

Strategies to Control *Listeria monocytogenes* in Ready-to-Eat Foods

Kathleen A. Glass, Ph.D.
Distinguished Scientist, Associate Director
Food Research Institute
University of Wisconsin-Madison
Madison, Wisconsin United States
Email: kglass@wisc.edu



**FOOD
RESEARCH INSTITUTE**
University of Wisconsin-Madison

Key Points

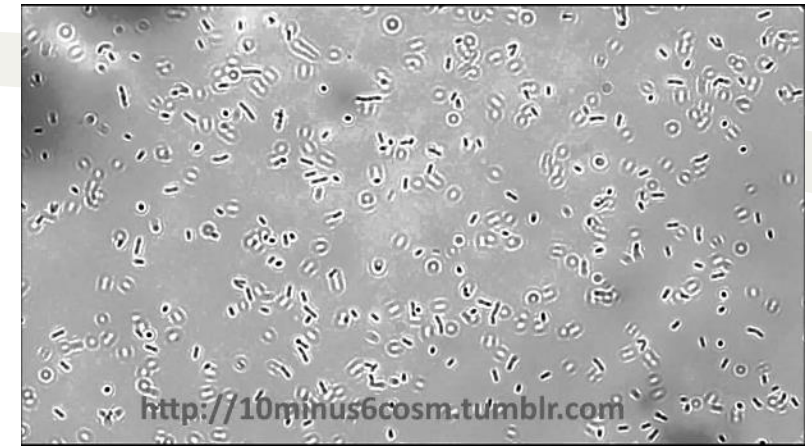
- Characteristics of Listeria
 - General information
 - Illness
 - Culprit foods and what we have learned
 - Contributing factors to listeriosis
- Intervention strategies
 - Environmental monitoring and control
 - Lethality
 - Growth inhibition
- Summary

What is
Listeria?



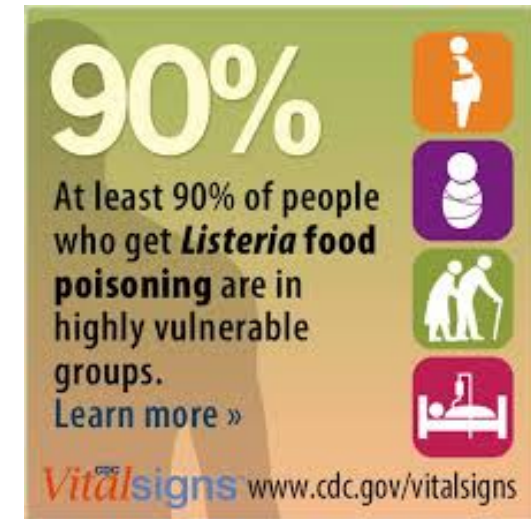
Listeria: Characteristics

- 17 *Listeria* species
- *L. monocytogenes* human pathogen
- Prevalence
 - Bulk tank milk: 3.8% *Lm*; ~30% *Listeria* sp.
 - Seed sprouts: 3-83% *Lm*
 - Lettuce: 0-23% *Lm*
 - Smoked finfish & shellfish: 14%
- Widespread in food processing environment
 - Can be resident in processing plants
- Thrives in moist, cold, high salt environments



Impact of *Listeria monocytogenes*

- ~1,600 illnesses in US annually, 90% hospitalizations, ~20% mortality,
 - 85% not associated with pregnancy; median age ~70 years
 - Economic burden (2014) ~US\$ 1.8M per case
 - Hospitalizations, premature death, disability
- 3-4 weeks lag between consumption of contaminated food and illness
- 2013: whole genomic sequencing able to detect smaller outbreaks and link multistate outbreaks
- Illness rate stable for past 15+ years despite advances in sanitation and formulation strategies



High risk foods

- High moisture, high pH with extended shelf-life
 - Particularly those without growth inhibitors
- 1998-2019 CDC report (Wise, 2019, ISOPOL)
 - Post Whole Genome Sequence (WGS) era:
 - 37.5% cheese (particularly soft cheeses)
 - 31.6% produce items
 - 15.8% ice cream
 - 11.8% deli items (including RTE meats, smoked seafood)
 - 5.3% unpasteurized milk



Most recent outbreaks

Vehicle	Year	States affected	illnesses	deaths	source
Frozen RTE chicken products	2021	1	3	1	Identified
Queso Fresco	2021	4	13	1	Identified
Italian deli meats	2020	4	12	1	??
enoki mushrooms	2020	17	36	4	Identified
hard boiled eggs	2020	5	8	1	Identified
unknown vehicle	2019	13	24	2	??
deli meat/cheeses	2019	5	10	1	??

Unexpected Vehicles

Deadly Outbreak Linked to Caramel Apples; CDC Warns Americans 'Not To Eat ANY'

Craig Bannister | January 9, 2016 5:22pm ET

2.3K



2014-15: 35 illnesses, 7 deaths
2017: 3 illnesses

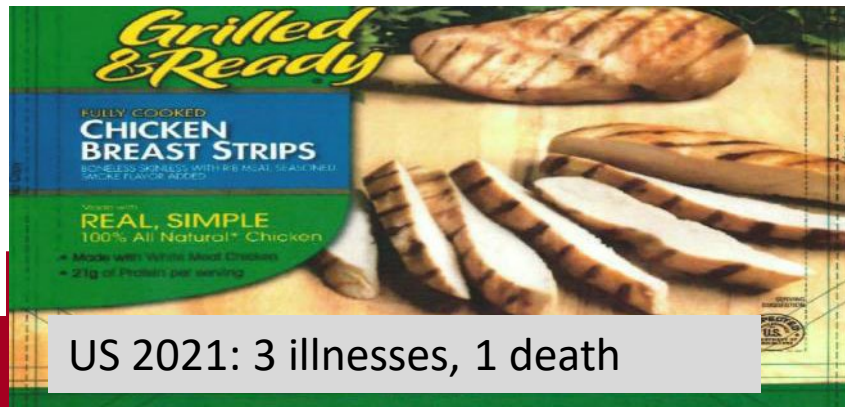


2010-15: 10 illnesses, 3 deaths
2014+2015: 2 +1 illnesses



Canada 2019: 7 illnesses

Listeria outbreak in Canada linked to frozen chicken; recall underway



US 2021: 3 illnesses, 1 death



US 2011: 139 illnesses, 29 deaths
Australia 2018: 16 illnesses, 6 deaths

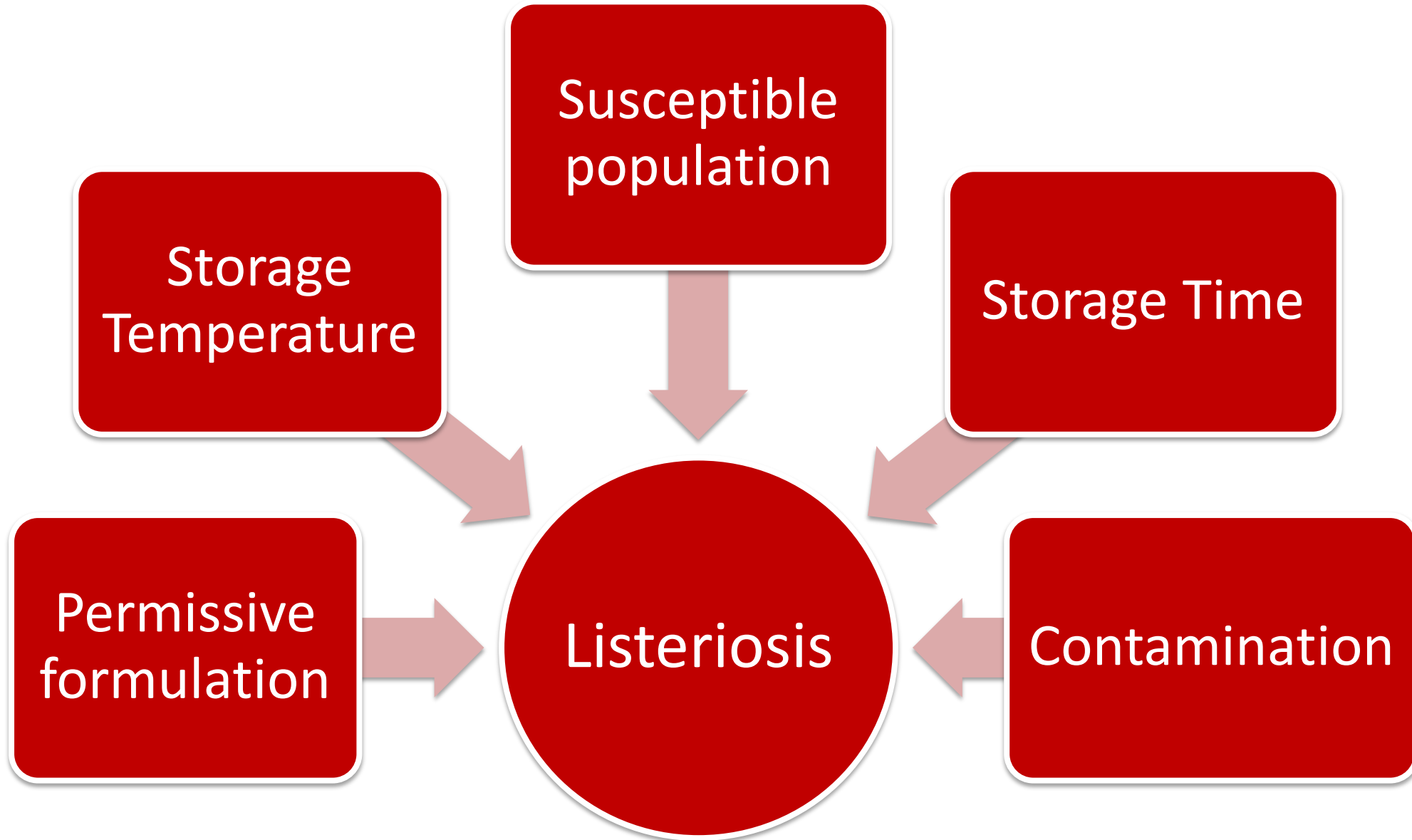


US 2013-2016: 9 illnesses, 3 deaths
EU 2015-2018: 47 illnesses, 9 deaths

Unexpected recalls (no illnesses reported)

- 2021: dry seasoning blends
- 2021: frozen raw dog food (*Listeria* and *Salmonella*)
- 2021: muffins
- 2017: frozen pancakes
- 2016: frozen waffles

Contributing factors to listeriosis



Characteristics of *Listeria* that causes problems

- Common and persists in the environment
- More heat tolerant than Gram negative pathogens at normal pasteurization temps
 - More heat tolerant with high fat, low water activity
- Salt tolerant (grows at 13% salt; survives at 30% salt)
- Long survival in frozen and dry foods
- Grows at refrigeration temperatures

No single practice will control *Lm* contamination in foods

Control Strategy	Reduction of predicted illness
Environmental Control	
Enhanced sanitation	5%
Control contamination at its source	22%
Control cross-contamination	34%
Reformulation with growth inhibitors	96%
Rigorous temperature control (<4°C)	9%

Source: FSIS Best Practices Guidance for Controlling
Listeria monocytogenes (*Lm*) in Retail Delicatessens April 2014



LETHALITY

Kill all you can

Lethality of *Listeria monocytogenes*

- Thermization of cheese milk
- Pathogen control in cheese brine

Thermization of cheesemilk

- Cheese made with raw milk must be aged for at least 60 days
 - Soft cheeses can support growth of pathogens during aging
- FDA/Health Canada risk assessment recommends a 3-log decrease in raw milk before cheesemaking
 - Reduce risk up to 10-fold compared to milk made from non-thermized milk
- Published thermal inactivation in milk variable due to experimental design and strains used
- UW-FRI modeled and validated data to create temperature-time tables for use by artisan cheesemakers (in press, J. Dairy Sci.)
 - App in development

Cheesemilk Thermization App (*in development*)

Calculate:

☒ Hold Time from Temperature and Log Reduction

Bacteria

Reduction:

Temperature (°F)

140 141 142 143 144 145 146 147 148 149 150 151 152 153 154 155

Hold Time (s) for 3-log reduction *Listeria monocytogenes* at 142°F:

290 s (= 4.83 min)

Provides: >6.5 log reduction Shiga-toxin producing E. coli

Disclaimers:

- Temperature must be taken at the coldest point of the system.
- Milk must be stirred/agitated during thermization to ensure each particle is heated.
- Time output is for hold time and does not include integrated lethality for come-up and cool down times.
- Values are valid for bovine milk with typical fat content 5% fat or less. Milk of higher fat content may require greater thermization.
- Data derived from D- and z-values from experiments Engstrom & Glass 2021, J. Dairy Science.

Confirms the greater heat stability of *Listeria* compared to STEC

Listeria monocytogenes in Cheese Brine

- Brine/salting important step in manufacturing cheese
 - Pure salt solutions: correlation between salt and water activity
- Brine can serve as a reservoir of salt tolerant pathogens
 - *Listeria monocytogenes*
 - “zero tolerance” pathogen
 - Growth at 13% salt; survive in up to 30% salt; lower limit for growth a_w 0.92
 - Survive in untreated cheese brine for up to 270 days
 - Brine continually reused
 - No “clean break”
- Inactivation of brine with sodium hypochlorite, hydrogen peroxide

% NaCl	A_w
0.9	0.995
1.7	0.99
3.5	0.98
7.0	0.96
10.0	0.94
13.0	0.92
16.0	0.90
22.0	0.86

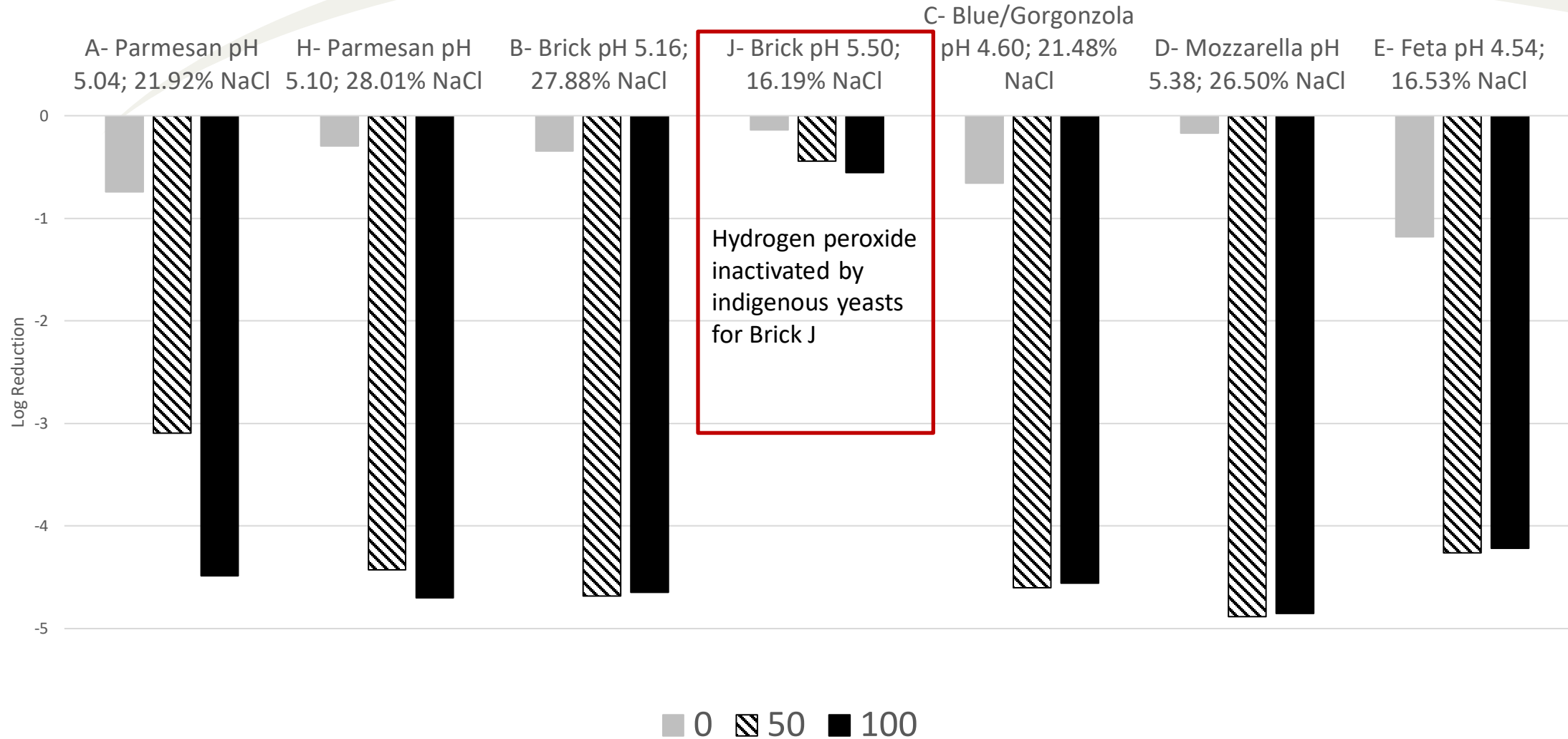
Commercial brines used for *Listeria* inactivation

Designation	Cheese type	Use temperature	pH range for brine received	Salt range for brine received	A _w range for brine received
A	Parmesan	50-53°F	5.00-5.10	21-22%	0.838-0.819
H	Parmesan	53-55°F	5.05-5.20	27-29%	0.909-0.867
B	Brick	71-73°F	5.05-5.25	27-29%	0.777-0.766
J	Brick	40-45°F	5.40-5.55	10-19%	0.768-0.761
C	Gorgonzola	50-60°F	4.55-4.75	20-23%	0.827-0.822
D	Mozzarella	30-32°F	5.30-5.40	25-28%	0.797-0.788
E	Feta	55°F	4.50-4.60	15-18%	0.874-0.858

Brines received August 2019, November 2019, January 2020

A_w below growth range for *Listeria*, but can survive

Reduction of *Listeria monocytogenes* by hydrogen peroxide, cheese brine, stored at 12.8°C/55°F, 7 d



<1 log reduction at 1 day
 ~3 log reduction with 100 ppm H₂O₂ at 0 and 7.2°C (32 and 45°F)



ENVIRONMENTAL CONTROLS

Keep them out

Learning Lessons, the Hard Way:

Importance of environmental controls

Cheese	4 years	Switzerland
	3 years*	Switzerland
	7 years	Sweden
Ice cream	7 years	Finland
	5+ years	US
	1 year**	US
Poultry, cooked deli products	12 years	US

*2018-2020 34 confirmed cases, 10 deaths linked to soft brie, pasteurized sheep and goat milk cheese; *L. monocytogenes* found in 22% of environmental samples

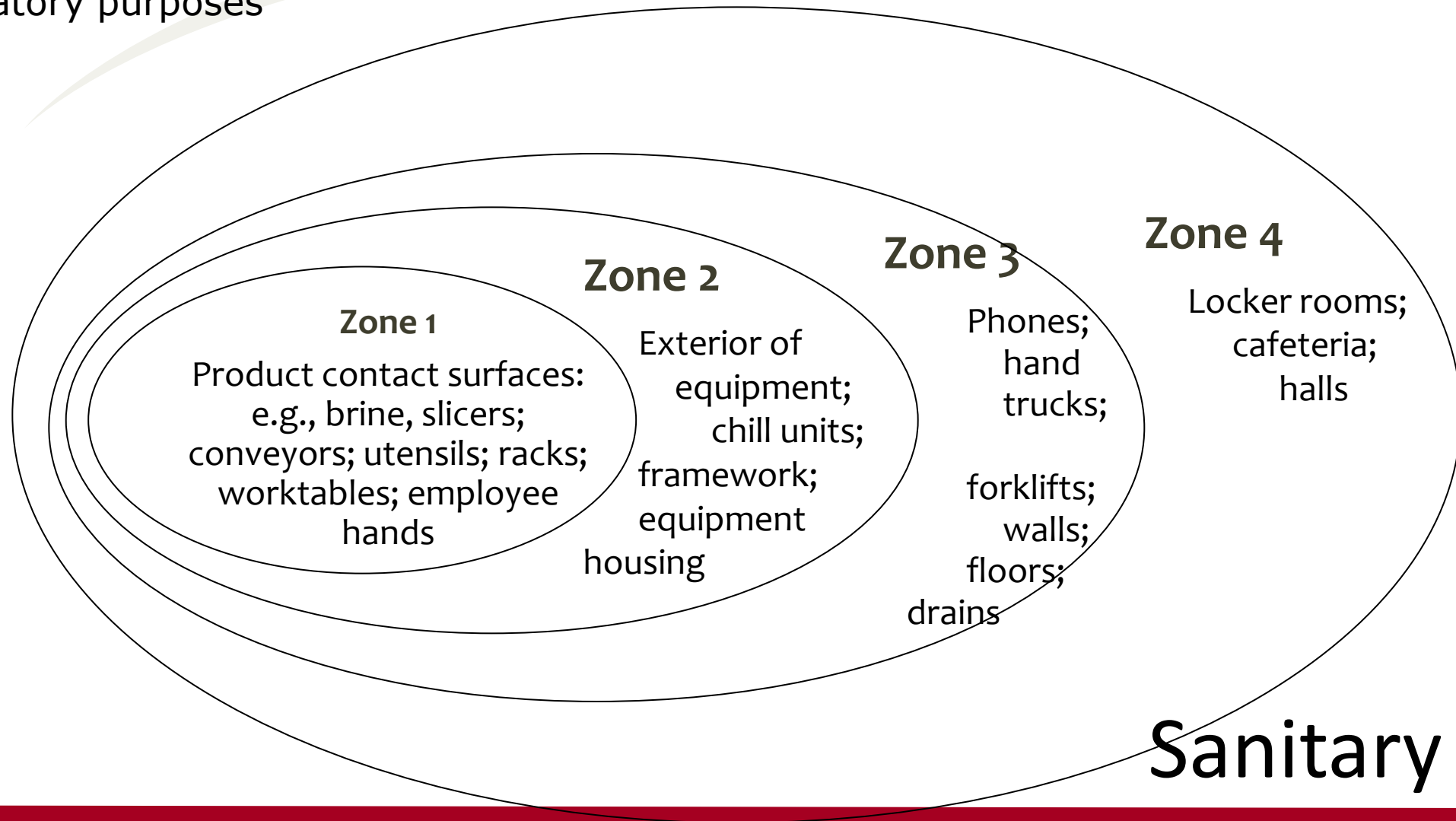
**2014-15 hospital shake freezer; disassembled, cleaned, sanitized after contamination/outbreak; additional cases post-clean

Environmental Control program

- Clean and dry; traffic control
- Sanitary design of facilities and equipment
 - Air handling
 - Cleanable
 - Disinfect/heat sanitize
- Find it and fix it
 - Zones of Control
 - Environmental monitoring
 - Indicator organisms
 - Targeted and random sampling
 - Hygiene checks



If Zone 2 or 3 positive for *Listeria* spp., consider sampling Zone 1
Zone 1 + for *Listeria monocytogenes* considered equivalent to finished product testing for regulatory purposes



2017 FDA Draft Guidance to Control Lm in RTE Foods

- Encourages robust *Listeria* control programs
 - “Expects” *Listeria* to be found
- “Seek and destroy” approach
 - Environmental samples from food contact surfaces (FCSs) and non-FCSs
 - Test several hours into production and preferably before clean up
- One *Listeria* spp. positive FCS test can continue production
 - Requirements for sanitation, retesting, investigation
 - For food that supports growth:
 - 2nd positive: “hold and test” food
 - 3rd positive: suspend production

NACMCF 2021

RTE Foods Testing to Verify Process Controls

- Substantial reference to FDA *Listeria* control document and specific industry guidance documents
- Rigorous environmental monitoring program for *Listeria* sp., particularly for foods that support growth
- Test finished product for *Listeria monocytogenes* if recurring positives in Zones 1 and 2
- Short shelf life foods (e.g., cut melon, prepared sandwiches) rely more on indicator organisms testing
- Less emphasis on *Listeria* spp. testing for environments of foods that don't support growth at refrigeration or ambient temperatures
 - Assumes that the food/ingredient is not used for other susceptible foods



GROWTH INHIBITION

Keep them from growing

Reduce growth Reduce risk

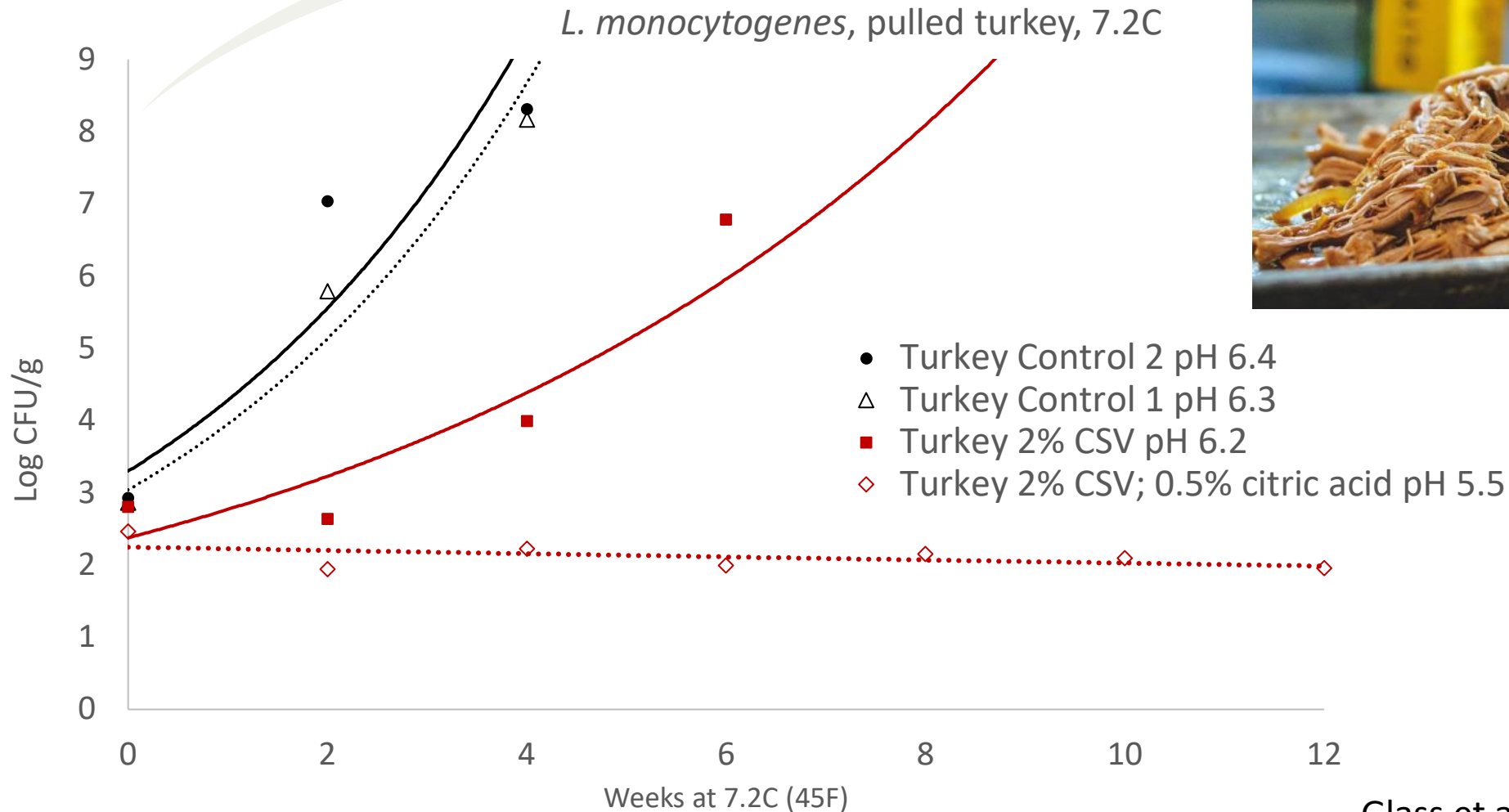
- FDA-CFSAN/USDA FSIS 2003 QRA
 - Foods that support growth had highest risk of causing listeriosis per serving
- FAO/WHO 2004
 - Listeriosis cause by consumption of foods with >100 CFU/g
- FSIS 2010 deli meats
 - Identified difference in rates with prepacked meats (with growth inhibitors) vs. sliced in deli (no growth inhibitors)
- Codex Alimentarius Commission
 - Limit 0.5 log increase during (labeled) shelf-life under “reasonably foreseeable conditions”

Defining growth/no growth conditions

- Freezing (-18°C)
 - Ingredients might be used in other foods that can support growth (milk shakes, salads) when refrigerated
- $\text{pH} \leq 4.4$
- Water activity ≤ 0.92
- Combination of $\text{pH} \leq 5.0$ and $a_w \leq 0.94$
- $\text{NaCl} \geq 16\%$
- Can reduce growth with addition of effective antimicrobials

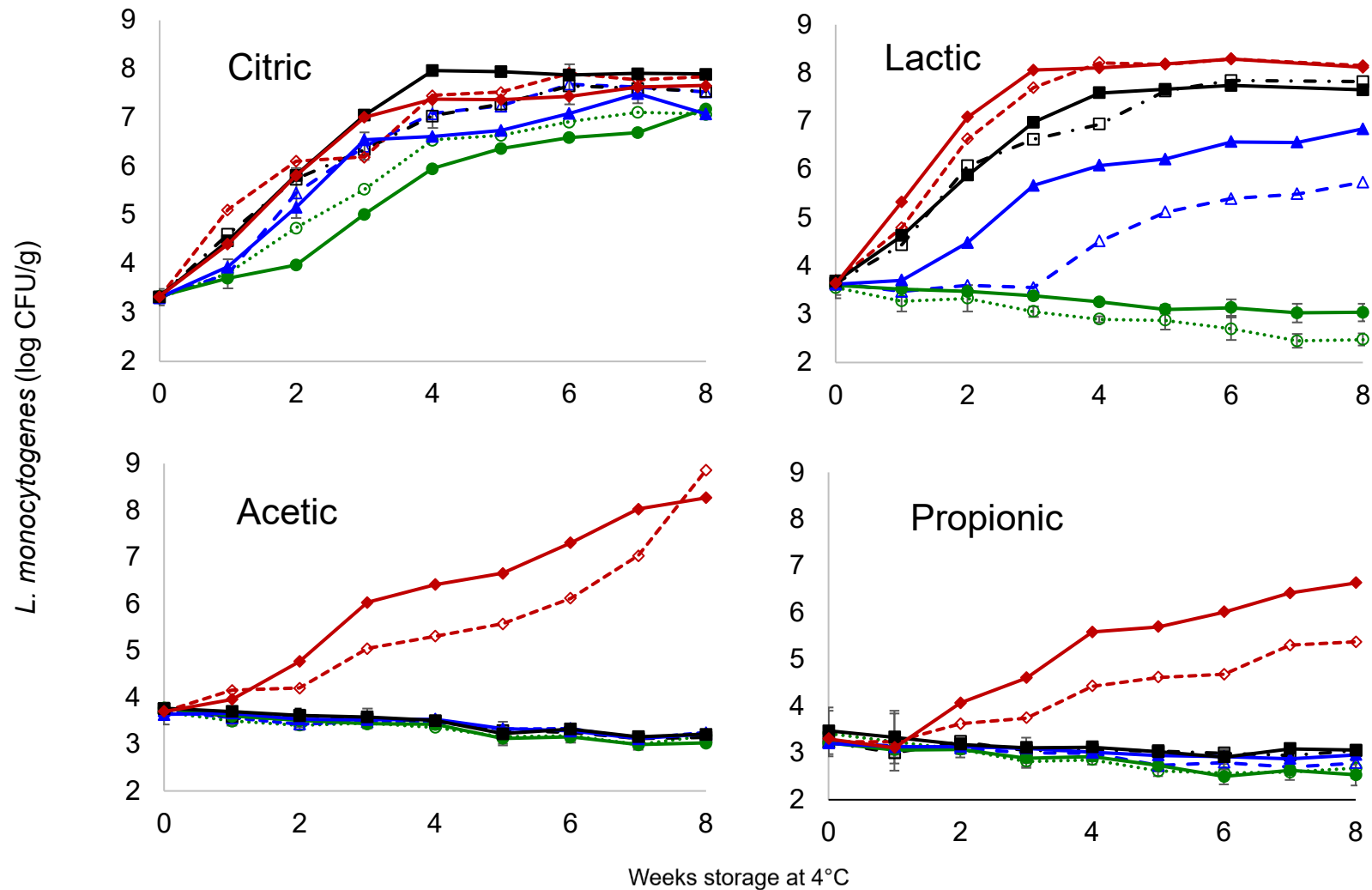


Validation: Effect of pH and antimicrobials in meats



Glass et al., 2021, unpublished data

Validation: Effect of acid type and pH on *L. monocytogenes* in cheese

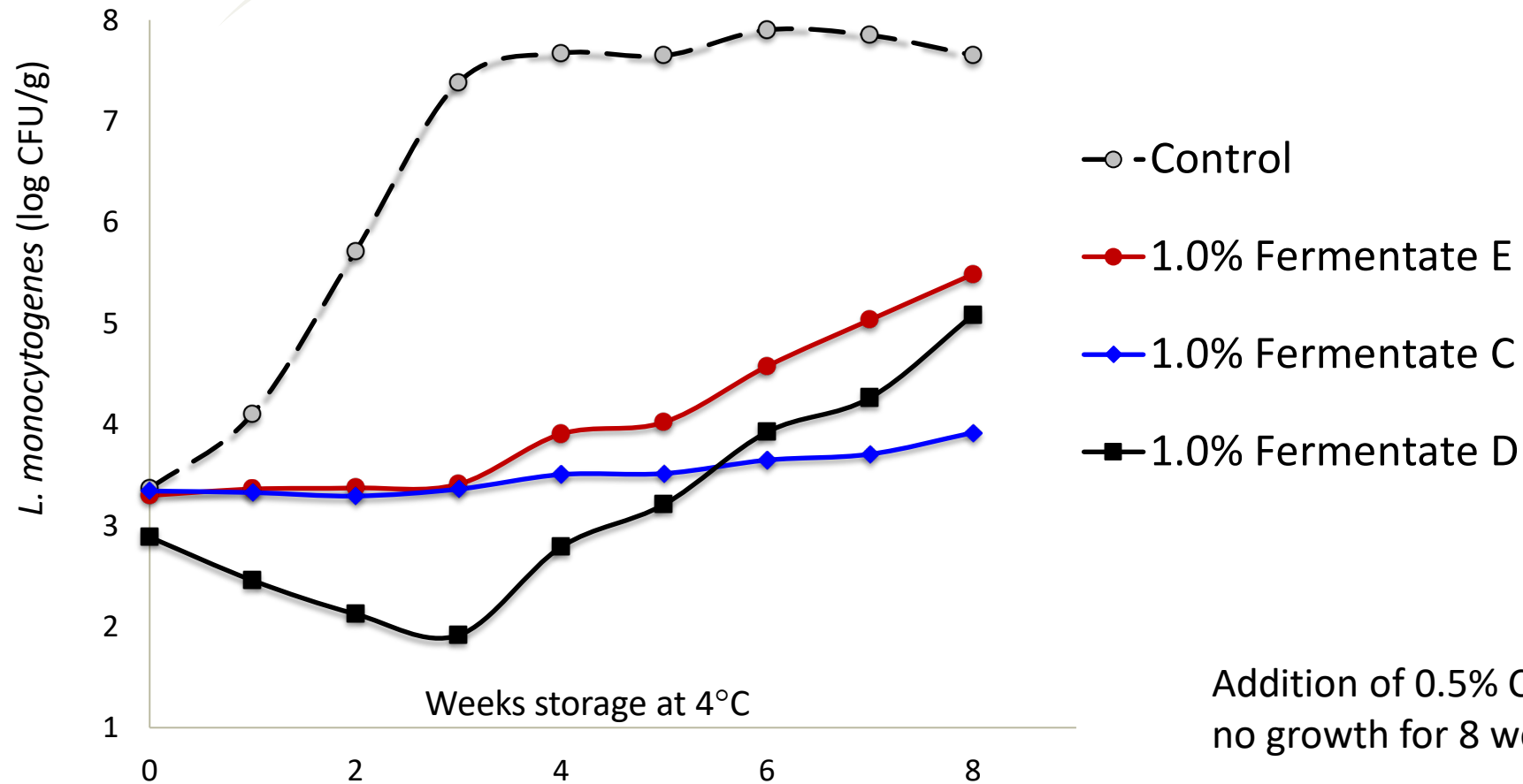


Test parameters:

- Moisture: 50, 56%
- NaCl: 1.25%
- pH: 6.0, 5.75, 5.5, 5.25
- Acid types: citric, lactic, acetic, propionic



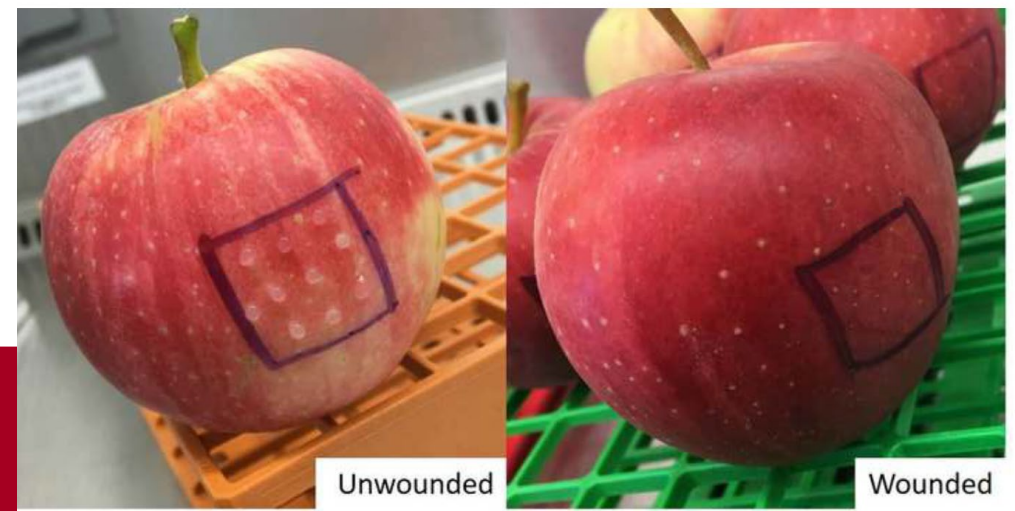
Validation: Effect of cultured milk solids or cultured sugar-vinegar *L. monocytogenes*, pH 6.0, lactic acid, 56% moisture cheese, 1.25% NaCl



Addition of 0.5% CSV and pH 5.5 lactic acid,
no growth for 8 weeks

Listeria persistence on apples

- Apples inoculated with *Listeria innocua*, w/ *Botrytis cinerea* or *Penicillium expansum*, wounded/no wound, controlled atmosphere chilled storage, up to 11 months, Gala and WA-38 apples
- Gradual decrease in populations of *Listeria*
- Greater persistence: Gala, wounded, with *Botrytis cinerea*
- Conclusion: remove apples with visible surface wound defects prior to storage or packing
 - Washing provides 1-3 log reduction



Learning lessons, the hard way:

Foods that don't support growth under normal storage, but harbor *Listeria*, can be a source of contamination to foods that can support growth



Summary: Intervention Strategies

- Kill all you can
 - Validated lethality step
 - Cooking; pasteurization; thermization
 - Brine treatments
 - Produce washes
 - HPP
- Keep them out
 - Expect to find *Listeria* in processing environment and raw ingredients
 - Persistent in equipment, facilities
 - Reintroduced by ingredients, workers
 - Requires rigorous cleaning, sanitation, monitoring to prevent from colonizing environment



Summary: Intervention Strategies

- Keep them from growing
 - Refrigeration/freezing temperatures alone insufficient
 - Prevent/reduce growth in foods by formulation
 - $\text{pH} < 4.4$
 - Water activity < 0.92 (will still have survival)
 - Combination of $\text{pH} \leq 5.0$ and $a_w \leq 0.94$
 - Adding synthetic and clean label “preservatives” provides an extra margin of safety
 - Target active components: acetic, propionic, lactic acid
 - Affected by processing, food components, temperature control, a_w , pH
 - Preventive controls must be validated in specific food/storage conditions



Resources

- FDA
 - Draft Guidance for Industry: Control of *Listeria monocytogenes* in Ready-To-Eat Foods January 2017
- Innovation Center for US Dairy
 - Control of *Listeria monocytogenes* Guidance for the U.S. Dairy 2015
 - Pathogen Control Guidance Document for U.S Dairy October 2019
 - Pathogen Control Check List October 2019
- 3M Environmental Monitoring Handbook
- AFFI Environmental Monitoring Plan
- Control of Listeria in RTE Meat Products (Comp Rev Food Safety 2006)
- Seek and Destroy: Process Controls RTE Meat/Poultry (JFP 2015)
- Universities (IL, Penn State, Cornell, WI, OR etc.).
 - Retail/delis
 - Validation guidance / challenge studies
- Virtual Workshop on Environmental Controls/Testing, Sept. 27-30
 - Platform presentations, small group exercises; registration link fri.wisc.edu



Food Research Institute
UNIVERSITY OF WISCONSIN-MADISON

Thank you for your attention

Contact: kglass@wisc.edu

Web: fri.wisc.edu



Applied Food Safety Laboratory, 2021