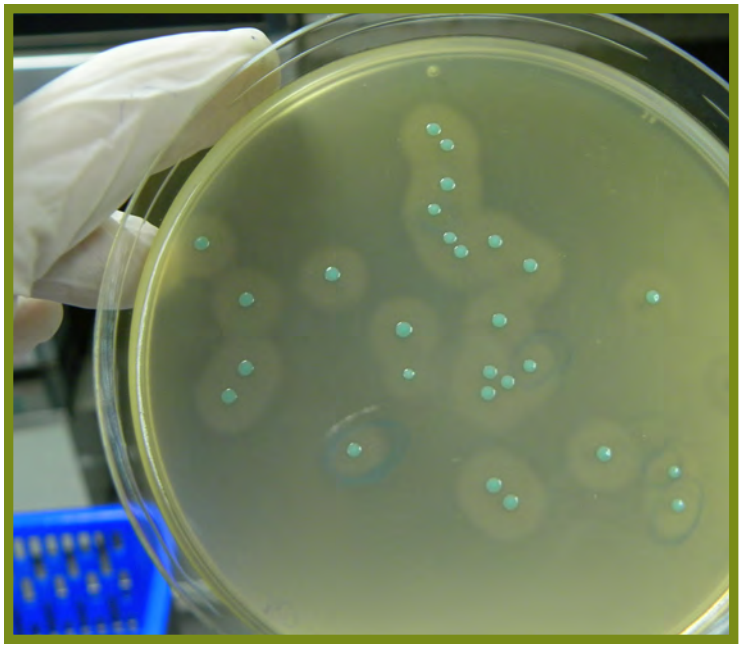
Ellen W. Evans[1] and Elizabeth C. Redmond[1]

[1]ZERO2FIVE Food Industry Centre, Cardiff Metropolitan University, Western Ave., Llandaff, Cardiff, Wales CF5 2YB

PEER-REVIEWED ARTICLE

Food Protection Trends, Vol 39, No. 2, p. 225–236

6200 Aurora Ave., Suite 200W, Des Moines, IA 50322-2864



Laboratory Re-enactment of Storage Practices of Older Adults to Determine Potential Implications for Growth of *Listeria monocytogenes*

ABSTRACT

Older adults are particularly susceptible to listeriosis, and many frequently consume ready-to-eat (RTE) foods associated with *Listeria monocytogenes*. Consequently, safe storage of RTE-food is essential to reduce the risks of listeriosis. This study aimed to re-enact domestic food-storage malpractices of older adult consumers in a laboratory, to assess the potential impact on *L. monocytogenes*. Observed and self-reported data relating to domestic food-storage malpractices included prolonged storage of RTE foods and/or refrigeration temperatures exceeding recommendations (> 5.0°C). Re-enactment was performed using soft-cheese and RTE meat inoculated with ~3.7 log CFU *L. monocytogenes*, stored at recommended temperatures (2.5°C) (n = 110); temperatures exceeding recommendations (7.8°C) (n = 110), and ambient temperature (19.5°C) (n = 55). Samples were analyzed every 24 h for up to 21 days. Results indicated that *L. monocytogenes* grew at all storage temperatures. Average generation times indicated slower growth of *L. monocytogenes* at 2.5°C (94 h t^{-1}) than at either 7.8°C (21.5 h t^{-1}) or 19.5°C (11 h t^{-1}), suggesting that prolonged storage of RTE foods resulted in increased *L. monocytogenes* populations (< 7.6 log CFU/g), potentially making such foods unsafe for consumption. Findings indicate that storage practices contrary to consumer recommendations, which are intended to reduce the risk of foodborne disease, increase *L. monocytogenes* populations, thus increasing the potential for foodborne disease.

INTRODUCTION

Listeria monocytogenes has the ability to survive and grow slowly at refrigeration temperatures *(1, 6)* and extended storage of food may allow the pathogen to reach high populations *(8)*. Many ready-to-eat (RTE) food products are associated with *L. monocytogenes* contamination or listeriosis incidence. These foods often have an extended refrigerated shelf life *(41)*, have the ability to support the growth of the pathogen to reach high populations *(29)*, and require no further processing by consumers prior to consumption *(40)*. Food products that have been associated with listeriosis include RTE fish products *(30, 32, 33, 69)*, RTE meat

*Author for correspondence: Phone: +44 (0.2920.205836; Fax: +44 0.2920.416982; Email: elevans@cardiffmet.ac.uk

products *(15, 19, 53, 58)*, pre-packed sandwiches containing meat or dairy products and/or salad ingredients *(45, 51, 71)*, soft cheese *(2, 4, 11, 39, 42)*, and cooked ham *(16, 35, 67)*.

Although contamination can result from consumer malpractices in the domestic environment, *L. monocytogenes* contamination of RTE foods may also occur as a result of post-processing cross-contamination, such as transfer from a slicing machine *(12, 48)* during manufacturing or during operations at retail *(9)*. Consequently, there is a need to ensure that practices that prevent the growth of *L. monocytogenes* in foods stored in the domestic kitchen are followed. Adequate storage temperatures and avoiding prolonged storage of opened RTE food products being stored in the domestic kitchen are essential to safeguard food from the potential growth of *L. monocytogenes* and the possible risk of listeriosis to consumers. Such practices are included in consumer food safety initiatives to reduce the risks associated with *L. monocytogenes (31)*.

The Codex Alimentarius Commission *(13)* has microbiological criteria for the presence of *L. monocytogenes* in RTE foods that can support the growth of the pathogen. Consequently, 'use-by' dates of foods that will support the growth of *L. monocytogenes* are established to ensure that food products remain safe for consumers to the point of consumption *(70)*, by ensuring that a limit of 100 CFU/g will not be exceeded at any point between their production and consumption *(10, 21)* . However, if modified atmosphere packaging and/or refrigeration are utilized to achieve extended shelf life in RTE food products, there is a need to ensure the safety of refrigeration temperatures and to ensure that after opening, when the integrity of the packaging changes and all antimicrobial properties are lost *(3, 66)*, such foods are consumed promptly. Although the consumer relies on food manufacturers and suppliers to ensure that the RTE food products they purchase are safe for consumption, the post-purchase responsibility is in the hands of the consumer to implement safe food storage and handling practices *(14)*.

Consumer recommendations to reduce the risks associated with listeriosis in the domestic environment include (i) following use-by dates on unopened prepackaged RTE food products, (ii) avoiding prolonged storage of leftover fresh RTE foods and opened prepackaged RTE food products, and (iii) ensuring safe operating temperatures of domestic refrigerators (≤ 5.0°C) *(25)*. Following these recommendations to reduce the risk of listeriosis is essential, particularly for consumer groups with weakened immunity, including older adults, pregnant women, people living with HIV and patients receiving chemotherapy, all of whom are known to be at an increased risk of foodborne disease, particularly listeriosis *(47)*.

The majority of cases of listeriosis in recent years (since 2000) have predominantly been associated with adults ≥ 60 years *(1, 20)*, and recent data indicate that 65% of reported listeriosis cases were among adults aged over 60 years *(61)*. The UK Food Standards Agency (FSA) *(54)*, the Advisory Committee on the Microbiological Safety of Food (ACMSF) in Europe *(1)* and the U.S. FDA *(68)* have identified the need for research to determine domestic food-handling and storage behaviors of consumers ≥ 60 years so as to better understand the behavioral risk factors potentially associated with listeriosis. Market intelligence reports suggest that older adults in the UK may consume more RTE foods associated with listeriosis than other consumer groups *(17, 55–57)* and that older adult consumers (aged ≥ 60 years) fail to adhere to recommendations designed to reduce the risk of listeriosis *(22–24)*.

Cognitive food safety research *(27)* has determined that although older adult consumers have expressed positive attitudes toward refrigeration, most are unaware of recommended temperatures and report that refrigeration temperatures are not checked. Similarly, although most know that "use-by" dates indicate food safety, the majority believe it safe to eat food beyond use-by dates and report doing so. Additionally, attitudes toward consuming foods within the recommended 2 days of opening were neutral, with the vast majority reporting that they consume RTE foods beyond that recommendation *(27)*. A survey of older adult consumers' domestic kitchens determined that the majority had opened RTE foods, associated with outbreaks of listeriosis, which had been or were intended to be stored beyond the recommended 2 days after opening. Older adults failed to ensure safe refrigeration temperatures, with the majority of refrigerators operating at temperatures exceeding recommendations *(26)*. A model kitchen observational study established that a small number of older adults may fail to implement refrigerated storage when handling leftover RTE food in the domestic kitchen *(22)*. Furthermore, time-temperature profiling of domestic refrigerators in consumer kitchens established that no refrigerators operated at recommended temperatures for the entire duration of the study *(28)*.

Given that unsafe refrigeration temperatures and prolonged storage of opened RTE foods are common, and that such storage malpractices are more widespread than the isolation of *L. monocytogenes* in older adult consumers' domestic kitchens (2% of kitchens *(26)*); it may be suggested that storage malpractices may be a greater risk factor for listeriosis than the presence and potential cross-contamination of the pathogen *(24)*. However, there is a need to determine the potential risk of such malpractices, utilizing a laboratory-based re-enactment of identified storage malpractices to ascertain the impact of such practices on the survival and growth of *L. monocytogenes* in RTE food *(23)*.

Many research studies have considered the potential influence of different storage conditions on the growth of *L. monocytogenes*, frequently evidenced and achieved by use of food models. To determine the impact of such consumer food storage practices on the safety of RTE

food products, there is a need to determine potential microbiological risks by re-enacting observed and self-reported domestic practices. Performing a laboratory-based re-enactment study allows for the replication of domestic kitchen food safety scenarios, and this facilitates obtaining of data on microbiological risk factors. Laboratory-based re-enactment of specific consumer food safety behaviors has not been widely used; however, previous research has linked consumer food hygiene and preparation behavior to microbiological cross-contamination *(62)*.

The aim of the study was to re-enact food safety malpractices observed in domestic kitchens of older adults, or self-reported by older adult consumers, that are contrary to food safety recommendations intended to reduce the risk of listeriosis. Such data will determine and quantify the potential risk associated with older adult consumer food storage malpractices with respect to the survival and growth of *L. monocytogenes* in RTE foods.

METHODS

Identification of older adult consumers' food storage malpractices

Data on cognitive and behavioral risk factors associated with storage malpractices of RTE food products associated with listeriosis were reviewed *(26–28)*. Observed and self-reported storage practices from a survey of older adults' domestic kitchens *(26)* as indicated in *Table 1* informed determination of storage lengths and temperatures used for reenactments, which include prolonged storage at recommended temperatures and storage at temperatures exceeding recommendations. In addition, two consumers reported that ham or soft cheese would be stored for 5 or 6 days, at ambient temperatures. Based on these findings, the re-enactment study was conducted with RTE ham and and soft cheese, which were stored at three storage temperatures for up to 21 days.

Laboratory-based re-enactment of storage malpractices

Refrigerated packs of 'Brie style soft cheese' and packets of pre-packed RTE sliced cooked ham were purchased from a large supermarket and transported to the laboratory within 20 min of purchase in a cool bag with ice packs. The method of transportation was validated on the basis of procedures used in previous validation work conducted by Slader *(64)*. The temperature within the cool bag was monitored on five occasions using calibrated dataloggers (SL52T self-contained single channel, button temperature logger; Signatrol Ltd Gloucestershire UK. Range: -40°C to +85°C. Accuracy: ±0.5°C); the temperature within the cool bag remained below 5.0˚C for up to 3 hours, thus ensuring that the microbiological safety of the food products was not compromised during transportation. Preliminary validation work was conducted along with controls on the uninoculated food products.

A suspension culture of *L. monocytogenes* serotype 1/2a (L002, isolated from a drain in a food processing plant from a previous research study *(59)*), was prepared. A single cryobead (Technical Service Consultants, Lancashire, UK), from frozen stock stored at -80°C (New Brunswick Scientific Innova® U535 ultra-low temperature lab freezer, Connecticut, USA) was aseptically placed in 100 mL tryptone soya broth (TSB; CM0129, Oxoid Ltd, UK) in a 250 mL Erlenmeyer flask. The flask was incubated for 24 hours on a shaking platform (MaxQ* 4000 Benchtop Orbital Shaker; Thermo Scientific Asheville, USA), set at 250 rpm and 35°C.

The inoculated broth was placed into two 50 mL sterile centrifuge tubes (Corning® 50 mL PP Centrifuge Tubes, Conical Bottom with CentriStar™ Cap; New York, USA). Cells were harvested by centrifugation at 3000 RPM (×1068g) for 20 min at ambient temperature (21°C) in a centrifuge (DuPont Sorvall Superspeed RC-5B refrigerated centrifuge; Thermo Scientific Asheville, USA). The pellet was re-suspended in 40 mL sterile peptone saline diluent (PSD; Oxoid Ltd, UK), achieving an initial inoculum of approximately 5.2×10^9 CFU/mL (9.7 log CFU/mL). Serial decimal dilutions were prepared aseptically using the initial inoculum and peptone saline to achieve a 10^{-5} dilution.

Food products were weighed aseptically, placed in sterile petri dishes (Petri dish 663102, Greiner bio-one Ltd. Stonehouse, UK) and inoculated with *L. monocytogenes*. A 12.5 g portion of RTE ham (quantity determined according to pack size and ease of portioning to ensure minimal handling) was inoculated by means of a 0.1 mL spot inoculation, in the middle of the ham, of approximately 3.7 log CFU *L. monocytogenes*, thus giving a concentration of 2.6 log CFU per gram of RTE ham. A 10 g portion of soft cheese was inoculated by means of a 0.1 mL spot inoculation in the center of two slices of cheese, giving a concentration of 2.7 log CFU per gram of soft cheese. Sufficient food products were inoculated to allow five replicates of each food product to be performed at each time and temperature point, to allow for sampling variability. Food products were left at ambient temperature for 20 min to ensure that the inoculum was absorbed into the food products prior to storage.

Two domestic type refrigerators (Gram K400 (h) climate class N, Denmark, and Scandinova LF 136 D Larder refrigerator, Vestfrost, Denmark) were selected to replicate typical domestic storage and were validated prior to the study to be operating at a recommended operating temperature (≤ 5.0°C) and a temperature exceeding this (> 5.0°C). The operating temperatures of the domestic refrigerators were monitored using two calibrated thermometers (P300 handheld thermometer; Industrial Temperature Sensors Co. Kildare Ireland; measuring range: -40°C to +200°C, accuracy: ± 1.0°C) and calibrated dataloggers (SL52T self-contained single channel, button temperature logger; Signatrol Ltd. Gloucestershire UK; measuring range: -40°C to +85°C, accuracy: ± 0.5°C).

TABLE 1. Observed and self-reported storage practices in older adults' domestic kitchens

Participant ID	Refrigerator operating temperature (°C)	Food product observed stored	Self-reported storage duration (days)
Prolonged storage at recommended temperatures (≤ 5.0°C)			
MP028	4.1	RTE Ham	5
MP018	5.0	RTE Ham	7
MP037	4.6	RTE Ham	10
MP082	3.6	RTE Ham	14
MP038	4.3	Soft Cheese	15
MP056	3.5	Soft Cheese	15
Storage at temperatures exceeding recommendations (> 5.0°C)			
MP073	13.3	RTE Ham	2
MP071	9.0	Soft Cheese	2
MP087	7.2	RTE Ham	4
MP023	9.5	RTE Ham	4
MP083	5.2	RTE Ham	4
MP014	9.0	RTE Ham	4
MP091	5.2	RTE Ham	6
MP087	8.3	RTE Ham	6
MP029	8.7	Soft Cheese	7
MP070	8.0	Soft Cheese	7
MP080	5.8	RTE Ham	9
MP006	8.3	RTE Ham	10
MP010	9.8	Soft Cheese	14
MP088	5.1	Soft Cheese	14
MP070	8.1	Soft Cheese	18
MP088	5.1	RTE Ham	21
MP072	Ambient temperature	Soft Cheese	6
MP038	Ambient temperature	RTE Ham	5

Uninoculated food products were stored up to the maximum length of storage at the three different temperatures to determine the presence and growth of *L. monocytogenes*. Inoculated and uninoculated control food products were stored in the two refrigerators, one operating at a recommended (≤ 5.0°C) temperature (mean temperature: 2.5°C, SD ± 2.2) and one operating at an 'abuse' temperature (> 5.0°C) exceeding recommendations (mean temperature: 7.8°C, SD ± 0.4) for up to 21 days, and at ambient temperature (mean: 19.5°C, SD ± 1.2) for 10 days. Following 48 hours at the

TABLE 2. Changes in average food product pH and a_w at start and end of storage

Food product	a_w		pH	
	Day 0	Day 21	Day 0	Day 21
Soft Cheese	0.97	0.99	7.7	6.9
RTE Ham	0.96	0.99	6.7	5.6

three different storage temperatures, both food products at all storage temperatures were analyzed at 24-hour intervals to determine *L. monocytogenes* growth. Analysis was conducted following methods based on the National Standard Method (F2) for preparation of samples and dilutions *(36)* and the National Standard Method (F19) for enumeration of *Listeria monocytogenes (38)* to determine the number of *L. monocytogenes* in the food samples.

A dilution was prepared by adding nine times the weight of the food of peptone saline diluent (PSD) (CM0733, Oxoid Limited, Hampshire, UK), to make a 100 g sample of soft cheese and 125 g sample of RTE ham, thus giving a 1 in 10 suspension. Decimal dilutions were prepared aseptically using the homogenate and ambient temperature PSD. The number of decimal dilutions was determined by the pilot study and by consideration of growth increase over previous days.

From the five replicate samples of each food product at each time point and temperature, duplicate 0.1 mL samples of each dilution were inoculated onto the surface of Chromogenic agar and spread, using single-use sterile spreaders. Plates were incubated (IP20 Controlled Environment Chamber, Binder GmbH, Tuttlingen, Germany) at 37°C for 24 hours and up to 48 hours if necessary.

Following incubation, plates were examined for typical colonies of *L. monocytogenes*, according to the Standard Operating Procedure to identify *Listeria* colonies *(37)*, whereby plates with up to 150 colonies were counted and cell counts were converted to log CFU/g of *L. monocytogenes*.

Physical properties of the food products were analyzed at the start and end of storage. The pH of the soft cheese and RTE ham stored were measured using a calibrated portable pH meter (HI-99161 Handheld Food and Dairy pH Meter; Hanna Instruments. Range: 0.00 pH to 14.00 pH. Accuracy: ±0.02 pH) on the first and last day of storage. The a_w was also measured in soft cheese and RTE ham at the start and end of the storage period, samples of the food products were placed in the base of a disposable sample cup inside a sealed chamber of a water activity meter (Pawkit Portable Water Activity Measurement system; AquaLab. Range: 0.00 – 1.00 a_w. Accuracy: ±0.02).

Data analysis

Data were entered into a specially designed Microsoft Excel 2010 (Microsoft; Redmond, WA, USA) dataset, and means of the 5 replicates were determined for calculation of population increase per day (log CFU); growth rate constant (from start of exponential phase (day 0 in RTE ham, and day 2 in soft cheese), to the end of exponential phase, giving the proportion of cells growing every hour); and generation time (time taken for population to double). Data analyses were conducted using SPSS Statistics 20 (IBM® Software Group; Chicago, IL, USA) and Minitab 15 (Minitab Inc.; State College, PA, USA). Statistical tests selected included *t*-tests and Analysis of Variance (ANOVA), with post-hoc comparison using the Tukey HSD test.

RESULTS

In total, over six hundred samples of soft cheese and RTE ham inoculated with *L. monocytogenes* were stored at three different temperatures, to reenact domestic food storage practices of older adults and analyzed to determine the influence of time and temperature on survival and growth of *L. monocytogenes*. Findings are presented according to storage temperature and are cumulatively compared against results obtained with recommended storage practices, using statistical analysis.

Uninoculated food products were stored up to the maximum storage time at the three different domestic storage temperatures. *L. monocytogenes* was not isolated in the RTE ham or soft cheese immediately after purchase, or in the RTE ham following the maximum storage time at all three temperatures. However, the uninoculated soft cheese samples stored at 19.5°C for 11 days contained *L. monocytogenes* at levels of 3.6 log CFU/g.

Initial inoculation was 2.6 log CFU *L. monocytogenes* per gram of RTE ham, and 2.7 log CFU *L. monocytogenes* per gram of soft cheese. After inoculation on day zero, analysis determined that initial levels of *L. monocytogenes* were < 1.8 log CFU/g in the food products.

Following 21 days of storage, an increase in a_w of the RTE ham and soft cheese was detected. The a_w increased from 0.97 in soft cheese and 0.96 in RTE ham to 0.99 in both products

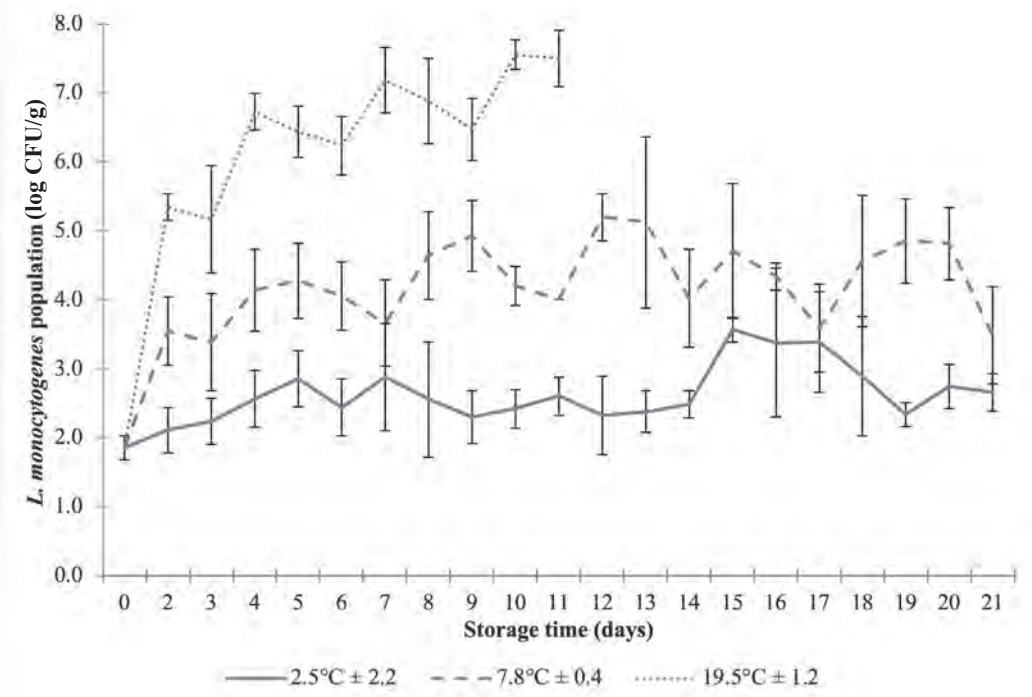


FIGURE 1. Mean *L. monocytogenes* growth (log CFU/g) in RTE ham at three different domestic storage temperatures. Recommended refrigeration temperature (2.5°C ± 2.2) (continuous line); refrigeration temperature exceeding recommendations (7.8°C ± 0.4) (broken line); ambient temperature (19.5°C ± 1.2) (dotted line).

(Table 2); changes were also identified in the pH of food products, with the pH of all food products becoming more acidic, a mean pH change of 0.8 in soft cheese, to 6.9, and of 1.1 in RTE ham, to 5.6. Although changes occurred in the pH and a_w of the RTE ham and soft cheese during re-enacted domestic storage, the values remained within the optimal growth ranges for *L. monocytogenes*.

Growth of *L. monocytogenes* in food products stored at recommended temperature

The maximum population of *L. monocytogenes* achieved during storage at 2.5°C was 5.5 log CFU/g in soft cheese after 16 days of storage. The maximum population of *L. monocytogenes* was 3.6 log CFU/g in RTE ham following 15 days of storage. The average daily increase in *L. monocytogenes* populations was 0.04 log/day in RTE ham and 0.3 log/day in soft cheese.

Growth of *L. monocytogenes* in food products stored at a temperature exceeding recommendations

During storage at 7.8°C, the maximum population of *L. monocytogenes*, 6.8 log CFU/g, was achieved after 12 days of storage in soft cheese and the maximum population, 5.2 log CFU/g, was also achieved after 12 days in RTE ham. The average daily population increase was 0.1 log/day in RTE ham stored at 7.8°C and was 0.3 log/day in soft cheese.

Growth of *L. monocytogenes* in food products stored at ambient temperature

Growth of *L. monocytogenes* in food products stored at (19.5°C ± 1.2) was determined. The maximum *L. monocytogenes* population, 7.6 log CFU/g, was achieved after 5 days of storage in soft cheese and after 11 days in RTE ham. The average daily population increase was 0.6 log/day in RTE ham and 0.7 log/day in soft cheese. After 3 days, organoleptic changes that included changes in appearance of texture, color and odor were detectable in both the soft cheese and RTE ham.

Comparison of *L. monocytogenes* growth at different domestic storage temperatures

As indicated in *Fig. 1* and *Fig. 2*, populations of *L. monocytogenes* increased more at ambient temperature (19.5°C) than at refrigerated temperatures (2.5°C and 7.8°C) and more at 7.8°C than at 2.5°C. Furthermore, higher counts were achieved at 19.5°C and 7.8°C than at 2.5°C. The levels of *L. monocytogenes* in RTE ham stored for two days at a temperature exceeding recommendations were greater than that achieved after 15 days storage at 2.5°C *(Fig. 1)*.

Similarly, in soft cheese, increased storage temperature resulted in an increase in maximum achieved population. As illustrated in *Fig. 2*, after seven days, *L. monocytogenes* in soft cheese stored at 7.8°C was 6.2 log CFU/g, compared with

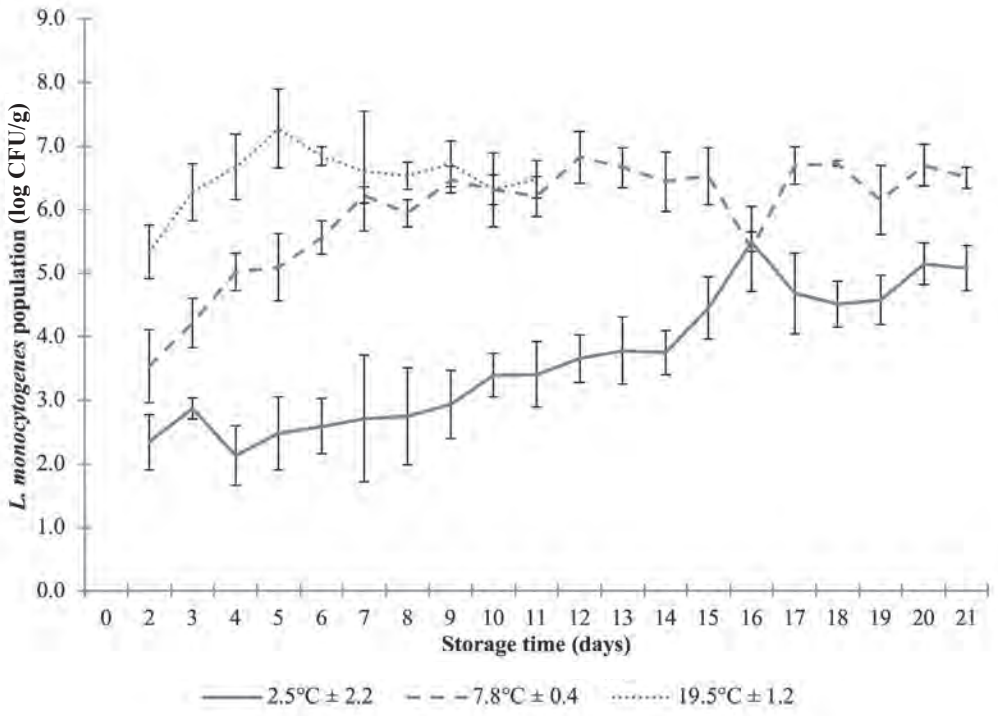


FIGURE 2. Mean *L. monocytogenes* growth (log CFU/g) in soft cheese at three different domestic storage temperatures. Recommended refrigeration temperature (2.5°C ± 2.2) (continuous line); refrigeration temperature exceeding recommendations (7.8°C ± 0.4) (broken line); ambient temperature (19.5°C ± 1.2) (dotted line).

2.7 log CFU/g when stored at 2.5°C. Maximum populations achieved in soft cheese stored at 7.8°C and 19.5°C were not achieved in soft cheese stored at 2.5°C. Data presented in *Fig. 2* indicate that no *L. monocytogenes* was isolated on day zero following inoculation. Very little difference is observed in populations of *L. monocytogenes* from day seven on in the soft cheese, whether stored at 7.8°C or 19.5°C; this suggests that maximum population densities that have been reached varied according to storage temperature.

A two-way between-groups analysis of variance was conducted to explore the impact of storage temperature and the type of food products on the population increase of *L. monocytogenes* (log CFU/g) per day. Both variables were determined to have a statistically significant interaction effect on *L. monocytogenes* populations (F (2, 97) = 4.602, P = 0.012). A statistically significant difference (P < 0.001) was observed for *L. monocytogenes* populations at the three different temperatures (F (2, 97) = 96.99, P = 0.000). The effect size (partial eta squared = 0.67) indicated a large effect in the strength of the association of temperature on population size. Post-hoc comparison using the Tukey HSD test indicated that the mean log CFU/g *L. monocytogenes* was significantly greater at higher temperatures, as indicated in *Table 3*. The distribution of mean *L. monocytogenes* populations according to storage temperature is illustrated in *Fig. 3*.

Furthermore, a significant difference was observed for *L. monocytogenes* between the two food products (F (1, 97) = 32.430, P = 0.000) with greater mean values for *L. monocytogenes* in soft cheese than in RTE ham, as indicated in *Table 3*.

Growth rates of *L. monocytogenes* in food products stored at different domestic storage temperatures

As indicated in *Table 4*, the growth rate (μ) was greatest in RTE ham stored at 19.5°C. In both products, the growth rates at temperatures exceeding recommendations (7.8°C and 19.5°C) were greater than those at 2.5°C. The greater the storage temperature, the greater the growth rate of *L. monocytogenes* in both foods. The generation time of *L. monocytogenes* at 2.5°C was 1.3 – 2.5 days (58.9 hours t^{-1} for RTE ham and 32.1 hours t^{-1} for soft cheese) whereas at 7.8°C, generation times were less than one day (23.7 – 22.0 hours t^{-1}). The generation time of *L. monocytogenes* at 19.5°C was 11 hours t^{-1}. The higher the temperature, the shorter the generation time.

DISCUSSION

As seen in previous research *(34)*, a period of inactivation (lag phase) was observed at the start of the re-enactment sampling following initial inoculation of food products with *L. monocytogenes*. Survival and growth of *L. monocytogenes* was determined in both food products (soft cheese and

TABLE 3. Post-hoc comparisons of *L. monocytogenes* mean populations (log CFU/g) using Tukey HSD test

Temperature	Total	Soft Cheese	RTE Ham
2.5°C ± 2.2	*M* = 3.1 log CFU/g	*M* = 3.6 log CFU/g	*M* = 2.6 log CFU/g
	SD = 0.93	*SD* = 1.04	*SD* = 0.43
7.8°C ± 0.4	*M* = 5.0 log CFU/g	*M* = 6.0 log CFU/g	*M* = 4.1 log CFU/g
	SD = 1.23	*SD* = 0.90	*SD* = 0.76
19.5°C ± 1.2	*M* = 6.3 log CFU/g	*M* = 6.5 log CFU/g	*M* = 6.1 log CFU/g
	SD = 1.20	*SD* = 0.50	*SD* = 1.61

TABLE 4. Growth rate of *L. monocytogenes* (μ hour^{-1}) and generation times (hours t^{-1}) at 2.5°C, 7.8°C and 19.5°C

Temperature	Growth rates (μ hour^{-1})		Generation times (hours t^{-1})	
	RTE Ham	Soft Cheese	RTE Ham	Soft Cheese
2.5°C ± 2.2	0.012	0.022	58.9	32.1
7.8°C ± 0.4	0.029	0.031	23.7	22.0
19.5°C ± 1.2	0.061	0.036	11.4	11.2

sliced cooked ham) at two domestic refrigeration storage temperatures (2.5°C and 7.8°C) and at ambient temperature (19.5°C). All of these have been found to be storage conditions existing in older adults' domestic kitchens *(22, 26–28)*.

Previous research has reported the generation time for *L. monocytogenes* incubated at 4.0°C is 1–2 days *(1)*, the same as found in the current study when foods were stored at 2.5°C. Storage of soft cheese at 7.8°C resulted in a maximum population of 6.8 log CFU/g and a generation time of 22 hours. McClure et al. reported that the generation time for *L. monocytogenes* in soft cheese stored at 6°C was 18 hours *(50)*.

A study in which soft cheese products were inoculated with 2.7 log CFU/mL demonstrated an increase of up to 5 log CFU/mL after 28 days at 6–8°C *(49)*. The soft cheese in this study was inoculated with 2.7 log CFU *L. monocytogenes* per gram of soft cheese; however, the maximum population of *L. monocytogenes* in soft cheese achieved in this study was 5.5 log CFU after 16 days at 2.5°C, while more than 6.23 log CFU was achieved after 6 days at 7.8°C, reaching a maximum population of 6.8 log CFU after 12 days.

Studies have shown that *L. monocytogenes* in meat products can increase during refrigerated storage by 0.5 to 3.0 log CFU per day (Ikeda et al., 2003; Barbosa et al., 1995; Grau et al., 1992; Grau et al., 1990; Gouet et al., 1978 as cited by Beverly, R. *(6)*). In this study, the average daily population increase of *L. monocytogenes* in RTE food products during refrigeration was 0.04–0.3 log CFU/day, while an increase of 0.6–0.7 log CFU/day was determined in RTE foods stored at ambient temperature.

The generation time of *L. monocytogenes* in dairy products stored at 21.0°C has previously been determined to be 1.8 h *(44)*, while in this study, generation times of *L. monocytogenes* in soft cheese and RTE ham were determined to be 11 h. The generation time of *L. monocytogenes* decreases substantially as the storage temperature increases *(44)*. The generation time of *L. monocytogenes* in this study were shorter at refrigeration temperatures above the recommendations and when stored at ambient temperatures rather than at recommended temperature.

The growth rate of *L. monocytogenes* was significantly greater in soft cheese than in RTE ham ($P < 0.001$). Additionally, at the recommended storage temperature ≤ 5°C, the generation time of *L. monocytogenes* in RTE ham was significantly greater than that in soft cheese. The characteristics/matrix of the food products or processing methods may have influenced this, as the use of growth inhibitors in RTE meat

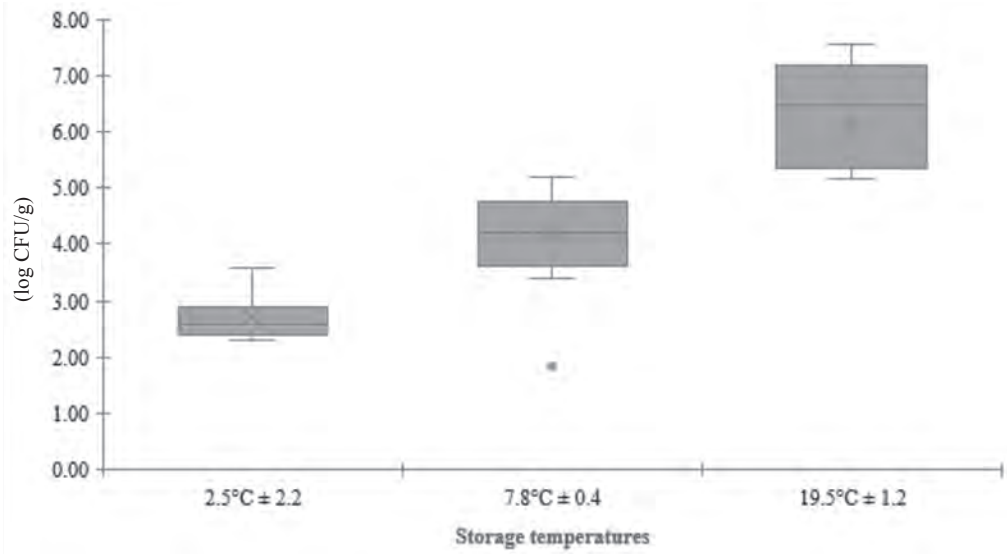


FIGURE 3. Boxplot illustrating the mean *L. monocytogenes* growth (log CFU/g) in RTE ham at three different domestic storage temperatures. Recommended refrigeration temperature (2.5°C ± 2.2) (21 days); refrigeration temperature exceeding recommendations (7.8°C ± 0.4) (21 days); ambient temperature (19.5°C ± 1.2) (10 days).

products can reduce the growth rate of *L. monocytogenes* *(41)*. The RTE ham used for the re-enactment contained sodium nitrite ($NaNO_2$) for preservation purposes. Sodium nitrite was traditionally used to control *Clostridium botulinum* in processed meat products; however, more recently it has been reported that the growth of *L. monocytogenes* is also reduced in the presence of nitrite in meat products *(63)*. It is reported that $NaNO_2$ in RTE meat products can affect the detection and recovery of *L. monocytogenes* as a result of nitrite-induced injury *(60)*. Furthermore, sodium nitrite has been determined to reduce the growth rate and increase the lag time of *L. monocytogenes* in sliced cooked meat *(18)*, as nitrite concentration, along with salt content, a_w and pH, can contribute to microbiological stability in meat products *(7)*. Combined treatment by listericidal antimicrobials can be an effective tool for control of *L. monocytogenes (65)*.

The pH of RTE ham was decreased at the end of maximum storage; research suggests that the growth of lactic acid bacteria during refrigerated storage can decrease the pH of cooked meat products from pH 6.5 to 5.3, although the initial pH of cooked meat products will not restrict microbial growth *(7)*. However, this is not the case with *L. monocytogenes*, as only food products with pH ≤ 4.4 (or pH ≤ 5.0 if the $a_w \leq 0.94$) are reportedly unable to support *L. monocytogenes* growth *(10)* as defined in the Commission Regulation (EC) No 2073/2005 on Microbiological Criteria for Foodstuffs *(21)*. Additionally, in research that detected pH changes in cooked meats similar to those found in this study, with a decrease in pH from 6.9 to 5.9, an increase in the lag time and a reduced growth rate of *L. monocytogenes* at 5°C was also observed *(18)*. In the present study, the lag time was not considered, as the microbial change over long-term storage was the focus of the research.

The a_w of RTE ham by the end the maximum storage time increased; however, previous research has reported the opposite occurring, with a_w in cooked meats decreasing from 0.993 to 0.960 *(18)*. Additionally, different bacterial growth rates under the same environmental conditions can result from the food matrix *(43)*, which can also have an effect on growth and inactivation of *Listeria* spp. *(46)*.

The kinetics of growth of *L. monocytogenes* are dependent on storage temperature, packaging and level of inoculum *(34)*. As discussed by Membré et al., understanding the effects of different temperatures on growth of *L. monocytogenes* is important, particularly in the case of post-processing contamination of RTE foods *(52)*, as up to a quarter of RTE foods have been determined to be contaminated with *L. monocytogenes (32, 33, 53)*. Because *L. monocytogenes* is a psychrotrophic pathogen, it has the ability to grow slowly at refrigeration temperatures, and if prolonged storage is provided, the population of *L. monocytogenes* can reach a high level, although the food remains unspoiled and appears acceptable for consumption *(8)*. It is reported that up to 10^6 CFU/g *L. monocytogenes* can be present without obvious signs of spoilage *(5)* in the foods, which the consumer may consequently consume.

Given the increased risk of foodborne infection to vulnerable consumer groups such as older adults, there is a need to effectively communicate through educational interventions the importance of safe domestic refrigeration temperatures

to limit the growth of *L. monocytogenes* so as to reduce the risk of listeriosis.

CONCLUSIONS

The data from this study adds to the existing microbiological risk-based data by means of realistic re-enactment of domestic storage. Previous research has shown that older adults often fail to store RTE foods commonly associated with *L. monocytogenes* at recommended temperatures and do so for prolonged periods. Consequently, this study has specifically examined such storage malpractices and has successfully determined that microbiologically, such practices increase the risks associated with listeriosis. Storage of RTE foods at temperatures exceeding recommendations for prolonged periods has been determined to increase *L. monocytogenes* populations.

This study determined that storage of RTE ham and soft cheese at temperatures exceeding recommendations had a statistically significant impact on the growth rate, generation rate and maximum population of *L. monocytogenes*, compared with storage at recommended storage temperatures. If such food products contain *L. monocytogenes* as a result of post-processing contamination, domestic refrigeration practices that are not in line with recommendations increase the time and temperature opportunities required by *L. monocytogenes* to grow to potentially unsafe levels, thus increasing the potential risk of listeriosis.

Re-enactment results determined that domestic storage under conditions exceeding recommended consumer refrigeration temperatures alone increased *L. monocytogenes* more than prolonged storage at recommended temperatures. Findings suggest that there is a need for consumers to ensure that refrigerated storage at temperatures ≤ 5.0°C is used for storing RTE foods associated with *L. monocytogenes* in the domestic kitchen. In addition, following storage guidance to use such RTE foods within two days of opening is also essential to reduce the risks associated with listeriosis.

Findings from this study suggest the need for targeted consumer food safety education to increase awareness of the importance of safe refrigeration practices and improve food storage practices, particularly among vulnerable consumer groups such as older adults, to reduce the risks associated with microbiological growth in the domestic environment.

ACKNOWLEDGMENTS

This study was supported by the Society for Applied Microbiology (SfAM) Research Development Fund Grant and the Vice Chancellor's Doctoral Award from Cardiff Metropolitan University. The authors acknowledge Prof. Adrian Peters (employed at Cardiff Metropolitan University at the time of the study) and Prof. Louise Fielding (1968 – 2014) for their support throughout the project.

REFERENCES

1. Advisory Committee on the Microbiological Safety of Food. Date, 2008, Ad Hoc Group on Vulnerable Groups, Report on the Increased Incidence of Listeriosis in the UK. Available at: https://acmsf.food.gov.uk/sites/default/ files/multimedia/pdfs/committee/acmsflisteria.pdf. Accessed 1st April 2015.
2. Amato, E., V. Filipello, M. Gori, S. Lomonaco, M. N. Losio, A. Parisi, P. Huedo, S. J. Knabel, and M. Pontello. 2017. Identification of a major *Listeria monocytogenes* outbreak clone linked to soft cheese in Northern Italy – 2009–2011. *BMC Infect. Dis.* 17:342.
3. Ballantyne, A., R. Stark, and J. D. Selman. 1988. Modified atmosphere packaging of shredded lettuce. *Int. J. Food Sci. Technol.* 23:267–274.
4. Bemrah, N., M. Sanaa, M. H. Cassin, M. W. Griffiths, and O. Cerf. 1998. Quantitative risk assessment of human listeriosis from consumption of soft cheese made from raw milk. *Preventive Veterinary Medicine* 37:129–145.
5. Berrang, M. E., R. E. Brackett, and L. R. Beuchat. 1989. Growth of *Listeria monocytogenes* on fresh vegetables stored under controlled atmosphere. *J. Food Prot.* 52:702–705.
6. Beverly, R. 2004. The control, survival and growth of *Listeria monocytogenes* on food products. Ph.D. Thesis. The Department of Food Science. Louisiana State University and Agricultural and Mechanical College.
7. Borch, E., M.-L. Kant-Muermans, and Y. Blixt. 1996. Bacterial spoilage of meat and cured meat products. *Int. J. Food Microbiol.* 33:103–120.
8. Brackett, R. 1992. Microbiological safety of chilled foods: current issues. *Trends Food Sci. Tech.* 3:81–85.
9. Brasileiro, I. S., M. Barbosa, M. C. Igarashi, V. Biscola, D. F. Maffei, M. Landgraf, and B. D. G. d. M. Franco. 2016. Use of growth inhibitors for control of *Listeria monocytogenes* in heat-processed ready-to-eat meat products simulating post-processing contamination. *LWT – Food Science and Technol.* 74:7–13.
10. British Retail Consortium, Chilled Food Association, and Food Standards Agency. 2010. Shelf life of ready to eat food in relation to *L. monocytogenes* — Guidance for food business operators. First Edition.
11. Cartwright, E. J., K. A. Jackson, S. D. Johnson, L. M. Graves, B. J. Silk, and B. E. Mahon. 2013. Listeriosis outbreaks and associated food behicles, United States, 1998–2008. *Emerg. Infect. Dis.* 19:7–9.
12. Chaitiemwong, N., W. C. Hazeleger, R. R. Beumer, and M. H. Zwietering. 2014. Quantification of transfer of *Listeria monocytogenes* between cooked ham and slicing machine surfaces. *Food Control* 44:177–184.
13. Codex Alimentarius Commission. Date, 2007, Guidelines on the application of general principles of food hygiene to the control of *Listeria monocytogenes* in foods CAC/GL 61-2007. Available at: http:// www. codexalimentarius.net/download/ standards/10740/CXG_061e.pdf. Accessed 24th October 2013.
14. Communicable Diseases Network Australia and New Zealand. 1997. Foodborne disease: Towards Reducing Foodborne Illness in Australia. *In* Communicable diseases intelligence technical report series no. 2, Commonwealth of Australia.
15. Cornelius, A. J., J. A. Hudson, and T. L. Wong. 2008. Enumeration and growth of naturally occurring *Listeria* spp. in unpackaged ham. *Food Microbiol.* 25:407–412.
16. Dahl, V., L. Sundqvist, I. Hedenström, M. Löfdahl, E. Alm, H. Ringberg, M. Lindblad, A. Wallensten, S. Thisted Lambertz, and C. Jernberg. 2017. A nationwide outbreak of listeriosis associated with cold-cuts, Sweden 2013–2014. *Infect. Ecol. Epidemiol.* 7:1324232.
17. Department for Environment Food and Rural Affairs. 2001. Annual report on food expenditure. National Food Survey 2000.
18. Duffy, L., P. Vanderlinde, and F. Grau. 1994. Growth of *Listeria monocytogenes* on vacuum-packed cooked meats: effects of pH, aw, nitrite and ascorbate. *Int. J. Food Microbiol.* 23:377–390.

19. European Commission. Date, 2001, *Listeria* in food. Trends and sources of zoonotic agents in the European Union and in Norway, 2001. Available at: http://ec.europa.eu/food/food/biosafety/salmonella/07_listeria_ 2001.pdf. Accessed 24th October 2013.

20. European Food Safety Authority Panel on Biological Hazards. 2007. Request for updating the former SCVPH opinion on *Listeria monocytogenes* risk related to ready-to-eat foods and scientific advice on different levels of *Listeria monocytogenes* in ready-to-eat foods and the related risk for human illness — Scientific opinion of the panel on biological hazards. *The EFSA J.* 599:1–42.

21. European Parliament. Date, 2005, Commission Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs. Official Journal of the European Union. Available at: http://eurlex.europa.eu/LexUriServ/ LexUriServ.do?uri=OJ:L:2005:338:0001:0026:EN:PDF. Accessed 24 October 2013.

22. Evans, E. W. 2015. Older adults' domestic food handling and storage practices associated with the risk of listeriosis. PhD Thesis. Cardiff School of Health Sciences, Cardiff Metropolitan University.

23. Evans, E. W. 2016. Domestic kitchen risk factors of listeriosis among older-adult consumers. *Microbiologist* 17:14–17.

24. Evans, E. W. 2016. Older adults' domestic kitchen practices associated with an increased risk of listeriosis. *Perspectives in Publ. Hlth.* 136:199–201.

25. Evans, E. W., and E. C. Redmond. 2014. Behavioural risk factors associated with listeriosis in the home: A review of consumer food safety studies. *J. Food Prot.* 77:510–521.

26. Evans, E. W., and E. C. Redmond. 2015. Analysis of older adults' domestic kitchen storage practices in the United Kingdom: Identification of tisk gactors sssociated with Listeriosis. *J. Food Prot.* 78:738–745.

27. Evans, E. W., and E. C. Redmond. 2016. Older adult consumer knowledge, attitudes and self-reported storage practices of ready-to-eat food products and risks associated with listeriosis. *J. Food Prot.* 79:263–272.

28. Evans, E. W., and E. C. Redmond. 2016. Time-temperature profiling of United Kingdom consumers' domestic refrigerators. *J. Food Prot.* 79:2119–2127.

29. Food and Agriculture Organization of the United Nations, and World Health Organization. 2004. Risk assessment of *Listeria monocytogenes* in ready-to-eat foods: interpretative summary. Available at: http://www.who.int/foodsafety/publications/micro/en/mra4.pdf. Accessed 2 October 2013.

30. Food Standards Agency. 2008. Retail survey of *Listeria monocytogenes* in smoked. FishFood survey information sheet 05/08. Available at: http://www.food.gov.uk/science/surveillance/fsisbranch2008/fsis0508. Accessed 24th October 2013.

31. Food Standards Agency, and Department of Health. Date, 2008, *Listeria* — keeping food safe. Food Standards Agency and Department of Health., [Leaflet]. Available at: http://www.food.gov.uk/multimedia/pdfs/ publication/listeriafactsheet0708.pdf. Accessed 24th October 2013.

32. Garrido, V., A. I. Vitas, and I. García-Jalón. 2009. Survey of *Listeria monocytogenes* in ready-to-eat products: Prevalence by brands and retail establishments for exposure assessment of listeriosis in Northern Spain. *Food Control* 20:986–991.

33. Gianfranceschi, M., A. Gattuso, S. Tartaro, and P. Aureli. 2003. Incidence of *Listeria monocytogenes* in food and environmental samples in Italy between 1990 and 1999: Serotype distribution in food, environmental and clinical samples. *Euro. J. Epidemiol.* 18:1001–1006.

34. Gounadaki, A. S., P. N. Skandamis, E. H. Drosinos, and G.-J. E. Nychas. 2007. Effect of packaging and storage temperature on the survival of *Listeria monocytogenes* inoculated postprocessing on sliced salami. *J. Food Prot.* 70:2313–2320.

35. Hächler, H., G. Marti, P. Giannini, A. Lehner, M. Jost, J. Beck, F. Weiss, B. Bally, M. Jermini, R. Stephan, and A. Baumgartner. 2013. Outbreak of listerosis due to imported cooked ham, Switzerland 2011. *Eurosurveillance* 18:20469.

36. Health Protection Agency. 2005. Preparation of samples and dilutions (F2). Available at: http://www.hpa-standardmethods.org.uk/documents/food/pdf/F23.pdf. Accessed 24 October 2013.

37. Health Protection Agency. 2007. British standard operating procedure ID 3: Identification of *Listeria* species, and other non-sporing gram-positive rods (except *Corynebacterium*). Accessed 24 October 2013.

38. Health Protection Agency. 2009. Detection and enumeration of *Listeria monocytogenes* and other *Listeria* species. National dtandard method F19. Standards unit, evaluations and standards laboratory. Available at: http://www.hpa-standardmethods.org.uk/documents/food/pdf/F10.pdf. Accessed 24 October 2013.

39. Heiman, K. E., V. B. Garalde, M. Gronostaj, K. A. Jackson, S. Beam, L. Joseph, A. Saupe, E. Ricotta, H. Waechter, A. Wellman, M. Adams-Cameron, G. Ray, A. Fields, Y. Chen, A. Datta, L. Burall, A. Sabol, Z. Kucerova, E. Trees, M. Metz, P. Leblanc, S. Lance, P. M. Griffin, R. V. Tauxe, and B. J. Silk. 2015. Multistate outbreak of listeriosis caused by imported cheese and evidence of cross-contamination of other cheeses, USA, 2012. *Epidemiol. Infect.* 144:2698–2708.

40. Hoffman, A. D., K. L. Gall, D. M. Norton, and M. Wiedmann. 2003. *Listeria monocytogenes* contamination patterns for the smoked fish processing environment and for raw fish. *J. Food Prot.* 66:52–60.

41. ILSI Research Foundation, and Risk Science Institute. 2005. Achieving continuous improvement in reductions in foodborne listeriosis – A risk-based approach. *J. Food Prot.* 68:1932–1994.

42. Knight, A., M. Worosz, E. Todd, L. Bourquin, and C. Harris. 2008. Listeria in raw milk soft cheese: a case study of the risk governance in the United States using the IRGC framework. *In* O. Renn, and K. Walker (ed.), Global Risk Governance, vol. 1.

43. Koutsoumanis, K., A. Pavlis, G. J. E. Nychas, and K. Xanthiakos. 2010. Probabilistic model for *Listeria monocytogenes* growth during distribution, retail storage, and domestic storage of pasteurized milk. *Appl. Environ. Microbiol.* 76:2181–2191.

44. Lado, B., and A. Yousef. 2007. Characteristics of *Listeria monocytogenes* important to food processors. *In* E.T. Ryser, and E.H. Marth (ed.), *Listeria*, listeriosis, and food safety CRC Press, Boca Raton.

45. Little, C., S. Sagoo, I. Gillespie, K. Grant, M. J, and F. w. a. E. s. network. 2008. LACORS/HPA Coordinated food liaison group studies: Assessment of the microbiological safety of selected retail ready-to-eat foods with a focus on *Listeria monocytogenes.* LACORS/HPA Co-ordinated Food Liaison Group Studies.

46. Lorentzen, G., E. Ytterstad, R. L. Olsen, and T. Skjerdal. 2010. Thermal inactivation and growth potential of *Listeria innocua* in rehydrated salt-cured cod prepared for ready-to-eat products. *Food Control* 21:1121–1126.

47. Lund, B., and S. O'Brien. 2011. The occurrence and prevention of foodborne disease in vulnerable people. *Foodborne Pathog. Dis.* 8:961–973.

48. Lundén, J. M., T. J. Autio, and H. J. Korkeala. 2002. Transfer of persistent *Listeria monocytogenes* contamination between food-processing plants associated with a dicing machine. *J. Food Prot.* 65:1129–1133.

49. Malheiros, P. d. S., D. J. Daroit, and A. Brandelli. 2012. Inhibition of *Listeria monocytogenes* in minas frescal cheese by free and nanovesicle-encapsulated nisin. *Braz. J. Microbiol.* 43:1414–1418.

50. McClure, P. J., A. L. Beaumont, J. P. Sutherland, and T. A. Roberts. 1997. Predictive modelling of growth of *Listeria monocytogenes* The effects on growth of NaCl, pH, storage temperature and NaNO2. *Int. J. Food Microbiol.* 34:221–232.

51. Meldrum, R. J., P. W. Ellis, P. T. Mannion, D. Halstead, J. Garside, and F. The Welsh Food Microbiological. 2010. Prevalence of *Listeria monocytogenes* in ready-to-eat foods sampled from the point of sale in Wales, United Kingdom. *J. Food Prot.* 73:1515–1518.

52. Membré, J., M. Kubaczka, J. Dubois, and C. Chèné. 2004. Temperature effect on *Listeria monocytogenes* growth in the event of contamination of cooked pork products. *J. Food Prot.* 67:463–469.

53. Mena, C., G. Almeida, L. Carneiro, P. Teixeira, T. Hogg, and P. Gibbs. 2004. Incidence of *Listeria monocygoenes* in different food products commercialized in Portugal. *Food Microbiol.* 21:213–216.

54. Microbiological Safety of Food Funders Group. Date, 2005. UK publicly funded research relating to *Listeria monocytogenes*, Report to the microbiological safety of food funders group, May 2005. Available at: http://www.food.gov.uk/multimedia/pdfs/msffglisteria.pdf. Accessed 24th October 2013.

55. Mintel Group Ltd. Date, 2009, Deli Meats, Bacon and Sausages — UK — June 2009. Available at: http://academic.mintel.com/display/466633/. Accessed 24th October 2013.

56. Mintel Group Ltd. Date, 2010, Red Meat — UK — October 2010. Available at: http://oxygen.mintel. com/display/479939/. Accessed 24th October 2013.

57. Mintel Group Ltd. Date, 2012, Targeting the over-55s through ready meals. Mintel Market Report. Available at: http://academic.mintel.com.ezproxy.uwic.ac.uk/display/624429/. Accessed 8th November 2014.

58. Morris, I., and C. Ribeiro. 1989. *Listeria monocytogenes* and pate. *Lancet.* 2:1285–1286.

59. Nicholas, R., P. Dunton, A. Tatham, and L. Fielding. 2013. The effect of ozone and open air factor on surface-attached and biofilm environmental *Listeria monocytogenes*. *J. Appl. Microbiol.* 115:555–564.

60. Nyachuba, D., C. Donnelly, and A. Howard. 2007. Impact of nitrite on detection of *Listeria monocytogenes* in selected ready-to-eat (RTE) meat and seafood products. *J. Food Sci.* 72:267–275.

61. Public Health Laboratory Service. 2015. Listeriosis in England and Wales in 2014: summary report. *Health Protection Report. Infection Reports* 9.

62. Redmond, E., C. Griffith, J. Slader, and T. Humphrey. 2004. Microbiological and observational analysis of cross-contamination risks during domestic food preparation. *Brit. Food J.* 106:581–597.

63. Sindelar, J., and A. Milkowski. 2011. Sodium nitrite inprocessed meat and poultry meats: a review of curing and examining the risk or benefit of its use. *In*, AMSA White Paper Series American Meat Science Association.

64. Slader, J. 2003. The potential for spread and persistence of *Salmonella* and *Campylobacter* during domestic food preparation. PhD Thesis. School of Health Sciences, University of Wales Institute, Cardiff.

65. Soni, K. A., M. Desai, A. Oladunjoye, F. Skrobot, and R. Nannapaneni. 2012. Reduction of *Listeria monocytogenes* in queso fresco cheese by a combination of listericidal and listeriostatic GRAS antimicrobials. *Int. J. Food Microbiol.* 155:82–88.

66. Stollewerk, K., A. Jofré, J. Comaposada, G. Ferrini, and M. Garriga. 2011. Ensuring food safety by an innovative fermented sausage manufacturing system. *Food Control* 22:1984–1991.

67. Tasara, T., J. Klumpp, J. Bille, and R. Stephan. 2016. Genome sequences of *Listeria monocytogenes* strains responsible for cheese- and cooked ham product-associated Swiss listeriosis outbreaks in 2005 and 2011. *Genome Announcements.* 4.

68. U.S. Food and Drug Administration. 2003. *Listeria monocytogenes* risk assessment: Interpretive summary, research assessment/safety assessment,. Available at: http://www.fda.gov/Food/ScienceResearch/ ResearchAreas/RiskAssessmentSafetyAssessment/ucm185291.htm. Accessed 24th October 2013.

69. Wagner, M., B. Auer, C. Trittremmel, I. Hein, and D. Schoder. 2007. Survey on the *Listeria* contamination of ready-to-eat food products and household environments in Vienna, Austria. *Zoonoses Publ. Hlth.* 54:16–22.

70. Wilbey, R. A. 1997. Estimating shelf life. *Int. J. Dairy Technol.* 50:64–67.

71. Wilson, I. G. 1996. Occurrence of *Listeria* species in prepacked retail sandwiches. *Epidemiol. Infect.* 117:89–93.

In Memory

Sue Snider

Newark, Delaware

We extend our deepest sympathy to the family of Sue Snider who recently passed away. Dr. Snider was a member of the Association since 2001. IAFP will always have sincere gratitude for her contribution.