

***Listeria monocytogenes* in Food Products other than Meat & Poultry**

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Unique Challenges with *Listeria monocytogenes*

- Changes in ways foods are prepared, distributed, and consumed
 - Increased consumption of ready-to-eat (RTE) foods with extended refrigerated shelf-life
 - Increased purchase of prepared foods
 - Food handling and storage practices
- Survival and growth under conditions used to control microbial populations
 - Refrigeration, high salt, low pH

Epidemiology of Listeriosis

- 2,500 illnesses and 500 deaths (Mead et al., 1999)
 - High hospitalization (20-30%) and case fatality (90%) rates
 - Long incubation period (7-60 days)
- Most cases not associated with large epidemics
 - Small clusters and sporadic cases
 - Food responsible for these cases rarely identified
- Identification of food source
 - Outbreak detection and extensive investigation
 - Food source not always identified
- Full range of food sources associated with listeriosis not likely known
- Strain specific virulence characteristics

Food Categories Used in the *L. monocytogenes* Risk Assessment

SEAFOOD	Smoked Seafood (finfish and mollusks)
	Raw Seafood (finfish, mollusks, and crustaceans)
	Preserved Fish (dried, pickled, and marinated finfish)
	Cooked Ready-to-Eat Crustaceans (shrimp and crab)
PRODUCE	Vegetables (raw)
	Fruits (raw, dried)
DAIRY	Fresh Soft Cheese (queso fresco, queso de Crema, Queso de Puna)
	Soft Unripened Cheese, >50% moisture (cottage cheese, cream cheese, ricotta)
	Soft Ripened Cheese, >50% moisture (brie, camembert, feta, mozzarella)
	Semi-soft Cheese, 39-50% moisture (blue, brick, Monterey, muenster)
	Hard Cheese, <39% moisture (cheddar, Colby, parmesan)
	Processed Cheese (cheese foods, spreads, slices)
	Pasteurized Fluid Milk
	Unpasteurized Fluid Milk
	Ice Cream and Other Frozen Dairy Products
	Cultured Milk Products (yogurt, sour cream, buttermilk)
	High Fat and Other Dairy Products (butter, cream, other milk products)
MEAT	Frankfurters (reheated)
	Frankfurters (not reheated)
	Dry/Semi-Dry Fermented Sausages
	Deli Meats (cooked, ready-to-eat)
	Pot and Meat Spreads
COMBINATION FOODS	Deli-type Salads (fruit, vegetable, meat, pasta, egg, or seafood salads)

L. monocytogenes Risk Assessment

- Relative risk of serious illness and death associated with consumption of RTE foods
 - Hazard identification:
 - Identifies health effects associated with *L. monocytogenes*
 - Exposure assessment:
 - Frequency and level of *L. monocytogenes* ingested via contaminated foods
 - Hazard characterization:
 - Relationship between dose and frequency of disease or mortality
 - Risk characterization:
 - Likelihood of adverse effect upon exposure to *L. monocytogenes*

Predicted No. Listeriosis Cases Per Annum for 23 Food Categories

Risk Ranking	Risk	Food	Cases
1	Very high	Deli Meats	1598.7
2	High	Pasteurized Fluid Milk	90.8
3	High	High Fat and Other Dairy Products	56.4
4	High	Frankfurters, not reheated	30.5
5	Moderate	Soft Unripened Cheese	7.7
6	Moderate	Pâté and Meat Spreads	3.8
7	Moderate	Unpasteurized Fluid Milk	3.1
8	Moderate	Cooked Ready-to-Eat Crustaceans	2.8
9	Moderate	Smoked Seafood	1.3
10	Low	Fruits	0.9
11	Low	Frankfurters, reheated	0.4
12	Low	Vegetables	0.2
13	Low	Dry/Semi-dry Fermented Sausages	<0.1
14	Low	Fresh Soft Cheese	<0.1
15	Low	Semi-Soft Cheese	<0.1
16	Low	Soft Ripened Cheese	<0.1
17	Low	Deli-type Salads	<0.1
18	Low	Raw Seafood	<0.1
19	Low	Preserved Fish	<0.1
20	Low	Ice Cream and Other Frozen Dairy	<0.1
21	Low	Processed Cheese	<0.1
22	Low	Cultured Milk Products	<0.1
23	Low	Hard Cheese	<0.1

Trends in *L. monocytogenes* Prevalence and Listeriosis

- Risk Assessment implicates RTE deli meats as major food vehicle
- Declines in prevalence of *L. monocytogenes* in RTE deli items and incidence of listeriosis not parallel
 - Prevalence of *L. monocytogenes* in RTE meat and poultry declined from 2.54% in 1998 to 0.55% in 2004 (USDA:FSIS)
 - Incidence of listeriosis declined from 5 cases/1,000,000 in 1996 to 3 cases/1,000,000 (CDC)
- Other food products (i.e., dairy, seafood, and produce) more common vehicles
- Changes in ecology of *L. monocytogenes* within food processing plant

Human Listeriosis Epidemics

Year	Location	Serotype	Ribotype	Food source
1976	Anjou, France	4b	DUP-1038B	-
1979	Boston, USA	Unknown	-	Raw vegetables
1980	New Zealand	Unknown	-	Raw shellfish
1981	Carlisle, England	1/2a	DUP-1030A	-
1981	Nova Scotia, Canada	4b	DUP-1038B	Coleslaw
1983	Massachusetts, USA	4b	DUP-1042B	Pasteurized milk
1985	Los Angeles, USA	4b	1038B	Jalisco soft cheese
1988-1990	United Kingdom	4b, 4bx	DUP-1042B	Pate
1987	Philadelphia, USA	4b	DUP-1038, DUP-1042	Ice cream, Salami
1992	France	Unknown	-	-
1988	Oklahoma, USA	1/2a	DUP-1053A	Turkey franks
1983-1987	Switzerland	4b	DUP-1038B	Cheese
1994	Illinois, USA	1/2b	DUP-1051B	Chocolate milk
1998	USA	4b	DUP-1044A	Hot dogs, packaged meat
1999	Finland	Unknown	-	Butter
2000	USA	1/2a	DUP-1053A	Delicatessen sliced turkey
2000	North Carolina, USA	4b	DUP-1042B	Mexican-style cheese
2002	Northeastern, USA	4b	DUP-1044A	Sliced deli meat

Loading of the Human Food Chain with *L. monocytogenes*

- Relationship between listeriosis in animals & humans not clear
 - Direct transmission very rare
 - 99% of human listeriosis cases attributed to foodborne transmission
- Animal-derived products other than meat and poultry (e.g., butter, chocolate milk, ice cream, soft cheeses) linked to human listeriosis outbreaks
- Agricultural practices have been associated with human listeriosis outbreak attributed to produce

Unpasteurized Milk Linked to Human Listeriosis Outbreak

- 1985 Los Angeles County, CA (Linnan et al., 1988)
 - 20 fetal, 10 maternal/neonatal, and 18 non-pregnant adult deaths
 - 82% isolates belonged to serotype 4b
 - 73% belonged to same phage type
- Case-control study implicated Mexican-style soft cheese
- Laboratory confirmed serotype 4b and epidemic phage type *L. monocytogenes* in cheese
- Investigation of cheese plant showed cheese commonly contaminated with unpasteurized milk

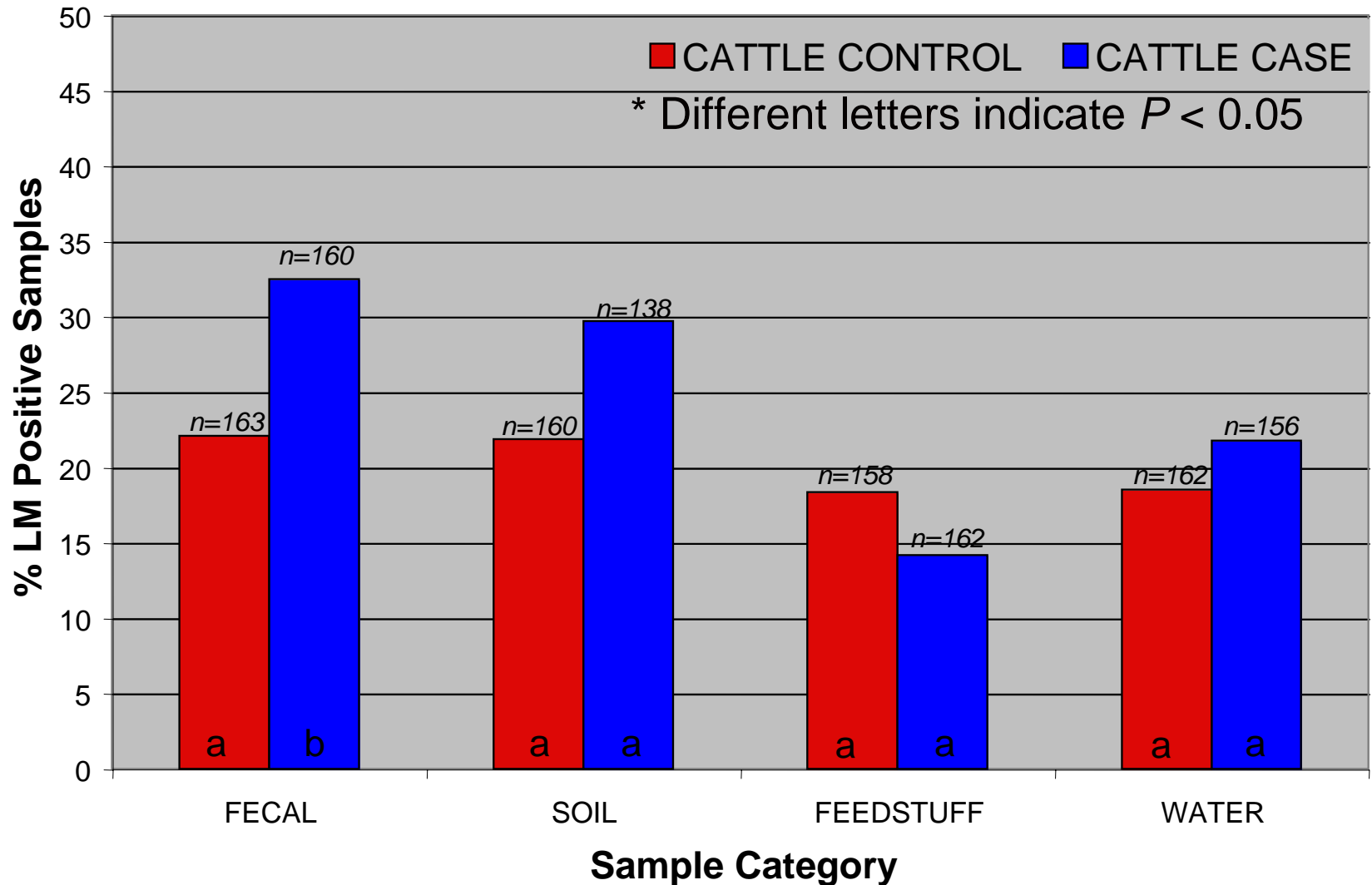
Agricultural Practices Linked to Human Listeriosis Outbreak

- 1981 Maritime Provinces, Canada (Schlech, 1984)
 - 7 adult cases and 34 perinatal cases
- Food preference survey to assess risk factors
 - Coleslaw identified as vehicle
 - *L. monocytogenes* serotype 4b in patient's blood detected in coleslaw from patient's refrigerator
- Coleslaw produced with cabbage from a farm with history ovine listeriosis
 - Contamination via fertilization with raw manure
 - Cabbage stored in large cold storage shed for prolonged period
- Indirect link established between invasive human listeriosis and pre-harvest food production system

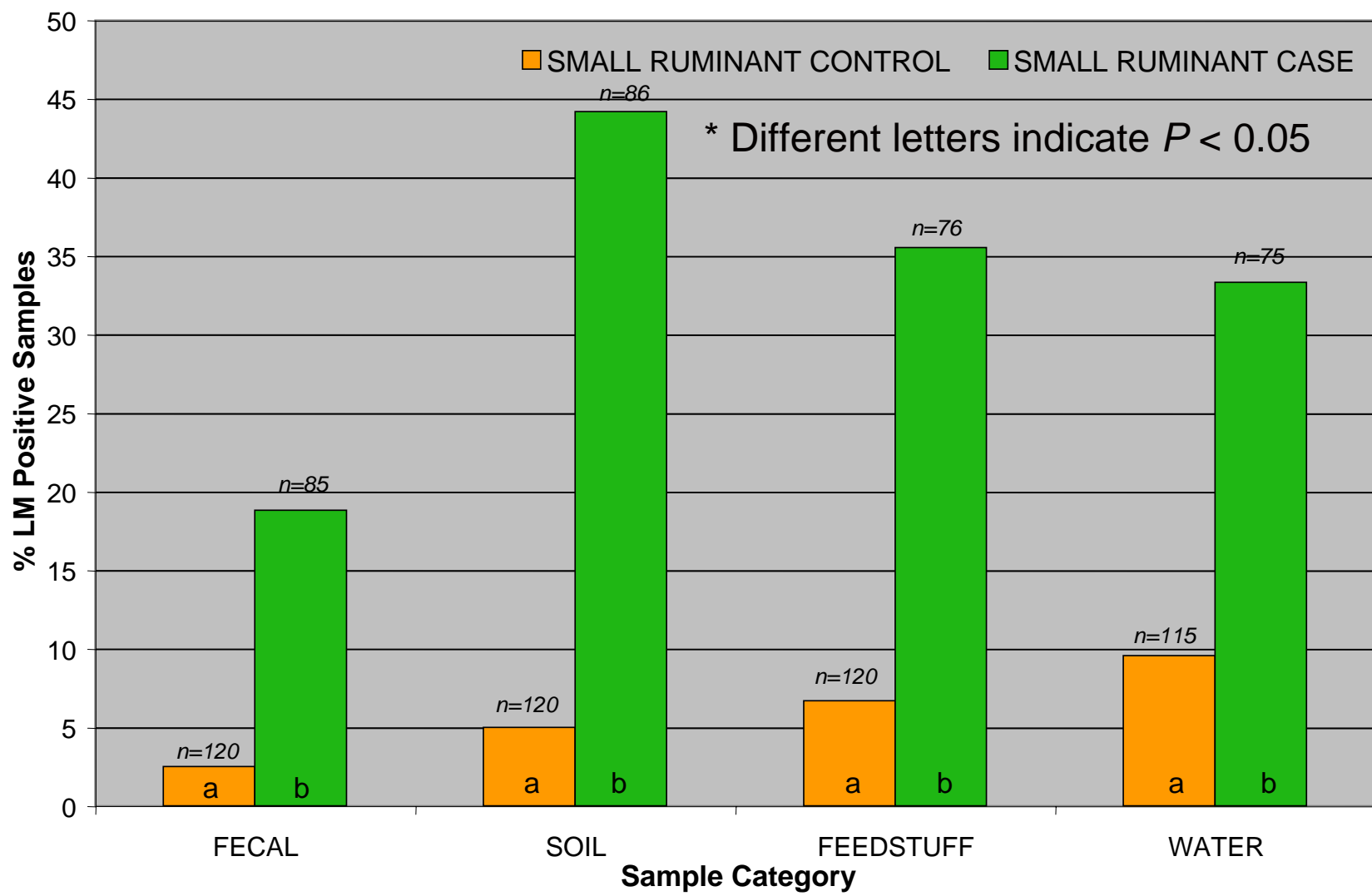
***L. monocytogenes* at the Pre-harvest Food Safety Level**

- Case-control study of ruminant listeriosis
 - Case farm definition
 - Clinical isolate, gross pathology or histopathology, or veterinarianian diagnosis
 - Control farm by matching for species, herd size, breed, location, and management system
- Fecal, feedstuffs, soil, and water samples collected and cultured for *L. monocytogenes*
- Molecular subtyping (*EcoRI* ribotyping) to characterize a single *L. monocytogenes* isolate to represent each positive sample
- Herd survey questionnaire

High Prevalence in Dairies with and without Disease



High Prevalence in Small Ruminant Farms with Disease



Epidemic Subtypes Present in Farms

No. subtype (% *monocytogenes* positive samples in case or control farms)

Subtype (Lineage)	Case farms					Control farms				
	Fecal	Feed	Soil	Water	Total	Fecal	Feed	Soil	Water	Total
DUP-1023A (II)	2	0	4	2	8 (3.1)	0	1	2	1	4 (2.5)
DUP-1030B (II)	0	1	2	4	7 (2.7)	2	0	2	0	4 (2.5)
DUP-1038B (I)	6	2	1	1	10 (3.9)	1	0	0	2	3 (1.9)
DUP-1039A (II)	1	2	3	2	8 (3.1)	1	3	1	3	8 (5.1)
DUP-1039C (II)	9	4	15	11	39 (15.2)	11	4	5	2	22 (13.9)
DUP-1039E (II)	7	3	2	3	15 (5.9)	5	5	4	10	24 (15.2)
DUP-1042B (I)	13	9	18	5	45 (17.6)	0	4	4	7	15 (9.5)
DUP-1045A (II)	0	1	3	2	6 (2.3)	3	5	6	4	18 (11.4)
DUP-1045D (II)	1	6	1	1	9 (3.5)	1	1	1	2	5 (3.2)
DUP-1045E (II)	2	3	4	6	15 (5.9)	0	0	0	0	0
DUP-1062E (II)	2	1	0	3	6 (2.3)	1	1	1	1	4 (2.5)
Other ribotype	8	6	13	12	88 (34.3)	7	5	6	6	51 (32.3)
Lineage I	32	24	34	12	102 (39.8)	9	9	10	10	38 (24.1)
Lineage II	35	26	45	47	153 (59.8)	30	28	29	30	117 (74.1)
Lineage III	1	0	0	0	1 (0.4)	0	0	2	1	3 (1.9)
Total	68	50	79	59	256	39	37	41	40	158

Persistence of *L. monocytogenes* in Food Processing Plants

- Followed environmental *Listeria* contamination patterns in smoked fish and seafood processing plants for at least 6 months to more than 5 years (e.g., Lappi et al., 2004)
- Environmental isolates were characterized by molecular subtyping
- Most plants had one or more plant specific *L. monocytogenes* subtype(s) that persisted in environment
 - Plants with dry environment and seasonal operation did generally NOT show persistent *L. monocytogenes* contamination

Plant A1	3/1/01	3/21/01	4/18/01	5/15/01	6/13/01	7/9/01	8/7/01	10/2/01	11/1/01	12/4/01	2/14/02	3/11/02	4/9/02	5/7/02	6/5/02	7/1/02	8/1/02	8/28/02	9/24/02	#####	12/2/02	#####	
Raw Product																							
	L.spp	L.spp	1052A	1042B	-	1039A	1039C	1039C	-	-	1038B	L.spp	L.spp	L.spp	-	L.spp	L.spp	L.spp	1042C	1027A	1052A	1042B	
	1 of 6	3 of 6	2 of 6	1 of 6	6 of 6	1 of 6	1 of 6	1 of 6	6 of 6	6 of 6	1 of 6	3 of 6	2 of 6	2 of 6	6 of 6	2 of 6	1 of 6	2 of 6	1 of 6	1 of 6	1 of 6	1 of 6	
Raw Environment																							
E2: Drain	-	1043A	-	1052A	1045B	1045B	1039C	1039C	1043A	1043A	L.spp	1052A	1039C	1048A	1039C	1043A	1043A	-	1043A	1062A	1027A	1052A	
E8: Apron	1062A	1062A	-	-	-	-	1052A	-	1043A	-	-	-	-	-	-	-	-	-	L.spp	1044A	-	-	
Fillet knife						1043A																	
Finished Enviroment																							
E1: Drain	1039C	1043A	1042B	1039C	L.spp	L.spp	L.spp	L.spp	1043A	L.spp	1043A	-	1043A			-	1043A	-	L.spp	-	1039C	1039C	
E3: Drain	1043A	-	1043A	-	1043A	1039C	-	L.spp	-	1043A	1042C	1042C	1042C	L.spp	1043A	1042C		1043A	1052A	1038B	1052A	1052A	
E4: Cooler Floor	1062A	L.spp	-	-	1043A	-	L.spp	L.spp	1052A	1043A	L.spp	-	-	L.spp	1052A	L.spp	-	-	-	1058B	-	L.spp	
Floor																							
Floor mat											L.spp	1052A											
E6: Cart wheels	L.spp	1043A	-	1052A	1027A	1043A	L.spp	1043A	1043A	1052A	1052A	-	-	-	-	-	-	-	-	-	-	-	
E5: Under Slice	-	-	-	-	-	-	L.spp	L.spp	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
E9: Sliding Door	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Food Contact Surfaces																							
E7: Gloves	-	-	-	-	-	-	-	-	L.spp	1043A	-	-	-	-	-	-	-	-	-	-	-	-	
E10: Slicer	-	-	-	-	-	-	-	-	-	-	1027A	-	-	-	-	-	-	-	-	-	-	L.spp	
E11: Skinner	-	-	-	-	L.spp	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1039C	-	-	
E12: DeBoner	-	-	-	1042B	-	-	-	-	-	-	-	-	-	-	L.spp	-	1043A	1044A	1044A	1044A	1044A	1044A	
E13: Sal. Table	-	-	-	-	-	-	-	-	-	-	-	L.spp	-	-	-	L.spp	L.spp	L.spp	-	-	1044A	-	
Tubs-dirty									1062A														
Tubs-clean									1043A	1044A													
New Mixer															-	-	-	L.spp	L.spp	L.spp	-	-	1044A
New Table															-	-	-	-	-	-	-	-	
Finished Product																							
	1 of 6	1 of 6	6 of 6	6 of 6	6 of 6	1 of 6	1 of 6	1 of 6	6 of 6	1 of 6	1 of 6	6 of 6	1 of 6	1 of 6	1 of 6	1 of 6	6 of 6	1 of 6	6 of 6	1 of 6	6 of 6	1 of 6	
	L.spp	L.spp	-	-	-	1062A	L.spp	L.spp	-	1043A	1042C	-	L.spp	1042C	L.spp	1052A	-	L.spp	-	L.spp	-	L.spp	

Summary

- Food products other than meat and poultry have commonly been linked to listeriosis outbreaks
 - May represent a more common vehicle
- Ruminant farms may constitute a significant natural reservoir for *L. monocytogenes*
- *L. monocytogenes* molecular subtypes have ability to persist in food processing plant environment

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