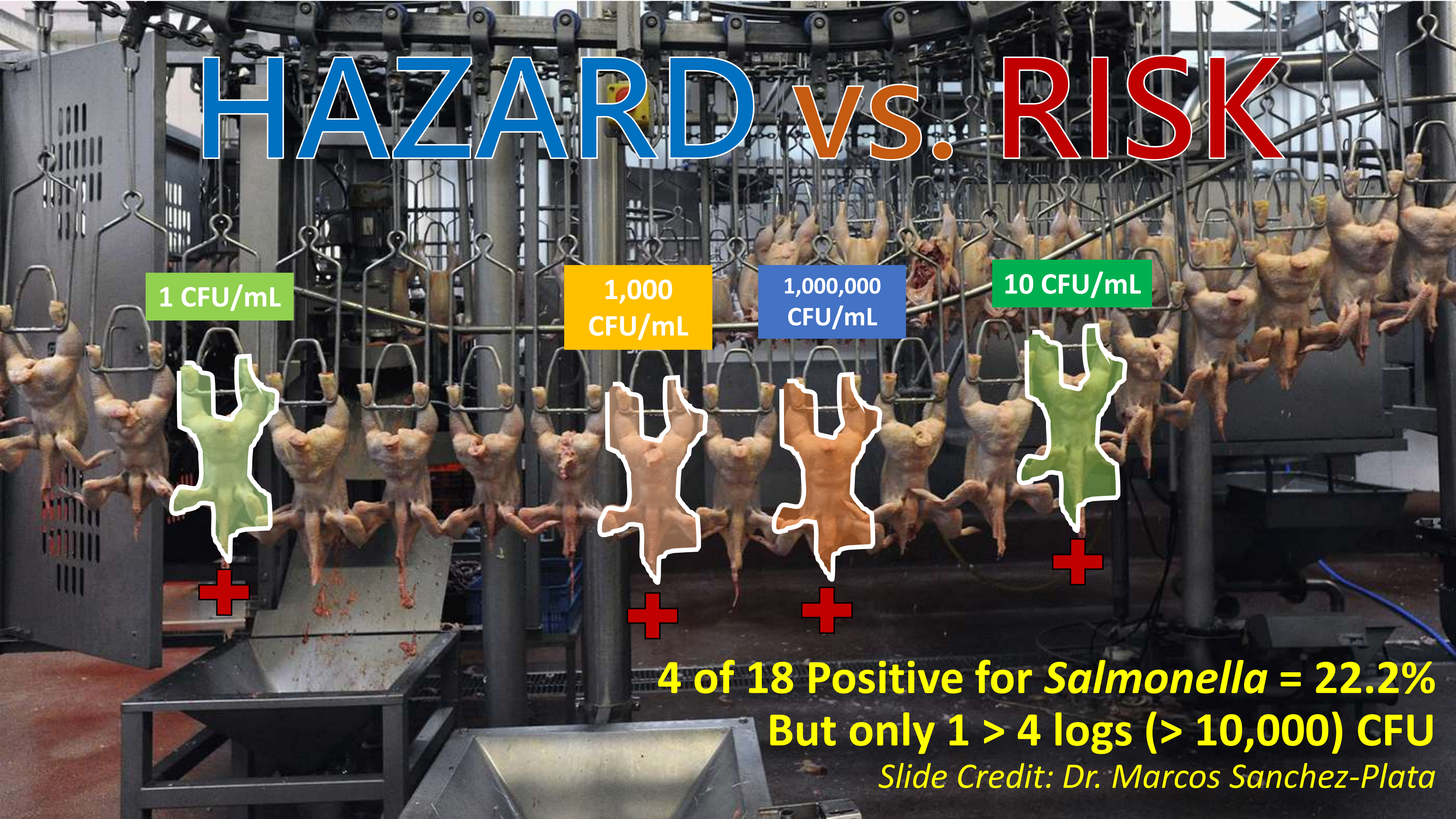
Salmonella as Adulterant

“Figure it Out” Approach (Not Science-based or Data-Driven)

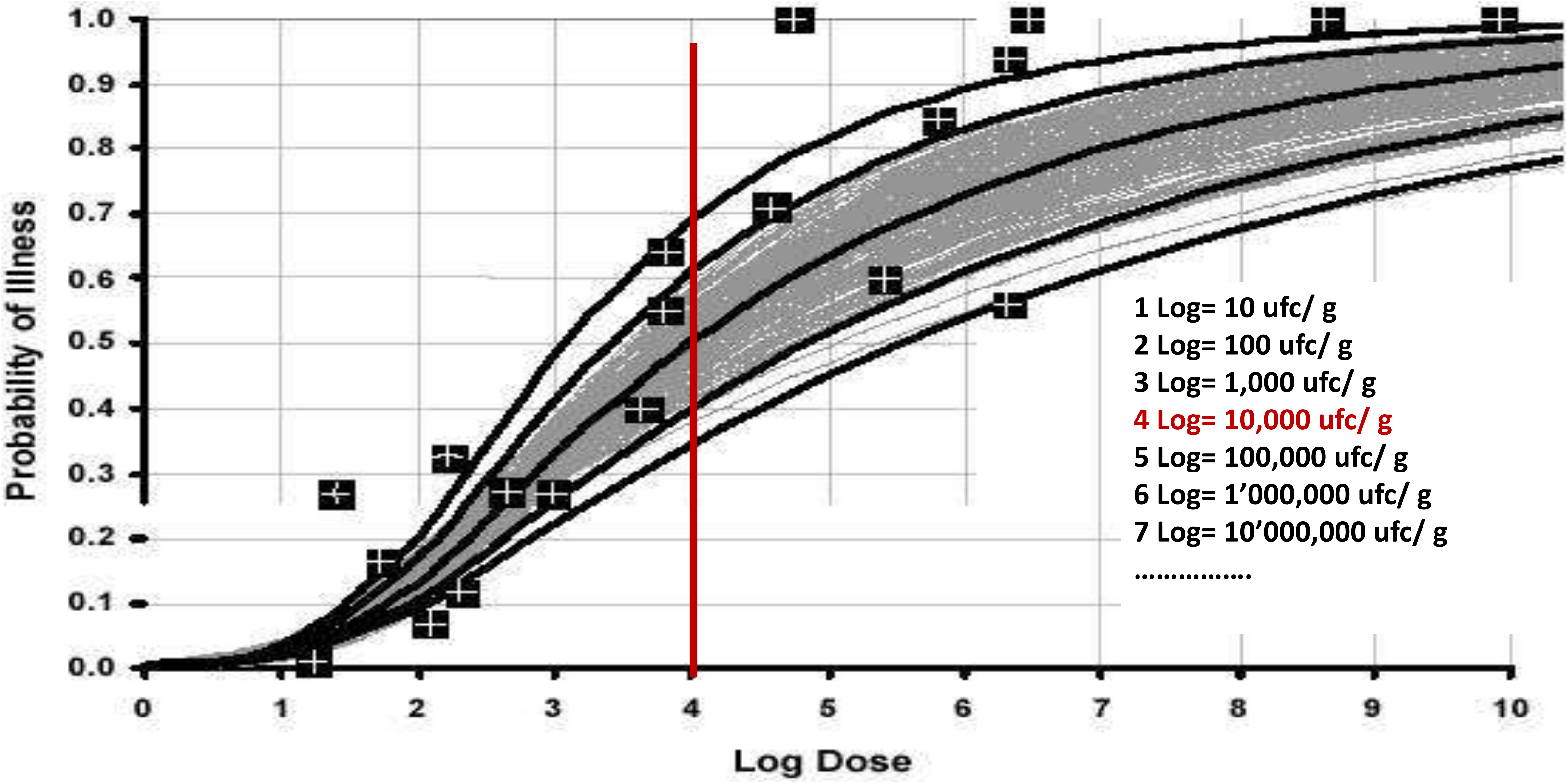
Not specific to virulent strains or in a highly consumed product

State of the Science

- Quantification (Dose-Response)
- Serotyping
- Highly-Pathogenic *Salmonella*


HAZARD vs. RISK
1 CFU/mL
1,000 CFU/mL
1,000,000 CFU/mL
10 CFU/mL
4 of 18 Positive for *Salmonella* = 22.2%
But only 1 > 4 logs (> 10,000) CFU
Slide Credit: Dr. Marcos Sanchez-Plata

Probability of Illness vs. Log Dose



1 Log= 10 ufc/ g
2 Log= 100 ufc/ g
3 Log= 1,000 ufc/ g
4 Log= 10,000 ufc/ g
5 Log= 100,000 ufc/ g
6 Log= 1’000,000 ufc/ g
7 Log= 10’000,000 ufc/ g
……………

Rapid Quantification Methods

AOAC Approved

Time to Results is no Difference than Detection

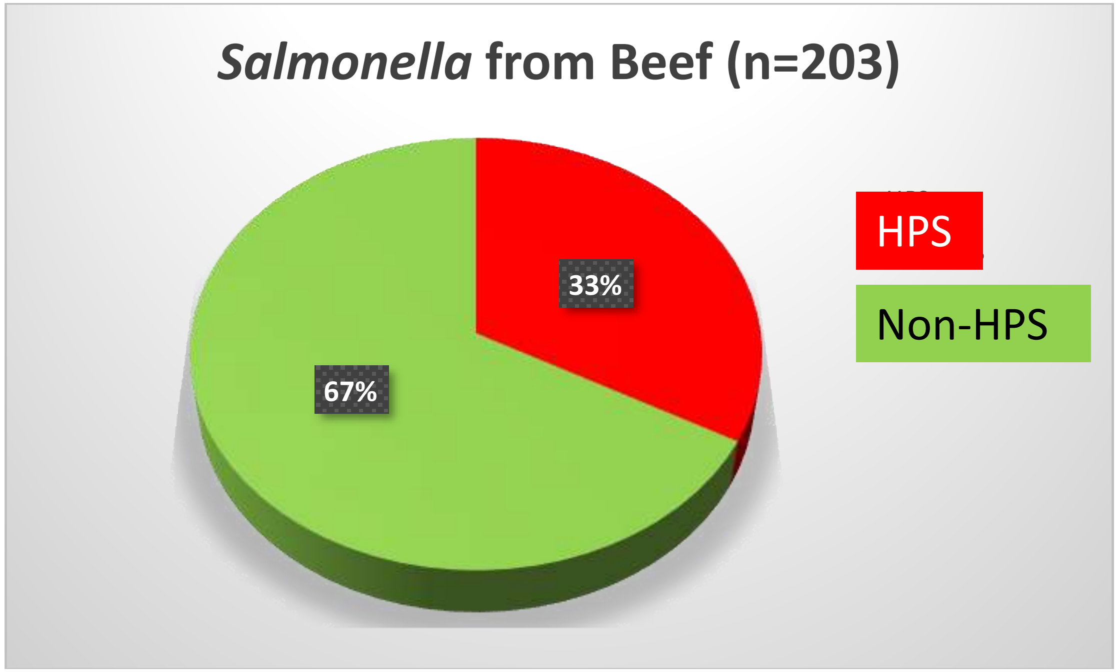
SalQuant-Hygiena

Quant Sal-bioMerieux

Pathogenicity

- Dayna Harhay, PhD
- USMARC

Beef Results
FSIS-ARS-IAA 2021-22

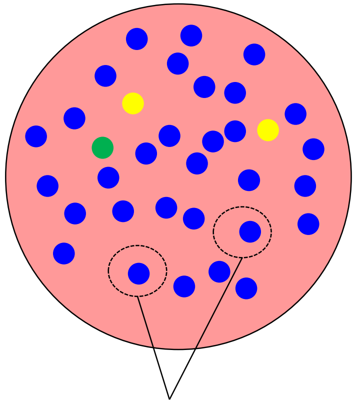


Beef (n=203; 42 Serotypes)					
HPS ≥ 5 markers amplified			Non-HPS ≤ 4 markers amplified		
Serotype	n	%	Serotype	n	%
Enteritidis	**5**	**2.5**	**Montevideo**	**29**	**14.3**
Newport	**9**	**4.4**	Paratyphi B	1	0.5
Typhimurium	**7**	**3.4**	Uganda	8	3.9
Infantis	**15**	**7.4**	Poona	1	0.5
B:i:-	**5**	**2.5**	Agona	6	3.0
Muenchen	**9**	**4.4**	Anatum	24	11.8
Berta	2	1.0	Mbandaka	2	1.0
Heidelberg	1	0.5	Muenster	9	4.4
Dublin	10	4.9	Give	8	3.9
Virginia	3	1.5	Meleagridis	7	3.4
Manhattan	1	0.5	Cerro	6	3.0
Total	**67**	**33.0**	Derby	5	2.5
			Kentucky	3	1.5
			Lubbock	3	1.5
			Adelaide	2	1.0
			London	2	1.0
			Altona	2	1.0
			Reading	3	1.5
			Liverpool	2	1.0
			Brandenburg	2	1.0
			Mississippi	1	0.5
			Appa	1	0.5
			Amsterdam	1	0.5
			B:d:-	1	0.5
			Bredeney	1	0.5
			Eastbourne	1	0.5
			Johannesburg	1	0.5
			Panama	1	0.5
			Schwarzengrund	1	0.5
			Kiambu	1	0.5
			Orion	1	0.5
			Total	**136**	**67.0**

Multiple Serotypes

- Nikki Shariat, PhD
- University of Georgia

Picking a few colonies limits *Salmonella* surveillance



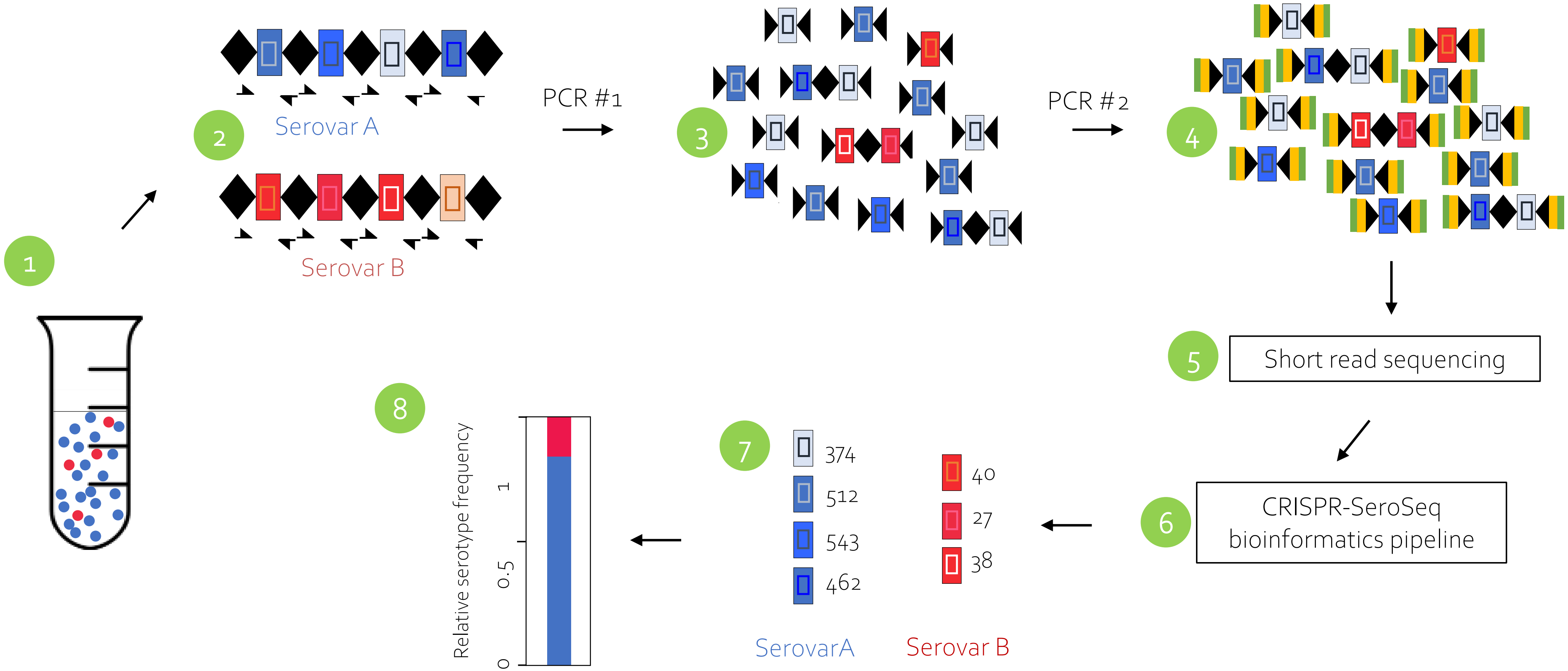
1-2 colonies picked

- Picking 1-2 colonies only identifies the most abundant serovars
- Background serovars remain undetected
- Limitations:

  Source tracking and *Salmonella* control
  Hidden serovars = hidden phenotypes (risk assessment)
  Prevents understanding of serovar dynamics

CRISPR-SeroSeq: Amplicon-based NGS tool for "deep serotyping" to identify multiple serovars within a *Salmonella* population

Deep Serotyping using CRISPR-SeroSeq



Thompson *et al.* Appl. Environ. Microbiol. 2018

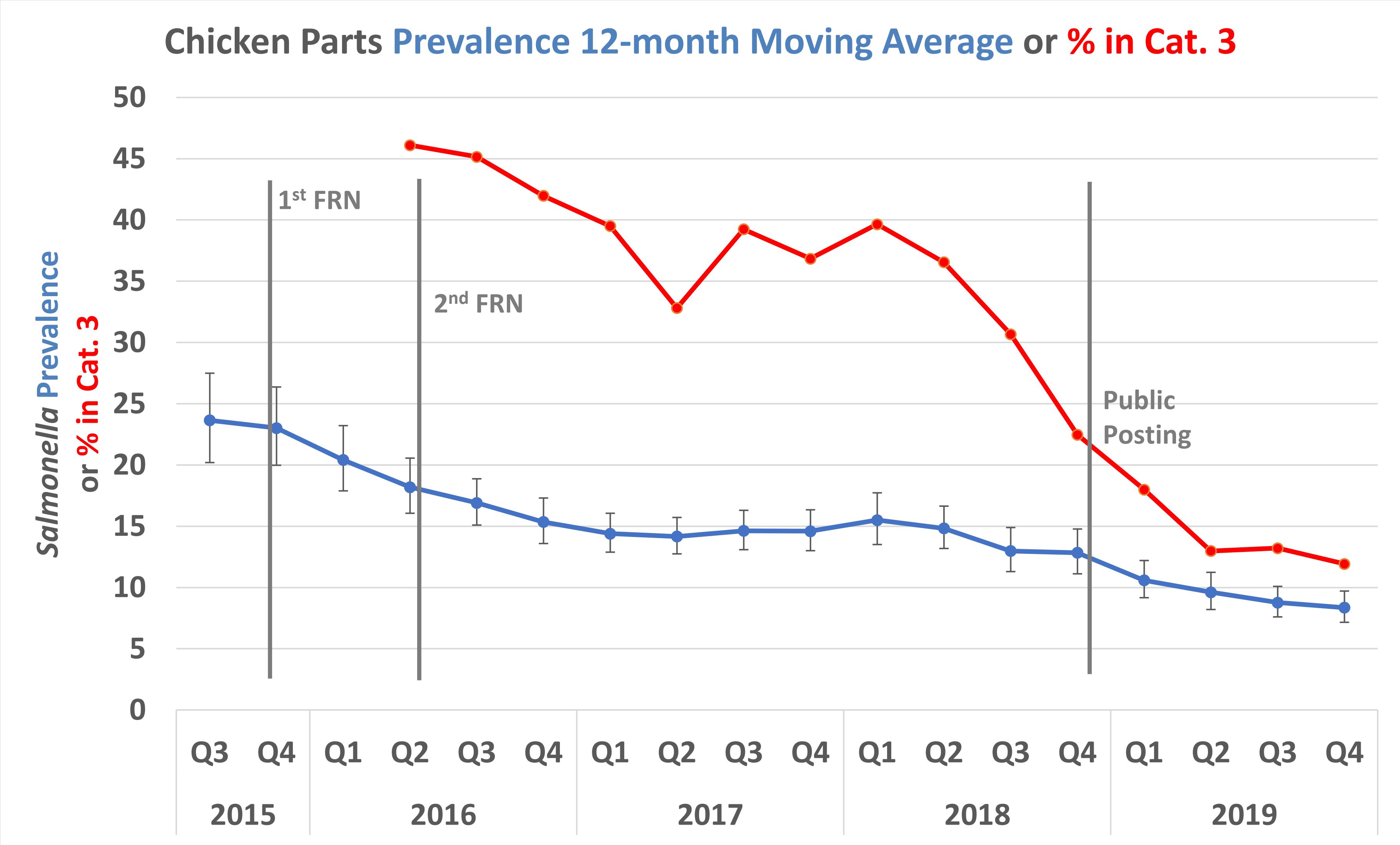
What about Performance Standards?

- *Salmonella* Baselines of Percent Positive
- Human Illness Data
- HP2030 Reduction (25%)
- Reduction of the Percentage Positive to achieve HP2030 Goals

Points to Consider-Performance Standards

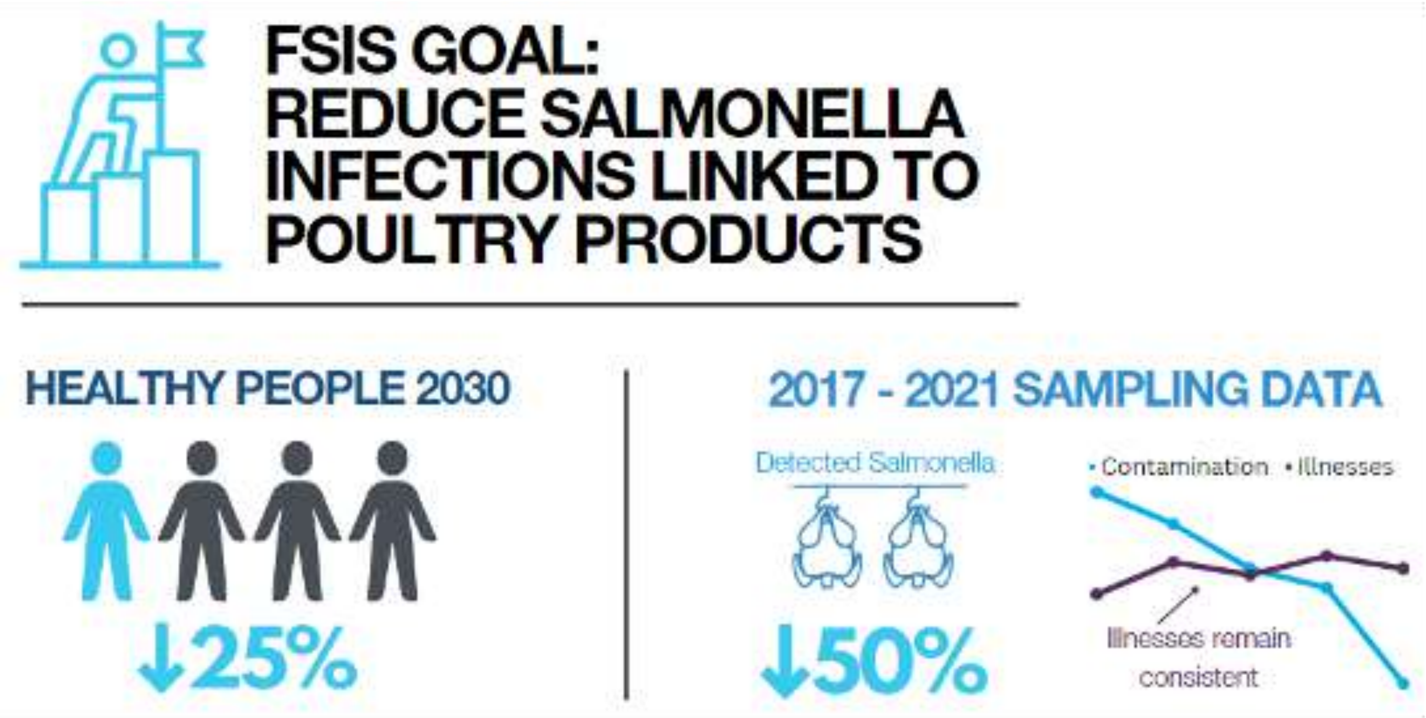
- Most Isolates are Kentucky
- Most fall below enumeration limits
- No information on Pathogenicity

Have performance standards been effective?



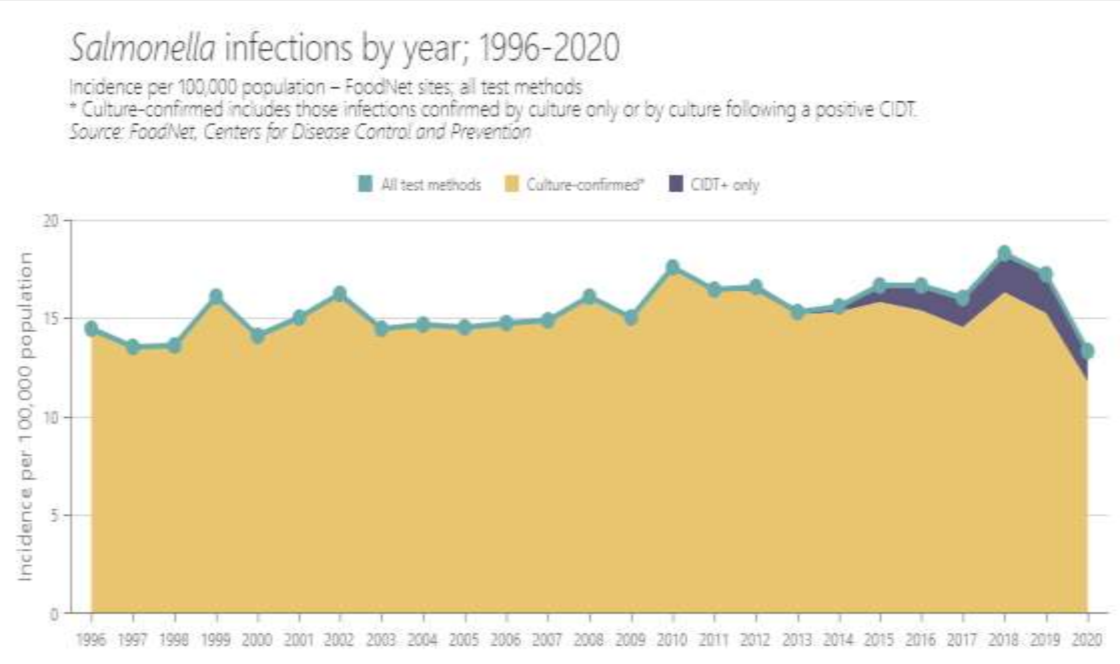
Source: FSIS

“Despite FSIS sampling data showing reductions in Salmonella contamination in poultry products, the current approach to Salmonella has not led to a demonstrable reduction in Salmonella infections.”
– FSIS Leadership, 2022



STAKEHOLDERS STATE THAT PERFORMANCE STANDARDS AREN'T WORKING…

- *Salmonella* in Poultry
  - Declines in product have been steady



Too soon to tell….

- *E. coli* O157:H7 Adulterant in 1994

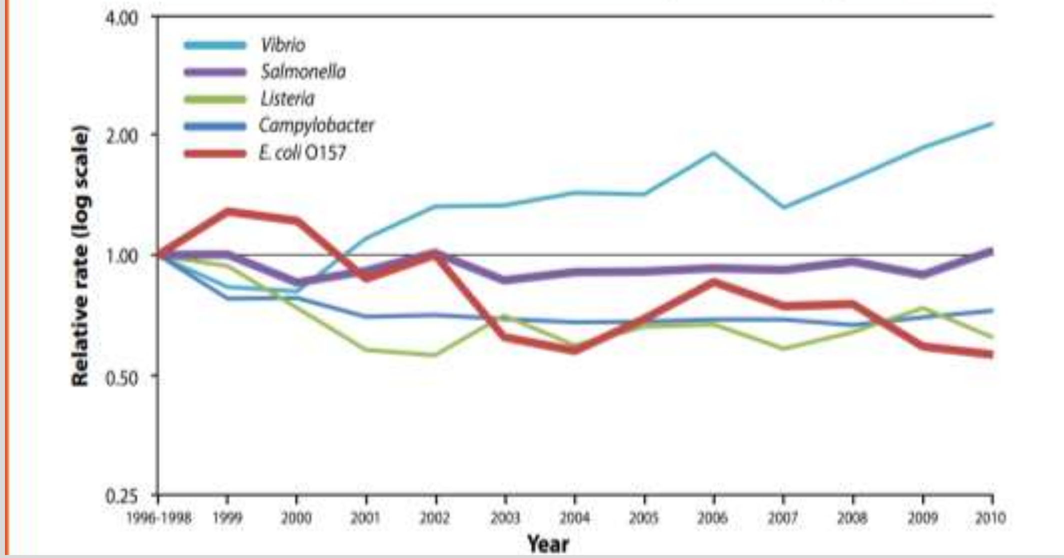


FIGURE 1. Relative rates of laboratory-confirmed infections with *Campylobacter*, *E. coli* O157, *Listeria*, *Salmonella*, and *Vibrio*, compared with 1996--1998 rates, by year --- Foodborne Diseases Active Surveillance Network, United States, 1996--2010[1]

Attribution Data Impact in 2003…

Limitations of Attribution Data

- Culture-Independent Diagnostic Tests
- Epidemiological
- Biased Consumer Perceptions
- Doesn’t consider Cross-Contamination
- Assumptions

Decision-Making

Data-Driven

- Evaluation of Process
- Strategic
- *Salmonella* Quantification
- *Salmonella* Serotypes/HP *Salmonella*
- Targeted Decision-Making based on Science and Data
- DRIVE THE NARRATIVE!

Regulatory

- Focused on One Product for Adulterant Status
- All “parts” are equal
- *Salmonella* Detection
- Little focus on Serotypes or Pathogenicity
- Changes take a LONG time

Conclusions

- Three Components of Proposed Regulatory Framework have some merit.
- Data and Science are missing to support some of the assumptions
- “They will figure it out” is a misinformed statement and is insulting to the scientific community.
- There is a discrepancy in stating that reducing *Salmonella* in poultry hasn’t resulted in a reduction in human illnesses and then implementing strategies to reduce Salmonella in poultry.
- Adulterant status in raw, breaded, stuffed chicken breasts will not impact human, public health data.

Questions