

Changes of *Listeria monocytogenes* Counts in a Gastrointestinal Model following Inoculation onto Salami or Bologna Slices and Storage at 4°C in Vacuum Packages

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ABSTRACT

Little is known regarding the influence of the food matrix on the ability of *Listeria monocytogenes* to survive passage through the gastrointestinal tract. We examined the fate of *L. monocytogenes* during simulated digestion of inoculated and stored salami and bologna. *L. monocytogenes* counts were determined (PALCAM agar; two replications, two samples each) during storage (82 days, 4°C) in vacuum-packages of inoculated (4.0-5.0 log CFU/g; 10-strain composite) salami or bologna slices, following exposure to a gastrointestinal model (37°C). Parameters included gastric emptying and gastrointestinal fluid secretion rates, gradual gastric acidification (pH reduction to 2.0 within 88 min) and intestinal pH maintenance (6.50±0.30). *L. monocytogenes* counts decreased on stored salami (pH 4.49±0.41) and increased on bologna (pH 6.13±0.09), reaching 1.4±0.4 and 8.7±0.1 log CFU/g, respectively (day-82). Major reductions in counts of both products occurred after 90 min of gastric challenge. At early stages of storage, cells appeared more acid-resistant on salami than on bologna. From day 1 to 27, contamination levels on salami decreased by approximately 2.0-3.0 log CFU/g after the 120-min gastric challenge; on bologna, reductions on corresponding days were 4.9-7.7 log CFU/g. The cell acid-resistance was not evident in salami as storage progressed (42-82 days) because counts present decreased (< 2.0 log CFU/g). Gastric survivors increased (P < 0.05) with storage of bologna (42-82 days) due to growth and possibly an increasing acid-tolerance of cells, which, at that time, had reached stationary-phase. Since emptying began when gastric pH was still high, contamination levels at the time of digestion affected numbers reaching the intestine more than gastric survival. Populations reaching the intestine increased with bologna storage; reductions after the 240-min intestinal exposure were approximately 1.0 log CFU/g. Prolonged storage may result in increased or decreased *L. monocytogenes* numbers during digestion depending on whether the product allows (bologna) or depresses (salami) growth of the pathogen.

OBJECTIVE

The objective of this study was to examine the survival properties of *Listeria monocytogenes* during simulated digestion of inoculated bologna and salami during refrigerated storage.

INTRODUCTION

Presence of *Listeria monocytogenes* in ready-to-eat (RTE) meat products poses a major public health concern as such products have been linked to fatal listeriosis outbreaks and numerous product recalls in the United States (CDC, 1999; 2000; 2002). Among 23 categories of RTE foods, the highest relative risk for listeriosis, on both per serving and per annum bases, was attributed to deli meats, while dry and semidry fermented sausages were characterized of low risk for the disease (HHS-FDA/USDA-FSIS, 2003). Nevertheless, although pathogen levels on dry and semidry fermented sausages are generally expected to be low, their consumption may still pose a food safety risk if surviving *L. monocytogenes* cells have been 'stress hardened' (Samelis and Sofos, 2003) due to their exposure to stress conditions (e.g., high acidity) prevailing on the surface of such foods. Consequently, stresses imposed to *L. monocytogenes* present on fermented dried products during their commercial life could lead to greater survival under subsequent host-related stresses. Existing scientific information regarding the contribution of the food matrix to the outcome of the *L. monocytogenes* infection is not enough for its consideration as a variable in the hazard characterization component of the *L. monocytogenes* risk assessment (Rocourt et al., 2003). Properties of the food vehicle that may be considered in hazard characterization include those that could affect survival of pathogens in the human gastrointestinal (GI) tract (HHS-FDA/USDA-FSIS, 2003), creating the need for research to advance the understanding of potential food matrix-related effects on the GI survival of *L. monocytogenes*.

MATERIALS AND METHODS

Meat products:

- ♦ Bologna (60% beef, 40% pork; no antimicrobials included; Samelis et al., 2001).
- ♦ Commercial salami (pork, beef, nonfat dry milk, salt, sugar, corn syrup solids, spices, wine, garlic powder, sodium ascorbate, lactic acid starter culture, sodium nitrite, butylated hydroxyanisole, butylated hydroxytoluene, and citric acid).
- ♦ Bologna (65 mm diameter) and salami (61 mm diameter) sticks were sliced (Globe slicer, Mozley Manufacturing, Stamford, CT) into 4 mm thick slices.

Inoculum:

- ♦ *L. monocytogenes* 10-strain composite:
 - ✓ N1-225 and N1-227 (serotype 4b, human and food isolate, respectively)
 - ✓ R2-500 and R2-501 (serotype 4b, food and human isolate, respectively)
 - ✓ R2-763, R2-764, and R2-765 (serotype 4b, human, food, and environmental isolate, respectively).
 - ✓ NA-1 (serotype 3b, pork sausage isolate)
 - ✓ N-7150 (serotype 3a, meat isolate)
 - ✓ 558 (serotype 1/2, pork meat isolate)
- ♦ Strains N1-225, N1-225, R2-500, R2-501, R2-763, R2-764, and R2-765 (Fugett et al., 2006) were kindly provided by Dr. Martin Wiedmann (Cornell University, Ithaca NY).

Product inoculation and storage:

- ♦ Inoculum level: 4.0 to 5.0 log CFU/g.
- ♦ Six slices of each product were vacuum packaged and stored at 4°C for 82 days.

Simulated GI fluids:

Artificial saliva:

- ♦ 6.2 g NaCl, 2.2 g KCl, 0.22 g CaCl₂, and 1.2 g NaHCO₃ were added to 1 liter of distilled water (Minekus et al., 1995).
- ♦ Sterilized by autoclaving and cooled to approximately 25°C before use.

Simulated gastric fluid:

- ♦ 0.4 g/liter glucose, 3.0 g/liter yeast extract (Acumedia, Lansing, MI), 1.0 g/liter Bacto Peptone (Difco, Becton, Dickinson, Sparks, MD), 4.0 g/liter porcine mucin (Sigma-Aldrich, St. Louis, MO), 0.5 g/liter cysteine, 0.08 g/liter NaCl, 0.4 g/liter NaHCO₃, 0.04 g/liter K₂HPO₄, 0.04 g/liter KH₂PO₄, 0.008 g/liter CaCl₂·2H₂O, 0.008 g/liter MgSO₄·7 H₂O, 1.0 g/liter xylan (Sigma-Aldrich), 3.0 g/liter soluble starch (Sigma-Aldrich), 2.0 g/liter pectin (Sigma-Aldrich), and 1 ml/liter Tween 80 (Naim et al., 2004).
- ♦ Autoclaved and cooled to approximately 25°C.
- ♦ Addition of 3 liter pepsin from porcine stomach mucosa (Sigma-Aldrich) and pH adjustment to 2 (5N HCl) before use.

Artificial intestinal fluid:

- ♦ 0.1 g trypsin from porcine pancreas (type IX-S; Sigma-Aldrich) and 3.5 g pancreatin from porcine pancreas (Sigma-Aldrich) were mixed in 1 liter distilled water (Koo et al., 2001).
- ♦ Filter-sterilized (0.45-µm pore-diameter cellulose filter; Millipore Corp., Bedford, MA).

Biliary secretions:

- ♦ Bile (porcine bile extract, Sigma-Aldrich) solutions (2% and 4%) prepared in distilled water (Minekus et al., 1995) and filter-sterilized by use.

GI challenge:

- ♦ Artificial saliva (70 g) was added to six slices of each product (contained in a single vacuum bag; 50-55 g total) and blended for 3 min at high speed.
- ♦ Gastric pH was adjusted (Dressman et al., 1990) to: 5.0 at 10 min, 4.0 at 28 min, 3.0 at 58 min, and 2.0 at 88 min (5N HCl).
- ♦ Intestinal pH was maintained at 6.5±0.3 (0.3 M NaHCO₃).

Microbiological analyses:

- ♦ At 0, 30, 60, 90, and 120 min in the gastric compartment (GC).
- ♦ At 30, 60, 90, 120, and 240 min in the intestinal compartment (IC).
- ♦ Plating on:
 - ✓ Tryptic soy agar (Difco) plus 0.6% yeast extract (TSAYE).
 - ✓ PALCAM agar (Difco).

- ♦ Plate incubation at 25°C for 72 h (TSAYE) or 30°C for 48 h (PALCAM agar).

Statistical analyses:

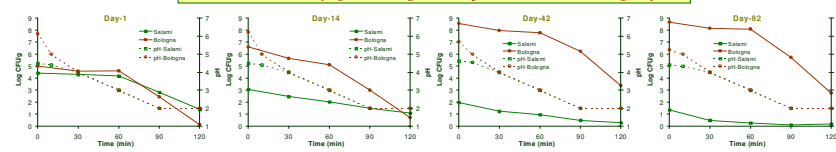
- ♦ Two replicate experiments; two samples per product and storage day.
- ♦ Cell counts were divided by dilution factors (DF; Koo et al., 2001):

$$DF (GC) = \frac{\text{Remaining sample (ml)}}{\text{Remaining sample (ml)} + HCl (ml) + \text{Gastric fluid (ml)}}$$

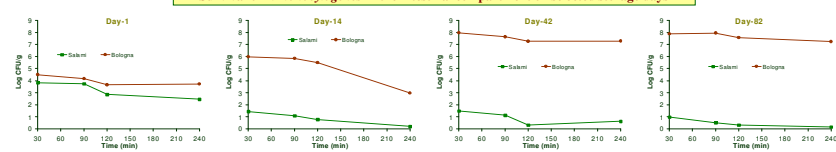
$$DF (IC) = \frac{\text{Remaining sample (ml)}}{\text{Total IC contents (ml)} + \text{Gastric contents (ml)} + \text{NaHCO}_3 (\text{ml}) + \text{Bile (ml)} + \text{Intestinal fluid (ml)}}$$

- ♦ Numbers obtained were converted into log CFU/g and analyzed using the Mixed Procedure of SAS version 9.1 (SAS Institute, Cary, NC).
- ♦ *L. monocytogenes* counts were used to determine death rates (DR) for each strain in each GI compartment with the model of Baranyi and Roberts (1994) and the DMFit program (Institute of Food Research, Norwich, UK).

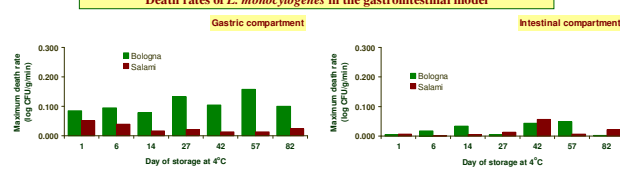
Survival of *L. monocytogenes* in the gastric compartment on selected storage days



Survival of *L. monocytogenes* in the intestinal compartment on selected storage days



Death rates of *L. monocytogenes* in the gastrointestinal model



♦ A, B: 500-ml Erlenmeyer flasks representing the gastric (GC) and intestinal compartment (IC).

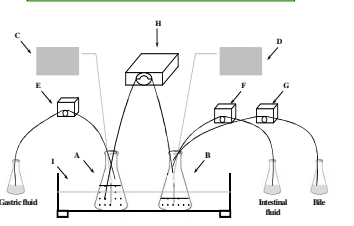
♦ C, D: pH meters (Ultra Basic, Denver Instrument, Arvada, CO) monitoring the pH in the GC and IC.

♦ E, F, G: peristaltic pumps (Variable speed Pump Low Flow, Fisher Scientific) delivering gastric fluid (flow rate: 0.33 ml/min) in the GC and intestinal fluid (flow rate: 0.33 ml/min) and 2 or 4% bile (flow rate: 0.5 ml/min) in the IC.

♦ H: peristaltic pump (205U, Watson-Marlow Limited, Cornwall, England) transferring (flow rate: 1.1 ml/min) the gastric contents to the IC.

♦ I: shaking water bath (Shaking Water bath 50, Precision Scientific, Chicago, IL) stabilized at 37°C.

Schematic diagram of the dynamic GI model



CONCLUSIONS

Under the conditions of this study:

- ♦ Type of product and duration of storage influenced the GI survival of *L. monocytogenes*.
- ♦ Enhanced gastric survival of *L. monocytogenes* inoculated on salami may have been due to adaptation of bacterial cells to the acidic conditions of the product, the high fat content of the product, or the combination of both factors.
- ♦ However, any effects of the food matrix *per se* on the GI survival of the pathogen were overshadowed by the high and low contamination levels reached on bologna and salami, respectively, during storage.
- ♦ Thus, the potential for *L. monocytogenes* growth, as affected by attributes of each product (i.e., pH, water activity), and the length of the storage period were the most influential factors, in terms of *L. monocytogenes* levels being present in the compartments of the GI system.
- ♦ Results also highlight the importance of simulating the dynamics of gastric emptying and gradual acidification in studies designed to investigate GI aspects of the *L. monocytogenes* infection.

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RESULTS

Chemical and physical properties of products:

- ♦ pH on day-0: 6.16±0.004 (bologna) and 4.47±0.13 (salami).
- ♦ a_w on day-0: 0.968±0.006 (bologna) and 0.892±0.014 (salami).
- ♦ The fat content (%) of bologna and salami was 18.8±0.7 and 29.1±2.1, respectively, and the moisture content was 61.8±0.5 and 35.6±1.0, respectively.

Changes in microbial populations during storage:

- ♦ *L. monocytogenes* populations increased on bologna and decreased on salami, reaching 8.7 and 1.4 log CFU/g, respectively, on day-82.
- ♦ Total microbial populations on bologna followed growth trends similar to those of *L. monocytogenes* during storage.
- ♦ Colonies of salami samples on TSAYE consisted mostly of colonies of lactic acid bacteria-like organisms.

Gastric survival:

- ♦ Effects of product type and storage duration on gastric survival being obvious mainly at 120 min of gastric exposure.
- ♦ Reductions in populations:
 - ✓ On bologna: 4.9 (day-1) to 7.6 (day-27) log CFU/g.
 - ✓ On salami: 1.2 (day-82) to 3 (day-1) log CFU/g.

- ♦ Gastric DR (log CFU/g/min) of *L. monocytogenes* on bologna or salami varied from 0.079 (day-14) to 0.158 (day-57), and 0.013 (day-42) to 0.051 (day-1), respectively.
- ♦ Populations declined faster on bologna than on salami during the gastric challenge, on corresponding days.

- ♦ In general, reductions of total microbial populations during digestion of bologna followed similar trends with those of *L. monocytogenes*.
- ♦ Reductions in total microbial populations during gastric exposure of salami samples ranged from 3.3 to 6.1 log CFU/g during storage.

- ♦ Although *L. monocytogenes* populations on bologna decreased more rapidly than those on salami during gastric exposure, initial contamination levels reached during bologna storage resulted in higher (P < 0.05) numbers of survivors being detected in the GC:
 - ✓ For the first 90 min of gastric challenge, on days 6 to 27 of storage.
 - ✓ Throughout gastric challenge, on days-42 to 82, as compared to those recovered during gastric challenge of salami.

- ♦ Throughout gastric challenge, on days-42 to 82, as compared to those recovered during gastric challenge of salami.

Intestinal survival:

- ♦ Delivery of gastric contents in the IC began while the gastric pH was still high (> 4.41).
- ♦ Thus, *L. monocytogenes* populations transferred from the GC to the IC within the first 30 min of gastric emptying depended on the initial contamination levels on each product.
- ♦ As a result, the duration of the storage period in combination with the type of product (supportive vs. not supportive of growth) had a major effect on the number of pathogenic cells being present in the IC, as overall initial (30 min) *L. monocytogenes* populations in the IC were ≤ 1 log CFU/g lower than initial levels on each product.
- ♦ Subsequently, DR ranged from 0.003 to 0.048 (bologna) and 0.002 to 0.056 (salami), throughout storage.

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