Beef Safety Breakout: Salmonella Risk Assessment & Lymph Node Research



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Impact of Peripheral Lymph Nodes on *Salmonella* Contamination in Ground Beef in the U.S.: Quantitative Microbial Risk Assessment Aided by Systematic Review and Meta-analysis

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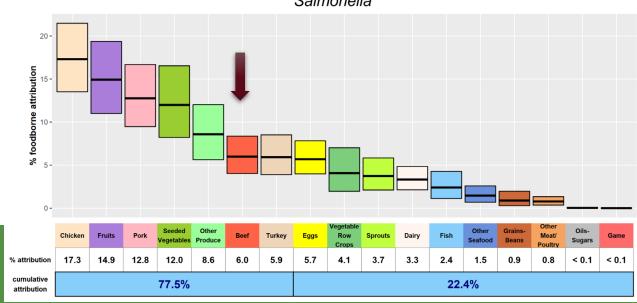
¹Department of Food Science and Technology, University of Nebraska-Lincoln ²U.S. Meat Animal Research Center, USDA ARS, Clay Center ³Department of Large Animal Clinical Sciences, College of Veterinary Medicine, Michigan State University ⁴Department of Nutrition and Food Sciences, The University of Vermont ⁵Department of Animal Science, University of California

Salmonellosis in the U.S.



- Salmonellosis in the U.S.:
 - Among the top 5 foodborne pathogens causing illness, hospitalizations, and deaths
 - 1.35 million illnesses, more than 26,000 hospitalizations, 420 deaths yearly
 - Total annual costs attributable to Salmonella: \$4.1 billion
- Source attribution (IFSAC 2020)
 - A wide variety of foods
 - Beef among the 7 highest contributing food commodities
 - Beef contribution: 6%





Salmonella

Health Risks of Ground Beef

- Not all beef products of equal risk
 - CDC, *Salmonella* outbreaks linked to beef, 2012 2019 (Canning *et al.* 2023)

	Beef (Total)	Ground Beef
Outbreaks	27	12 (44%)
Illnesses	1103	800 (73%)
Deaths	2	2 (100%)

- Outbreaks associated with ground beef have not decreased
 - Illnesses since 2016 as many as during previous 36 years
- Urgent needs in interventions at production, at slaughter and processing, and during preparation



Salmonella in Ground Beef



• Main sources: hide and peripheral lymph nodes (Koohmaraie et al. 2012)

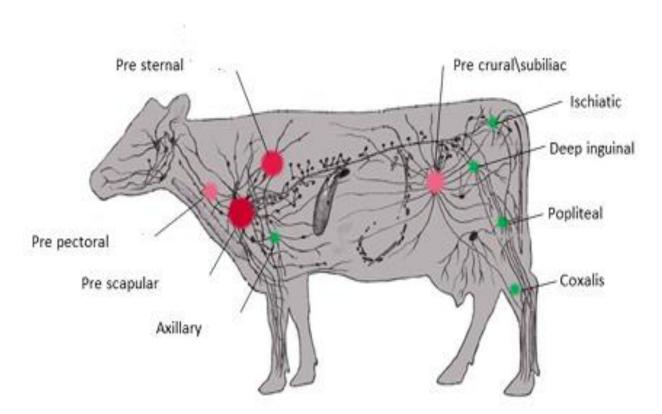
Samples	Prevalence	
Hide	96%	
Carcasses before interventions	47%	Genetic Re
Carcasses after interventions	0%	Î
Peripheral lymph nodes	18%	
Trim	7%	
Ground beef	2%	

 Minimize the hide-to-carcass transfer and inclusion of lymph nodes



Salmonella in Peripheral Lymph Nodes

- Important part of immune system, filters for foreign particles
- Mesenteric lymph nodes vs PLNs.
- PLNs can harbor pathogens, including *Salmonella*.
- Important contamination source of *Salmonella* in ground beef.





Goal and Specific Objectives



- **Risk management goal:** Determine the contribution of endogenous extraintestinal sources of *Salmonella* (i.e., lymph nodes) to reduce foodborne infections caused by *Salmonella*.
- **Objective 1.** Characterize the distribution of both prevalence and concentration of *Salmonella enterica* in bovine PLNs by lymph node type, production source, region, and season.
- **Objective 2.** Assess the relative contribution of PLNs to the public risks imposed by the consumption of ground beef, and identify critical control points in the slaughter-to-table continuum in reducing human salmonellosis associated with the consumption of ground beef in the US.



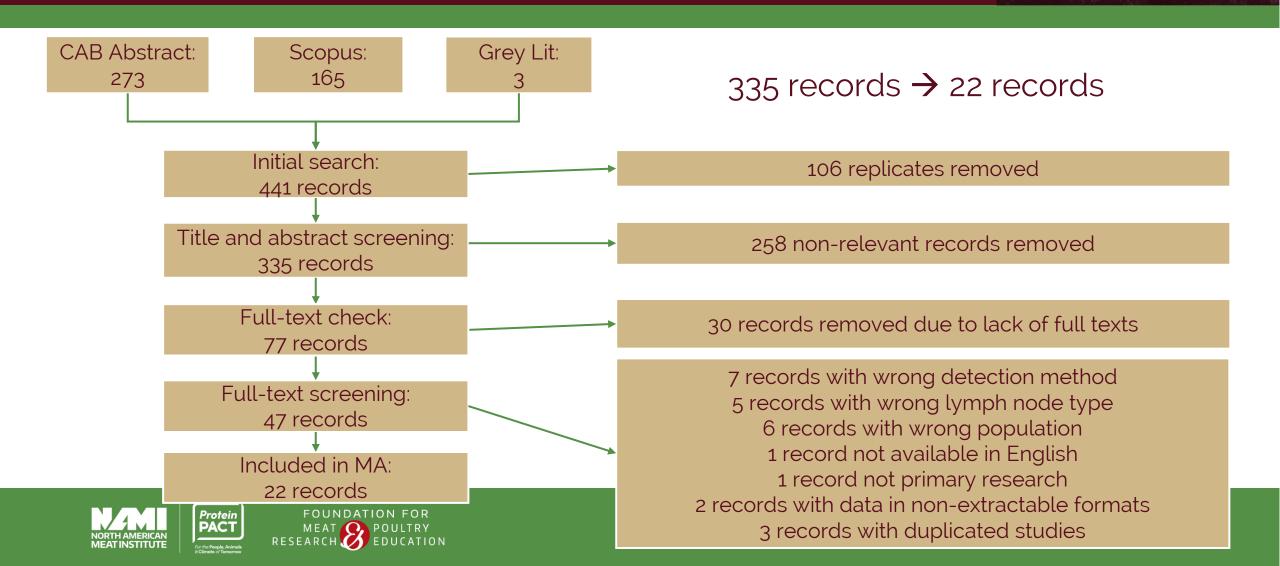


Obj. 1 Distribution of Salmonella in PLNs

- Method: Systematic review and meta-analysis
- **Review question**: "*What are the prevalence and levels of* Salmonella *in PLNs of beef and dairy cattle*?"
- Searching strategy and eligibility determination
 - CoCoPop: Salmonella, PLNs, beef and dairy cattle, in the U.S.
 - Electronic databases (Scopus and CAB Abstracts) and grey literature
 - Stepwise screening
 - Quality assessment: appraisal tool for prevalence studies with modified questions
- Data synthesis \rightarrow Obj. 2
 - Random-effects meta-analysis using 'metaphor' in R

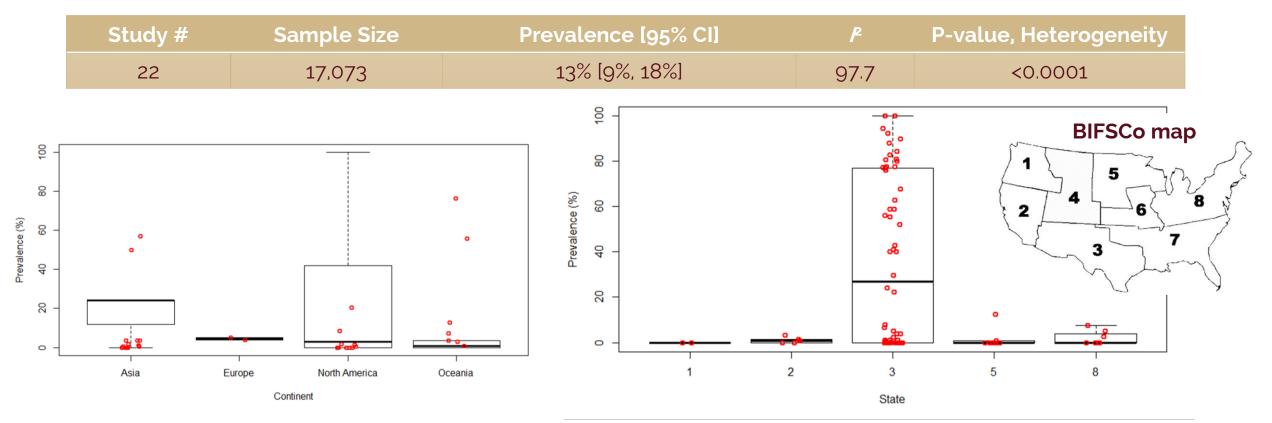


Flowchart of Systematic Review



Overall Prevalence in PLNs





Majority of studies (14/22, 63.6%) were in the U.S., showing a prevalence of 16% (12%,23%).



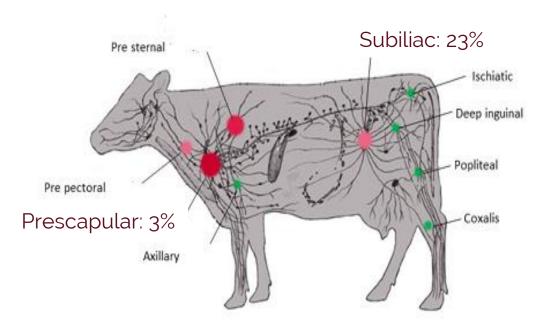
Prevalence by LN Type and Season

LN Type	Study #	Sample Size	Prevalence %
Subiliac	10	7019	23 (13, 33)
Iliofemoral	2	184	34 (0, 100)
Retropharyngeal	1	28	4 (0, 15)
Prescapular	1	240	3 (0, 10)
Prefemoral	1	240	2 (0, 2)
Unspecified	4	6073	7 (1, 15)

Season	Study #	Sample Size	Prevalence %
Cool	7	5827	3 (1, 6)
Warm	10	5717	11 (5, 18)







Serotype and Antibiotic Resistance

Serotype	Study #	Sample Size	Serotype %
<i>S</i> . Enteritidis	1	283	25%
<i>S</i> . Cerro	6	806	17%
<i>S</i> . Montevideo	6	869	17%
S. Reading	2	347	16%
<i>S</i> . Anatum	7	887	15%
<i>S</i> . Mbandaka	4	726	14%
<i>S</i> . Typhimurium	6	383	13%
<i>S</i> . Muenchen	4	780	10%
<i>S</i> . Kentucky	4	787	9%
<i>S.</i> Lille	2	12	8%
<i>S</i> . Brandenburg	2	148	6%
<i>S</i> . Give	2	38	5%
<i>S</i> . Infantis	2	345	1%

Antibiotics	Study #	Sample Size	Resistance %
Neomycin	1	5	60%
Multi-drug resistance	3	290	37%
Tetracycline	3	290	36%
Streptomycin	3	290	35%
Chloramphenicol	3	290	33%
Ampicillin	3	290	25%
Sulfa-trimethoprim	2	23	24%
Cefoxitin	3	290	19%
Ceftiofur	3	290	18%
Gentamycin	3	56	17%
Ceftriaxone	3	290	16%
Kanamycin	2	51	10%
Sulfioxazile	1	266	9%

Lack of Data on Concentration

- Only three studies (vs 22 reporting prevalence data).
- Diverse reporting styles: tabular vs graphical, on gram or LN basis.
- The *Salmonella* concentration of overall studies was found as 1.5 log cfu/g (95%CI: 0.5, 2.8 log cfu/g) according to 838 sample size.
- Not enough data for a stratified analysis.



Obj. 2 Impact of PLNs

- Quantitative microbial risk assessment
 - Modular Process Risk Model
- Slaughter to table
 - Fabrication and trimming
 - Grinding and partitioning
 - Transport and storage
 - Cooking

MEATINSTITUTE

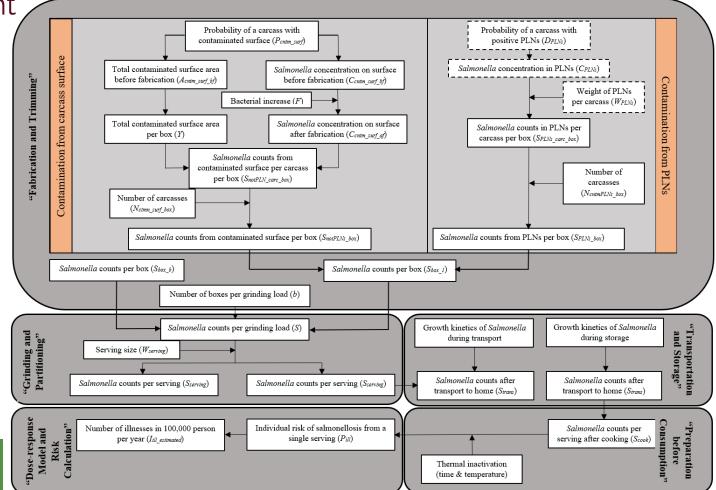
• Dose-response model

Protein PACT

- Risk characterization
 - # of illnesses / 100,000 population

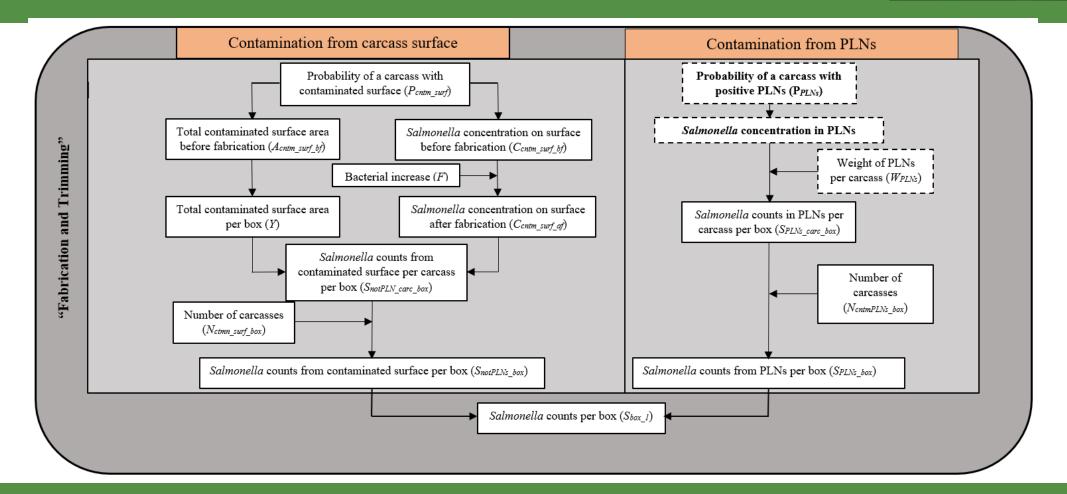
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MEAT POULTRY RESEARCH BOUCATION



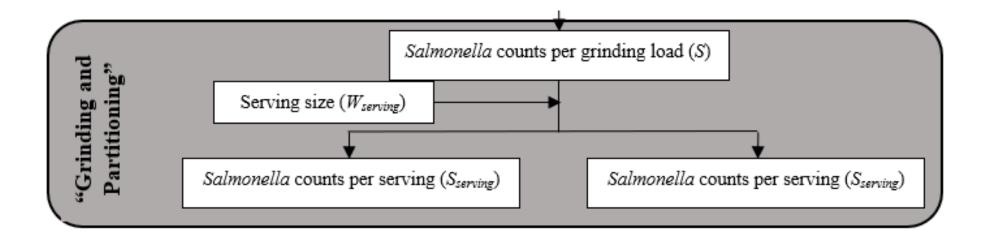


Module – Fabrication & Trimming





Module – Grinding & Portioning

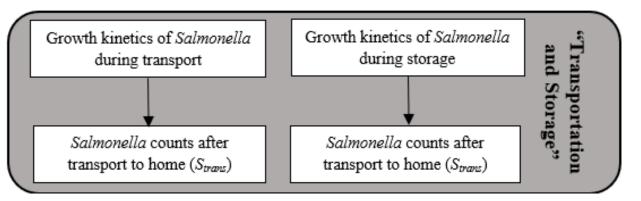


Assumptions

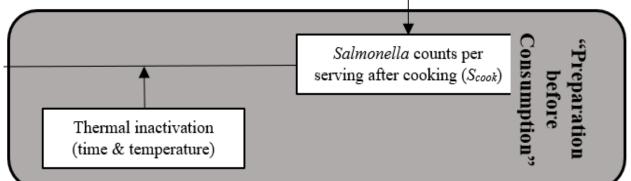
- *Salmonella* population remained constant due to qualified control of the cold temperature during processing.
- The distribution of *Salmonella* in ground beef is homogeneously distributed.



Modules – Transport, Storage & Cooking



- *Salmonella* population remained constant due to qualified control of the cold temperature during
 - Transport from plant to retail
 - Storage at retail



- No effect of cross-contamination
 - Ground beef is generally cooked without cutting or washing steps



Baseline Risk Estimate

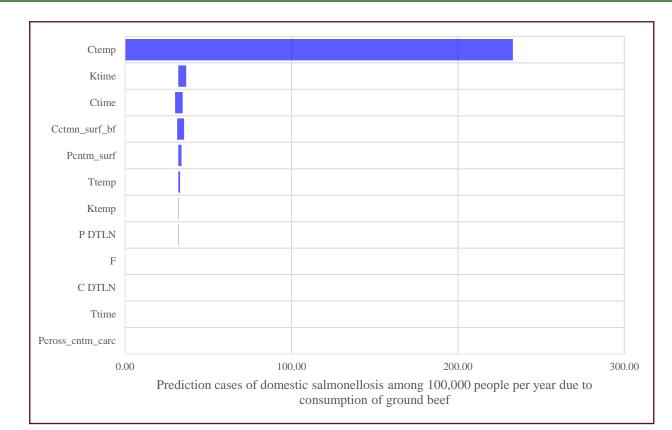


- Population risk of the predicted number of salmonellosis cases among 100,000 persons in U.S. because of consumption of ground beef was estimated as 45.0 cases (90% CI: 22.9-67.2).
 - Highly skewed to the right with the 25th percentile, median, and 75th percentile values of 0, 0, and 0, resulting in a great difference between median and mean values.
- Model validation: Back calculation from CDC estimate, 1.35 million cases and 6% attributable to beef → 24 / 100,000.



Influential Factors

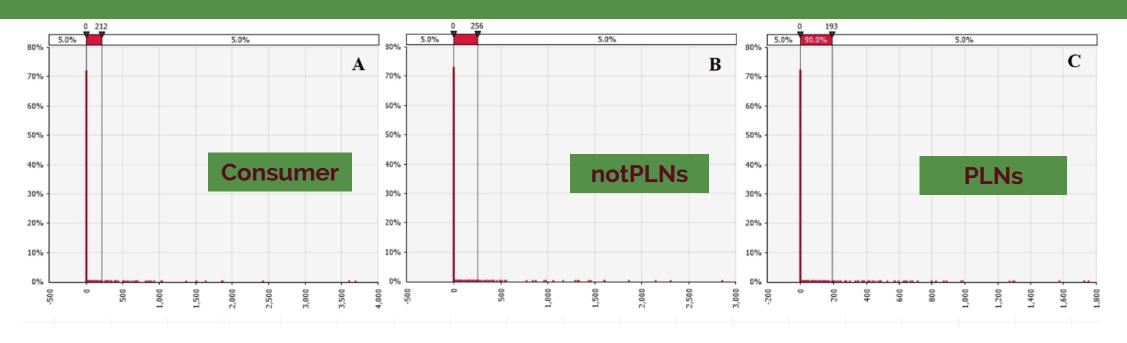




- Sensitivity analysis
- Top factors: Cooking Temperature and time, Storage time
- Surface contam > PLNs



Impact of PLNs and Other Sources



Ranges: 0-4000 for consumer

0-3000 for notPLNs

0-1800 for PLNs

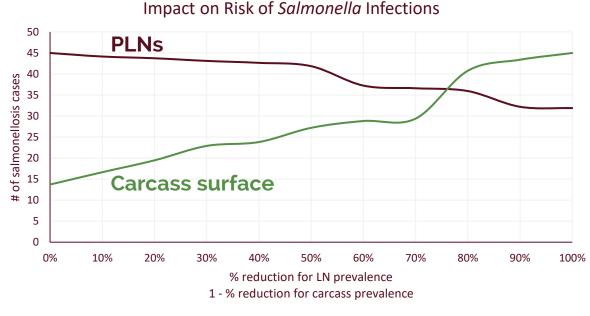
The frequency distributions of the predicted average number of human salmonellosis cases due to the variation of input variables grouped by different sources of contamination



Impact of PLNs and Carcass Surface

Based on our model,

- ~30% of salmonellosis cases related to ground beef can be attributable to the contamination in PLNs.
- 0.5 log \downarrow in PLNs \rightarrow 20% \downarrow in cases.
- 0.5 log ↓ on carcass surface → 40% ↓ in cases.



- Reduction in LN Prevalence - Reduction of Carcass Prevalence



Take Home Messages



- Detection of Salmonella in bovine PLNs is not uncommon.
- Reduction of *Salmonella* in PLNs can be associated with noticeable reduction in salmonellosis cases posed by ground beef consumption.
- Interventions to control *Salmonella* on carcass surface may be more effective.
- More factors need to assessed, such as costs, technology, and feasibility.
- Model improvement: more concentration data, data allowing for differentiation in virulence.



Acknowledgements



 This study was supported by a grant from the Beef Checkoff administered by the Foundation for Meat and Poultry Research and Education

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• Dr. Bing Wang bing.wang@unl.edu FOUNDATION FOR MEAT POULTRY RESEARCH EDUCATION



The University of Vermont UNIVERSITY OF CALIFORNIA

Salmonella control in beef production risk-based methods and virulence

Dr. Francisco J Zagmutt DVM, MPVM, PhD

Dr. Jane G. Pouzou MPH, PhD





ANALYTICS Salmonella serovars exhibit different

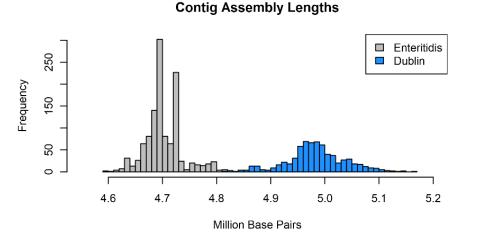
"virulence"

Hospitalization %	Mortality %
<i>S.</i> Dublin ≈ 67%	<i>S.</i> Dublin ≈ 3.0%
S. Enteritidis ≈ 21%	S. Enteritidis ≈ 0.6%

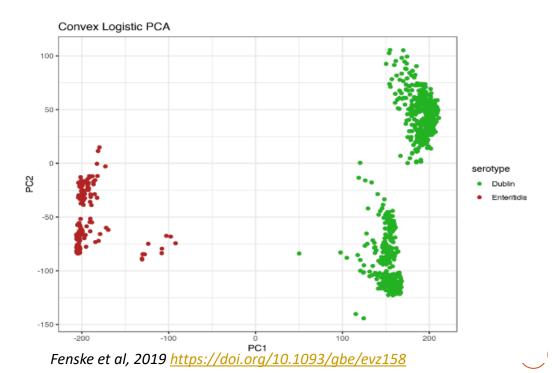
Jones et al, 2008 https://doi.org/10.1086/588823

Between and within serovar variation in genome composition

Virulence Factors (VF) - Genes that facilitate pathogenesis. Mechanisms not fully understood. Interactions even less.



Pangenome PCA S. Dublin vs Enteriditis



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Why don't we just target top serovars in reported foodborne illnesses?

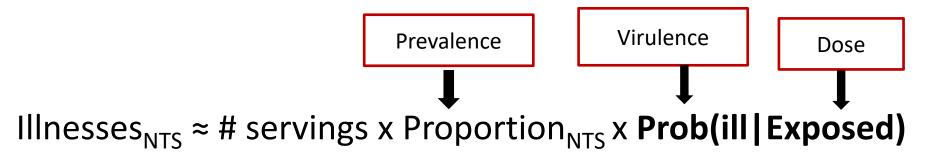
"Foodborne illness surveillance and monitoring systems **only detect a fraction of all cases** that occur, and **most** reported cases do not have a known source. Also, infrequent large events can have a marked effect on the case count in a given year. This adds statistical "noise" to the numerical signal needed to detect temporal changes in foodborne salmonellosis trends and its main food sources."

NACMCF (2023) Response to Questions Posed by the Food Safety and Inspection Service: Enhancing Salmonella Control in Poultry Products https://www.fsis.usda.gov/sites/default/files/media_file/documents/NACMCF%20Salmonella-Poultrv17Mar2023.pdf

Ebel et al (2017)

- 4% confidence detect 10% \downarrow ill **1 yr**
- <53% chance detecting 30% \downarrow ill sustained **4 years**

https://www.sciencedirect.com/science/article/abs/pii/S2352352217301354



Takeaways:

- Detecting risk trends takes time
- Prevalence and load matters

... can we use risk-based methods to achieve greatest public health gains?

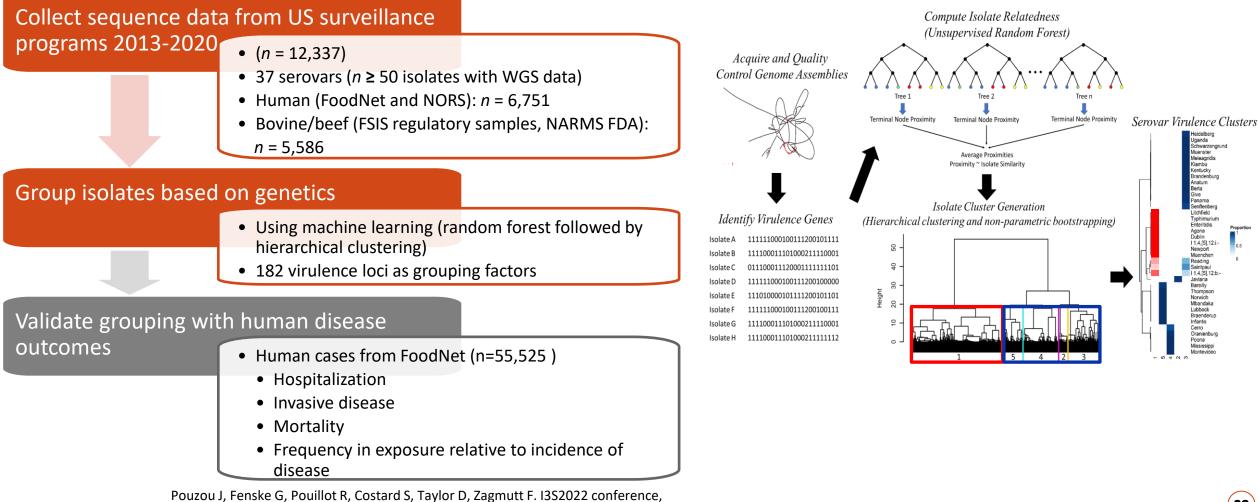


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Grouping isolates/serovars by virulence (Patent pending)

Results using the beef foodborne pathway shown here,

upcoming work incorporating other food sources



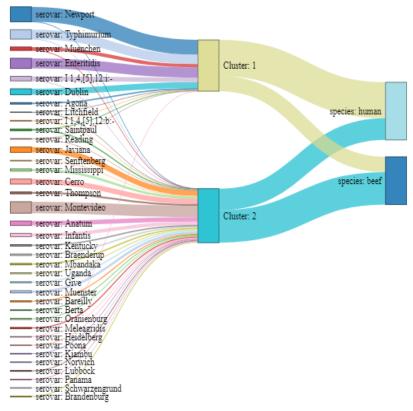
St Malo, France. https://www.i3s2022.com/content.php?PAGE=11

serovar: Norwich serovar: Lubbock serovar: Panama serovar: Schwarzengrund serovar: Brandenburg © EpiX Analytics LLC

ANALYTICS Higher (HV) and Lower Virulence (LV) groups Epi validation resulted in HV and LV groups, allocated isolates to

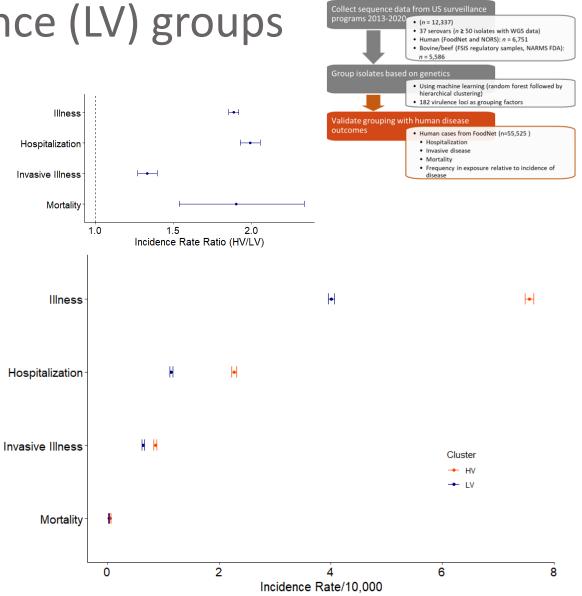
- serovar using majority rule Most often implicated in foodborne outbreaks of any cause AND
- In beef-associated outbreaks but not most common in beef

Relative appearance in beef (HV/LV): 0.44 (0.37 - 0.53)

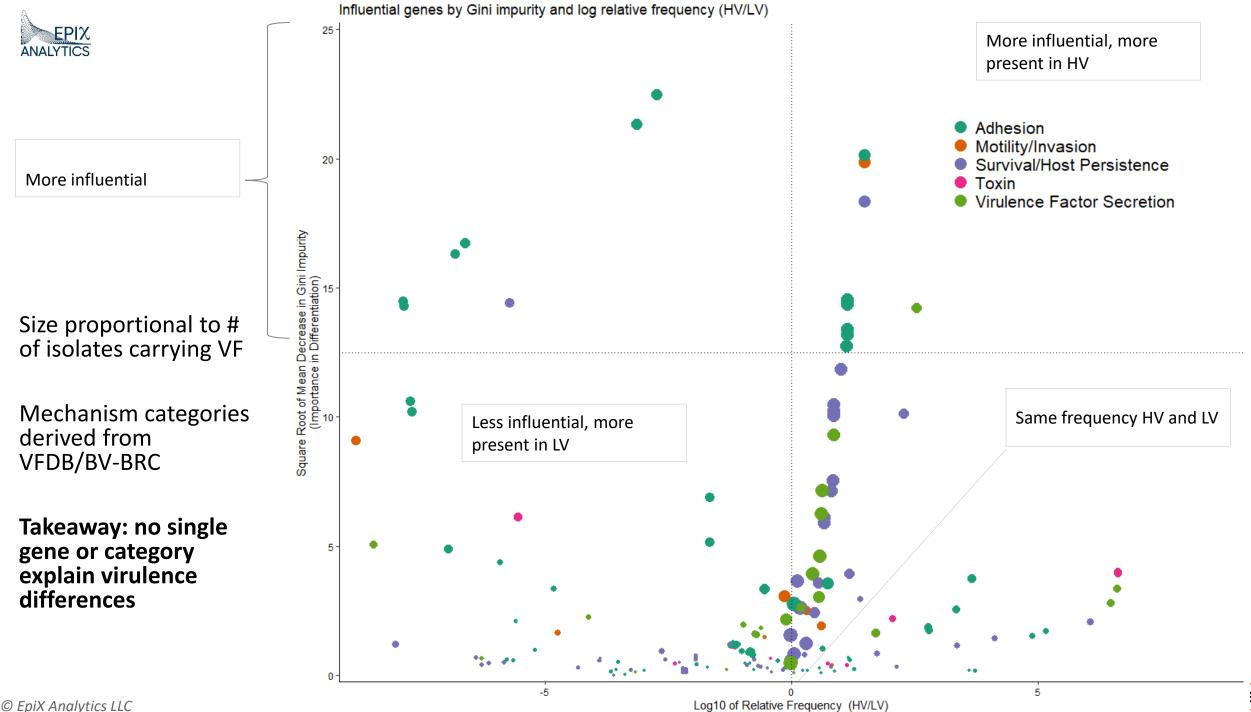


Higher virulence

Typhimurium Enteritidis | 1,4,(5),12:i:-Newport Muenchen Paratyphi B Dublin Minority groups of St. Paul and Reading Agona Litchfield



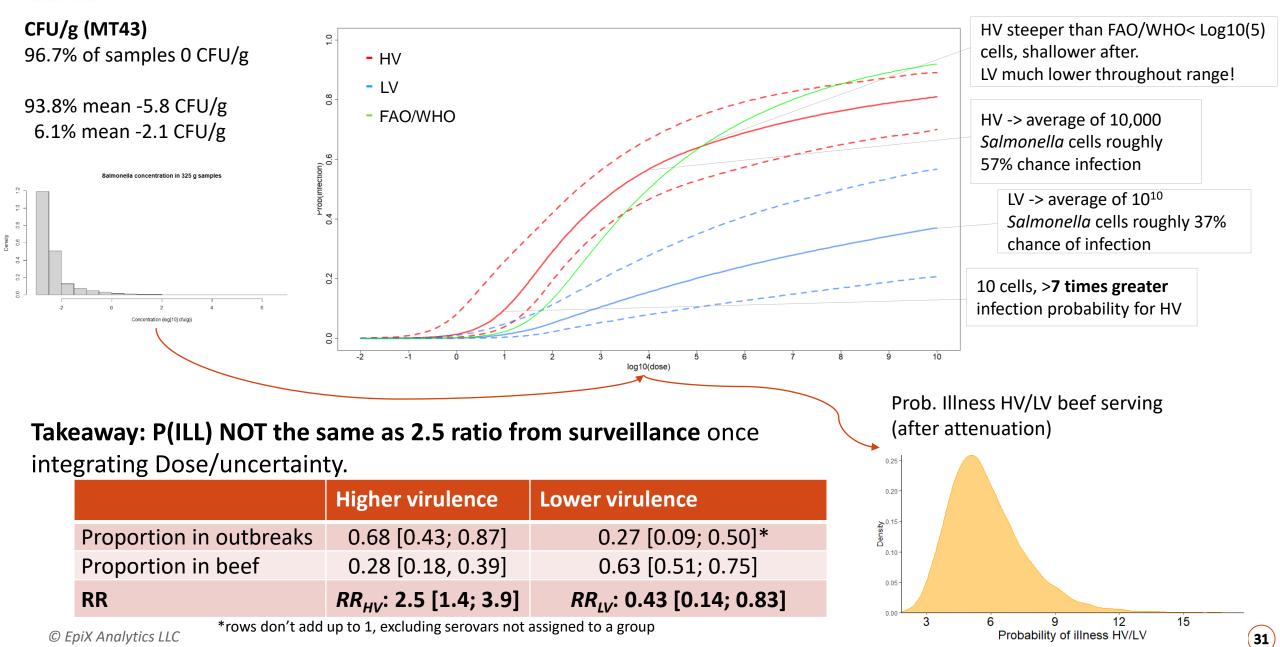
Gavin J. Fenske, Jane G. Pouzou, Régis Pouillot, Daniel D. Taylor, Solenne Costard, Francisco J. Zagmutt medRxiv 2022.12.13.22283417; doi: https://doi.org/10.1101/2022.12.13.22283417



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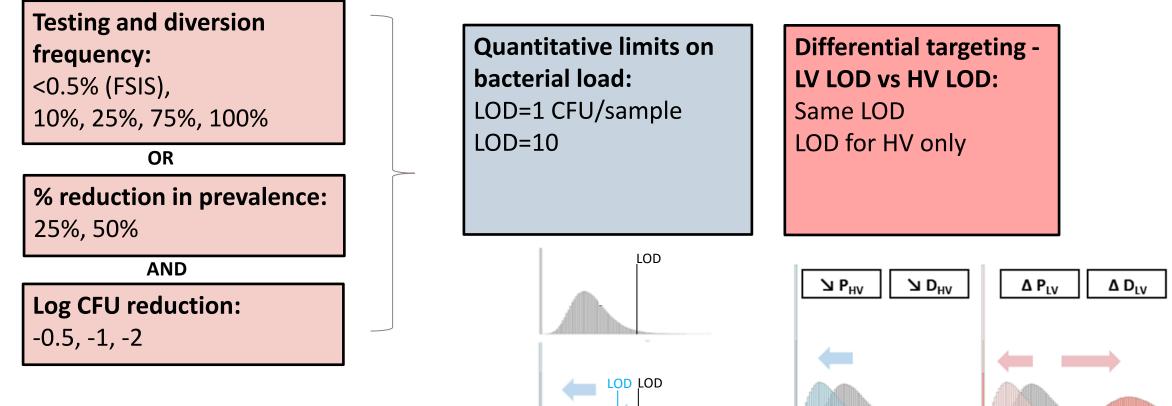
Connecting load to illnesses, by virulence



Modeling salmonella interventions and illness reduction

Quantitative microbial risk assessment using empirical prevalence and load combined with virulence-specific DR to (ground beef in US as model case)

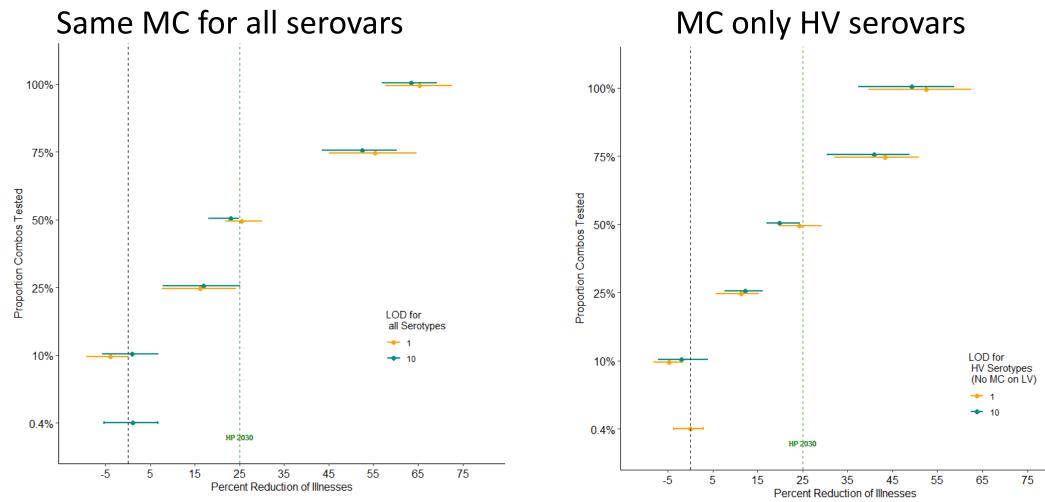
- Outcomes: reduction of illnesses vs baseline, diversion of positive products
- Scenarios combine:



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ANALYTICS

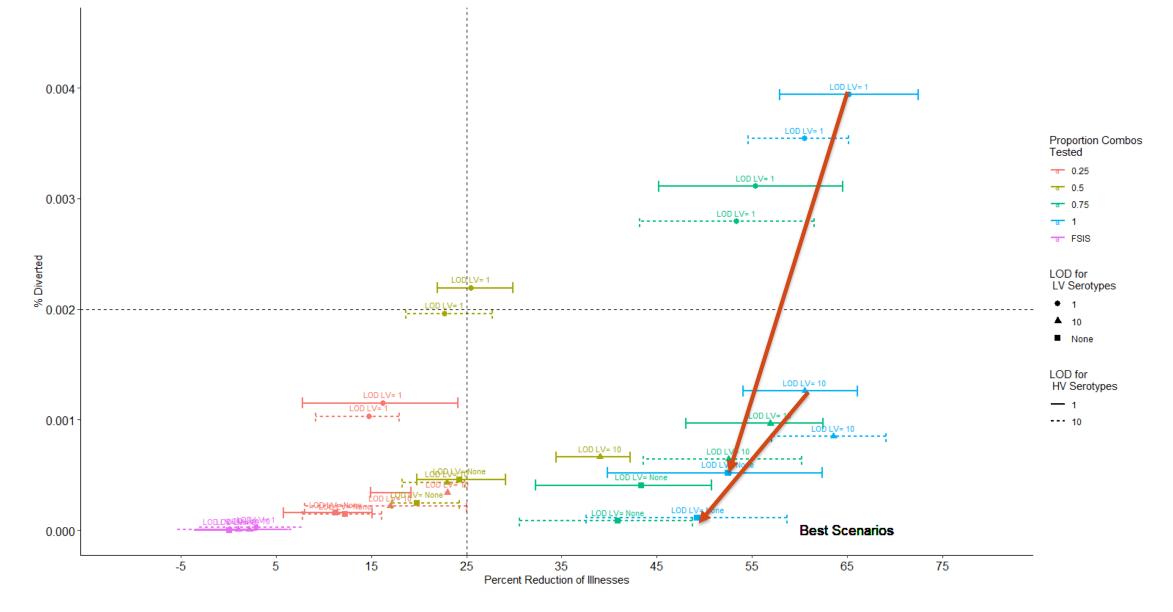
Test and diversion scenarios Targeting levels, HV/LV, sampling proportions Baseline: any Salmonella sp. positive diverted (stricter than current FSIS MC)



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ANALYTICS Illness reduction vs diversions

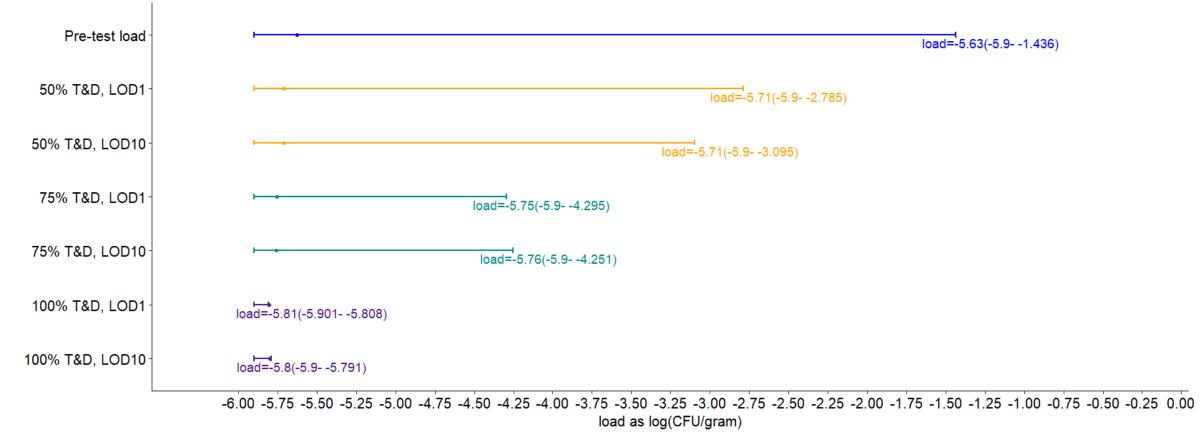
Takeaway: targeting HV equivalent risk reduction with less diversions



Effect of test and diversion on salmonella load

Test and diversion can achieve > 25% illness reduction, but diversions may not be feasible for all processors

Can we achieve similar/better risk reduction by lowering prevalence and/or load via other methods?

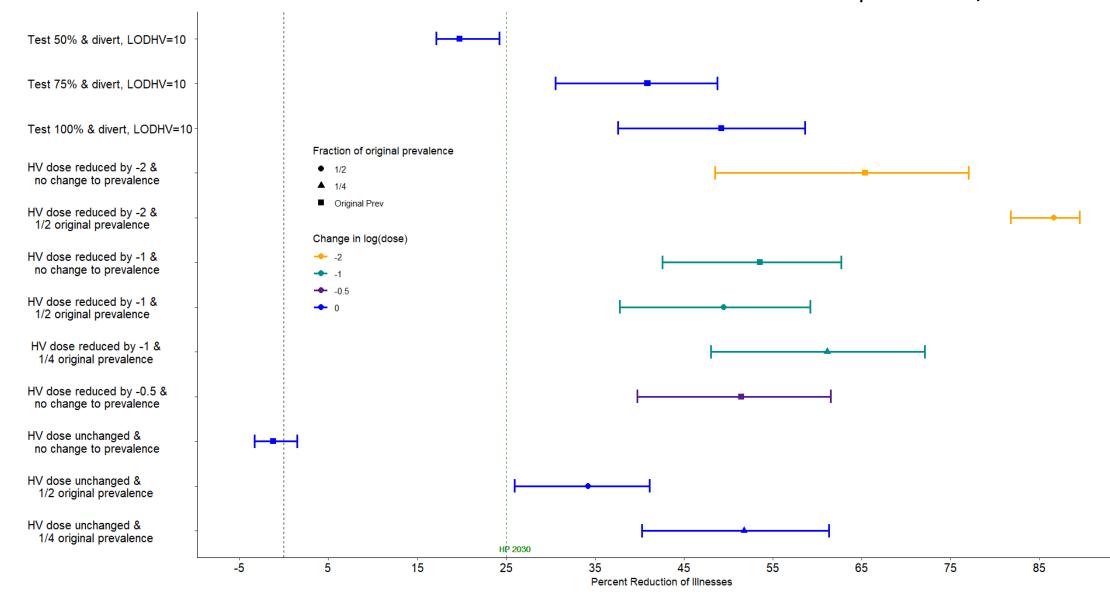


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ANALYTICS

ANALYTICS Prevalence/load reduction scenarios

Takeaway: equivalent results to test/divert by targeting prevalence/load



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NALYTICS Conclusions/practical applications

- Genomic identification of highest-risk serovars is feasible, faster than waiting to see changes in public health surveillance data
- Test and diversion works at higher testing proportions, but other strategies reducing prevalence/load can also result in significant public health gains

Targeting highest risk sources and adapting programs to evolving risks (emerging, seasonal, source-based) can be more cost-effective while achieving significant PH gains

 Diagnostic tests that can be quickly deployed to identify emergent threats will be important, but also data sharing efforts to best detect and manage risks

Predicting and devising **optimal plans at individual plant level might provide** highest salmonellosis prevention while being cost-effective



EpiX team Dr. Jane Pouzou Dr. Dan Taylor Dr. Régis Pouillot Dr. Solenne Costard Collaborator: Dr. Gavin Fenske For any questions or comments, please contact:

Dr. Francisco Zagmutt Managing Director EpiX Analytics <u>fzagmutt@epixanalytics.com</u>

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- FoodNet Data: The findings and conclusions in this report are those of the author(s) and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

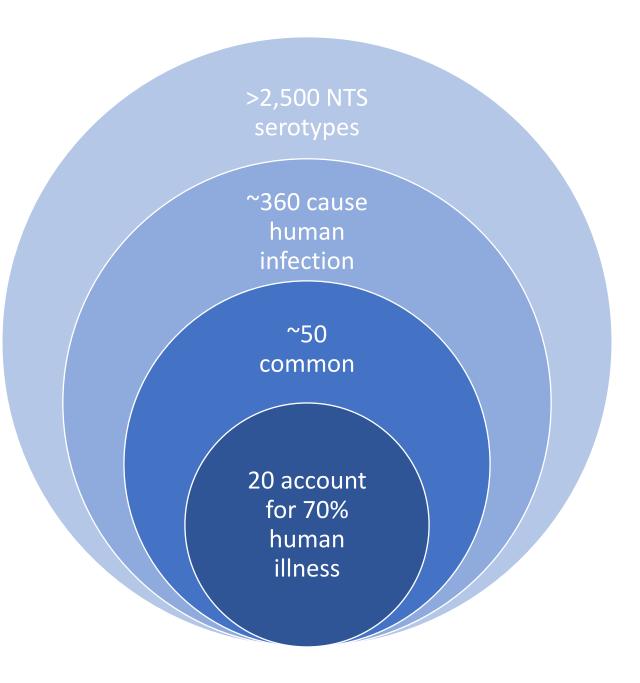
Beef Safety- Salmonella Outbreaks and Risk Assessment

Ali Strickland, Fernando Sampedro, Craig Hedberg UMN School of Public Health

> MINNESOTA INTEGRATED FOOD SAFETY CENTER OF EXCELLENCE

> UNIVERSITY OF MINNESOTA . MINNESOTA DEPARTMENT OF HEALTH

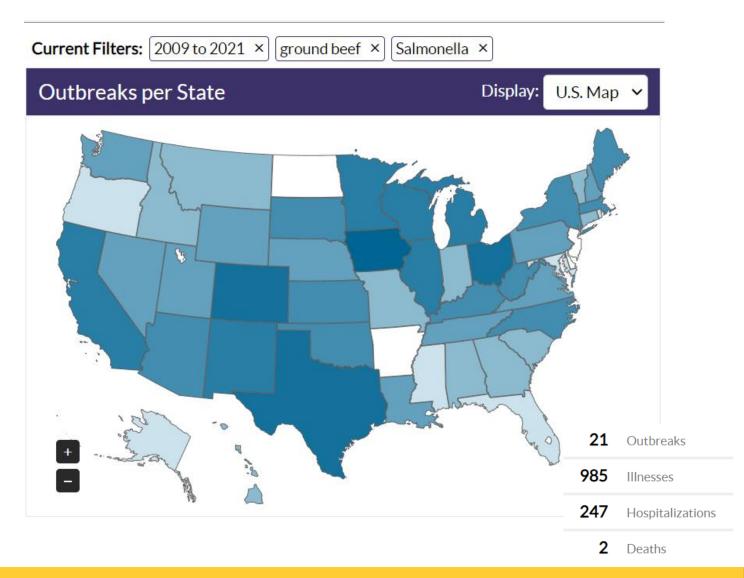
CDC. National *Salmonella* Surveillance Overview. Atlanta, Georgia: US Department of Health and Human Services, CDC, 2011.





Salmonella Outbreaks and Risk Assessments

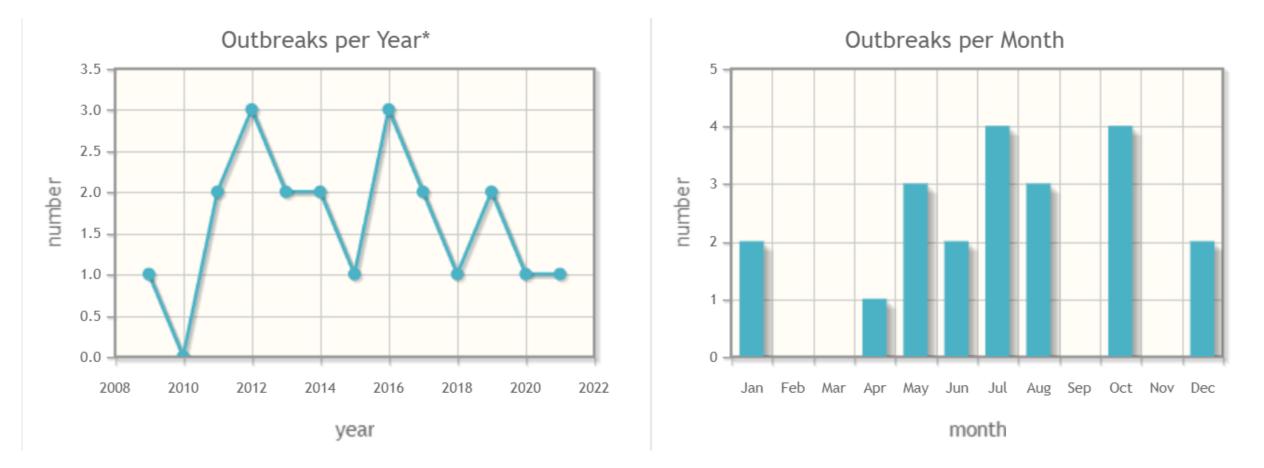
- Overview of public health surveillance
 - Transformative effect of whole genome sequencing
- Importance for source attribution and risk assessments
- Impact for industry





https://wwwn.cdc.gov/norsdashboard/

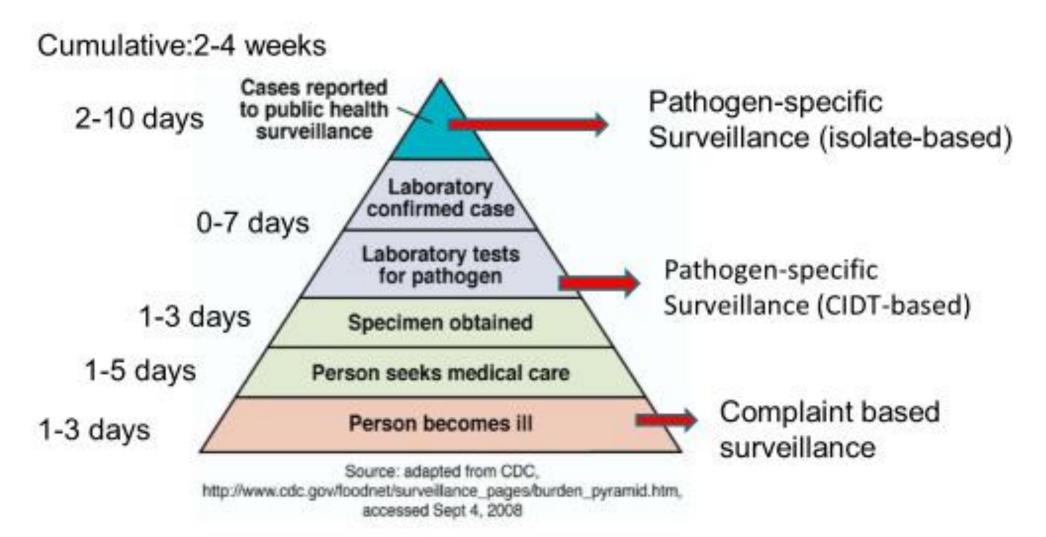
Salmonella Outbreaks Associated with Ground Beef, US, 2009-2021





https://wwwn.cdc.gov/norsdashboard/

Primary Sources for Outbreak Detection

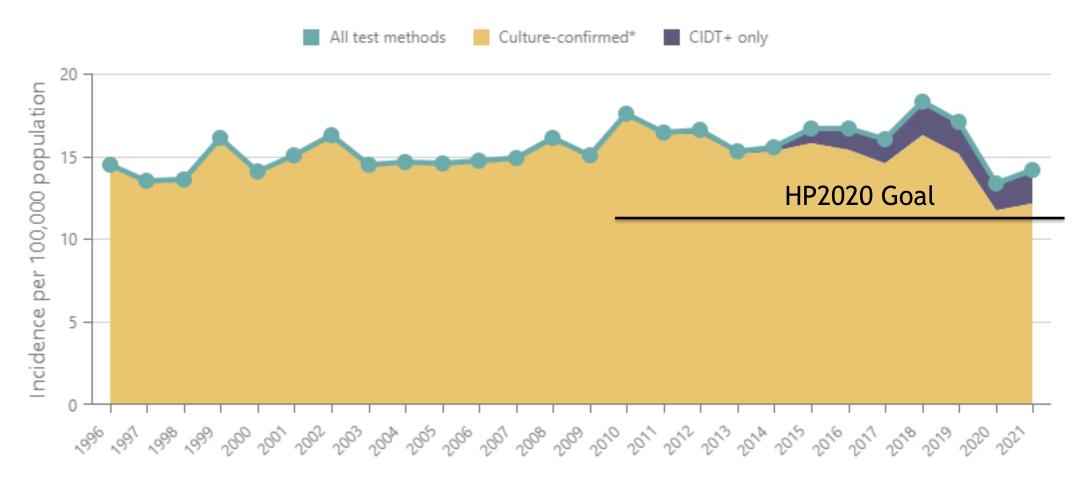




Salmonella infections by year; 1996-2021

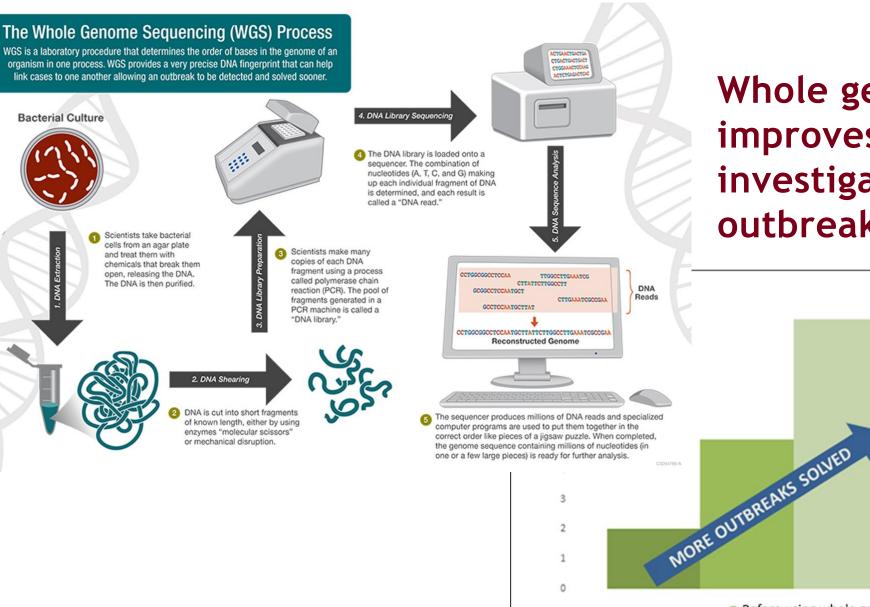
Incidence per 100,000 population – FoodNet sites; all test methods * Culture-confirmed includes those infections confirmed by culture only or by culture following a positive CIDT.

Source: FoodNet, Centers for Disease Control and Prevention





https://www.cdc.gov/foodnet



Whole genome sequencing improves the detection and investigation of foodborne outbreaks



Before using whole genome sequencing (WGS) (Sept 2012-Aug 2013)

Year 1 of WGS (Sept 2013-Aug 2014)

Year 2 of WGS (Sept 2014-Aug 2015)







Increasing the specificity of food exposure information provided by casepatients is as important as increasing the specificity of the case definition.

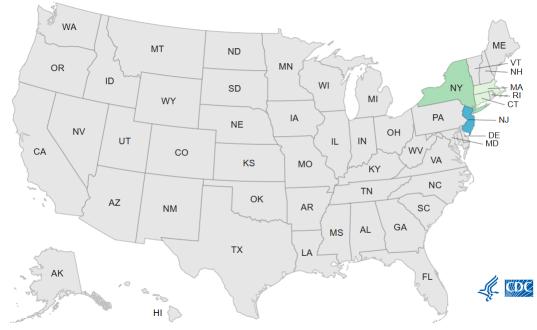


Team Diarrhea

The National Molecular Subtyping Network for Foodborne Disease Surveillance

Julse Vetus 17 au 18 he National Molecular Subtyping Ne r Foodborne Disease Surv WASHINGTON NORTH DAKOTA OREGON Philadelphia IDAHO City SOUTH DAKOTA IOW/ NEVADA NEBRASKA CO ILLINOIS ()City CALIFORNIA KANSAS Los Angeles KENTUCKY County OKLAHOMA San Diego County National Pattern NEW MEXICO ARKANSA CDC CDC Database TEXAS Area Laboratories (7) Tan E 00 State Laboratories (50) City of Houston County/ City Laboratories(5) 😥 USDA-FSIS Laboratory 🛛 兽 FDA-CFSAN Laboratory 🔶 FDA-CVM Laboratory 🔘 FDA-ORA Laboratory FoodNet Participant

Salmonella Outbreak Linked to Ground Beef



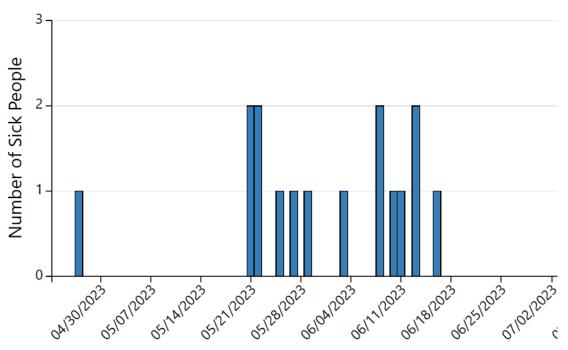
August 2002





Salmonella Outbreak Linked to Ground Beef

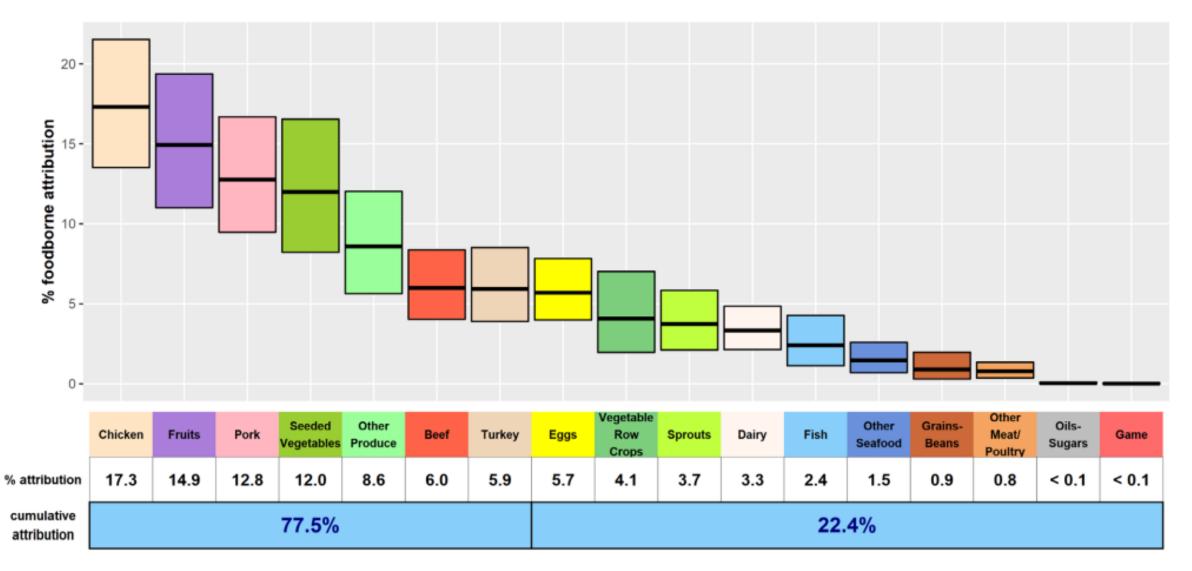
- 16 ill
- Age 0 to 97 years, 19% under 5 years
- 56% female
- 14 interviewed, 9 (64%) ate ground beef, all named same grocery store chain.
- Routine FSIS ground beef surveillance sample in March 2023 was closely related to bacteria from sick people's samples.





https://www.cdc.gov/salmonella/i45-10-21

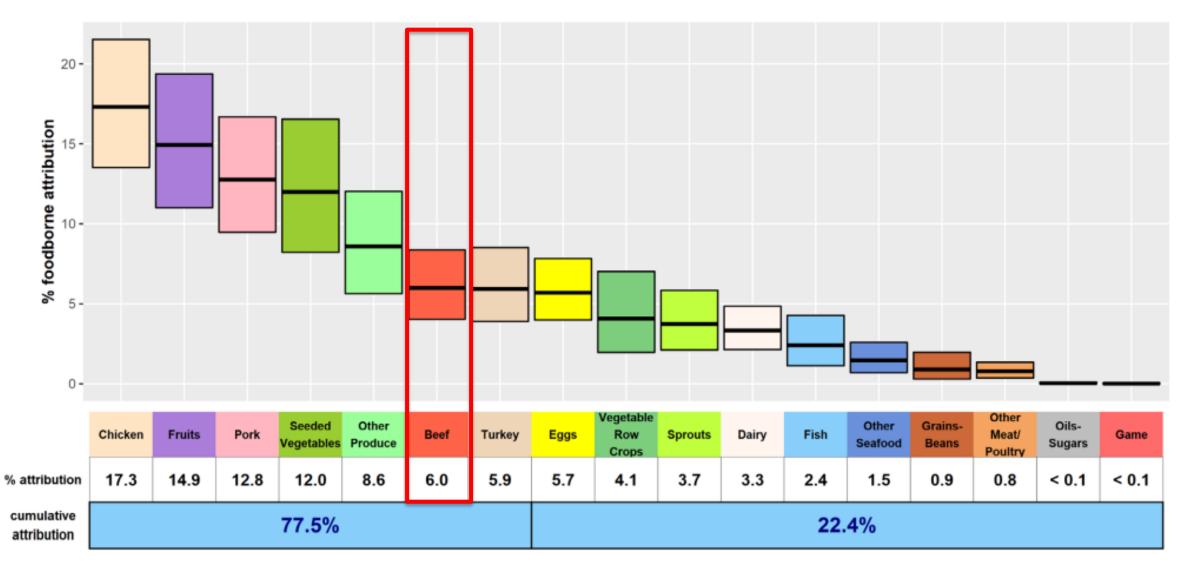
Salmonella Attribution to Food Categories, 2020





https://www.cdc.gov/foodsafety/ifsac/pdf/P19-2020-report-TriAgency-508.pdf

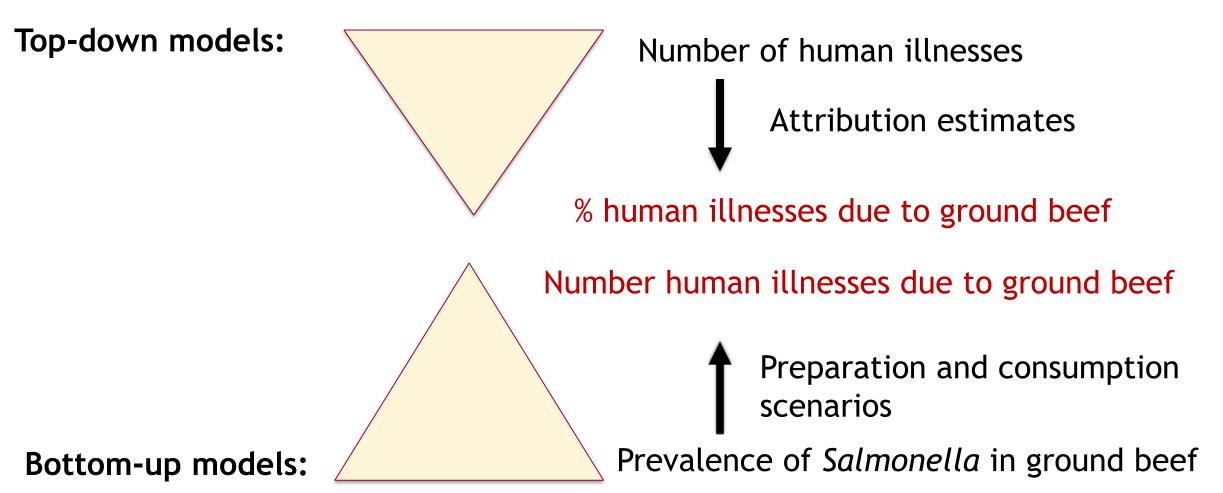
Salmonella Attribution to Food Categories, 2020





https://www.cdc.gov/foodsafety/ifsac/pdf/P19-2020-report-TriAgency-508.pdf

Risk Assessment Models





FSIS Raw Product Sampling and Testing

- 52 week "moving window" testing approach
- Frequency dependent upon daily production volume
- Increased NTS prevalence in ground products
- HACCP Plans







USDA FSIS Quarterly Sampling Reports on Salmonella. Q1, 2023

Product	Number of Samples	Number of Positives	Percent Positive
Young Chicken Carcasses	2442	117	4.79 %
Chicken Parts (legs/breast/wings)	3698	306	8.27%
Comminuted Chicken	471	127	26.96 %
Mechanically Separated Chicken	30	26	86.67%
Total for Raw Chicken	6663	580	8.70%
Young Turkey Carcasses	412	0	0.00%
Comminuted Turkey	301	50	16.61%
Mechanically Separated turkey	22	12	54.55%
Total for Raw Turkey	735	62	8.44%
Raw ground beef - Retail	126	5	3.97 %
Raw ground beef	2617	28	1.07%
Total for Raw Beef	4302	76	1.77%
Comminuted pork	1545	246	15.92%
Pork Cuts	576	41	7.12%
Total for Raw Pork	2121	287	13.53%



https://www.fsis.usda.gov/science-data/data-sets-

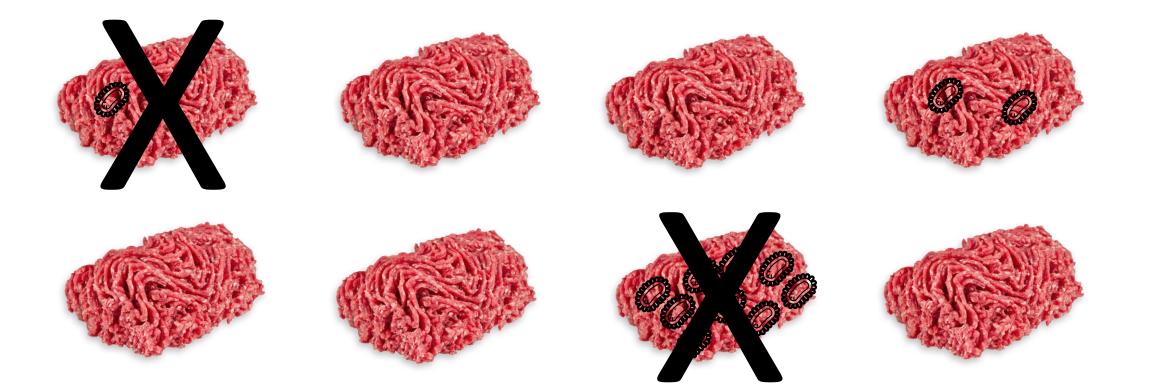
visualizations/microbiology/microbiological-testing-program-rte-meat-and-7

Prevalence: 3/8 = 37.5%





Prevalence: 1/6 = 16.7%

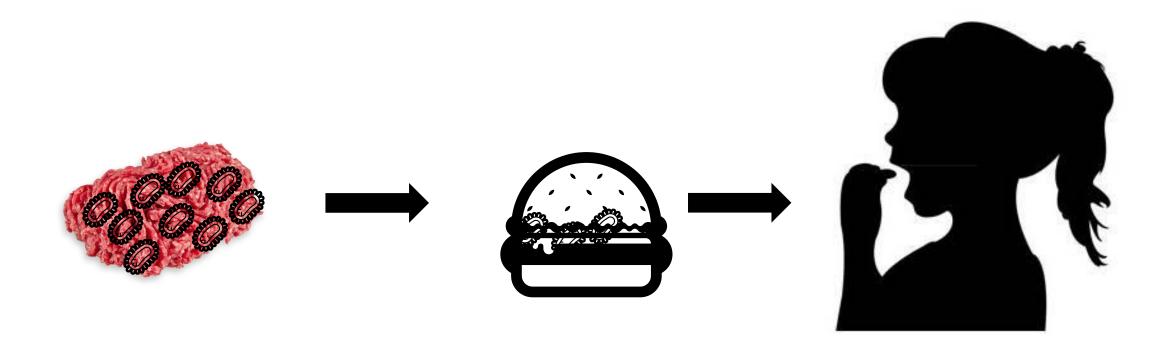




Prevalence: 1/6 = 16.7%









Ground Beef Risk Assessment

- Estimate annual reductions in *Salmonella* infections when highly contaminated ground beef lots were diverted from consumption.
- Estimate contribution of high and low-virulent and multi-drug resistant (MDR) serotypes on the total number of illnesses and burden of disease.
- Prioritize risk-based pathogen mitigation strategies.



FSIS Enumeration Data

1060 Salmonella enumerated samples (2010-2020)

- Salmonella prevalence in models varied from 1.43 1.47%
- 13.7% met high virulence criteria
- 15.9% MDR

Very low Salmonella prevalence in production lots sampled

- >1 MPN/g = 2.4% production lots
- >10 MPN/g = 0.4% production lots

Average concentration = 0.017 MPN/g (4.07 MPN/g)



Risk Assessment Process - Ground Beef

Initial prevalence & concentration

Handling, cooking, and consumption practices

Ingested dose and dose-response

Effect of removing highly contaminated lots

Baseline

Effect of removing highly virulent serotypes

Effect of removing drug resistant serotypes



High Virulence Criteria

• Listed as a top 10 serotype isolated from human illnesses according to the most recent CDC *Salmonella* Annual Report

<mark>OR</mark>

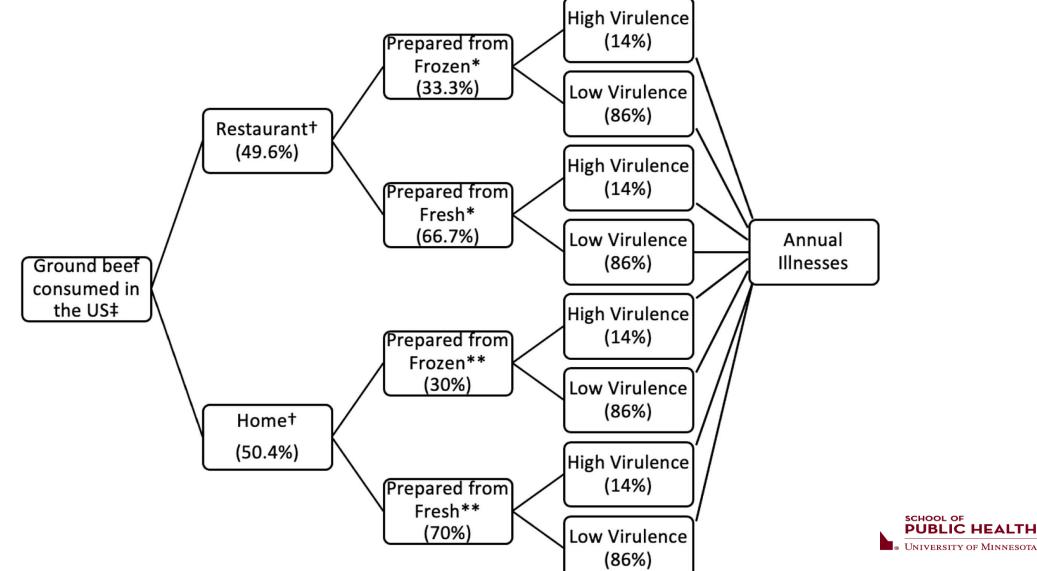
 Identified as an outbreak causing serotype by the National Outbreak Reporting System

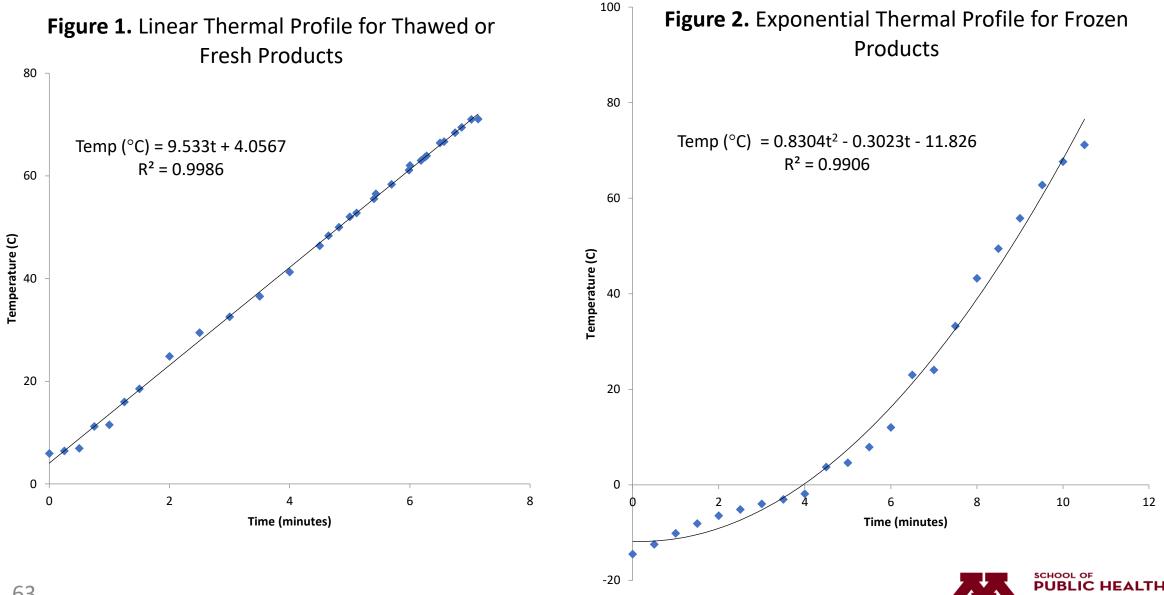
<mark>AND</mark>

 Was not individually over-represented in risk estimates using CDC and FoodNET serotype reporting data



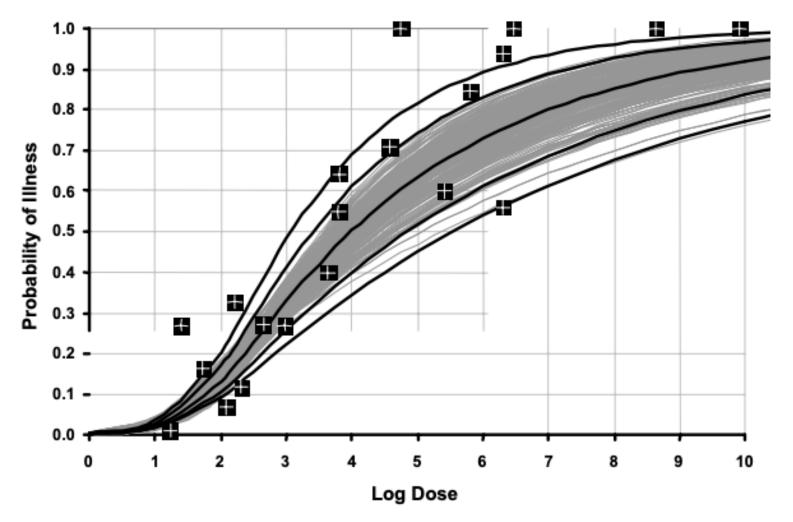
Consumption Scenarios and Proportion of High- and Low-Virulence Serotypes





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High Virulence NTS Salmonella Dose-Response



Source data: World Health Organization, Food and Agriculture Organization of the United Nations, 2002



Results

Table 1. Risk estimate comparisons after removal of lots based on relativeSalmonella characteristics

Model	Annual Illnesses*	Reduction from Baseline (%)
Baseline	8,980	_
>10 MPN/g removed	7,759	13.6
>1 MPN/g removed	5,686	36.7
Highly virulent lots removed	300	96.7

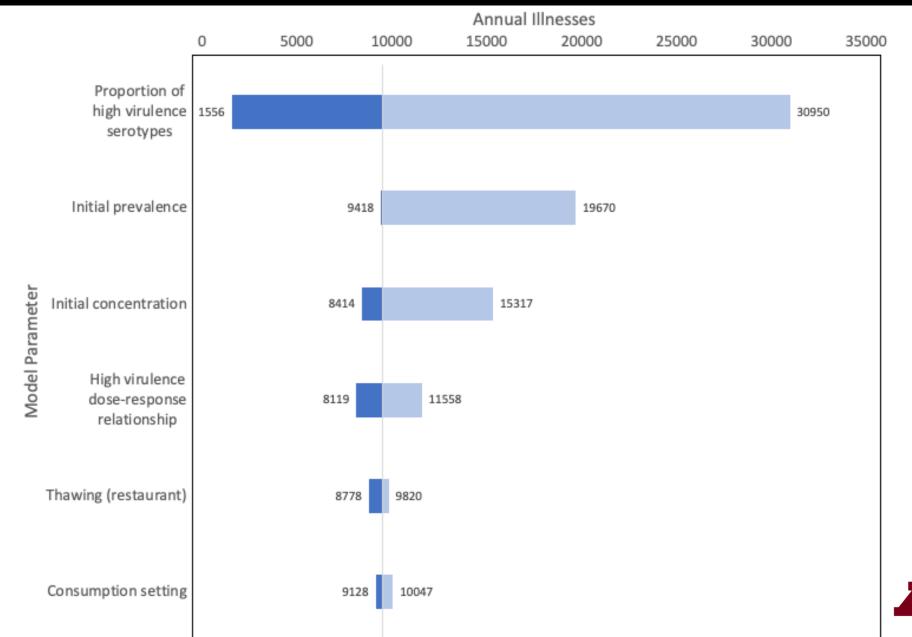
*Unadjusted for under-reporting



Table 2. Annual salmonellosis illness estimates separated by consumption scenarios and virulence profile at baseline

		Annual Illnesses by Virulence Profile				
	Consumption Scenario	High-virulence (90% CI)	Low-virulence (90% CI)	Total		
	Home, Fresh (n = 3.2×10^9)	3360 (2360, 4480)	116 (43, 1020)	3476 (2403, 5500)		
	Home, Frozen (n = 1.6x10 ⁹)	2690 (1900, 3590)	93 (35, 819)	2783 (1935, 4409)		
	Restaurant, Fresh $(n = 3.5 \times 10^9)$	1250 (882, 1670)	43 (16, 379)	1293 (898, 2049)		
	Restaurant, Frozen (n = 1.5x10 ⁹)	1380 (968, 1840)	48 (18, 417)	1428 (986 <i>,</i> 2257)		
	Total	8680 (6110, 11580)	300 (112, 2635)	8980 (6222, 14215)		

Tornado diagram illustrating sensitivity analysis of ground beef baseline model





MDR Salmonella Removal

Removal of MDR Salmonella:

- 21% decrease in Years of Life Disabled
- 56% decrease in Years of Life Lost

• 45% reduction in Disability Adjusted Life Years



Project Highlights:

~9,000 annual cases of salmonellosis attributable to ground beef

Removing >1 MPN/g resulted in a 36.7% reduction in illnesses Removing >10 MPN/g resulted in a 13.6% reduction in illnesses Removing MDR Salmonella reduces burden of disease by 45%

Presence of highly virulent *Salmonella* was the most impactful model parameter



Research Highlights

Consumption Model	Baseline	Removal of lots >10 MPN/g (% decrease)	Removal of lots >1 MPN/g (% decrease)	After Cross- contamination (% increase)
Ground Beef	8,980	7,759 (13.6)	5 <i>,</i> 686 (36.7)	15,310 (70.5)
Ground Pork	10,590	_	5,632 (46.8)	11,851 (11.9)

- >90% annual illnesses attributable to high virulence NTS serotypes
- Significant illness reduction at each pathogen concentration threshold
- Cross-contamination effectively managed after removal of highly contaminated production lots



Impacts for Industry

- Most ground beef is contaminated at low concentrations and majority of Salmonella serotypes not highly virulent.
- Human illnesses are driven by high levels of contamination and highly virulent *Salmonella* serotypes.
- To reduce Salmonella illnesses due to consumption of ground beef, identify and remove products
 - contaminated above threshold of 1MPN/g
 - contaminated with virulent Salmonella serotypes, MDR Salmonella



Data Gaps to Improve Risk Assessment Models

- Dose-response relationships for Salmonella strains
- AMR-specific burden of disease estimates
- Levels of detection for testing
- Cross-contamination coefficients









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