

1 **LABORATORY RE-ENACTMENT OF STORAGE PRACTICES OF OLDER**
2 **ADULTS TO DETERMINE POTENTIAL IMPLICATIONS FOR GROWTH OF**
3 ***LISTERIA MONOCYTOGENES***

4
5 **Running title: Implication of domestic storage malpractices on *Listeria monocytogenes***

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ABSTRACT

1
2 Older adults are more susceptible to listeriosis, and many frequently consume ready-to-eat (RTE)
3 foods associated with *Listeria monocytogenes*. Consequently, safe storage of RTE-food is essential to
4 reduce the risks of listeriosis. This study aimed to re-enact domestic food-storage malpractices of older
5 adult consumer in a laboratory to assess the potential impact on *L. monocytogenes*. Observed and self-
6 reported data relating to domestic food-storage malpractices included prolonged storage of RTE foods
7 and/or refrigeration temperatures exceeding recommendations ($>5.0^{\circ}\text{C}$). Re-enactment occurred using
8 soft-cheese and RTE meat inoculated with ~ 3.7 log CFU *L. monocytogenes*, stored at recommended
9 temperatures (2.5°C)($n=110$); temperatures exceeding recommendations (7.8°C)($n=110$) and ambient-
10 temperature (19.5°C)($n=55$). Samples were analyzed every 24h for <21 d. Results indicated *L.*
11 *monocytogenes* grew at all storage temperatures. Average generation times indicated slower growth of
12 *L. monocytogenes* at 2.5°C (94h t^{-1}) than at 7.8°C (21.5h t^{-1}) and 19.5°C (11h t^{-1}), suggesting prolonged
13 storage of RTE foods at increased *L. monocytogenes* populations (< 7.6 log CFU/g), potentially making
14 such foods unsafe for consumption. Findings indicate storage malpractices contrary to consumer
15 recommendations intended to reduce the risk of foodborne disease, increase *L. monocytogenes*
16 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 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 occur as a result of post-processing 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 practices that prevent the growth of *L. monocytogenes* when stored in the domestic kitchen are followed. Adequate storage temperatures and avoiding prolonged storage of opened RTE food products when 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, the ‘use-by’ date 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 its production and consumption (10, 21) . However, if modified atmosphere packaging and/or refrigeration is utilized to achieve extended shelf life in RTE food products, there is a need to ensure safe refrigeration temperatures, and ensure that after opening, when the integrity of the packaging changes and all antimicrobial properties are lost (3, 66), that such

1 foods are consumed promptly. Although the consumer relies on food manufacturers and suppliers to
2 ensure the RTE food products that they purchase are safe for consumption, the post-purchase
3 responsibility is in the hands of the consumer to implement safe food storage and handling practices
4 (14).

5 Consumer recommendations to reduce the risks associated with listeriosis in the domestic
6 environment include; (i) following use-by dates on unopened prepackaged RTE food products, (ii)
7 avoiding prolonged storage of leftover fresh RTE foods and opened prepackaged RTE food products,
8 and (iii) ensuring safe operating temperatures of domestic refrigerators ($\leq 5.0^{\circ}\text{C}$) (25). Implementation
9 of these recommendations to reduce the risk of listeriosis are essential, particularly for consumer groups
10 with weakened immunity including older adults, pregnant women, people living with and patients
11 receiving chemotherapy that are known to be at an increased risk of foodborne disease, particularly
12 listeriosis (47).

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

22 Cognitive food safety research (27) has determined that although older adult consumers have
23 expressed positive attitudes toward refrigeration, most are unaware of recommended temperatures and
24 report that refrigeration temperatures are not checked. Similarly, although most know that “use-by”
25 dates indicate food safety, the majority believe it safe to eat food beyond use-by dates and report doing
26 so. Additionally, attitudes towards consuming foods within the recommended 2 days of opening were
27 neutral, with the vast majority reporting that they consume RTE foods beyond recommendation (27). A

1 survey of older adult consumers' domestic kitchens determined the majority had opened RTE foods,
2 associated with outbreaks of listeriosis, which had been or were intended to be stored beyond the
3 recommended 2 days after opening. Older adults failed to ensure safe refrigeration temperatures with
4 the majority of refrigerators operating at temperatures exceeding recommendations (26). A model
5 kitchen observational study established that a small number of older adults may fail to implement
6 refrigerated storage when handling leftover RTE food in the domestic kitchen (22). Furthermore, time-
7 temperature profiling of domestic refrigerators in consumer kitchens established that no refrigerators
8 operated at recommended temperatures for the entire duration of the study (28).

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

16 Many research studies have considered the potential influence of different storage conditions on the
17 growth of *L. monocytogenes*, frequently evidenced and achieved using food models. To determine the
18 impact of such consumer food storage practices on the safety of RTE food products, there is a need to
19 determine potential microbiological risks by re-enacting observed and self-reported domestic practices.
20 Implementation of a laboratory-based re-enactment study allows for the replication of domestic kitchen
21 food safety scenarios and this facilitates determination of microbiological risk based data. The use of
22 laboratory based re-enactment of specific consumer food safety behaviors has not been widely used;
23 however, previous research has linked consumer food hygiene and preparation behavior microbiological
24 cross-contamination (62).

25 The aim of the study was to re-enact food safety malpractices observed in domestic kitchens of older
26 adults or self-reported by older adult consumers, that are contrary to food safety recommendations
27 intended to reduce the risk of listeriosis. Such data will determine and quantify the potential risk

1 associated with older adult consumer food storage malpractices on the survival and growth of *L.*
2 *monocytogenes* in RTE foods.

3 **METHODS**

4 **Identification of older adult consumers' food storage malpractices.**

5 Data regarding cognitive and behavioral risk factors associated with storage malpractices of RTE
6 food products associated with listeriosis were reviewed from previous research (26-28). Observed and
7 self-reported storage practices from a survey of older adults' domestic kitchens (26) as indicated in
8 Table 1 informed determination of storage length and temperatures used for reenactments, which include
9 prolonged storage at recommended temperatures and storage at temperatures exceeding
10 recommendations. In addition, two consumers reported that ham or soft cheese would be stored for 5 or
11 6 days, at ambient temperatures. Based upon these findings, RTE ham and soft cheese were selected for
12 the re-enactment study which were stored at three storage temperatures for up to 21 days.

13 **Laboratory-based re-enactment of storage malpractices.**

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

23 A suspension culture of *L. monocytogenes* serotype 1/2a (L002, isolated from a drain in a food
24 processing plant from a previous research study (59)), was prepared. A single cryobead (Technical
25 Service Consultants, Lancashire, UK), from frozen stock stored at -80°C (New Brunswick Scientific

1 Innova® U535 ultra-low temperature lab freezer, Connecticut, USA) was aseptically placed in 100 mL
2 tryptone soya broth (TSB; CM0129, Oxoid Ltd, UK) in a 250 mL Erlenmeyer flask. The flask was
3 incubated on a shaking platform (MaxQ* 4000 Benchtop Orbital Shaker; Thermo Scientific Asheville,
4 USA), set at 250 rpm and at 35°C for 24 hours.

5 The inoculated broth was placed into two 50 mL sterile centrifuge tubes (Corning® 50 mL PP
6 Centrifuge Tubes, Conical Bottom with CentriStar™ Cap; New York, USA). Cells were harvested by
7 centrifugation at 3000 RPM (x1068g) for 20 min at ambient temperature (21°C) in a centrifuge (DuPont
8 Sorvall Superspeed RC-5B refrigerated centrifuge; Thermo Scientific Asheville, USA). The pellet was
9 re-suspended in 40 mL sterile peptone saline diluent (PSD; Oxoid Ltd, UK), achieving an initial
10 inoculum of approximately 5.2×10^9 CFU/mL (9.7 log CFU/mL). Serial decimal dilutions were prepared
11 aseptically using the initial inoculum and peptone saline to achieve a 10^{-5} dilution.

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

22 Two domestic type refrigerators (Gram K400 (h) climate class N, Denmark, and Scandinova LF
23 136 D Larder refrigerator, Vestfrost, Denmark) were selected to replicate typical domestic storage and
24 were validated prior to the study to be operating at a recommended operating temperature ($\leq 5.0^\circ\text{C}$) and
25 a temperature exceeding this ($> 5.0^\circ\text{C}$). The operating temperatures of the domestic refrigerators were
26 monitored using two calibrated thermometers (P300 handheld thermometer; Industrial Temperature

1 Sensors Co. Kildare Ireland; measuring range: -40°C to +200°C, Accuracy: $\pm 1.0^\circ\text{C}$) and calibrated
2 dataloggers (SL52T self-contained single channel, button temperature logger; Signatrol Ltd
3 Gloucestershire UK; measuring range: -40°C to +85°C. Accuracy: $\pm 0.5^\circ\text{C}$).

4 Uninoculated food products were stored up to the maximum length of storage at the three different
5 temperatures to determine the presence and growth of *L. monocytogenes*. Inoculated and uninoculated
6 control food products were stored in the two refrigerators. One operating at a recommended ($\leq 5.0^\circ\text{C}$)
7 temperature (mean temperature: 2.5°C , $\text{SD} \pm 2.2$) and one operating at an ‘abuse’ temperature ($> 5.0^\circ\text{C}$)
8 exceeding recommendations (mean temperature: 7.8°C , $\text{SD} \pm 0.4$) for up to 21 days, and at ambient
9 temperature (mean: 19.5°C , $\text{SD} \pm 1.2$) for 10 days. Following 48 hours at the three different storage
10 temperatures, both food products at all storage temperatures were analyzed at 24-hour intervals to
11 determine *L. monocytogenes* growth. This was conducted following methods based on the National
12 Standard Method (F2) for preparation of samples and dilutions (36) and the National Standard Method
13 (F19) for enumeration of *Listeria monocytogenes* (38) to determine the number of *L. monocytogenes* in
14 the food samples.

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

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

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

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

8 **Data analysis.**

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

17 **RESULTS**

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

23 Uninoculated food products were stored up to the maximum length of storage at the three different
24 domestic storage temperatures. *L. monocytogenes* was not isolated in the RTE ham or soft cheese
25 immediately after purchase or following the maximum storage time at all three temperatures in RTE

1 ham. However, the uninoculated soft cheese control samples stored at 19.5 °C for 11 days were found
2 to contain *L. monocytogenes* at levels of 3.6 log CFU/g.

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

6 Following 21 days of storage an increase in a_w of the RTE ham and soft cheese was determined.
7 The a_w increased from 0.97 in soft cheese and 0.96 in RTE ham to 0.99 in both products (Table 2),
8 changes were also identified in the pH of food products, with all food products pH changing to become
9 more acidic. A mean pH change of 0.8 in soft cheese to be 6.9 and 1.1 in RTE ham to be 5.6. Although
10 changes occurred to the pH and a_w of the RTE ham and soft cheese during re-enacted domestic storage,
11 they remain within the optimal growth ranges for *L. monocytogenes*.

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

13 Growth of *L. monocytogenes* was determined when RTE ham and soft cheese was stored at 2.5°C.
14 The maximum population of *L. monocytogenes* achieved during storage at 2.5°C was 5.5 log CFU/g in
15 soft cheese after 16 days of storage. The greatest population of *L. monocytogenes* in RTE ham (3.6 log
16 CFU/g) was achieved following 15 days storage. The average increase in *L. monocytogenes* populations
17 each day in RTE ham was 0.04 log/day and 0.3 log/day in soft cheese.

18 **Growth of *L. monocytogenes* in food products stored at a temperature exceeding** 19 **recommendations.**

20 During storage at 7.8°C growth of *L. monocytogenes* was determined, the maximum population of
21 *L. monocytogenes* of 6.8 log CFU/g, was achieved after 12 days of storage in soft cheese and 5.2 log
22 CFU/g in RTE ham also after 12 days. The average population increase per day in RTE ham stored at
23 7.8°C was 0.1 log/day and in soft cheese was 0.3 log/day.

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

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

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

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

13 Similarly in soft cheese, increase in storage temperature resulted in an increase in maximum
14 achieved population. As illustrated in Figure 2, after seven days, *L. monocytogenes* in soft cheese stored
15 at 7.8°C was 6.2 log CFU/g compared to 2.7 log CFU/g when stored at 2.5°C. Maximum population
16 achieved in soft cheese stored at 7.8°C and 19.5°C were not achieved by soft cheese stored at 2.5°C.
17 Data presented in Figure 2, indicates that no *L. monocytogenes* was isolated on day zero following
18 inoculation. Very little difference is observed in population of *L. monocytogenes* in the soft cheese from
19 day seven onwards in both soft cheese stored at 7.8°C and 19.5°C , this may suggest that maximum
20 population densities have been reached varied according to storage temperature.

21 A two-way between groups analysis of variance was conducted to explore the impact of storage
22 temperature and the type of food products on the population of *L. monocytogenes* (log CFU/g) per day.
23 Both variables were determined to have a significantly significant interaction effect on *L.*
24 *monocytogenes* populations ($F(2, 97) = 4.602, p = 0.012$). A statistically significant difference
25 ($p < 0.001$) was determined for *L. monocytogenes* populations at the three different temperatures ($F(2,$
26 $97) = 96.99, p = 0.000$). The effect size (partial eta squared = 0.67) indicated a large effect in the strength

1 of the association of temperature on population size. Post-hoc comparison using Tukey HSD test
2 indicated that the mean log CFU/g *L. monocytogenes* was significantly greater at higher temperatures
3 as indicated in Table 3. The distribution of mean *L. monocytogenes* populations according to storage
4 temperature are illustrated in Figure 3.

5 Furthermore, a significant difference was determined for *L. monocytogenes* according to the two
6 different food products ($F(1, 97) = 32.430, p = 0.000$) with greater mean *L. monocytogenes* in soft
7 cheese than RTE ham as indicated in Table 4.

8 **Growth rates of *L. monocytogenes* in food products stored at different domestic storage** 9 **temperatures.**

10 The growth rate constant (μ) for *L. monocytogenes* was calculated and as indicated in Table 4, the
11 growth rate was greatest in RTE ham stored at 19.5°C. In both products, the growth rate at temperatures
12 exceeding recommendations (7.8°C and 19.5°C) were greater than at 2.5°C. The greater the storage
13 temperature the greater the growth rate of *L. monocytogenes* in RTE foods. The generation time of *L.*
14 *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
15 cheese) whereas at 7.8°C, generation times were less than one day (23.7 – 22.0 hours t^{-1}). The generation
16 time of *L. monocytogenes* at 19.5°C was determined to be 11 hours t^{-1} . The higher the temperature, the
17 shorter the generation time.

18 **DISCUSSION**

19 As seen in previous research (34), a period of inactivation (lag phase) was determined at the start of
20 the re-enactment sampling following initial inoculation of food products with *L. monocytogenes*.
21 Survival and growth of *L. monocytogenes* was determined in both food products (soft cheese and sliced
22 cooked ham) at both domestic refrigeration storage temperatures (2.5°C and 7.8°C), and at ambient
23 temperature (19.5°C). All of which were found to be storage conditions implemented in older adults'
24 domestic kitchens in previous studies (22, 26-28).

1 Previous research reported the generation time for *L. monocytogenes* incubated at 4.0°C is 1 - 2 days
2 (1); which was the same as that found in the current study when foods were stored at (2.5°C). Storage
3 of soft cheese at 7.8°C achieved a maximum population growth of 6.8 log CFU/g) and the generation
4 time of *L. monocytogenes* in soft cheese stored at 7.8°C was determined to be 22 hours. Findings by
5 McClure *et al.*, report that the generation time for *L. monocytogenes* in soft cheese stored at 6°C was 18
6 hours (50).

7 A study in which soft cheese products were inoculated with 2.7 log CFU/mL demonstrated an
8 increase of up to 5 log CFU/mL after 28 days at 6 – 8°C (49). The soft cheese in this study was inoculated
9 with 2.7 log CFU *L. monocytogenes* per gram of soft cheese; however, the maximum population of *L.*
10 *monocytogenes* in soft cheese achieved in this study was 5.5 log CFU after 16 days at 2.5°C. Whilst
11 >6.23 log CFU was achieved after 6 days at 7.8°C, reaching a maximum population of 6.8 log CFU after
12 12 days.

13 Studies have shown *L. monocytogenes* in meat products can increase during refrigerated storage by
14 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;
15 Gouet *et al.*, 1978 as cited by Beverly, R. (6)). In this study, the average population increase of *L.*
16 *monocytogenes* in RTE food products per day during refrigeration was 0.04 – 0.3 log CFU/day; whilst
17 an increase of 0.6 – 0.7 log CFU/day was determined in RTE foods stored at ambient temperature.

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

24 The growth rate of *L. monocytogenes* was determined to be significantly greater in soft cheese than
25 in RTE ham ($p < 0.001$). Additionally, at a recommended storage temperature $\leq 5^\circ\text{C}$, the generation time
26 of *L. monocytogenes* in RTE ham was significantly greater to that of soft cheese. The

1 characteristics/matrix of the food products or processing methods may have influenced this, as the use
2 of growth inhibitors in RTE meat products can reduce the growth rate of *L. monocytogenes* (41). The
3 RTE ham used for the re-enactment contained Sodium Nitrite (NaNO_2) for preservation purposes.
4 Sodium nitrite was traditionally used to control *Clostridium botulinum* in processed meat products;
5 however, in more recent years it is reported that the growth of *L. monocytogenes* is reduced in the
6 presence of nitrite in meat products (63). It is reported that NaNO_2 in RTE meat products can affect the
7 detection and recovery of *L. monocytogenes* as a result of nitrite induced injury (60). Furthermore,
8 sodium nitrite has been determined to reduce the growth rate and increase the lag time of *L.*
9 *monocytogenes* in sliced cooked meat (18), as nitrite concentration along with salt content, a_w and pH
10 can contribute to microbiological stability in meat products (7). Combined treatment of listericidal
11 antimicrobials can be an effective tool for control of *L. monocytogenes* (65).

12 A decrease in the pH of RTE ham was determined following maximum storage; research suggests
13 that resulting from the growth of lactic acid bacteria during refrigerated storage the pH of cooked meat
14 products may decrease from pH 6.5 to 5.3, the initial pH-value of cooked meat products will not restrict
15 microbial growth (7). However, this is not the case with *L. monocytogenes*, as only food products with
16 $\text{pH} \leq 4.4$ (or $\text{pH} \leq 5.0$ if the $a_w \leq 0.94$) are reportedly unable to support *L. monocytogenes* growth (10)
17 as defined in the Commission Regulation (EC) No 2073/2005 on Microbiological Criteria for Foodstuffs
18 (21). Additionally, in research which determined similar pH changes in cooked meats as this study, with
19 a decrease in pH from 6.9 to 5.9, an increase in the lag time and reduced the growth rate of *L.*
20 *monocytogenes* at 5°C was also determined (18). In the present study, the lag time was not considered,
21 as the microbial change over long-term storage was the focus of the research.

22 The a_w of RTE ham following the maximum storage length increased, however, previous research
23 has determined the opposite occurring with a_w in cooked meats decreasing from 0.993 to 0.960 (18).
24 Additionally, different bacterial growth rates under the same environmental conditions can result from
25 the food matrix (43), the matrix of food can also have an effect on growth and inactivation of *Listeria*
26 spp. (46).

1 The kinetics of growth of *L. monocytogenes* are dependent on storage temperature, packaging and
2 level of inoculum (34). As discussed by Membré *et al.*, understanding the effects of different
3 temperatures on growth of *L. monocytogenes* are important particularly in the case of post-processing
4 contamination of RTE foods (52), as up to a quarter of RTE foods have been determined to be
5 contaminated with *L. monocytogenes* (32, 33, 53). Due to *L. monocytogenes* being a psychrotrophic
6 pathogen, it has the ability to grow at refrigeration temperatures slowly, and if prolonged storage is
7 provided the population of *L. monocytogenes* can reach a high population, but remains unspoiled and
8 acceptable for consumption (8). It is reported that up to 10⁶ CFU/g *L. monocytogenes* can be present
9 without adverse signs of spoilage (5), which the consumer may consequently consume.

10 Given the increased risk of foodborne infection to vulnerable consumer groups such as older adults,
11 there is a need to effectively communicate through educational interventions the importance of safe
12 domestic refrigeration temperatures to limit the growth of *L. monocytogenes* to reduce the risk of
13 listeriosis.

14 CONCLUSIONS

15 The data presented in this study adds to existing microbiological risk based data by realistic re-
16 enactment of domestic storage. Previous research has identified that older adults fail to store RTE foods
17 commonly associated with *L. monocytogenes* at recommended temperatures and do so for prolonged
18 periods. Consequently, this study has specifically selected such storage malpractices and has
19 successfully determined that microbiologically, such practices increase the risks associated with
20 listeriosis. Storage of RTE foods at temperatures exceeding recommendations for prolonged periods has
21 been determined to increase *L. monocytogenes* populations.

22 This study determined that storage of RTE ham and soft cheese at temperatures exceeding
23 recommendations had a statistically significant impact on the growth rate, generation rate and maximum
24 population of *L. monocytogenes* when compared to storage at recommended storage temperatures. In
25 the event of such food products becoming contaminated with *L. monocytogenes* as a result of post-
26 processing contamination, this study has determined that domestic refrigeration practices that are not in

1 line with recommendations facilitate the time and temperature opportunities required by *L.*
2 *monocytogenes* to grow to potentially unsafe levels, thus increasing the potential risk of listeriosis..

3 Re-enactment results determined that domestic storage conditions exceeding recommended
4 consumer refrigeration temperatures alone increased *L. monocytogenes* more so than prolonged storage
5 at recommended temperatures. Findings suggest that there is a need for consumers to ensure that
6 refrigerated storage at temperatures $\leq 5.0^{\circ}\text{C}$ are implemented when storing RTE foods associated with
7 *L. monocytogenes* in the domestic kitchen. In addition, following storage guidance to use such RTE
8 foods within two days of opening is also essential to reduce the risks associated with listeriosis.

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

13 **ACKNOWLEDGEMENTS**

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

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- 20

FIGURE LEGENDS

1

2 **Figure 1 Mean *L. monocytogenes* growth (log CFU/g) in RTE ham at three different domestic**
3 **storage temperatures. Recommended refrigeration temperature ($2.5^{\circ}\text{C} \pm 2.2$) (continuous line);**
4 **refrigeration temperature exceeding recommendations ($7.8^{\circ}\text{C} \pm 0.4$) (broken line) and ambient**
5 **temperature ($19.5^{\circ}\text{C} \pm 1.2$) (dotted line).**

6 **Figure 2 Mean *L. monocytogenes* growth (log CFU/g) in soft cheese at three different domestic**
7 **storage temperatures. Recommended refrigeration temperature ($2.5^{\circ}\text{C} \pm 2.2$) (continuous line);**
8 **refrigeration temperature exceeding recommendations ($7.8^{\circ}\text{C} \pm 0.4$) (broken line) and ambient**
9 **temperature ($19.5^{\circ}\text{C} \pm 1.2$) (dotted line).**

10 **Figure 3 Boxplot illustrating the mean *L. monocytogenes* growth (log CFU/g) in RTE ham at three**
11 **different domestic storage temperatures. Recommended refrigeration temperature ($2.5^{\circ}\text{C} \pm 2.2$)**
12 **(21 days); refrigeration temperature exceeding recommendations ($7.8^{\circ}\text{C} \pm 0.4$) (21 days) and**
13 **ambient temperature ($19.5^{\circ}\text{C} \pm 1.2$) (10 days).**

1

TABLES

2

1

Table 1 Observed and self-reported storage practices in older adult domestic kitchens.

Participant ID	Refrigerator operating temperature (°C)	Food product observed stored	Self-reported storage duration (days)
Prolonged storage at recommended temperatures ($\leq 5.0^{\circ}\text{C}$)			
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 ($> 5.0^{\circ}\text{C}$)			
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

2

1 **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

2

1 **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	<i>M</i> = 3.1 log CFU/g <i>SD</i> = 0.93	<i>M</i> = 3.6 log CFU/g <i>SD</i> = 1.04	<i>M</i> = 2.6 log CFU/g <i>SD</i> = 0.43
7.8°C ± 0.4	<i>M</i> = 5.0 log CFU/g <i>SD</i> = 1.23	<i>M</i> = 6.0 log CFU/g <i>SD</i> = 0.90	<i>M</i> = 4.1 log CFU/g <i>SD</i> = 0.76
19.5°C ± 1.2	<i>M</i> = 6.3 log CFU/g <i>SD</i> = 1.20	<i>M</i> = 6.5 log CFU/g <i>SD</i> = 0.50	<i>M</i> = 6.1 log CFU/g <i>SD</i> = 1.61

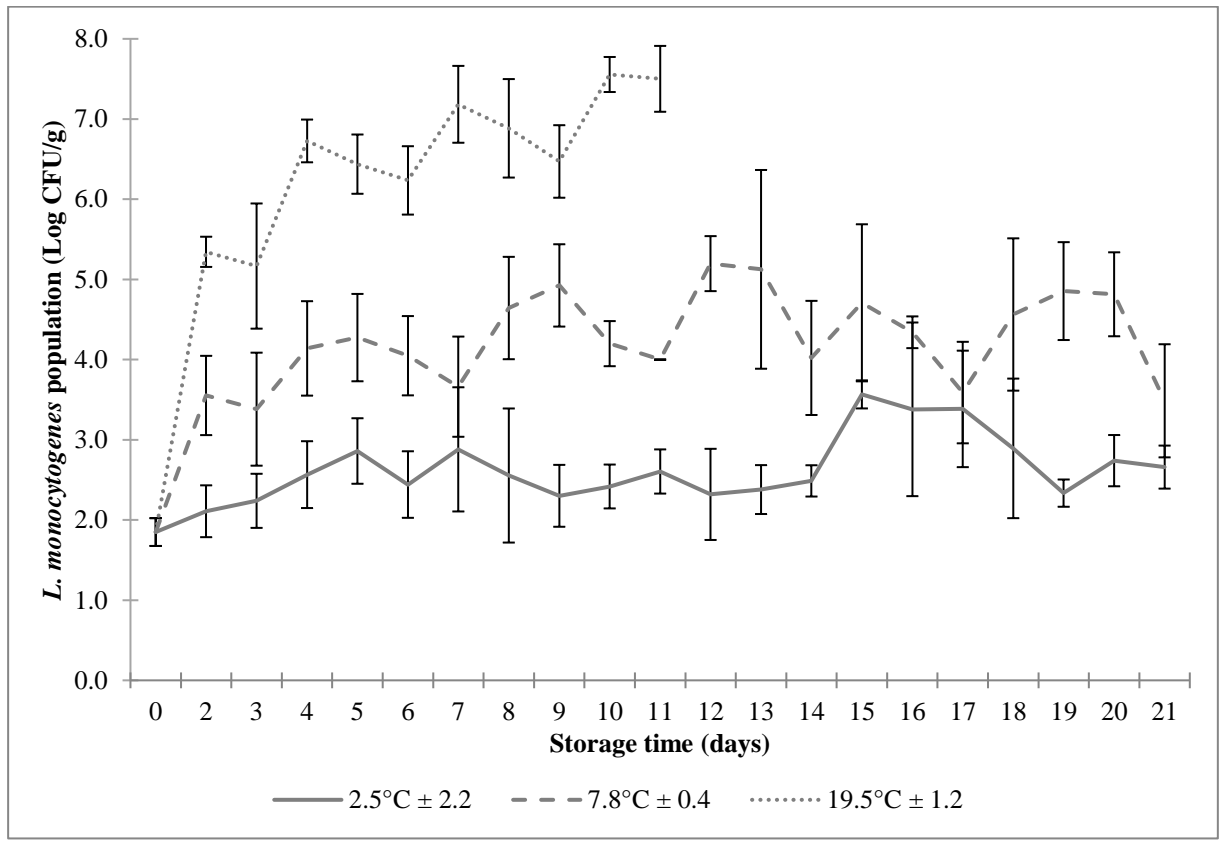
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1 **Table 4 Growth rate of *L. monocytogenes* (μ hour⁻¹) and generation times (hours t⁻¹) at 2.5°C 7.8°C and 19.5°C.**

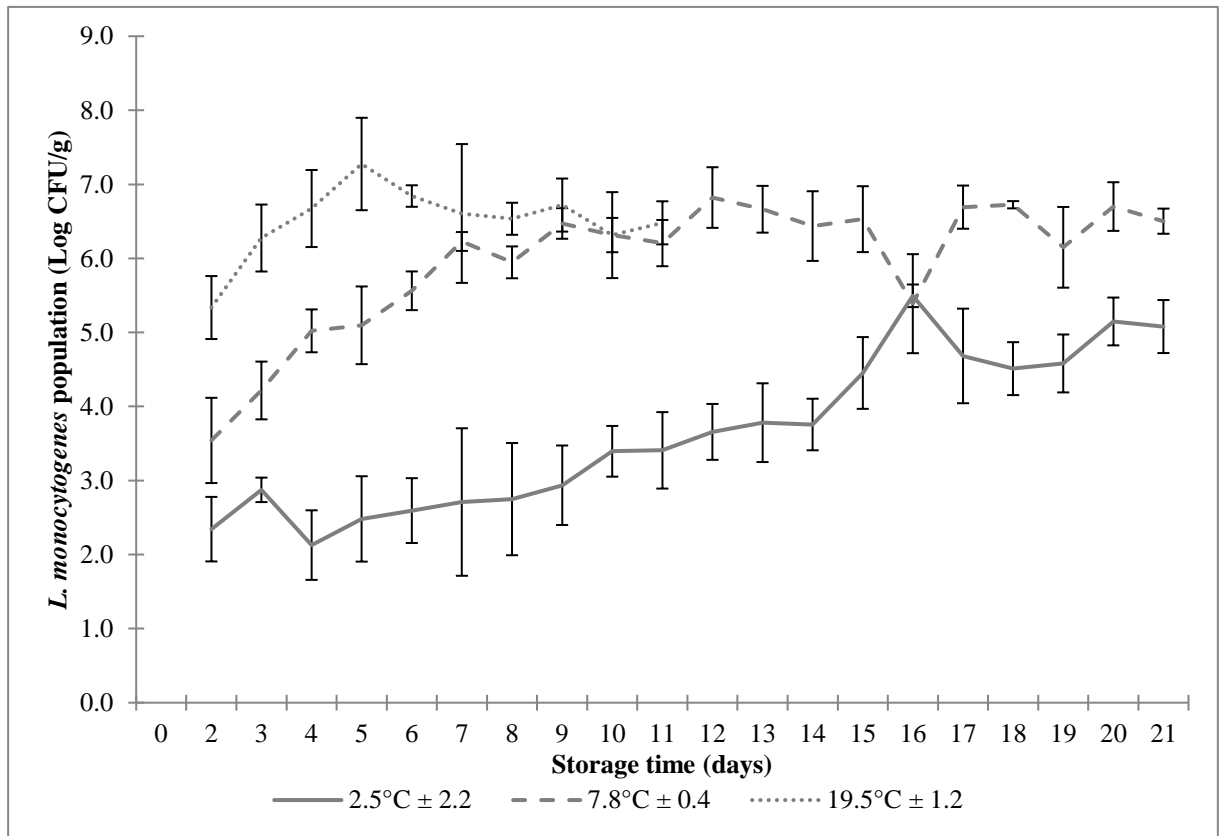
Temperature	Growth rates (μ hour ⁻¹)		Generation times (hours t ⁻¹)	
	RTE Ham	Soft cheese	RTE Ham	Soft cheese
2.5°C± 2.2	0.012	0.022	58.9	32.1
7.8°C± 0.4	0.029	0.031	23.7	22.0
19.5°C± 1.2	0.061	0.036	11.4	11.2

2



1

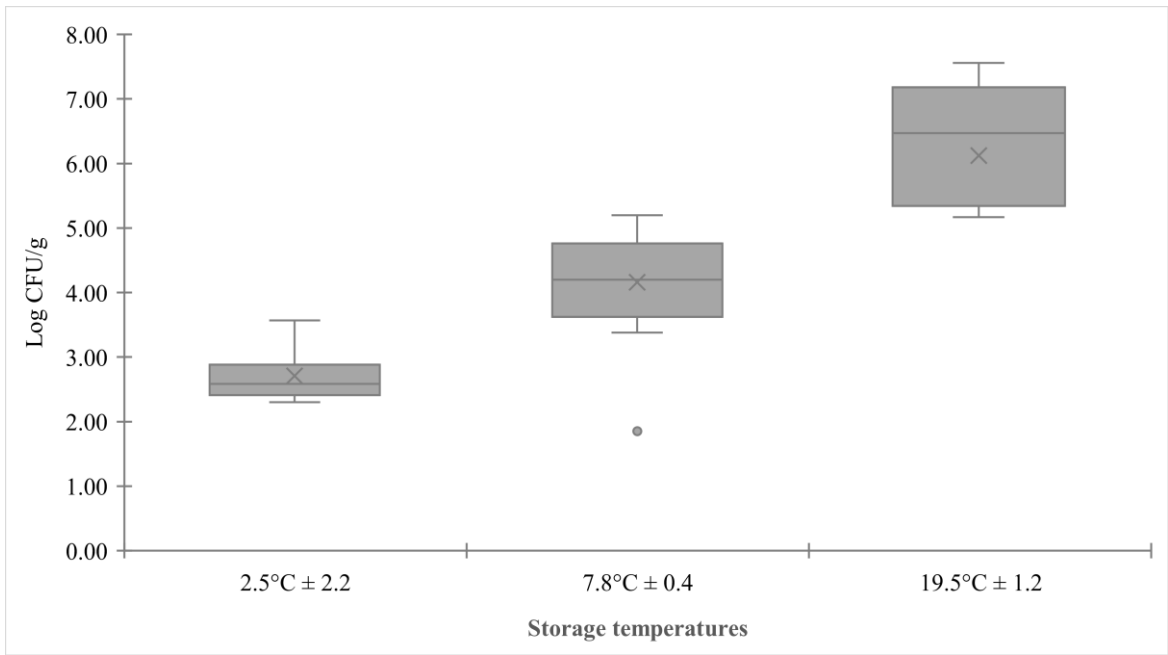
FIGURE 2



2

3

FIGURE 3



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