

Sources and survival of *Listeria monocytogenes* on fresh, leafy produce

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DOI: <https://doi.org/10.1111/jam.14025>



Smith, A., Moorhouse, E., Monaghan, J., Taylor, C. and Singleton, I. 2018. Sources and survival of *Listeria monocytogenes* on fresh, leafy produce. *Journal of Applied Microbiology*.

23 July 2018

1 Sources and Survival of *Listeria monocytogenes* on Fresh, Leafy Produce

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6 Abbreviated running headline: Sources & Survival of *L.mono* on FLP

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10 Summary

11 *Listeria monocytogenes* is an intracellular human pathogen which enters the body through
12 contaminated food stuffs and is known to contaminate fresh leafy produce such as spinach, lettuce
13 and rocket. Routinely, fresh leafy produce is grown and processed on a large scale before reaching
14 the consumer through various products such as sandwiches and prepared salads. From farm to fork,
15 the fresh leafy produce supply chain is complex and contains a diverse range of environments where
16 *L. monocytogenes* is sporadically detected during routine sampling of produce and processing areas.
17 This review describes sources of the bacteria in the fresh leafy produce supply chain and outlines the
18 physiological and molecular mechanisms behind its survival in the different environments associated
19 with growing and processing fresh produce. Finally, current methods of source tracking the bacteria
20 in the context of the food supply chain are discussed with emphasis on how these methods can
21 provide additional, valuable information on the risk that *L. monocytogenes* isolates pose to the
22 consumer.

23 Keywords: *Listeria*, *monocytogenes*, Food Safety, Food Processing, Microbial Contamination, Soil,

24 Introduction

25 *Listeria monocytogenes* is a Gram-positive, facultative anaerobic, opportunistic bacterial pathogen. It
26 is the causative agent of listeriosis, a disease which predominantly affects immunocompromised
27 people including the elderly, immunosuppressed and pregnant women together with their unborn or
28 new-born babies. Contaminated foodstuffs are the main cause of infection and there have been
29 several well-documented, high-profile outbreaks from this source over recent years (Garner and
30 Kathariou 2016). Because of the risk of infection from food, safety authorities impose limits on the
31 number of *L. monocytogenes* cells that can contaminate food products. Guidelines in the USA advise
32 that *L. monocytogenes* should not be present (<1 CFU 25g^{-1}) in ready to eat (RTE) foods that support
33 the growth of *L. monocytogenes* and should not be equal to or above 100 CFU g^{-1} for foods that do not
34 support the growth of *L. monocytogenes* (Center for Food Safety and Applied Nutrition 2017).
35 Legislation on *L. monocytogenes* contamination of RTE foods in the EU requires that *L. monocytogenes*
36 number remains less than 100 CFU g^{-1} for the shelf life of the product unless it has been demonstrated
37 that *L. monocytogenes* has the potential to exceed this number (European Commission 2005). In such
38 cases the food producer must demonstrate *L. monocytogenes* absence in raw materials and the
39 production environment (i.e. there is no potential for contamination of the final product). Limits are
40 set on the number of *L. monocytogenes* allowed in RTE food due to the risk of *L. monocytogenes*
41 infection in highly susceptible individuals coupled with the bacterium's ability to grow in a range of
42 food substrates (Leong *et al.* 2013; Jami *et al.* 2014). Although incidence of listeriosis is relatively low
43 compared to other foodborne bacteria, the disease outcome is often more serious, making it a priority
44 pathogen for many countries. Furthermore, *L. monocytogenes* can grow at refrigeration temperatures
45 (Chan and Wiedmann 2009), meaning it presents an added danger to consumers over other food
46 pathogens such as *Salmonella* and *E. coli*.
47 Foods which have been previously implicated in *L. monocytogenes* infections include milk, soft
48 cheeses, deli or sandwich meats and fresh produce, which encompasses both fresh fruit and
49 vegetables (Cartwright *et al.* 2013). Several reports have demonstrated *L. monocytogenes* presence in

50 a wide variety of fresh produce samples (Zhu *et al.* 2017) and other minimally processed foods. Other
51 than a potentially tragic loss of life, the economic consequences of a *L. monocytogenes* outbreak are
52 significant due to a loss of consumer confidence and subsequent drop in product sales and related
53 value (McCollum *et al.* 2013). This review focuses on *L. monocytogenes* contamination of fresh leafy
54 produce lines, such as salad ingredients (lettuces, wild rocket etc.) and leafy brassicas (kale, spinach
55 etc.), which account for a significant proportion of the UK market and are “high-risk” in terms of
56 bacterial contamination because of their leaf structures and proximity to the ground. The fresh leafy
57 produce supply chain (FLPSC), from farm to fork, is complex and contains a diverse range of
58 environments where *L. monocytogenes* can be detected during routine sampling of fresh leafy
59 produce throughout the supply chain. For example, in soil, recently harvested crops, the processing
60 environment and in the final the product itself, although detection tends to be sporadic.

61 *L. monocytogenes* is more likely to be detected in environments where soil contamination is present
62 due to its ubiquity in the environment and presence in soil. Owing to this ubiquitous nature,
63 companies that operate in the supply chain have difficulty determining the source of contaminating
64 *L. monocytogenes* on fresh leafy produce. For source tracking, an increasing array of tools are
65 becoming available with the gold-standard being whole genome sequencing (WGS). However, use of
66 these tools on a day to day basis in the FLPSC is not yet feasible due to their cost, complexity of
67 analysis, and expertise required to interpret data. In contrast, during outbreaks of disease, the advent
68 of subtyping techniques has enabled source tracking of *L. monocytogenes* after an outbreak has been
69 identified (Pichler *et al.* 2011; Gaul *et al.* 2013). Once a contamination source is located or indicated,
70 regulatory bodies and companies that operate in the supply chain take appropriate precautionary
71 measures to avoid further contamination (e.g. increased sanitation regimes or avoidance of the
72 contaminated area). Subtyping can also indicate the potential risk of *L. monocytogenes* isolates. The
73 species can be split into four evolutionary lineages (I, II, III, IV), where most of human clinical cases are
74 caused by lineages I & II (Orsi *et al.* 2011). Despite the added benefits that subtyping provides in terms

75 of potential risk assessment, routine sampling in the FLPSC often only characterises isolates down to
76 the species level as currently, all *L. monocytogenes* are treated equally for regulatory purposes.
77 Owing to the potential risk of foodborne illness from this bacterium, source tracking, risk assessment
78 and understanding the ability of *L. monocytogenes* to survive in the FLPSC should be considered key
79 factors in tackling *L. monocytogenes* contamination of fresh leafy produce and reducing risk to the
80 consumer. In this review, possible sources of *L. monocytogenes* contamination in the FLPSC and the
81 mechanisms behind *L. monocytogenes* survival in this environment are discussed and the benefits of
82 subtyping *L. monocytogenes* isolates found in the FLPSC in the context of source tracking and risk
83 evaluation are outlined.

84 The Structure of the Fresh Leafy Produce Supply Chain

85 Fresh leafy produce types include but are not limited to, baby spinach (*Spinacia oleracea*), lettuce
86 (*Lactuca sativa*), rocket (*Eruca sativa*), kale (*Brassica oleracea*) and herbs such as coriander
87 (*Coriandrum sativum*). These crops are distributed to the consumer through a variety of end products
88 such as whole head crops, mixed bagged salads and sandwich ingredients. The FLPSC has been
89 summarised (see Monaghan and Beacham, 2017), but it is useful to provide a brief overview. The
90 chain starts in the field where a crop typically takes between 3-24 weeks to grow before being
91 harvested mechanically or by hand. After harvest, a crop may be packaged in field (as is the case with
92 whole head lettuce) where the product is cooled and transported to the retailer, or subjected to
93 further processing such as washing, cutting and packaging in a dedicated facility (Figure 1).
94 Protocols have been developed and applied to the growing process to reduce the risks of microbial
95 contamination of fresh produce supplied to retail outlets. These include preventing farmers from
96 growing crops on land that has been amended with raw manure and not irrigating crops in the
97 immediate period before harvest to reduce the risk of contamination from irrigation water.
98 Microbiological testing for *L. monocytogenes* throughout the FLPSC is obligatory through regulation
99 and/or customer specifications. The presence of *L. monocytogenes* or those of the *Listeria* genus in

100 sampled product or surrounding areas results in 1) an increase in the scope and frequency of testing
101 and 2) a review of the risk assessment with emphasis on possible sources of the bacteria (Monaghan
102 and Hutchinson 2015). Despite these measures, *L. monocytogenes* continues to sporadically
103 contaminate fresh leafy produce. A detailed review of *L. monocytogenes* outbreaks and prevalence
104 associated with fresh produce is provided by Zhu, Gooneratne and Hussain, (2017).

105 **Potential Sources of *L. monocytogenes* Contamination in the Fresh Leafy Produce**

106 **Supply Chain**

107 *L. monocytogenes* has been isolated from soil, waterways and vegetation where it exists as a
108 saprophyte (Welshimer 1968; Locatelli *et al.* 2013a) from domestic and wild animals where it is
109 harboured in the intestine and shed in faeces (Hellström *et al.* 2008; Hellström 2011) and from food
110 contact surfaces in processing facilities (Leong *et al.* 2014). Such environments are significantly
111 associated with production and processing of leafy produce and the pathogen can potentially be
112 transferred to the product surface through several transmission routes (Table 1). Survival of *L.*
113 *monocytogenes* in these environments is key to its transmission to foodstuffs. For example, *L.*
114 *monocytogenes* can persist in a food processing facility for months and re-contaminate product
115 passing through that facility (Leong *et al.* 2017).

116 ***L. monocytogenes*: An Organism Adapted to Survive in the Fresh Leafy Produce**

117 **Supply Chain**

118 To survive in the FLPSC *L. monocytogenes* must withstand various environmental pressures such as,
119 competition with other microbes, cleaning, desiccation, nutrient starvation and fluctuation in
120 temperatures. *L. monocytogenes* can grow between temperatures of 0 - 45°C and a pH of 4.1 to 9.6
121 (Liu 2008; Shabala *et al.* 2008). Liu *et al.* (2005) also showed that *L. monocytogenes* recovers well
122 after treatment with a pH 12 solution and was resistant to saturated (40% v/v) NaCl for at least 20h.

123 This ability to withstand physiochemical stresses is a major factor in *L. monocytogenes* ability to
124 contaminate chilled and minimally processed foods.

125 Exposure to environmental stresses induces the *L. monocytogenes* stress response, mediated by the
126 alternative sigma factor σ^B which regulates several stress, virulence and transporter associated
127 genes (e.g. *lmo2230*, *ltrC*, *ctc*, *inlA-E* & *opuC operon*) and related proteins (Kazmierczak *et al.* 2003).
128 Phenotypic investigations with strains lacking *sigB* demonstrate the important role that σ^B plays in
129 protecting against osmotic, oxidative, acid and detergent stresses (Ferreira *et al.* 2001, 2003). PrfA,
130 another important *L. monocytogenes* regulatory protein, plays a central role in the bacterium's
131 transition from soil to gut environments by activating and deactivating key virulence factors from a
132 set of environmental cues (Heras *et al.* 2011). Cold-adaptation is especially important for *L.*
133 *monocytogenes* survival in the FLPSC as low temperatures are readily encountered in the growing
134 and processing environments and during storage of products. *L. monocytogenes* has an innate ability
135 for cold adaptation, partly regulated by σ^B using a variety of mechanisms including the uptake of
136 cryoprotective osmolytes and peptides and the maintenance of cell surface fluidity (Tasara and
137 Stephan 2006). Biofilm production (Ferreira *et al.* 2014) and the ability to enter a protective, viable
138 but non-culturable (VBNC) state (Oliver 2010; Ayrapetyan and Oliver 2016) may also facilitate *L.*
139 *monocytogenes* survival in environments associated with the FLPSC.

140 These mechanisms ensure that *L. monocytogenes* has a more robust cross-stress tolerance
141 compared to other food-borne pathogens such as *E. coli* or *S. enterica* allowing it to survive in food
142 and food associated environments. For this reason, *L. monocytogenes* should not be considered in
143 the same way as other food-borne pathogens and comparatively stronger measures relating to
144 contamination of food and food associated environments are employed to control its presence.

145 *L. monocytogenes* Prevalence in Soil

146 Fresh produce begins its journey through the FLPSC as a seed or transplant in the soil. Soil is a
147 complex, nutritionally rich, heterogeneous environment which is in a state of 'dynamic equilibrium'

148 and contains an abundance of endogenous microbiota, mesofauna and macrofauna (Vivant *et al.*
149 2013). Soil is an environmental niche for *L. monocytogenes* and the bacterium has been readily
150 isolated from soil samples from different locations including meadows, mountainous regions and
151 forests (Linke *et al.* 2014a).

152 Whilst *L. monocytogenes* is nearly always found in low numbers, needing selective enrichment to be
153 detected (i.e. ISO 11290-1 for the presence/absence of *L. monocytogenes* in samples), the bacterium
154 can be found in around 17% of soil samples (Locatelli *et al.* 2013a). Data on the occurrence of *L.*
155 *monocytogenes* in soil from fresh leafy produce production fields indicate between 4% and 11% of
156 soil samples harbour the bacterium (Weller *et al.* 2015). To survive in soil, *L. monocytogenes* must
157 endure physiological stresses and competition from other soil dwelling microorganisms.

158 Factors Affecting *L. monocytogenes* Survival in Soil

159 Biotic factors have an important role in determining the size and growth characteristics of the *L.*
160 *monocytogenes* population in soil. McLaughlin *et al.*, (2011) showed an increase in the *L.*
161 *monocytogenes* population of over one log in 4 days from an initial inoculum of 10^7 CFU g^{-1} soil in
162 sterilised soil whilst the population decreased nearly two logs in the same time in unsterilised soil.
163 The suppressive effect of endogenous soil microbiota on *L. monocytogenes* survival in soil has been
164 demonstrated by many authors and reviewed expertly by Vivant, Garmyn and Piveteau, (2013). For
165 example, using a pathogen death rate model, Moynihan *et al.*, (2015) showed that the suppressive
166 effect on *L. monocytogenes* survival by the native soil microbiota increases with an increasingly
167 diverse population. Additionally, when a partial reconstruction of the soil microbiota is re-inoculated
168 into soil after sterilisation, it has a significant suppressive effect on *L. monocytogenes* survival
169 (McLaughlin *et al.* 2011).

170 *L. monocytogenes* survival in soil is variable by soil type, ranging from rapid decline to long-term
171 persistence, but generally, removing the bacterial population (sterilisation by autoclaving or other)
172 enables *L. monocytogenes* to survive for longer compared to the identical unsterilised soils (Locatelli

173 *et al.* 2013b). This effect could be due to competition for nutrients and space combined with
174 inhibitory bacteriocins which are produced by soil bacteria to kill or inhibit the growth of
175 competitors (Bruce *et al.* 2017), meaning that a large inoculum is not sustainable in the soil. Survival
176 has been shown to be dependent on soil type and abiotic factors such as soil texture (especially clay
177 content), pH and basic cation saturation ratio (BCSR) appear to be significant drivers of *L.*
178 *monocytogenes* survival in soil (Locatelli *et al.* 2013b). Owing to this variation in soil survival
179 (dependant on soil type), there is a need to determine how *L. monocytogenes* survives in soils
180 typically used in the intensive production of fresh leafy produce. This information will infer the risk
181 these commercially important soils pose to fresh leafy produce in terms of *L. monocytogenes*
182 contamination and may allow growers to consider alternative soils to reduce the likelihood of *L.*
183 *monocytogenes* survival.

184 [Mechanisms of *L. monocytogenes* Survival in Soil](#)

185 *L. monocytogenes* survival in the soil has been shown to be significantly affected by the response
186 regulator AgrA and corresponding genes; this regulator controls genes responsible for the transport
187 and metabolism of amino acids and related molecules, genes responsible for motility & chemotaxis
188 and genes that code for other regulators (Vivant *et al.* 2015). Emphasis has also been placed on the
189 role of transporters, which are upregulated by AgrA and allow *L. monocytogenes* to recruit an
190 extensive range of substrates for energy production in the soil (Piveteau *et al.* 2011). Interestingly,
191 *agrA* and *agrD* deletion mutants have altered ability to adhere to surfaces, suggesting the *agr*
192 system's involvement in the early stages of biofilm formation (Rieu *et al.* 2007). Biofilm production
193 and the ability of *L. monocytogenes* to survive in soil appear to be intimately linked as mutants
194 which lack *Lmo0753* (a *prfA* like transcription factor gene) form poor biofilms and show poor survival
195 in soil compared to wild-type strains (Salazar *et al.* 2013). Furthermore, *Lmo0753* is highly conserved
196 in lineage I & II strains, which are more commonly isolated from the soil than lineage III and IV
197 strains (Locatelli *et al.* 2013a; Linke *et al.* 2014b). *SigB* too plays an important role in soil survival – it

198 regulates the stress response after *L. monocytogenes* entry to the soil allowing the bacteria to stop
199 multiplying as a response to nutrient limitation, similar to entry to the stationary phase (Piveteau *et*
200 *al.* 2011). Entry to the soil also causes *prfA* to be down-regulated, subsequently de-activating key
201 virulence factors whilst genes involved with mobility, chemotaxis and the transport of carbohydrates
202 are up-regulated (Vivant *et al.* 2017).

203 **Is the Viable but Non Culturable (VBNC) State as a Potential *L. monocytogenes* Strategy for Soil Survival?**

204 VBNC cells are metabolically active bacteria that have lost the ability to develop colonies on rich
205 laboratory media and cannot therefore, be detected by conventional methods (i.e. direct plate
206 count). This state is believed to be a survival strategy to minimise energy requirements (Li *et al.*
207 2014). A variety of pathogenic bacteria including *L. monocytogenes* enter a protective VBNC state in
208 response to nutrient starvation, incubation outside the normal temperature, increased or reduced
209 osmotic concentrations and heavy metal exposure (Oliver 2010). Indeed, research has shown that a
210 large fraction of the *L. monocytogenes* population becomes VBNC in microcosms containing pig
211 manure and digestates from agricultural biogas plants (Desneux *et al.* 2016; Maynaud *et al.* 2016).
212 Given that the soil environment may result in nutrient deprivation and other stresses known to
213 induce VBNC, this may also cause *L. monocytogenes* to turn VBNC, but data on this characteristic of
214 the bacterium in the soil environment is missing. Overall, there is evidence to suggest that the VBNC
215 state of *L. monocytogenes* may be important for soil survival, but this whole area requires further
216 study.

217 **The Risk Posed from Soil Contaminated with *L. monocytogenes***

218 *L. monocytogenes* may be transferred from the soil to fresh produce through soil splash from
219 rainfall/irrigation or general soil contamination from mechanical or human activity. In an experiment
220 assessing the survival and transfer of the *L. monocytogenes* surrogate *L. innocua*, Girardin *et al.*,
221 (2005) demonstrated that transfer of this bacterium to the surface of parsley leaves occurred mostly
222 through soil splash from rain and irrigation after the bacterium was inoculated into the soil. The

223 authors also showed rapid decline of *L. innocua* numbers in soil and noted that when leaf surfaces
224 were contaminated with soil containing bacteria, the number of *L. innocua* was low.

225 Whilst only 1 *L. monocytogenes* cell per 25g⁻¹ of sample is required for detection of the bacteria on
226 fresh leafy produce (based on ISO 11290-1 methodology), illness caused by *L. monocytogenes* is
227 usually linked to consumption of food contaminated with a high number of the bacteria (European
228 Commission 1999). Using a dose response model Farber, Ross and Harwig, (1996) determined that
229 inoculum sizes of 10⁵ and 10⁷ *L. monocytogenes* cells would be required to cause listeriosis infection
230 in 10% and 90% of a 'high-risk' population respectively. These inoculum sizes contrast with the low
231 number of *L. monocytogenes* cells that survive in soil for extended periods and may suggest that
232 contamination of fresh leafy produce by soil borne bacteria is not likely to be a high risk to
233 consumers. However, recent evidence has shown that susceptible individuals can become ill after
234 consuming low levels of the bacteria (Pouillot *et al.* 2016) and infection with *L. monocytogenes* is
235 made more complicated due to the risk of repeated exposure and variation in susceptibility among
236 immunocompromised individuals (Buchanan *et al.* 2017). Therefore, whilst infection from low levels
237 of soil borne *L. monocytogenes* on leafy produce may not be high risk to consumers based on the
238 level of bacteria transferred, it is not possible to rule out infection of susceptible individuals from
239 this type of contamination.

240 Soil spoilage of product is common when growing leafy fresh produce, yet *L. monocytogenes*
241 outbreaks from this food type are rare, implying that soil is not a significant source of *L.*
242 *monocytogenes* in the FLPSC. When contamination does occur, the amount of *L. monocytogenes*
243 transferred to product is likely to be small/minimal based on previous data on the number of *L.*
244 *monocytogenes* present in soil (Locatelli *et al.* 2013a). Conversely, *L. monocytogenes* can proliferate
245 when in contact with a substrate such as cut produce (Salazar *et al.* 2017), but more research is
246 needed to determine its growth behaviours specifically for fresh leafy produce. Additionally, more

247 investigation is required to determine the effect that this change of environments has on the
248 culturability and infectiveness of this pathogen.

249 *L. monocytogenes* Association with Pre-harvest Fresh Leafy Produce

250 As discussed above, whilst growing in the field, fresh leafy produce may be subject to *L.*
251 *monocytogenes* contamination through soil splash where the bacteria is transferred to the surface of
252 the leaves. Opportunistic human pathogenic bacteria, including *L. monocytogenes*, can also interact
253 with fresh leafy produce through the root portion of the plant. For example, *E. coli* O157:H7
254 internalises to the root of lettuce and spinach plants (Wright *et al.* 2013). *L. monocytogenes* has
255 been shown to internalise both into lettuce seedlings and mature plants – the former after 5 days of
256 watering with contaminated water (10^5 CFU ml⁻¹) and the latter when the plant is grown
257 hydroponically with repeated exposure to the same level of *L. monocytogenes* contaminated water
258 (Standing *et al.* 2013). These conditions are unlikely to be encountered in the normal growing
259 environment and so the ability of *L. monocytogenes* to internalise into crop plants under field
260 conditions remains an open question. Opportunistic human pathogenic bacteria such as *E. coli*
261 O157:H7 and *Salmonella enterica* serovar Typhimurium have also been shown to be associated with
262 the rhizosphere – the narrow zone of soil influenced by the plant root. *L. monocytogenes* has a
263 supposed preference for the rhizosphere (Dowe *et al.* 1997), but research with *L. monocytogenes* in
264 this area is scarce. Crop plants produce root exudates, improve aeration in the soil and serve as a
265 source of nutrients to soil bacteria, thus improving soil microbial growth and activity. Based on
266 previous evidence this increase in microbial activity could have an increased suppressive effect on *L.*
267 *monocytogenes* survival. Overall, research is needed to determine how *L. monocytogenes* survives in
268 the soil in the presence of crop plants and whether this bacterium associates with the plant
269 rhizosphere like other opportunistic pathogens.

270 *L. monocytogenes* Presence in the Processing Environment

271 After harvest, fresh produce may be cut, washed and packaged in a dedicated processing facility
272 depending on customer requirements. The processing environment is kept clean through regular
273 sanitation and hygiene barrier systems, such as the segregation of pre- and post-wash product, aim
274 to prevent cross contamination. Despite these measures, *L. monocytogenes* enters the processing
275 facility, unintentionally, through contaminated product and personnel. Cross-contamination of food
276 from the processing environment does occur and research has highlighted that *L. monocytogenes*
277 can persist in the food processing environment and contaminate food products passing through a
278 food processing facility over time (Ferreira *et al.* 2014; Leong *et al.* 2017).

279 The fresh leafy produce processing environment is in some respects, a stark contrast to the soil –
280 nutritionally poor abiotic surfaces are abundant, detergent application is frequent and refrigeration
281 temperatures are typical. In spite of these different stresses, *L. monocytogenes* can be found in
282 difficult to clean harbourage sites, such as drains, cracks in surfaces and crevices in machinery where
283 disinfectants and sanitisers cannot properly reach (Jordan *et al.* 2015) and nutrients may be available
284 to the bacteria through product debris and factory run off (i.e. water containing leaf juices and soil
285 organic matter etc.). Evidence from factories suggests that *L. monocytogenes* can be introduced into
286 the food processing environment easily, grows at operational temperatures and is resistant to
287 several stresses which results in contamination of the processing environment.

288 Detection rates for *L. monocytogenes* in food processing facilities changes depending on the type of
289 food processing facility being sampled (Jordan *et al.* 2015). It is important to note that authors vary
290 in their sampling approach in the food processing environment and so differences in sampling
291 locations and detection methods may influence detection rates between studies. Interestingly, in
292 the largest study of its kind which monitored *L. monocytogenes* prevalence in food and
293 environmental samples across 54 small food businesses in Ireland, fish processing facilities returned
294 the lowest incidence of *L. monocytogenes* positive environmental samples (1.6%), followed by dairy

295 and meat processing facilities (both 4.1%) and vegetable (including fresh leafy produce) processing
296 facilities had the highest incidence of *L. monocytogenes* (9.5%) (Leong *et al.* 2017). Despite the
297 obvious presence of *L. monocytogenes* in the fresh produce processing environment, data on the
298 incidence of *L. monocytogenes* in processing facilities of this food group is scarce.

299 **Harbourage Sites and Persistent Strains in Food Processing Facilities**

300 Harbourage sites, also known as niches, reservoirs and hard to reach places, are areas in a
301 processing facility which are difficult to clean and may harbour *L. monocytogenes*. Harbourage sites
302 can arise from badly designed or worn equipment (e.g. hollow parts, cracks or crevices), and organic
303 matter from soil and product can be transferred to these areas and persist if not cleaned properly.
304 This process creates a supportive environment for bacterial growth and *L. monocytogenes* can be
305 introduced to harbourage sites from product contaminated outside the processing facility, or from
306 human carriers (Jordan *et al.* 2015). Low temperatures in processing facilities may inhibit the growth
307 of competitors, essentially selecting for *L. monocytogenes* in these niches. Additionally, these
308 harbourage sites may enable the selection of detergent resistant *L. monocytogenes* mutants through
309 ineffective cleaning due to the diluted levels of detergent that the harbourage site is exposed to
310 (Carpentier and Cerf 2011).

311 *L. monocytogenes* is known to persist in the processing environment and harbourage sites are
312 thought to play an important role in persistence of the bacterium in processing facilities. In addition,
313 inappropriate cleaning and sanitation can add to the spreading *L. monocytogenes* in a processing
314 facility through the creation of aerosols. A persistent strain can be defined as repeated isolation of
315 an identical *L. monocytogenes* subtype (as determined by molecular subtyping) from a single
316 processing facility over 6 months. Persistent strains in the food processing environment have been
317 identified by several authors (Sauders *et al.* 2009; Stasiewicz *et al.* 2015; Fagerlund *et al.* 2016)
318 because identifying and subsequently eliminating persistent strains in the processing environment is
319 a key step in reducing consumer risk from *L. monocytogenes* contamination. Leong *et al.*, (2017)

320 determined that out of 4 food groups tested, vegetable processing facilities had the highest number
321 of persistent strains and the highest diversity of pulsotypes which may reflect *L. monocytogenes*
322 presence and distribution in the growing environment for fruit and vegetables.

323 Potential Survival Mechanisms of *L. monocytogenes* in the Food Processing Environment

324 Persistent strains have been shown to exist in the processing environment, but studies which have
325 tried to explain the physiological characteristics which contribute to *L. monocytogenes* persistence
326 vary in their findings. For example, it has been reported that persistent strains show enhanced
327 adherence to food contact surfaces after short contact times (Lundén *et al.* 2000) with some studies
328 suggesting that persistent strains form better biofilms than sporadic strains (Nowak *et al.* 2017)
329 whereas others showing no difference in biofilm formation between persistent and sporadic strains
330 (Magalhães *et al.* 2017). Persistent strains have also been shown to be more resistant to detergent
331 stresses, although this attribute may be due to the characteristics of biofilms rather than intrinsic
332 resistance of the bacterial cell (Pan *et al.* 2006). Cheng *et al.*, (2015) determined that persistent
333 strains showed increased adherence and biofilm formation, but no difference was noted in sanitiser
334 resistance between persistent and transient strains, demonstrating the lack of consensus in the
335 literature. Whether persistent strains confer a physiological advantage compared to their non-
336 persistent counterparts remains an open question as so far, research has generated mixed results
337 which do not explain how persistent strains seem to be able to survive more readily in the
338 processing environment.

339 In *L. monocytogenes*, σ^B , the major transcriptional regulator of stress response genes, plays an
340 important role in resistance to detergent stresses at lethal levels (Ryan *et al.* 2008). In addition, *SigB*
341 has been shown to be activated in biofilms and appears to be an essential gene for the formation of
342 biofilms with increased resistance to disinfectants in *L. monocytogenes* (Van Der Veen and Abee
343 2010). *L. monocytogenes* biofilms contribute to persistence in the food processing environment as
344 biofilms can be formed on many different surfaces and serve as a source of subsequent

345 contamination (Colagiorgi *et al.* 2017). Another aspect of *L. monocytogenes* physiology which may
346 contribute to persistence in the food processing environment is the ability of the bacteria to enter
347 the VBNC state. The VBNC state may be triggered in response to numerous physiological cues as
348 mentioned previously. Importantly, in the context of the food processing environment, the
349 sanitation procedure (cleaning and disinfection) leads to a loss in culturability of *L. monocytogenes*
350 and appearance of VBNC populations (Overney *et al.* 2017). By entering a protective, VBNC state, *L.*
351 *monocytogenes* may be able to further resist environmental stresses in the food processing
352 environment (Ayrapetyan and Oliver 2016). Upon entry into a suitable environment (e.g. a
353 harbourage site) VBNC *L. monocytogenes* can subsequently regain culturability and begin to
354 proliferate. Further evidence outlining the potential importance of VBNC *L. monocytogenes* in the
355 food processing environment is demonstrated by work indicating that chlorine stress induces the
356 VBNC state in *L. monocytogenes* and that these VBNC cells remain infectious in a *Caenorhabditis*
357 *elegans* model (Highmore *et al.* 2018).

358 Recent evidence has shown that *L. monocytogenes* ST121, a sequence type commonly associated
359 with food and food environments, carries a stress survival islet (SSI-2) that confers increased survival
360 under oxidative and alkaline stresses which are common in the food processing environment (Harter
361 *et al.* 2017). Overall, *L. monocytogenes* is well suited to surviving the various stresses presented by
362 the fresh produce processing environment and may have a competitive advantage over other
363 contaminating bacteria, facilitated through harbourage sites. Moreover, due to its ubiquitous nature
364 in the growing environment, recontamination of a processing environment in the FLPSC after
365 cleaning and disinfection is possible, meaning that regular sanitation regimes must be undertaken to
366 combat its continuing presence.

367 *L. monocytogenes* Survival on the Product Surface: Post-harvest

368 It has been shown that *L. monocytogenes* survives and grows on a range of fresh products including
369 lettuce (Beuchat and Brackett 1990), mixed vegetable salads (García-Gimeno *et al.* 1996), green and

370 red peppers and avocado pulp (Salazar *et al.* 2017). Studies such as these have outlined the
371 importance of keeping produce at refrigeration temperatures to slow growth of *L. monocytogenes*
372 populations, but have also demonstrated that post-harvest, *L. monocytogenes* can survive on the
373 surface of fresh produce for extended periods. For example, *L. monocytogenes* can survive on the
374 surface of an apple for up to 12 weeks from an initial inoculum of 3.5 log CFU ml⁻¹ (Sheng *et al.*
375 2017).

376 Contamination events with relatively high levels of *L. monocytogenes* may be rare in the FLPSC,
377 however, a small bacterial contamination on an injured leaf may lead to growth and colonisation
378 similar to *Salmonella* and pathogenic *E. coli* (Koukkidis *et al.* 2016) increasing the risk to consumers.
379 Of concern to the companies operating within the FLPSC is that any *L. monocytogenes*
380 contamination (1 *L. monocytogenes* per 25g product as determined by ISO 11290-1) of the leaf
381 surface can ultimately lead to a positive detection during routine sampling creating an expensive
382 logistical issue and potential health threat.

383 Mechanisms of Survival on the Product Surface: Post-harvest

384 There is good awareness of the *L. monocytogenes* (plus other pathogens) contamination risk to fresh
385 produce and fresh leafy produce is subject to a wash/decontamination step before packaging (ready
386 to eat prepared products) or customers are advised to wash before use (non-prepared, whole head
387 products). The specific requirements for product processing and consumer labelling are controlled
388 by legislation with additional customer-specific demands. The wash step is intended to reduce
389 foreign bodes, dirt and microbial load on the product surface and process wash water contains
390 sanitisers to maintain the water quality during processing. The effectiveness of the wash step in
391 reducing bacterial loads on lettuce leaves that have recently been contaminated with *L.*
392 *monocytogenes* depends on the amount of time post contamination. Ölmez and Temur, (2010)
393 showed a 99.9% reduction in *L. monocytogenes* when green leaf lettuce was subject to sanitiser
394 treatments 6h after a contamination event. This efficacy was reduced to 90% after applying the

395 sanitiser treatments 48h post-contamination due to the formation of *L. monocytogenes* biofilms on
396 the leaf surface. Biofilms also facilitate resistance to desiccation, an environmental stress readily
397 encountered on the product surface. *L. monocytogenes* strains which are resistant to desiccation
398 stress may present an increased contamination risk to the consumer due to their ability to survive on
399 the leaf surface. Desiccation resistance has been shown to be influenced by serotype, origin,
400 genotype and virulence with strains of serotype 1/2b being more resistant to desiccation stress than
401 other serotypes (Zoz *et al.* 2017). A further contributing factor to *L. monocytogenes* contamination
402 of post-harvest product is the bacterium's ability to adhere to and persist on abiotic surfaces in the
403 processing environment such as stainless steel and polystyrene (Lee *et al.* 2017). *L. monocytogenes*
404 forms biofilm on a range of abiotic surfaces and it is hypothesised that this characteristic of the
405 bacterium aids in its persistence and subsequent recontamination of post-harvest produce.

406 [Source Tracking *L. monocytogenes* in the Fresh Leafy Produce Supply Chain](#)

407 [Using Subtyping to Source Track *L. monocytogenes* Through the Supply Chain and Identify](#)

408 [Persistent Strains](#)

409 An important step in tackling *L. monocytogenes* contamination in the FLPSC is to identify the source
410 of contaminating bacteria and persistent strains in environments where they may be subsequently
411 eradicated. To do this in food associated environments, subtyping methods such as pulsed gel field
412 electrophoresis (PGFE) and whole genome sequencing (WGS) must be employed. Once common
413 subtypes have been identified, investigators can begin to link separate contamination events and
414 search for commonality (source) between these events (e.g. a single processing facility, farm or deli
415 counter). Subtyping of *L. monocytogenes* during outbreak investigations has successfully revealed
416 sources of contamination including a celery processing environment (Gaul *et al.* 2013) and a
417 cantaloupe processing environment (McCollum *et al.* 2013). Importantly, in the cantaloupe example,
418 the authors did not find any evidence of *L. monocytogenes* in the raw material, establishing the

419 processing environment as the main source of contamination. These examples indicate the
420 contamination risk from *L. monocytogenes* presence in ‘bottle-neck’ areas of food supply chains.
421 The same rationale can be applied to contamination events in the FLPSC through regular sampling of
422 fresh produce and surrounding environments. Leong *et al.*, (2017) used PGFE to subtype isolates
423 from a variety of food processing facilities and were able to identify persistent strains in vegetable
424 processing facilities which subsequently contaminated produce, but also recognise that elucidation
425 of the specific source of contamination in a processing facility requires sampling over a longer time.
426 Nastasijevic *et al.*, (2017) applied single nucleotide polymorphism (SNP) analysis to WGS data to
427 determine the genetic relatedness of strains and trace contamination through a meat production
428 facility to a single line (slaughter line) demonstrating that the use of subtyping techniques enables
429 source tracking through a food processing environment. Identification of persistent strains and
430 contamination sources would enable companies who operate in the FLPSC to employ a “seek and
431 destroy” strategy (Stasiewicz *et al.* 2015) to eradicate the contaminating bacteria from contaminated
432 environments. However, routine commercial sampling of fresh produce and surrounding
433 environments is often infrequent and currently only identifies *L. monocytogenes* down to the species
434 level. Even with the advent of subtyping techniques, source tracking in a processing environment
435 remains difficult due to the risk of recontamination, i.e. if an indistinguishable strain is found in a
436 processing environment and on a raw material it doesn’t prove that the contamination came from
437 the raw material or vice versa. To elucidate the specific source of *L. monocytogenes* in this context,
438 companies in the FLPSC would have to embark on a regular sampling regime of both the processing
439 environment and raw/processed product combined with molecular subtyping which may currently
440 be beyond the scope (in terms of time and financial investment) of companies operating in this
441 sector. Source tracking with WGS relies on well-designed sampling plans as the difficulties in
442 distinguishing persistent and genetically similar, repeatedly reintroduced *L. monocytogenes* strains
443 in a given environment have been noted (Stasiewicz *et al.* 2015).

444 As the cost of WGS reduces year on year however, this molecular subtyping method becomes more
445 attractive. In terms of source tracking, WGS data gives a higher resolution (i.e. more distinction
446 between genetically similar isolates) than PGFE, making it a more powerful and reliable tool (Moura
447 *et al.* 2017). Implementation of WGS in the commercial microbiology laboratories which service the
448 FLPSC by testing produce for pathogens produce is limited by expertise in the field, data
449 interpretation and lack of infrastructure (Kwong *et al.* 2015). Implementation is also limited by cost,
450 and whilst the cost per sample is reducing it still remains a significant cost which is prohibitive for
451 such routine use in the FLPSC.

452 *Subtyping L. monocytogenes by WGS Can Infer the Potential Risk of Isolates*

453 In addition to being used as a source tracking tool, WGS can infer the risk posed by isolates found in
454 the FLPSC by allowing genome-wide mapping and phylogenetic analysis. WGS can be used to group
455 *L. monocytogenes* isolates based on their phylogenetic lineage. Other sequencing tools such as multi
456 locus sequence typing (MLST) also provide this advantage but unlike MLST, WGS also provides data
457 on the presence and intactness of specific and essential virulence associated genes in *L.*
458 *monocytogenes* such as internalins (*InIA*, *InIB*, *InIC* & *InIJ*) essential for host cell internalisation,
459 listeriolysin O (*hly*) essential for *L. monocytogenes* escape from phagosomes into the cytosol and
460 listeriolysin S (*IIsX*), essential for modifying host gut microbiota during infection (Wu *et al.* 2016;
461 Quereda *et al.* 2017). Determining the presence and functioning of these genes could indicate the
462 potential risk that *L. monocytogenes* isolates found in the FLPSC pose to the consumer, although it
463 should be said that missing or non-functioning genes do not necessarily confer reduced virulence or
464 avirulence in an isolate and more research is needed in this area.

465 Thus, when applied to *L. monocytogenes* isolates in the FLPSC, WGS is only able to give an indication
466 of risk. However, implementation of this technique combined with a *L. monocytogenes* surveillance
467 programme in the supply chain would give insight into the relatedness of the *L. monocytogenes*
468 population that exists in the FLPSC, outlining the frequency with which strains are isolated (thus
469 whether a strain is sporadic or persistent) and their source. WGS also provides phylogenetic

470 information on isolates and could therefore outline the potential risk they pose to the consumer.
471 This information may be valuable to the companies that operate in the FLPSC by informing risk
472 assessments associated with *L. monocytogenes* contamination, ultimately reducing the risk to the
473 consumer.

474 Conclusion

475 Several molecular and physiological mechanisms contribute to *L. monocytogenes* survival in the
476 FLPSC. There are many potential contamination routes in the growing environment of fresh leafy
477 produce that may be difficult or impossible to prevent (e.g. transfer from wild animal faeces) and we
478 suggest that whilst contamination from the soil is possible, it is of low risk to consumers due to the
479 small number of bacteria transferred. *L. monocytogenes* can persist in a processing facility,
480 facilitated by harbourage sites and recontaminate product passing through that facility, making this
481 environment a high priority for the elimination of the bacteria. Although currently expensive, WGS
482 should be used to identify persistent *L. monocytogenes* due to the additional valuable data it
483 provides compared to other subtyping methods. As the cost of WGS reduces, *L. monocytogenes*
484 isolates from the FLPSC should be characterised by this method to determine their source,
485 relatedness and evaluate the risk they pose to the consumer. The authors recommend that future *L.*
486 *monocytogenes* research should focus on; *L. monocytogenes* survival in soil, transfer to the product
487 surface and subsequent survival on the product surface of fresh leafy produce, *L. monocytogenes*
488 association with the product in the growing environment (i.e. in the soil), the VBNC state of *L.*
489 *monocytogenes* in the context of survival in the food supply chain, how *L. monocytogenes* biofilms
490 can be mitigated and removed and finally robust methods for determining sources of *L.*
491 *monocytogenes* in the FLPSC.

492 Conflict of Interest Statement

493 The authors declare that no conflict of interest exists.

494 **References**

- 495 Allende, A., Monaghan, J. (2015) Irrigation water quality for leafy crops: A perspective of risks and
496 potential solutions. *Int J Environ Res Public Health* **12**:7457–7477. doi: 10.3390/ijerph120707457
- 497 Ayrapetyan, M., Oliver, J.D. (2016) The viable but non-culturable state and its relevance in food
498 safety. *Curr Opin Food Sci* **8**:127–133. doi: 10.1016/j.cofs.2016.04.010
- 499 Beuchat, L.R., Brackett, R.E. (1990) Survival and Growth of *Listeria monocytogenes* on Lettuce as
500 Influenced by Shredding, Chlorine Treatment, Modified Atmosphere Packaging and Temperature. *J*
501 *Food Sci* **55**:755–758. doi: 10.1111/j.1365-2621.1990.tb05222.x
- 502 Bruce, J.B., West, S.A., Griffin, A.S. (2017) Bacteriocins and the assembly of natural *Pseudomonas*
503 *fluorescens* populations. *J Evol Biol* **30**:352–360. doi: 10.1111/jeb.13010
- 504 Buchanan, R.L., Gorris, L.G.M., Hayman, M.M., *et al.* (2017) A review of *Listeria monocytogenes*: An
505 update on outbreaks, virulence, dose-response, ecology, and risk assessments. *Food Control* **75**:1–
506 13. doi: 10.1016/j.foodcont.2016.12.016
- 507 Carpentier, B., Cerf, O. (2011) Review - Persistence of *Listeria monocytogenes* in food industry
508 equipment and premises. *Int J Food Microbiol* **145**:1–8. doi: 10.1016/j.ijfoodmicro.2011.01.005
- 509 Cartwright, E.J., Jackson, K.A., Johnson, S.D., *et al.* (2013) Listeriosis outbreaks and associated food
510 vehicles, United States, 1998-2008. *Emerg Infect Dis* **19**:1–9. doi: 10.3201/eid1901.120393
- 511 Center for Food Safety and Applied Nutrition. (2017) Control of *Listeria monocytogenes* in Ready-To-
512 Eat Foods: Guidance for Industry Draft Guidance. *Int J Pharm Biol Arch* **3**:1–49
- 513 Chan, Y.C., Wiedmann, M. (2009) Physiology and genetics of *Listeria monocytogenes* survival and
514 growth at cold temperatures. *Crit Rev Food Sci Nutr* **49**:237–253. doi: 10.1080/10408390701856272
- 515 Cheng, C., Yang, Y., Dong, Z., *et al.* (2015) *Listeria monocytogenes* varies among strains to maintain
516 intracellular pH homeostasis under stresses by different acids as analyzed by a high-throughput

517 microplate-based fluorometry MATERIALS AND METHODS. **6**:1–10. doi: 10.3389/fmicb.2015.00015

518 Colagiorgi, A., Bruini, I., Di Ciccio, P.A., *et al.* (2017) *Listeria monocytogenes* Biofilms in the
519 Wonderland of Food Industry. *Pathogens* **6**:41. doi: 10.3390/pathogens6030041

520 Desneux, J., Biscuit, A., Picard, S., Pourcher, A.M. (2016) Fate of viable but non-culturable *Listeria*
521 *monocytogenes* in pig manure microcosms. *Front Microbiol* **7**:1–13. doi: 10.3389/fmicb.2016.00245

522 Dowe, M., Jackson, E., Mori, J., Colin, B. (1997) *Listeria monocytogenes* Survival in Soil and Incidence
523 in Agricultural Soils. *J Food Prot* **10**:1158–1286. doi: 10.4315/0362-028X-60.10.1201

524 European Commission. (2005) Guidance Document on *Listeria monocytogenes* shelf-life studies for
525 ready-to-eat foods, under Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological
526 criteria for foodstuffs

527 European Commission. (1999) Opinion of the Scientific Committee on Veterinary Measures Relating
528 to Public Health on *Listeria monocytogenes*. *Foodborne Pathog Dis* 1–47. doi:
529 10.1089/fpd.2011.0830

530 Fagerlund, A., Langsrud, S., Schirmer, B.C.T., *et al.* (2016) Genome analysis of *Listeria monocytogenes*
531 sequence type 8 strains persisting in salmon and poultry processing environments and comparison
532 with related strains. *PLoS One* **11**:1–22. doi: 10.1371/journal.pone.0151117

533 Farber, J.M., Ross, W.H., Harwig, J. (1996) Health risk assessment of *Listeria monocytogenes* in
534 Canada. *Int J Food Microbiol* **30**:145–156. doi: 10.1016/0168-1605(96)01107-5

535 Fenlon, D.R. (1985) Wild birds and silage as reservoirs of *Listeria* in the agricultural environment. *J*
536 *Appl Bacteriol* **59**:537–543. doi: 10.1111/j.1365-2672.1985.tb03357.x

537 Ferreira, A., O’Byrne, C.P., Boor, K.J. (2001) Role of sigma(B) in heat, ethanol, acid, and oxidative
538 stress resistance and during carbon starvation in *Listeria monocytogenes*. *Appl Environ Microbiol*
539 **67**:4454–4457. doi: 10.1128/AEM.67.10.4454

540 Ferreira, A., Sue, D., O'Byrne, C.P., Boor, K.J. (2003) Role of *Listeria monocytogenes* sigma(B) in
541 survival of lethal acidic conditions and in the acquired acid tolerance response. *Appl Env Microbiol*
542 **69**:2692–2698. doi: 10.1128/AEM.69.5.2692

543 Ferreira, V., Wiedmann, M., Teixeira, P., Stasiewicz, M.J. (2014) *Listeria monocytogenes* Persistence
544 in Food-Associated Environments: Epidemiology, Strain Characteristics, and Implications for Public
545 Health. *J Food Prot* **77**:150–70. doi: 10.4315/0362-028X.JFP-13-150

546 García-Gimeno, R.M., Zurera-Cosano, G., Amaro-Lopez, M. (1996) Incidence, survival and growth of
547 *Listeria monocytogenes* in ready-to- use mixed vegetable salads in Spain. *J Food Saf* **16**:75–86

548 Garner, D., Kathariou, S. (2016) Fresh Produce–Associated Listeriosis Outbreaks, Sources of Concern,
549 Teachable Moments, and Insights. *J Food Prot* **79**:337–344. doi: 10.4315/0362-028X.JFP-15-387

550 Gaul, L.K., Farag, N.H., Shim, T., *et al.* (2013) Hospital-acquired listeriosis outbreak caused by
551 contaminated diced celery-texas, 2010. *Clin Infect Dis* **56**:20–26. doi: 10.1093/cid/cis817

552 Girardin, H., Morris, C.E., Albagnac, C., *et al.* (2005) Behaviour of the pathogen surrogates *Listeria*
553 *innocua* and *Clostridium sporogenes* during production of parsley in fields fertilized with
554 contaminated amendments. *FEMS Microbiol Ecol* **54**:287–295. doi: 10.1016/j.femsec.2005.04.003

555 Haase, J.K., Didelot, X., Lecuit, M., *et al.* (2014) The ubiquitous nature of *Listeria monocytogenes*
556 clones: A large-scale Multilocus Sequence Typing study. *Environ Microbiol* **16**:405–416. doi:
557 10.1111/1462-2920.12342

558 Harter, E., Wagner, E.M., Zaiser, A., *et al.* (2017) Stress survival islet 2, predominantly present in
559 *Listeria monocytogenes* strains of sequence type 121, is involved in the alkaline and oxidative stress
560 responses. *Appl Environ Microbiol* **83**:. doi: 10.1128/AEM.00827-17

561 Heaton, J.C., Jones, K. (2008) Microbial contamination of fruit and vegetables and the behaviour of
562 enteropathogens in the phyllosphere: A review. *J Appl Microbiol* **104**:613–626. doi: 10.1111/j.1365-
563 2672.2007.03587.x

564 Hellström, S. (2011) Contamination routes and control of *Listeria monocytogenes* in food production.
565 University of Helsinki

566 Hellström, S., Kiviniemi, K., Autio, T., Korkeala, H. (2008) *Listeria monocytogenes* is common in wild
567 birds in Helsinki region and genotypes are frequently similar with those found along the food chain. *J*
568 *Appl Microbiol* **104**:883–888. doi: 10.1111/j.1365-2672.2007.03604.x

569 Heras, A. De., Cain, R.J., Bielecka, M.K. (2011) Regulation of *Listeria* virulence : PrfA master and
570 commander. *Curr Opin Microbiol* 118–127. doi: 10.1016/j.mib.2011.01.005

571 Highmore, C.J., Warner, J.C., Rothwell, S.D., *et al.* (2018) Viable-but-Nonculturable *Listeria*
572 *monocytogenes* and *Salmonella enterica* Serovar Thompson Induced by Chlorine Stress Remain
573 Infectious. *MBio* **9**:1–12. doi: 10.1128/mBio.00540-18

574 Inoue, S., Tanikawa, T., Kawaguchi, J., *et al.* (1992) Prevalence of *Listeria* (spp.) in Wild Rats Captured
575 in the Kanto Area of Japan. *J Veterinary Med Sci* **54**:461–463

576 Jami, M., Ghanbari, M., Zunabovic, M., *et al.* (2014) *Listeria monocytogenes* in aquatic food
577 products-A review. *Compr Rev Food Sci Food Saf* **13**:798–813. doi: 10.1111/1541-4337.12092

578 Jordan, K., Leong, D., Alvarez-Ordóñez, A. (2015) *Listeria monocytogenes* in the Food Processing
579 Environment

580 Kazmierczak, M.J., Mithoe, S.C., Boor, K.J., Wiedmann, M. (2003) *Listeria monocytogenes* σ B
581 Regulates Stress Response and Virulence Functions. *J Bacteriol* **185**:5722–34. doi:
582 10.1128/JB.185.19.5722

583 Khan, I., Khan, J., Miskeen, S., *et al.* (2016) Prevalence and control of *Listeria monocytogenes* in the
584 food industry – a review. *Czech J Food Sci* **34**:469–487. doi: 10.17221/21/2016-CJFS

585 Koukkidis, G., Haigh, R., Allcock, N., *et al.* (2016) Salad leaf juices enhance *Salmonella* growth, fresh
586 produce colonisation and virulence. *Appl Environ Microbiol* AEM.02416-16. doi:

587 10.1128/AEM.02416-16

588 Kwong, J.C., Mccallum, N., Sintchenko, V., Howden, B.P. (2015) Whole genome sequencing in clinical
589 and public health microbiology. *Pathology* **47**:199–210. doi: 10.1097/PAT.000000000000235

590 Lee, B., Hébraud, M., Bernardi, T., Lee, B. (2017) Increased Adhesion of *Listeria monocytogenes*
591 Strains to Abiotic Surfaces under Cold Stress. *Front Microbiol* **8**:1–10. doi: 10.3389/fmicb.2017.02221

592 Leong, D., Alvarez-Ordóez, A., Guillas, F., Jordan, K. (2013) Determination of *Listeria monocytogenes*
593 Growth during Mushroom Production and Distribution. *Foods* **2**:544–553. doi:
594 10.3390/foods2040544

595 Leong, D., Alvarez-Ordóez, A., Jordan, K. (2014) Monitoring occurrence and persistence of *Listeria*
596 *monocytogenes* in foods and food processing environments in the Republic of Ireland. *Front*
597 *Microbiol* **5**:1–8. doi: 10.3389/fmicb.2014.00436

598 Leong, D., NicAogáin, K., Luque-Sastre, L., *et al.* (2017) A 3-year multi-food study of the presence and
599 persistence of *Listeria monocytogenes* in 54 small food businesses in Ireland. *Int J Food Microbiol*
600 **249**:18–26. doi: 10.1016/j.ijfoodmicro.2017.02.015

601 Li, L., Mendis, N., Trigui, H., *et al.* (2014) The importance of the viable but non-culturable state in
602 human bacterial pathogens. *Front Microbiol* **5**:1–1. doi: 10.3389/fmicb.2014.00258

603 Linke, K., Ruckerl, I., Brugger, K., *et al.* (2014a) Reservoirs of *Listeria* species in three environmental
604 ecosystems. *Appl Environ Microbiol* **80**:5583–5592. doi: 10.1128/AEM.01018-14

605 Linke, K., Ruckerl, I., Brugger, K., *et al.* (2014b) Reservoirs of *Listeria* species in three environmental
606 ecosystems. *Appl Environ Microbiol* **80**:5583–5592. doi: 10.1128/AEM.01018-14

607 Liu, D. (2008) Handbook of *Listeria Monocytogenes*

608 Liu, D., Lawrence, M.L., Ainsworth, A.J., Austin, F.W. (2005) Comparative assessment of acid, alkali
609 and salt tolerance in *Listeria monocytogenes* virulent and avirulent strains. *FEMS Microbiol Lett*

610 **243**:373–378. doi: 10.1016/j.femsle.2004.12.025

611 Locatelli, A., Depret, G., Jolivet, C., *et al.* (2013a) Nation-wide study of the occurrence of *Listeria*
612 *monocytogenes* in French soils using culture-based and molecular detection methods. *J Microbiol*
613 *Methods* **93**:242–250. doi: 10.1016/j.mimet.2013.03.017

614 Locatelli, A., Spor, A., Jolivet, C., *et al.* (2013b) Biotic and Abiotic Soil Properties Influence Survival of
615 *Listeria monocytogenes* in Soil. *PLoS One* **8**:1–8. doi: 10.1371/journal.pone.0075969

616 Lundén, J.M., Miettinen, M.K., Autio, T.J., Korkeala, H.J. (2000) Persistent *Listeria monocytogenes*
617 strains show enhanced adherence to food contact surface after short contact times. *J Food Prot*
618 **63**:1204–1207. doi: 10.4315/0362-028X-63.9.1204

619 Magalhães, R., Ferreira, V., Biscottini, G., *et al.* (2017) Biofilm formation by persistent and non-
620 persistent *Listeria monocytogenes* strains on abiotic surfaces. *Acta Aliment* **46**:43–50. doi:
621 10.1556/066.2017.46.1.6

622 Maynaud, G., Pourcher, A.M., Ziebal, C., *et al.* (2016) Persistence and potential viable but non-
623 culturable state of pathogenic bacteria during storage of digestates from agricultural biogas plants.
624 *Front Microbiol* **7**:. doi: 10.3389/fmicb.2016.01469

625 McCollum, J.T., Cronquist, A.B., Silk, B.J., *et al.* (2013) Multistate outbreak of listeriosis associated
626 with cantaloupe. *N Engl J Med* **369**:944–53. doi: 10.1056/NEJMoa1215837

627 McLaughlin, H.P., Casey, P.G., Cotter, J., *et al.* (2011) Factors affecting survival of *Listeria*
628 *monocytogenes* and *Listeria innocua* in soil samples. *Arch Microbiol* **193**:775–785. doi:
629 10.1007/s00203-011-0716-7

630 Monaghan, J., Hutchison, M. (2015) Monitoring microbial food safety of fresh produce. AHDB
631 Horticulture. Factsheet 13/10, 1-16.

632 Monaghan, J.M., Beacham, A.M. (2017) Salad Vegetable Crops. In Brian Thomas, Brian G Murray and

633 Denis J Murphy (Editors in Chief), Encyclopedia of Applied Plant Sciences, Vol 3, Waltham, MA:
634 Academic Press, pp. 262–267.

635 Monaghan, J.M., Hutchison, M.L. (2012) Distribution and decline of human pathogenic bacteria in
636 soil after application in irrigation water and the potential for soil-splash-mediated dispersal onto
637 fresh produce. *J Appl Microbiol* **112**:1007–1019. doi: 10.1111/j.1365-2672.2012.05269.x

638 Moura, A., Tourdjman, M., Leclercq, A., *et al.* (2017) Real-Time Whole-Genome Sequencing for
639 Surveillance of *Listeria monocytogenes*, France. *Emerg Infect Dis* **23**:. doi: 10.3201/eid2309.170336

640 Moynihan, E.L., Richards, K.G., Brennan, F.P., *et al.* (2015) Enteropathogen survival in soil from
641 different land-uses is predominantly regulated by microbial community composition. *Appl Soil Ecol*
642 **89**:76–84. doi: 10.1016/j.apsoil.2015.01.011

643 Nastasijevic, I., Milanov, D., Velebit, B., *et al.* (2017) Tracking of *Listeria monocytogenes* in meat
644 establishment using Whole Genome Sequencing as a food safety management tool: A proof of
645 concept. *Int J Food Microbiol* **257**:157–164. doi: 10.1016/j.ijfoodmicro.2017.06.015

646 Nowak, J., Cruz, C.D., Tempelaars, M., *et al.* (2017) Persistent *Listeria monocytogenes* strains isolated
647 from mussel production facilities form more biofilm but are not linked to specific genetic markers.
648 *Int J Food Microbiol* **256**:45–53. doi: 10.1016/j.ijfoodmicro.2017.05.024

649 Oliveira, M., Usall, J., Villas, I., *et al.* (2011) Transfer of *Listeria innocua* from contaminated compost
650 and irrigation water to lettuce leaves. *Food Microbiol* **28**:590–596. doi: 10.1016/j.fm.2010.11.004

651 Oliver, J.D. (2010) Recent findings on the viable but nonculturable state in pathogenic bacteria. *FEMS*
652 *Microbiol Rev* **34**:415–425. doi: 10.1111/j.1574-6976.2009.00200.x

653 Ölmez, H., Temur, S.D. (2010) Effects of different sanitizing treatments on biofilms and attachment
654 of *Escherichia coli* and *Listeria monocytogenes* on green leaf lettuce. *LWT - Food Sci Technol* **43**:964–
655 970. doi: 10.1016/j.lwt.2010.02.005

656 Orsi, R.H., Bakker, H.C. den., Wiedmann, M. (2011) *Listeria monocytogenes* lineages: Genomics,
657 evolution, ecology, and phenotypic characteristics. *Int J Med Microbiol* **301**:79–96. doi:
658 10.1016/j.ijmm.2010.05.002

659 Overney, A., Jacques-André-Coquin, J., Ng, P., *et al.* (2017) Impact of environmental factors on the
660 culturability and viability of *Listeria monocytogenes* under conditions encountered in food
661 processing plants. *Int J Food Microbiol* **244**:74–81. doi: 10.1016/j.ijfoodmicro.2016.12.012

662 Pan, Y., Breidt, F., Kathariou, S. (2006) Resistance of *Listeria monocytogenes* biofilms to sanitizing
663 agents in a simulated food processing environment. *Appl Environ Microbiol* **72**:7711–7717. doi:
664 10.1128/AEM.01065-06

665 Pichler, J.G., Appl, G., Pietzka, a., Allerberger, F. (2011) Lessons to be Learned from an Outbreak of
666 Foodborne Listeriosis, Austria 2009–2010. *Food Prot Trends* **31**:268–273

667 Piveteau, P., Depret, G., Pivato, B., *et al.* (2011) Changes in gene expression during adaptation of
668 *Listeria monocytogenes* to the soil environment. *PLoS One* **6**:. doi: 10.1371/journal.pone.0024881

669 Pouillot, R., Klontz, K.C., Chen, Y., *et al.* (2016) Infectious Dose of *Listeria monocytogenes* in Outbreak
670 Linked to Ice Cream, United States, 2015. *Emerg Infect Dis* **22**:2113–2119. doi:
671 10.3201/eid2212.160165

672 Quereda, J.J., Meza-Torres, J., Cossart, P., Pizarro-Cerdá, J. (2017) Listeriolysin S: A bacteriocin from
673 epidemic *Listeria monocytogenes* strains that targets the gut microbiota. *Gut Microbes* **8**:1–8. doi:
674 10.1080/19490976.2017.1290759

675 Rieu, A., Weidmann, S., Garmyn, D., *et al.* (2007) agr system of *Listeria monocytogenes* EGD-e: Role
676 in adherence and differential expression pattern. *Appl Environ Microbiol* **73**:6125–6133. doi:
677 10.1128/AEM.00608-07

678 Ryan, E.M., Gahan, C.G.M., Hill, C. (2008) A significant role for Sigma B in the detergent stress
679 response of *Listeria monocytogenes*. *Lett Appl Microbiol* **46**:148–154. doi: 10.1111/j.1472-

680 765X.2007.02280.x

681 Salazar, J.K., Sahu, S.N., Hildebrandt, I.M., *et al.* (2017) Growth Kinetics of *Listeria monocytogenes* in
682 Cut Produce. *J Food Prot* **80**:1328–1336

683 Salazar, J.K., Wu, Z., Yang, W., *et al.* (2013) Roles of a Novel Crp/Fnr Family Transcription Factor
684 Lmo0753 in Soil Survival, Biofilm Production and Surface Attachment to Fresh Produce of *Listeria*
685 *monocytogenes*. *PLoS One* **8**:. doi: 10.1371/journal.pone.0075736

686 Sauders, B.D., Sanchez, M.D., Rice, D.H., *et al.* (2009) Prevalence and molecular diversity of *Listeria*
687 *monocytogenes* in retail establishments. *J Food Prot* **72**:2337–49

688 Shabala, L., Lee, S.H., Cannesson, P., Ross, T. (2008) Acid and NaCl Limits to Growth of *Listeria*
689 *monocytogenes* and Influence of Sequence of Inimical Acid and NaCl Levels on Inactivation Kinetics. *J*
690 *Food Prot* **71**:1169–1177. doi: 10.4315/0362-028X-71.6.1169

691 Sheng, L., Edwards, K., Tsai, H.C., *et al.* (2017) Fate of *Listeria monocytogenes* on fresh apples under
692 different storage temperatures. *Front Microbiol* **8**:1–8. doi: 10.3389/fmicb.2017.01396

693 Standing, T.A., Du Plessis, E., Duvenage, S., Korsten, L. (2013) Internalisation potential of *Escherichia*
694 *coli* O157:H7, *Listeria monocytogenes*, *Salmonella enterica* subsp. *enterica* serovar Typhimurium and
695 *Staphylococcus aureus* in lettuce seedlings and mature plants. *J Water Health* **11**:210–223. doi:
696 10.2166/wh.2013.164

697 Stasiewicz, M.J., Oliver, H.F., Wiedmann, M., den Bakker, H.C. (2015) Whole-genome sequencing
698 allows for improved identification of persistent *Listeria monocytogenes* in food-associated
699 environments. *Appl Environ Microbiol* **81**:6024–6037. doi: 10.1128/AEM.01049-15

700 Tasara, T., Stephan, R. (2006) Cold stress tolerance of *Listeria monocytogenes*: A review of molecular
701 adaptive mechanisms and food safety implications. *J Food Prot* **69**:1473–84. doi: 10.4315/0362-
702 028X-69.6.1473

703 Van Der Veen, S., Abee, T. (2010) Importance of SigB for *Listeria monocytogenes* static and
704 continuous-flow biofilm formation and disinfectant resistance. *Appl Environ Microbiol* **76**:7854–
705 7860. doi: 10.1128/AEM.01519-10

706 Vivant, A.-L., Garmyn, D., Piveteau, P. (2013) *Listeria monocytogenes*, a down-to-earth pathogen.
707 *Front Cell Infect Microbiol* **3**:87. doi: 10.3389/fcimb.2013.00087

708 Vivant, A., Desneux, J., Pourcher, A., Piveteau, P. (2017) Transcriptomic Analysis of the Adaptation of
709 *Listeria monocytogenes* to Lagoon and Soil Matrices Associated with a Piggery Environment :
710 Comparison of Expression Profiles. *Front Microbiol* **8**:1–16. doi: 10.3389/fmicb.2017.01811

711 Vivant, A.L., Garmyn, D., Gal, L., et al. (2015) Survival of *Listeria monocytogenes* in soil requires AgrA-
712 mediated regulation. *Appl Environ Microbiol* **81**:5073–5084. doi: 10.1128/AEM.04134-14

713 Weis, J., Seeliger, H.P. (1975) Incidence of *Listeria monocytogenes* in nature. *Appl Microbiol* **30**:29–
714 32

715 Weller, D., Wiedmann, M., Strawn, L.K. (2015) Spatial and temporal factors associated with an
716 increased prevalence of *Listeria monocytogenes* in spinach fields in New York State. *Appl Environ*
717 *Microbiol* **81**:6059–6069. doi: 10.1128/AEM.01286-15

718 Welshimer, H.J. (1968) Isolation of *Listeria monocytogenes* from vegetation. *J Bacteriol* **95**:300–303

719 Wright, K.M., Chapman, S., McGeachy, K., et al. (2013) The endophytic lifestyle of *Escherichia coli*
720 O157:H7: quantification and internal localization in roots. *Phytopathology* **103**:333–40. doi:
721 10.1094/PHYTO-08-12-0209-FI

722 Wu, S., Wu, Q., Zhang, J., et al. (2016) Analysis of multilocus sequence typing and virulence
723 characterization of *Listeria monocytogenes* isolates from chinese retail ready-to-eat food. *Front*
724 *Microbiol* **7**:1–11. doi: 10.3389/fmicb.2016.00168

725 Zhu, Q., Gooneratne, R., Hussain, M. (2017) *Listeria monocytogenes* in Fresh Produce: Outbreaks,

726 Prevalence and Contamination Levels. *Foods* **6**:21. doi: 10.3390/foods6030021

727 Zoz, F., Grandvalet, C., Lang, E., *et al.* (2017) *Listeria monocytogenes* ability to survive desiccation:

728 Influence of serotype, origin, virulence, and genotype. *Int J Food Microbiol* **248**:82–89. doi:

729 10.1016/j.ijfoodmicro.2017.02.010

730

731 **Tables**

732 Table 1. Possible sources of *L. monocytogenes* on fresh leafy produce from the growing and

733 processing environments

Environment	Source	Reference
Farm	Soil splash	(Monaghan and Hutchison 2012)
	Contaminated irrigation water	(Heaton and Jones 2008; Hellström 2011; Allende and Monaghan 2015; Weller <i>et al.</i> 2015)
	Application of natural fertilisers	(Girardin <i>et al.</i> 2005; Oliveira <i>et al.</i> 2011)
	Wild animal faecal contamination	(Weis and Seeliger 1975; Fenlon 1985; Inoue <i>et al.</i> 1992; Hellström <i>et al.</i> 2008; Haase <i>et al.</i> 2014)
Processing Environment	Cross contamination from human carriers	(Buchanan <i>et al.</i> 2017)
	Cross-contamination from food surfaces	(Khan <i>et al.</i> 2016; Buchanan <i>et al.</i> 2017; Overney <i>et al.</i> 2017)
	Cross contamination from harbourage sites	(Leong <i>et al.</i> 2017)

734

735 **Figures**

736 Figure 1. Summary of the fresh produce supply chain