Listeria monocytogenes market basket survey: pathogen presence and/or viability on foods and in retail stores
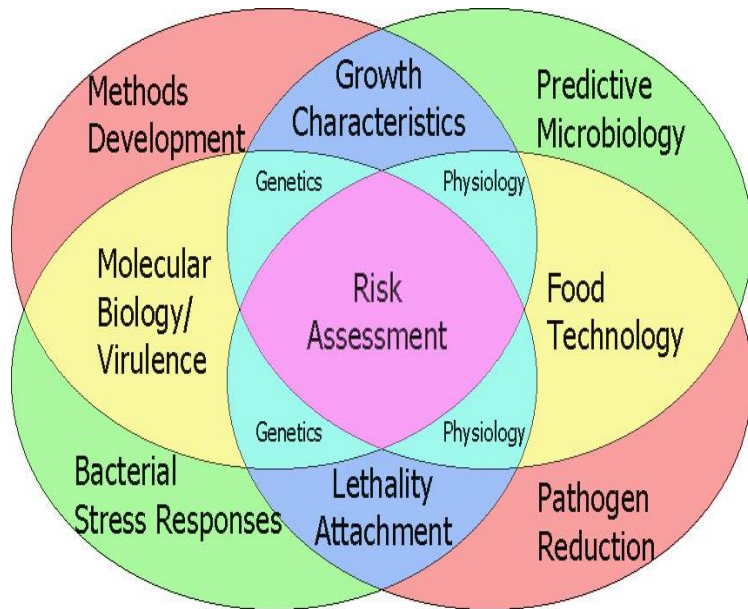
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Lm Market Basket Survey Team

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**FDA CFSAN 2012
Leveraging & Collaboration Award
Congratulations Everyone!**

Goals

- Assess if regulatory reforms coupled with directed modifications of food processes and formulations had an impact on the prevalence, levels, and/or types of Lm in RTE foods at retail over the last decade

- Determine if product formulation (e.g., ingredients, inhibitors, pH, a_w) affects the presence or persistence of Lm on representative RTE foods

- Conduct inoculated package studies of higher volume and/or higher risk RTE retail foods to validate the fate of Lm during refrigerated shelf life

- Identify actual and perceived food safety risks at grocery stores

- Compare what consumers "see" with what food safety professionals "see" as risky behaviors/practices to assist in developing appropriate interventions

Phases of the Lm MBS

- **Phases I and II (2010-2013; ARS, FDA, FSIS):**
  - sampling of retail foods for levels and presence of the pathogen

- **Phase III (2012-2014; ARS, FDA, FSIS):**
  - molecular subtyping of isolates
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- **Phase IV (2012-2013; ARS, FDA):**
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- **Phase V (2015-present; ARS, NCSU)**
  - risk factors at retail – behavioral change
  - what consumers see
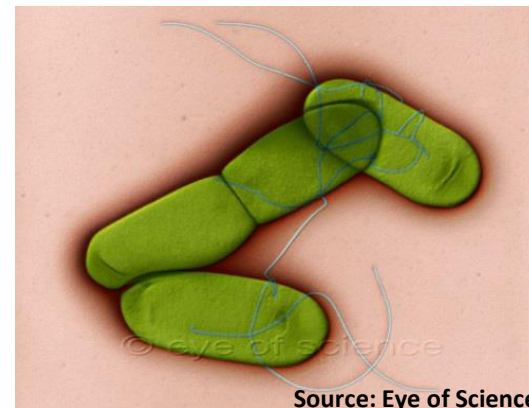
Source: Eye of Science

Funds Leveraged = $4.2M (December 2010 – present)

"Prevalence" of *Listeria monocytogenes*

- **Microbiological perspective:**
  - Samples yielding a viable isolate of Lm divided by the total number of samples tested…"recovery rate" vs "prevalence"
    - 532 samples from which a viable isolate was recovered divided by 32,800 total samples x 100 = 1.6% recovery rate/prevalence
- **Risk assessment perspective:**
  - "Prevalence" used only when in-depth modeling addresses potential clustering effects originating from the study design
    - Generalized linear mixed models considered clustering effects as follows: i) random effect not considered or including state as a fixed effect, ii) random effect considered with regards to states, and iii) random effect considered with regard to stores within states
- **Syntactic perspective:**
  - Recovery rate, occurrence, percent number of samples positive, percentage of samples positive, proportion of positive samples…

Phases of the Lm MBS

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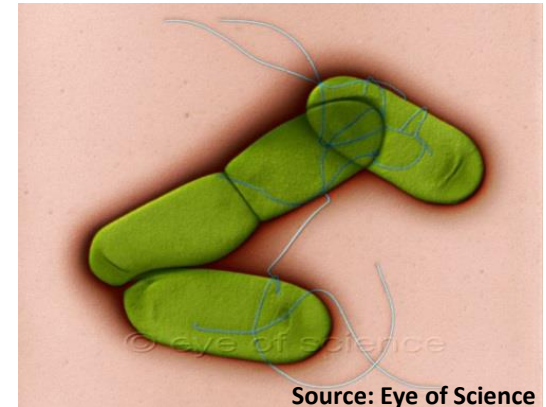
Source: Eye of Science

Funds Leveraged = $4.2M (December 2010 – present)

Questions addressed

- **How many food samples contain Lm *or Listeria*-like organisms?**

  - What is the chemical composition of food samples testing positive and representative samples testing negative?

- **How many cells of Lm are present in a positive food sample?**

  - What are the types/relatedness of these isolates?

"Listeria monocytogenes Market Basket Survey"

Key parameters	FDA		FSIS
Food categories	13		3
Packaging types	Deli pack vs. pre-pack		Deli pack
Samples tested	19,486		7,903
Sampling period	Weeks 1 to 100		Weeks 24 to 75
Sampling locations	4 States (GA, CT, CA, & MD) over 100 weeks		
Store types	Independent	Chain	Online
Meta data	Ingredients/antimicrobials, product temperature, store quality, domestic vs. imported, etc…		
Micro data	Prevalence, levels, & types of *L. monocytogenes*		
Risk Assessments	Yes		

Research Paper

Survey for *Listeria monocytogenes* in and on Ready-to-Eat
Foods from Retail Establishments in the United States
(2010 through 2013): Assessing Potential Changes of Pathogen
Prevalence and Levels in a Decade

JOHN B. LUCHANSKY,[1] YUHUAN CHEN,[2*] ANNA C. S. PORTO-FETT,[1] RÉGIS POUILLOT,[2] BRADLEY A. SHOYER,[1]
RACHEL JOHNSON-DeRYCKE,[3†] DENISE R. EBLEN,[3†] KARIN HOELZER,[2‡] WILLIAM K. SHAW, JR.,[3]
JANE M. VAN DOREN,[2] MICHELLE CATLIN,[3] JEEHYUN LEE,[4§] ROHAN TIKEKAR,[4§] DANIEL GALLAGHER,[5]
JAMES A. LINDSAY,[1] THE *LISTERIA* MARKET BASKET SURVEY MULTI-INSTITUTIONAL TEAM,‖
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Department of Agriculture, Food Safety and Inspection Service, Patriots Plaza III, 1400 Independence Avenue SW, Washington, D.C. 20250; [4]Department of
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Luchansky et al., JFP 80:903-921, 2017

FDA AND FSIS REGULATED FOODS

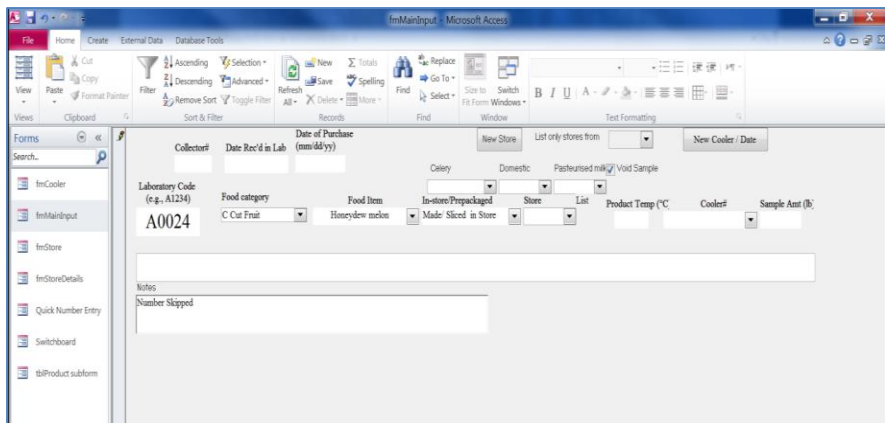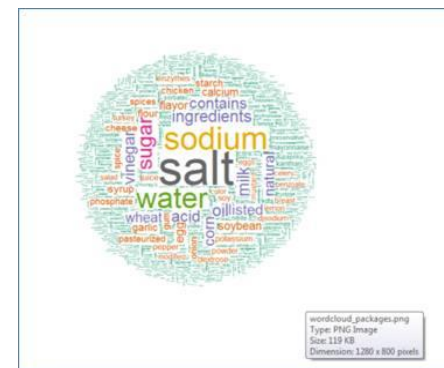| Food categories	
Smoked seafood	Low acid cut fruit
Seafood salad	Cut vegetables, raw
Fresh crab meat and/or sushi	Sprouts
Soft-ripened and semisoft cheese	Sandwiches
Cultured milk products	Deli salads (non-meat)
Artisanal cheese	Deli meats
Raw milk	Deli salads containing meat
Eggs	Dried/fermented sausage

How many food samples should be tested?

Food Category	Estimated # of samples	Estimated prevalence (%)
Smoked Seafood	700	4.3
Seafood Salad	600	4.7
Low Acid Cut Fruits	2,200*	2.0
Soft Ripened and Semi-Soft Cheese	2,000	1.4
Deli-type Salads (no meat)	2,300*	2.4
Sandwiches (prepared)	2,610*	6.0
Raw Milk (destined for retail market)	700*	2.0
Deli Meat Salads	800	3.8
Deli Meat	5,271	1.4
Dried/Fermented Sausages	460	6.4
Cut Vegetables, raw	2,000	1.5-1.7
Sprouts	3,000	1.0
Egg	700	2.0
Artisanal Cheese (online + retail)	2,600	1.2
Fresh Crab Meat and Sushi	700	4.3
FDA + FSIS regulated foods	**Total = 26,641**	**Range = 1.0-6.4**

Estimated number needed to obtain at least 30 Lm-positive samples

Data collected and mined

- **Meta – product and store info, etc.**
  - All food samples retained

- **Ingredients - info from product labels**

  - All labels photographed/retained

- **Microbiology – prevalence, levels, and types**
  - Multiple isolates from each positive sample retained

Total FDA Food Samples Collected per Food Category (Weeks 1-100)

State	Seafood Smoked	Seafood Salad	Crab\Sushi	Fruit	Cut Veggie	Sprout	Raw milk	Soft\Semi cheese	Artisanal cheese	Cultured milk	Eggs	Sandwich	Deli salad	Total samples / state
Maryland	187	283	150	673	524	-	-	516	626	144	168	649	690	4,610*
Connecticut	183	231	65	545	380	-	169	493	534	109	100	568	627	4,004*
Georgia	179	262	73	634	369	-	-	510	637	154	110	574	587	4,089*
California	196	217	69	556	416	-	308	509	640	61	78	528	553	4,131*
Total	745	993	357	2,408	1,689	2,652	477	2,028	2,437	468	456	2,319	2,457	**19,486 ****

* Does not include sprout samples
** Includes sprout samples

FDA-regulated foods = 19,486 samples

Total FSIS Food Samples Collected per Food Category (Weeks 24 – 75)

State	Food Categories			
	Deli salads	Deli meats	Fermented/dry sausage	Total samples/state
Maryland	390	1,481	179	2,050
Connecticut	351	1,662	179	2,192
Georgia	355	1,491	119	1,965
California	319	1,283	94	1,696
Total	1,415	5,917	571	**7,903**

FSIS-regulated foods = 7,903 samples

How many food samples contained *Lm?*

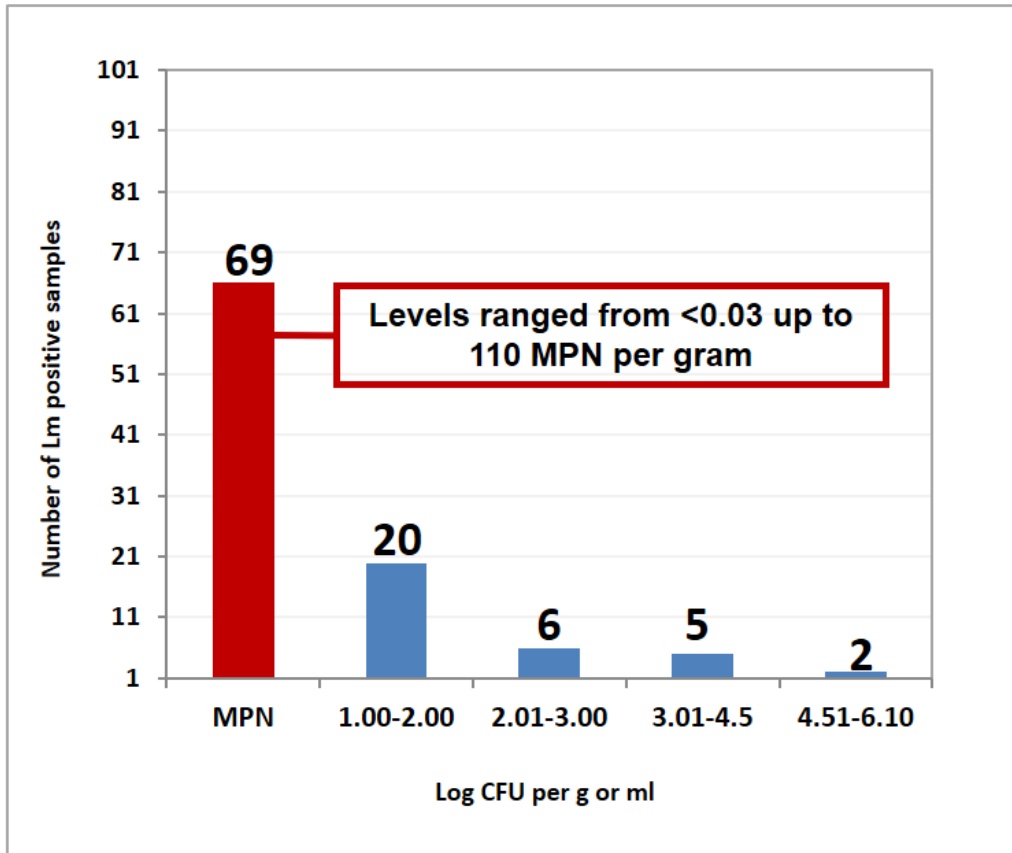We recovered a viable cell of *L. monocytogenes* from **102** of the **27,389** food samples tested

Average Recovery Rate = **0.37%** (range from **0.0 to 1.07%**)

[Recovery Rate of **1.0 to 6.4%** for studies conducted in mid-2000's]

Cluster analyses of presence/absence data will impact "true prevalence"

Number of Lm cells per positive sample

Levels ranged from <0.03 up to 110 MPN per gram

Total = 102 positive samples

FDA Lm positive = 37/100 weeks
FSIS Lm positive = 9/50 weeks
FDA and FSIS Lm positive = 42/100 weeks

Number and Percentage of Lm Positive Samples

Food Category	% of Positive Samples	# Positive Samples*
Smoked Seafood	0.40	3/745
Seafood Salad	0.91	9/993
Low Acid Cut Fruits	0.37	9/2,408
Soft Ripened and Semi-Soft Cheese	0.0	0/2,028
Deli-type Salads (no meat)	0.85	21/2,457
Sandwiches	0.47	11/2,319
Raw Milk	0.63	3/477
Deli Meat Salads	0.28	4/1,415
Deli Meat	0.25	15/5,917
Dried/Fermented Sausages	0.18	1/571
Cut Vegetables, raw	1.07	18/1,619
Sprouts	0.11	3/2,652
Egg	0.0	0/456
Artisanal Cheese	0.16	4/2,437
Cultured milk products	0.21	1/468
Fresh Crab Meat and Sushi	0.0	0/357
Number of Categories = 16	**Range = 0 to 1.07%** **Range expected = 1.0-6.4%**	**Total positives = 102/27,389**

Number of samples containing LLO = **571** [% of samples containing LLO = **0.79-3.32%**]

Proportion of positives for *L. monocytogenes* in RTE foods: Lm MBS study compared with previous studies in mid-2000's

Food Category	Lm MBS Study (2010-2013)			Previous studies: Gombas et al. , 2000-2001		
	No. samples	No. BAX positives	% Positive	No. samples	No. BAX or Gene-Trak positives	% Positive
Smoked Seafood	745	2	0.27	2644	114	4.31
Seafood Salads (exclude tuna salad)	683	7	1.02	2446	115	4.70
Soft Ripened and Semi-Soft Cheeses	2028	1	0.049	2970	37	1.25
Deli Meats	5917	15	0.25	2116	56	2.65
Deli-type Salads without meat (include tuna salad)	2767	26	0.94	8549	202	2.36

Prevalence is appreciably lower in Lm MBS than earlier NFPA (and NAFSS) study
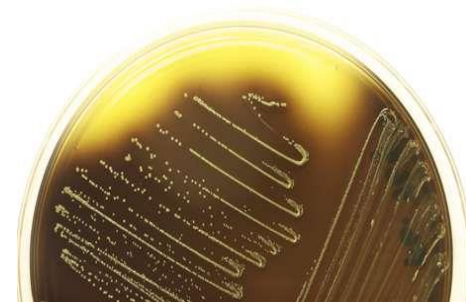
Representative Micro Data – Weeks with Lm Positive samples

| Week	Season	Food category	Food Subcategory	Pre /Deli	Made in Store	Store Type	Store	Brand	Level Log CFU/g
13	Spring	B	Shrimp salad	Deli	No	A	I-a	I	1.45
		B	Shrimp & pasta salad	Deli	No	A	I-a	I	1.0
		E	Turkey salad	Deli	Yes	A	II	II	1.3
		E	Chicken salad	Deli	Yes	A	II	II	1.45
20	Spring	F	Egg salad sandwich	Deli	Yes	A	I-b	I	MPN
		F	Chicken salad sandwich	Deli	Yes	B	III	III	MPN
25	Summer	E	Egg salad	Pre	No	A	I-c	I	MPN
		E	Egg salad	Pre	No	A	I-d	I	MPN
		F	Tuna salad sandwich	Deli	Yes	A	I-d	I	MPN
29	Summer	F	Egg salad sandwich	Deli	Yes	A	I-e	I	MPN
33	Summer	E	Potato salad	Deli	No	A	IV	IV	MPN
41	Fall	E	Egg salad	Deli	Yes	A	I-f	I	MPN
		F	Egg salad sandwich	Deli	Yes	A	I-f	I	MPN

Long story short for Phases I and II …

- **Most comprehensive survey for presence/absence and concentration of Lm in retail RTE foods in the U.S. conducted over the past decade**

- **In general, recovery rate of Lm in the RTE foods tested is lower than that determined for 5 categories of RTE products in studies of similar design, scope, and/or magnitude published in the 2000's**

- **Reduction in contamination may in part reflect regulatory reforms, monitoring efforts, directed modifications of food processes and formulations, and improved sanitation measures implemented by federal agencies and by the food industry**

No difference in % of samples positive for Lm based on store type, season, or pre-packed versus deli-packed foods

Phases of the Lm MBS

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  - chemical analyses of select foods

Source: Eye of Science

- **Phase IV (2012-2013; ARS, FDA):**
  - Inoculated package studies

- **Phase V (2015-present; ARS, NCSU)**
 - risk factors at retail – behavioral change
  - what consumers see
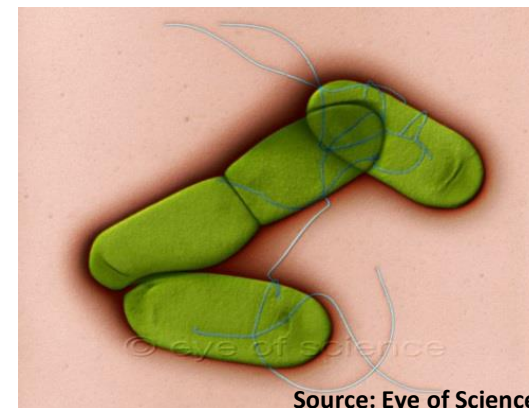
Funds Leveraged = $4.2M (December 2010 – present)

Typing of Isolates - Goals

- **Develop subtyping plan to complement and extend community-specific prevalence & enumeration data:**
  - Define distribution & diversity of Lm associated with foods and food production & storage environments, and retail
    - Identify harborage points and inform illness investigations
  - Identify subtypes associated with higher or lower risk of listeriosis, as well as genes that affect pathogenicity
  - Correlate subtype information and enumeration data with estimated risk of listeriosis

PFGE – PulseNet protocol & database
SNP and MLST typing – existing procedures
WGS – existing phylogenetic trees
inlA sequence analysis (or WGS)
Phage genes typing/sequencing
Additional assays?
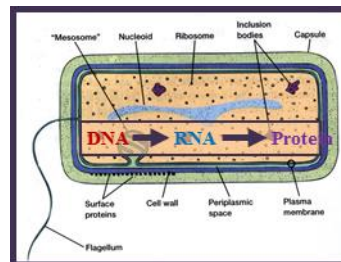
Proximate Compositional Analyses

- **Compare ingredient label information with proximate compositional analyses data for selected foods**

  - Gain insight on product formulation and its impact on presence and levels of Lm
  - Capture ingredients and "inhibitors" on the label and in the formulation

Snapshot of 11,584 (~42%) of labels:

- 285,010 words
- 2,780 unique words
- 1.85M characters w/o spaces

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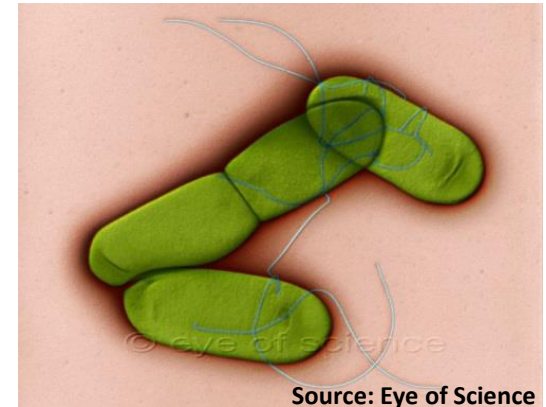
Funds Leveraged = $4.2M (December 2010 – present)

Effect of intrinsic and extrinsic factors on Lm in/on foods

- **RTE products generally do not support growth of Lm if:**

  – pH is ≤ pH 4.4 **or** a_w is ≤ a_w 0.92

  OR

  – pH, a_w, **and/or** inhibitors are used together

- **Conduct inoculated package studies (aka "challenge studies")**

  – Quantify the effect of pH, a_w, and/or use of inhibitors on Lm viability in RTE foods during shelf life
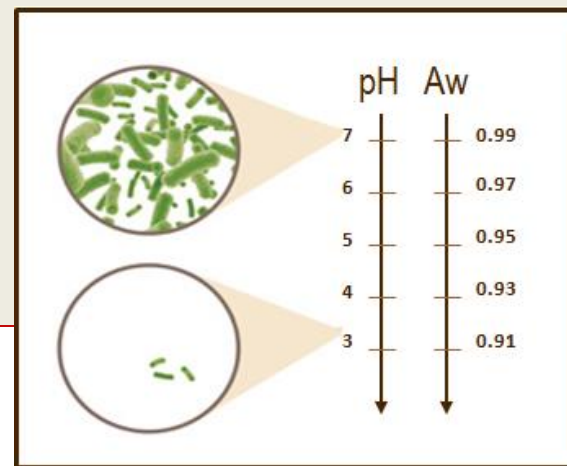
Data are illustrative pending completion of statistical analyses

Hurdle technology

pH   water activity   Inhibitors
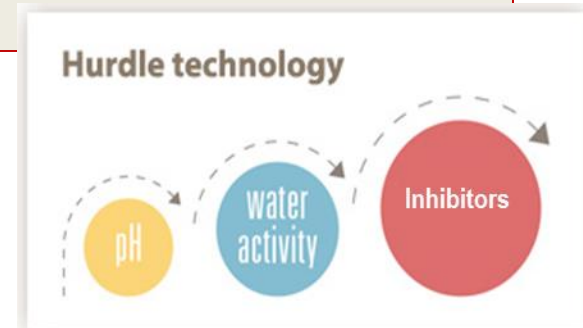
Examples of Challenge Studies
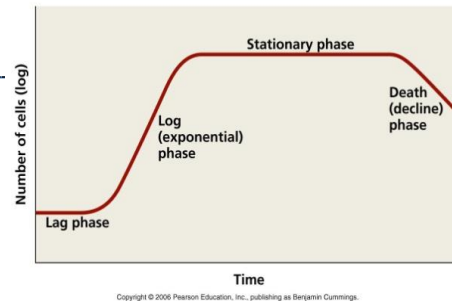
- **Fate of Lm during "cold" storage for 1.5x shelf life**
  - **18 treatments, pH based, 7 food categories, purchased at retail**
    - **Salads, fruits/veggies, & cheese**
    - **Shelf life ranged from 3 days (fresh fruit) to 90 days (eggs)**

- **Behavior of Lm on retail fruits/veggies – effect of pH and a_w**
  - **Strawberries, cantaloupe, & broccoli**
    - **Cantaloupe outbreak of 2011 – 28 states, 147 illnesses, 33 deaths**

- **Modeling pathogen viability on fruits**
  - **Cauliflower & honeydew**
  - **7 sampling intervals, 4 storage temperatures**

Data are illustrative pending completion of statistical analyses

# "Cold storage of inoculated retail foods for 1.5x shelf life"

"Experimental Matrix"	
Inoculation level	~3.0 log CFU/gram
Food Categories	Deli salad, Seafood salad, cut fruit, cut vegetable, artisanal cheese, and eggs
Food Source	Purchased at retail
Treatments	18 total pH-based
Sampling time lengths	At purchase, shelf life use-by-date, and 1.5X shelf life
Storage temperatures	4, 7, and 10°C

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Source: Eye of Science

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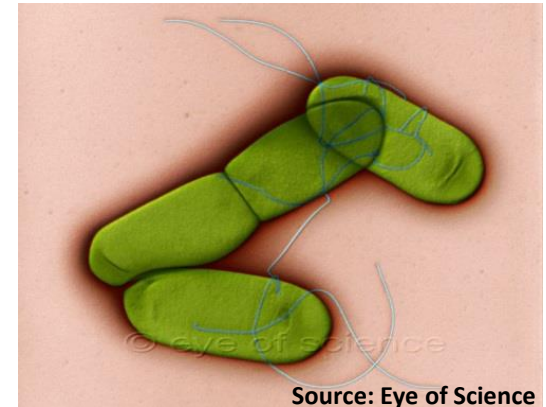
Funds Leveraged = $4.2M (December 2010 – present)

Consumer Perceptions on Safety of RTE Foods at Grocery Stores

- **Premise:**

  Consumers perceive risks differently than food safety experts and/or develop opinions and habits that are not typically science/reality based

- **Objective:**

  Gain insight on perceptions, attitudes, and self-reported behaviors related to observed food safety hazards of consumers who shop at grocery stores

- **Two studies:**
  - Scary things shoppers have seen (Food Prot. Trends 37:30-42, 2017).
  - Yuck factors vs risk factors at retail (J. Food Prot., Under Review, 2017).

Scary Things Shoppers Have Seen

- **Poor hygiene**
- **Cross contamination**
- **Poor sanitation**
- **Improper temperature**

Luchansky et al., Food Prot. Trends 37:30-42, 2017

Scary Things Shoppers Have Seen (2)

Conclusions

- Photo's provide real world teaching tools to better inform and engage a positive food safety culture among shoppers and employees as grocery stores

- Will assist in developing & implementing interventions that effect behavior changes

Yuck Factors vs. Risk Factors
(Perceived vs. Actual Risks)

- **Does the average consumer see what food safety professionals see in photos of "good" and "bad" scenarios taken at grocery stores?**

  – Target audience: age 18+ primary food shoppers that shop at grocery stores

- **Part I: Representative national survey (n=1,041)**

  – Survey recruitment and administration via Qualtrics (Jan-Feb 2016)

  – 12 photos of actual or perceived risks – from Lm MBS

- **Part II: Four NC focus groups (n=39)**

  – More info needed to understand WHY risk is perceived

  – Survey recruitment and administration via community fliers, social media, and NC State email listservs (Jun-Jul 2016)

  – 4 photos of actual or perceived risks

Experts and consumers don't see the same risks!

Imagine you are out at a store and are actually seeing what is shown in this picture. **On a scale of 1 to 5, with <u>1 being very unsafe</u> and <u>5 being very safe</u>**, what do you think about the situation shown in this picture in terms of **food safety**?
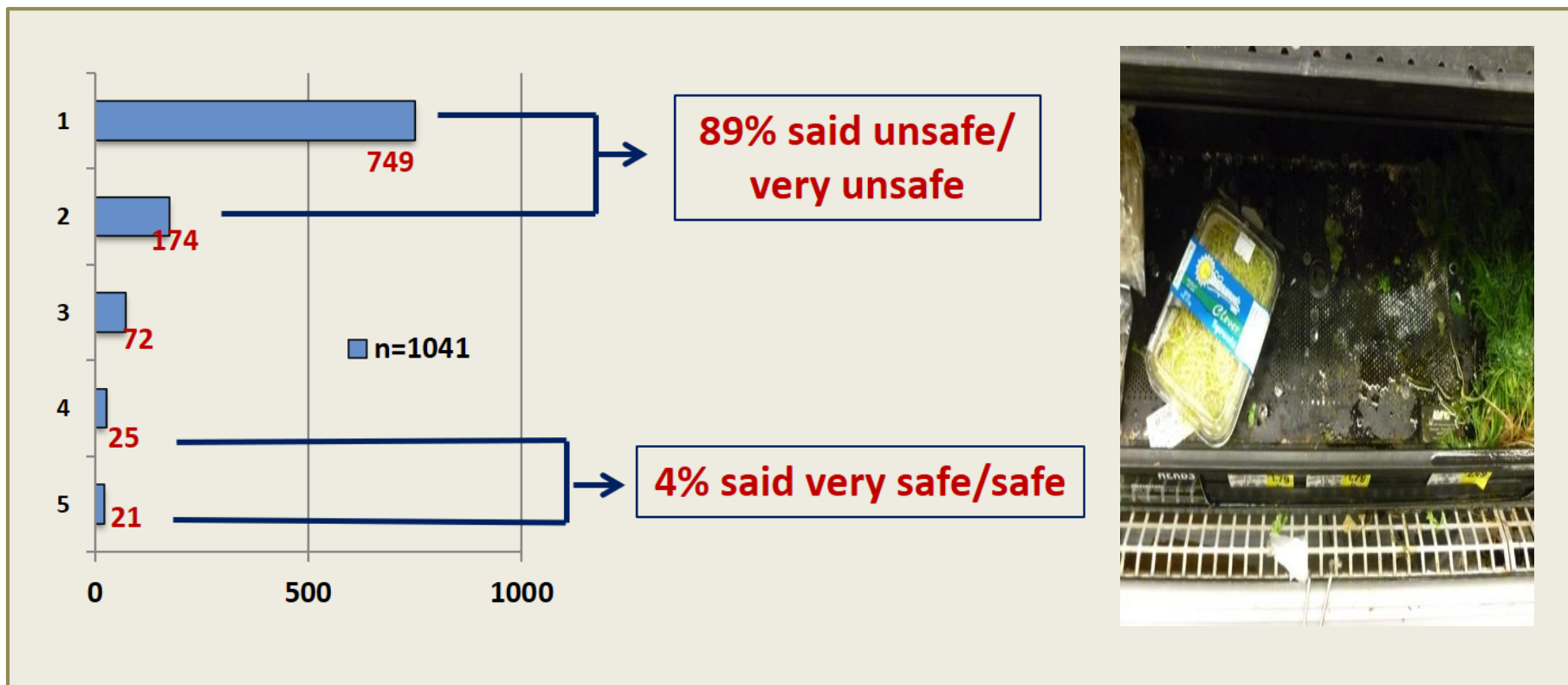
"Perceived Risk" = "Yuck Factor"

Imagine you are out at a store and are actually seeing what is shown in this picture. **On a scale of 1 to 5, with <u>1 being very unsafe</u> and <u>5 being very safe</u>**, what do you think about the situation shown in this picture in terms of **<u>food safety</u>**?

1	361
2	283
3	205
4	118
5	74

n=1041
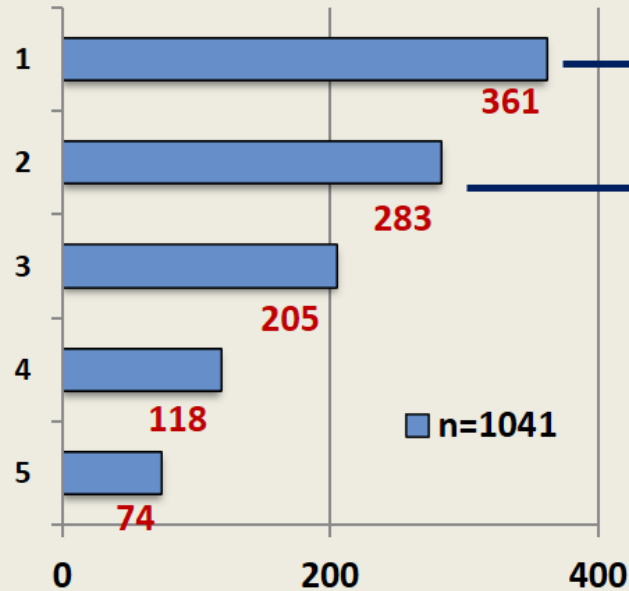
62% said unsafe/ very unsafe

38% said neutral/safe

"Actual Risk"

The take home points are …

- Consumers and food safety professionals don't see the same risks

- Many factors influence customer perceptions of food safety risks at groceries

  - Context, trust, loyalty/familiarity, & management/customer service

Research Paper

Consumer Perceptions of the Safety of Ready-to-Eat Foods in Retail Food Store Settings

KATRINA LEVINE,[1]* MARY YAVELAK,[1] JOHN B. LUCHANSKY,[2] ANNA C. S. PORTO-FETT,[2] AND BENJAMIN CHAPMAN[1]

Levine et al., JFP 80:1364-1377

The Road Ahead…

- Perform statistical analyses of all data to draft manuscripts
- Complete molecular characterization and subtyping of multiple isolates from all positive samples
- Analyze proximate compositional data and conduct additional growth studies on selected foods as needed
- Use data to support risk assessments, inform policy decisions, and improve product and processes
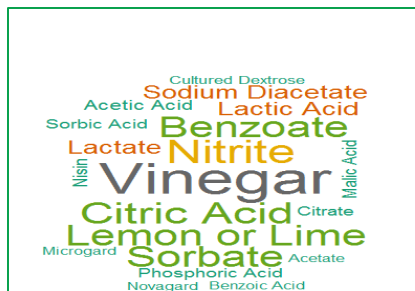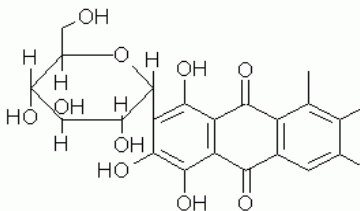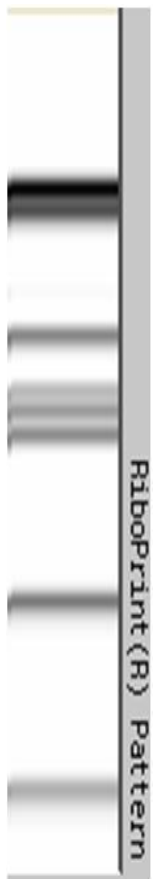
Thank you for your attention!

Kudos to the Lm MBS team!