



Transition to the Viable but Non-Culturable (VBNC) state for strains grown on parsley

LISTERIA MONOCYTOGENES

THE ORGANISM/TOXIN

- Listeria spp. are Gram positive, facultatively anaerobic, motile, rod-shaped coccobacilli (1).
- There are 17 species in the genus *Listeria*. *Listeria monocytogenes* (*L. monocytogenes*) is the most important species with respect to human health (2).
- Thirteen serotypes of *L. monocytogenes* are recognised with serotypes 1/2a, 1/2b and 4b accounting for more than 95% of human listeriosis cases (3).
- *L. monocytogenes* causes two forms of disease: Invasive listeriosis (referred to as listeriosis) and noninvasive gastrointestinal listeriosis (referred to as febrile gastroenteritis) (4).
- Food is considered to be the main transmission route of *L. monocytogenes* to humans (5).
- The organism does not produce toxins in food (5).

GROWTH AND ITS CONTROL

Unless otherwise stated, the information below was derived from the following references $_{(6, 7, 8)}$.

Growth:

	Minimum	Optimum	Maximum
Temperature*	-1.5°C	30-37°C	45°C
Water activity	0.90	0.97	-
pH**	4.3	7.0	9.6
Atmosphere	 Grows optimally under micro-aerobic conditions, but grows well both aerobically and anaerobically. Can grow in low-to-moderate (5-30%) CO₂-containing atmospheres but is inhibited at 100% CO₂. 		

*Growth of *L. monocytogenes* occurs more slowly at lower temperatures.

**Growth at low pH values is influenced by other factors including temperature of incubation and acidulant.

Survival:

Temperature	 Can survive freezing, although survival and injury during frozen storage depend on the substrate and the rate of freezing. Survives and grows slowly at refrigeration temperatures.
Water activity	 Can survive for extended periods at lower aw values (as low as 0.83).
рН	 Can survive, but not multiply, at pH values below 4.3.
Atmosphere	 Can survive in food packaged under vacuum, increased CO₂ (up to 40%) and N₂ (up to 70%) gas levels.
Biofilm production	 Produces biofilms that help the bacterium survive for prolonged periods in the environment (9). <i>L. monocytogenes</i> biofilms are resistant to chlorine, iodine and anionic acids.

VBNC	leaves (10) or isolated from salmon (11). Live/dead staining indicated continued cell viability (11).
Inactivation:	
Temperature	 Inactivated by exposure to temperatures above 50°C. Inactivation proceeds faster at higher temperatures.
D values	• A literature review suggests the following time/temperature (°C/minutes) in "all meats": <i>D</i> ₅₅ 95.6; <i>D</i> ₆₀ 15.2; <i>D</i> ₇₀ 0.4 (12).
рН	 Rate of inactivation is influenced by acidulant and temperature. Organic acids (e.g acetic) are more effective at <i>L. monocytogenes</i> inactivation than mineral acids (e.g. hydrochloric).
Radiation	 Low dose gamma irradiation eliminates some, but not all, <i>L. monocytogenes</i> at a dose of up to and including 3 kGy. <i>L. monocytogenes</i> is more resistant to UV- light compared with other foodborne pathogens (13).
Preservatives and other non- thermal processing technologies	 High Hydrostatic Pressure (HHP) used for <i>L.</i> monocytogenes inactivation with variation on effective treatments (13, 14). Bacteriocin-producing starter cultures, direct addition of bacteriocins to foods, lysozyme, sodium propionate, potassium sorbate, nitrite, sodium ascorbate, antimicrobial rinses, acidified sodium chlorite, trisodium phosphate, cetylpyridinium chloride, potassium lactate, peroxyacetic acid essential oils, in combination with other treatments such as nisin and irradiation, can all reduce the concentration of <i>L. monocytogenes</i> on certain RTE, manufactured food products and poultry (7, 8, 15, 16, 17) Post-packaging pasteurisation (considered a listericidal process) and hot water carcass sprays used in the meat and poultry industries to remove <i>L. monocytogenes</i> from carcass surfaces. The commercially-available bacteriophage preparation (ListexTM P100) has been demonstrated to eliminate <i>L. monocytogenes</i> on food preparation surfaces and on certain foods including fresh fish and cooked meats (in conjunction with chemical antimicrobials) (18).
THE ILLNE	SS

There are two types of disease associated with *L. monocytogenes* infection: invasive and non-invasive listeriosis (1, 19).

Invasive listeriosis: Incubation:

• Range 1-67 days, median 8 days (21).

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 Values differ significantly according to the clinical form of the disease with a longer incubation period observed for pregnancy-associated cases (17-67 days), compared with central nervous system (1-14 days) and bacteraemia cases (1-12 days) (1, 20).

Symptoms:

- *Elderly and immunocompromised patients*: Flu-like symptoms (e.g fever, headache), diarrhoea, vomiting, meningitis, septicaemia, encephalitis.
- Pregnant women: Miscarriage and stillbirth, perinatal infection, neonatal infection (sepsis or meningitis) (1, 19).

Condition: Listeriosis.

At Risk Groups:

- Pregnant women and their foetuses, neonates, the elderly and those with compromised immune systems (21).
- Highest incidence of non-perinatal listeriosis reported for adults > 65 years (19, 22).
- Main risk factor is through the consumption of contaminated RTE foods containing levels markedly above 100 colony forming units *per* gram (CFU/g) (21, 22, 23, 24).

Long-term effects:

- Neurological problems (cranial nerve palsies). Preterm infants may suffer from excess fluid in the brain and partial paralysis (21).
- Mortality rate is high, ranging from 20% to 30% (1).

Dose:

- Infective dose estimated to be 10⁷ to 10⁹ CFU in healthy hosts and 10⁵ to 10⁷ CFU in individuals at high risk of infection (25).
- Presence of *L. monocytogenes* in food at a concentration of <100 CFU/g carries a very low probability of causing disease (23, 24).

Incidence:

- New Zealand incidence rate (invasive and noninvasive listeriosis) of 0.8 /100,000 (2016) saw a slight increase from the 2015 rate of 0.6/100,000). The notification rate has been relatively stable for the past 18 years at around 0.6/100,000 (22).
- New Zealand incidence rates are higher than the EU (0.46/100,000 (2015)) (28), the USA (0.24/100,000 (2015)) (2015)) (29) and Australia (0.3/100,000 (2014)) (30).

Treatment:

 For invasive disease, ampicillin alone, or in combination with an aminoglycoside such as gentamicin, is considered to be the drug of choice (1).

Non-invasive listeriosis:

Incubation:

Range 6-240 hours, median 24 hours (20, 26).

Symptoms:

- Diarrhoea, fever, muscle pain, headache, and less frequently abdominal cramps and vomiting (26).
- Symptoms typically last between 1-3 days but can last up to 1 week (26).

Condition: Febrile gastroenteritis.

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At Risk Groups:

- Anyone consuming a high enough concentration of *L. monocytogenes*.
- Pre-existing gastrointestinal problems, such as irritable bowel syndrome and inflammatory bowel disease, may be a risk factor (27).

Long-term effects:

- Typically none in healthy individuals.
- Hospitalisation following illness is rare and is typically confined to young, elderly or immunocompromised patients (26).
- Invasive disease can be a rare complication (26).

Dose:

- Consumption of more than 10⁶ CFU required to cause febrile gastroenteritis at a high attack rate (24, 26).
- Foods contaminated with lower numbers of *L. monocytogenes* may also infrequently cause febrile, non-invasive gastrointestinal disease.

Treatment:

• Febrile gastroenteritis is typically self-limiting however supportive therapy (maintenance of hydration and electrolyte balance) and antibiotics may be given if necessary (26).

SOURCES

Human:

- Infected persons (placenta, stools).
- Faecal carriage rate of *L. monocytogenes* in healthy people is estimated at 1-10% of the population (1).
- Human shedders are not thought to contribute significantly to contamination of foods. However, patients with listeriosis (invasive and non-invasive) shed *L. monocytogenes* in their faeces with high levels (≥ 10⁴/g) recorded in some cases of non-invasive gastroenteritis (7, 31, 32).

Animal:

- Wild and domesticated animals, including birds, are common symptomless carriers acting as reservoirs of infection for humans (8).
- Higher rates of isolation from farm animals are reported in winter months as a result of increased feeding of spoiled silage (which can contain concentrations of up to 10⁶ CFU/g) and housing of ruminants in barns (7).

Food:

- *L. monocytogenes* should be considered as potentially present in all raw foods and ingredients (1, 24, 33, 34).
- The pathogen may also be present in cooked foods as a result of post-cooking contamination. Some foods are more likely to be contaminated with *L. monocytogenes* and are recognised as high risk. Examples of such foods include all RTE cooked foods with long shelf lives, uncooked, smoked or RTE seafood, cold, pre-cooked chicken, pate, hummusbased spreads and dips, ham and other pre-cooked meat products, salami, pre-prepared salads and coleslaws, raw milk, soft-serve ice creams, soft, semisoft or surface-ripened cheese (1, 24, 33, 34).
- Concentrations from <10 CFU/g to >10⁷ CFU/g have Updated September 2018

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been reported in foods (4).

Environment/Water:

• Widespread in the environment. Found in many niches including soil, vegetation, water, food-processing facilities and sewage (8, 9).

Transmission Routes:

- Consumption of food contaminated during production and processing is the main route of transmission to humans (7).
- Other transmission routes include animal to human (occupational exposure), human to human (most commonly via an infected mother to the foetus), nosocomial and plant/soil to human (7).

OUTBREAKS AND INCIDENTS

Outbreaks - Invasive listeriosis:

Most cases are sporadic. Outbreaks feature prominently in the media because of the associated high case fatality rate. Notable outbreaks of foodborne invasive listeriosis, in recent years, are included in Tables 1 and 2 below.

Outbreaks - Non-Invasive listeriosis:

Outbreaks of febrile, non-invasive listeriosis are relatively rare. In these outbreaks, large concentrations of *L. monocytogenes* are typically reported as being present in the foods concerned. Notable outbreaks of foodborne non- invasive listeriosis, in recent years, are included in Tables 3 and 4 below.

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Table 1: New Zealand foodborne outbreaks of invasive listeriosis

Year	Cases (deaths)	Suspected foods	Control measure failure
2012	6 (3)	Ready-to-eat meat products	Product and environmental contamination (22, 35)

Table 2: Worldwide outbreaks of foodborne invasive listeriosis

Year	Cases (deaths)	Suspected foods	Country	Control measure failure
2009	36 (4)	Chicken wraps	Australia	Pre-packaged chicken wraps sold on flights of a domestic airline. Supplier's food safety programme had numerous failures (36).
2011	147 (33)	Cantaloupe melons	USA (multistate)	Supply of contaminated melons (37).
2012	22 (4)	Ricotta Salata Frescolina cheese	USA (multistate)	Contaminated imported Italian cheese (38).
2013- 2014	32 (4)	Ready-to-eat salad	Switzerland	Product and environmental contamination (39).
2014	35 (7)	Caramel apples	USA (multistate)	Environmental contamination at the apple-packing facility (40).
2015	10 (3)	Ice-cream	USA (multistate)	Contaminated ice-cream mix (41).

Table 3: New Zealand outbreaks of foodborne non-invasive listeriosis

Year	Cases	Suspected foods	Control measure failure
2000	31	Cooked ham	Contamination of meat during manufacture or by under- processing of product. Supermarket failed to remove recalled product from shelves expediently (31).

Table 4: Worldwide outbreaks of foodborne non-invasive listeriosis

Year	Cases	Suspected foods	Country	Control measure failure
2001	86	Washed rind cheese	Japan	Product and environmental contamination (42).
2001	16	Delicatessen meat	USA	Temperature abuse of product during storage (43).
2008	12	Cold meat cuts, including jellied pork	Austria	Product contamination (44).

LIST OF NZ RISK PROFILES ON *L. MONOCYTOGENES* IN FOODS

Matrix	Date	Link
Ice-cream (update)	2009	http://www.foodsafety.govt.nz/elibrary/industry/Risk_Profile_Listeria- Science_Research.pdf
Soft cheeses	2005	http://www.foodsafety.govt.nz/elibrary/industry/Risk_Profile_Listeria_Monocytogene s_Soft-Science_Research.pdf
Low-moisture cheeses	2005	http://www.foodsafety.govt.nz/elibrary/industry/Risk_Profile_Listeria_Monocytogene s-Science_Research.pdf
Ready-to-eat salads	2005	http://www.foodsafety.govt.nz/elibrary/industry/Risk_Profile_Listeria_Monocytogene s_Ready-Science_Research.pdf
Processed ready-to- eat meats	2009	http://www.foodsafety.govt.nz/elibrary/industry/Risk_Profile_Listeria_Monocytogene s_Processed-Science_Research.pdf
Raw milk	2014	http://www.mpi.govt.nz/dmsdocument/1122-risk-profile-listeria-monocytogenes-in- raw-milk
Cheese (update)	2015	http://www.foodsafety.govt.nz/elibrary/industry/listeria-monocytogenes-cheese.pdf

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