

Listeria monocytogenes

Family *Listeriaceae*Genus *Listeria*

Bacterium

Zoonotic agent¹

Characteristics and sources of *Listeria monocytogenes*

Main microbiological characteristics

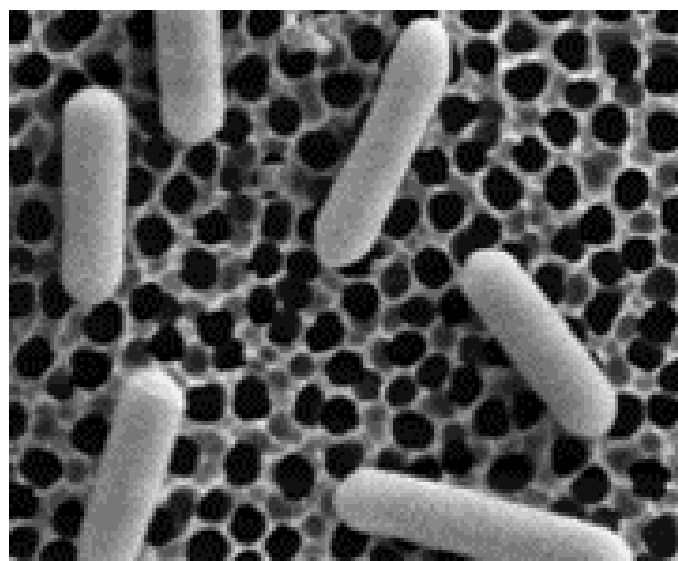
Listeria monocytogenes causes a disease called listeriosis that affects humans and animals. There are currently 21 species in the genus *Listeria*. Only two are pathogenic to humans and animals: *L. monocytogenes*, which is pathogenic to humans and animals, and *L. ivanovii*, which is pathogenic to animals but rarely to humans.

L. monocytogenes is a small (0.5-2 µm x 0.5 µm) Gram-positive bacillus, isolated or arranged in small chains, motile at 20-25°C, non-motile at 37°C and non-spore-forming. It is facultatively anaerobic and microaerophilic, catalase-positive except for a few rare strains, oxidase-negative and hydrolyses esculin. *Listeria* ferments many carbohydrates without producing gas. Strains of *L. monocytogenes* are always D-xylose-negative and produce lecithinase. They are generally β-haemolytic and L-rhamnose-positive. The species *monocytogenes* is divided into 13 serovars based on somatic and flagellar antigens. Since 2005, the French reference method has identified five serogroups determined by polymerase chain reaction (PCR): IIa (serovars 1/2a and 3a), IIb (serovars 1/2b and 3b), IIc (serovars 1/2c and 3c), IVb (serovars 4b, 4d and 4e) and L (other serovars). Of these, IVb followed by IIa and IIb are the serogroups most commonly associated with human cases. Reference molecular typing is achieved by pulsed field gel electrophoresis (PFGE) using the restriction enzymes *AscI* and *Apal* to obtain pulsotypes. This typing is replaced in France by a multilocus sequence typing (MLST) analysis of the core genome (cgMLST). Although studies report virulence progressing from hypovirulent to hypervirulent clones, the legislation currently considers all strains of *L. monocytogenes* to be pathogenic.

Table 1: Growth characteristics of *Listeria monocytogenes* under laboratory conditions (variable according to the strain)

Growth	Min.	Opt.	Max.
Temperature (°C)	-2	30 – 37	45
pH	4.0 – 4.3	7	9.6
a _w	0.92*	0.99	/
% NaCl inhibiting growth	/	/	12%

* 0.90 with glycerol



Listeria monocytogenes (SEM) © NRC-WHOCC *Listeria*, Institut Pasteur, Paris

L. monocytogenes is a psychrotrophic bacteria that can grow at refrigeration temperatures and has the ability to persist in food-processing plants and on equipment.

Hazard sources

L. monocytogenes is a ubiquitous soil bacterium that is widely distributed in the environment. Poorly produced silage (insufficient acidification) may contain *L. monocytogenes* in large quantities and can cause contamination in ruminants. In pig farms, feed in slop form is a risk factor for contamination. Environmental contamination is mainly due to the excreta of both healthy and diseased wild or livestock animals.

Transmission routes

Foodborne transmission is by far the most frequent route (99% of cases). Other routes of transmission (mucocutaneous) have also been observed in veterinarians and farmers during parturition of an infected animal or from abortions associated with animal listeriosis.

Recommendations for primary production

- Ensure the safety of silage by controlling acidification sufficiently and limiting contamination from soil.
- Strictly observe general hygiene rules with limitation of faecal contamination.
- Isolate sick animals, especially in dairy herds.
- Ensure hygiene during milking and chill milk rapidly.

¹ An agent responsible for disease or infection that can be transmitted from animals to humans, or from humans to animals.

Human foodborne illness

Nature of the disease (Table 2)

Listeriosis occurs in invasive (maternal-neonatal and non-maternal-neonatal) and non-invasive forms. Non-invasive forms are rarely detected: they are essentially febrile gastroenteritis, for which some outbreaks have been recorded.

A pregnant woman who is contaminated by food can transmit the bacteria to her foetus through placental transfer or during delivery as the foetus passes through the contaminated genital tract.

Susceptible population group²: the people most likely to develop a severe form of listeriosis are, in decreasing order of susceptibility: people with haematological cancers, people infected with HIV, organ transplant patients, people with kidney or liver failure, pregnant women, people with inflammatory diseases (Crohn's disease, rheumatoid arthritis, etc.) or non-haematological cancers, people over 65 years of age without other underlying conditions, diabetics (type 1 or 2) and people with heart disease.

Dose-effect³ and dose-response⁴ relationships

As the bacteria can contaminate different types of food, many people frequently ingest small amounts of *L. monocytogenes* without any symptoms appearing.

The relationship between the ingested dose and the probability of severe listeriosis (response) depends on the immune status of the host and the virulence of the strain.

According to the most recent models (2015), the probability of developing invasive listeriosis when an individual in the general population ingests a *L. monocytogenes* cell is 8.10^{-12} , and 3.10^{-9} for the most susceptible category of the population. The probability of listeriosis is 100 times higher with the most virulent strains.

Epidemiology

Listeriosis monitoring in France has been conducted by *Santé Publique France* through mandatory reporting since 1998, and by the National Reference Centre (NRC) for *Listeria*. Although rare, invasive listeriosis is a foodborne infection with extremely high mortality (20 to 30%) and hospitalisation rates (>97%), resulting in significant costs from treating patients. There are around 350 to 400 cases of listeriosis each year in France. These cases are mostly sporadic, but small outbreaks (2 to 20 cases) are also identified each year. Its incidence decreased until 2001, stabilised from 2001 to 2006, and has been steadily increasing since then, reaching 5.6 cases of listeriosis/million inhabitants in 2019. Since 2006, while the incidence of bacteraemic forms (the majority, around 50%), neuromeningeal forms (around 30%) and localised forms (< 10%) has been increasing, the incidence of maternal-neonatal forms (around 10%) has been falling.

Investigations of recent outbreaks using genomic tools have underlined the importance of combining continuous surveillance of human cases with surveillance of foodborne strains, in order to rapidly detect foods that were not known to be a source of human contamination and to cause outbreaks.

Table 2: Characteristics of human listeriosis

	Mean incubation period	Main symptoms	Duration of symptoms	Duration of the shedding period	Complications
Maternal-neonatal forms	17 to 67 d (median: 28 d)	- Flu-like symptoms (fever, chills, back pain) - Miscarriage - Death <i>in utero</i> , prematurity - Neonatal infection	Several days	Unknown	- 20% to 30% mortality rate among newborns
Non-maternal-neonatal forms	Bacteraemic forms: 1 to 12 d (median: 2 d) Neuromeningeal forms: 2 to 14 d (median: 9 d)	- Septicaemia / bacteraemia - Meningitis, meningoencephalitis, rhombencephalitis, brain abscess - Local infections	Several days	Unknown	- Neurological sequelae - Local infections - 20% to 30% mortality
Gastroenteric forms	6 to 4 d (median: 24 h)	- Fever - Nausea, vomiting, diarrhoea	One to several days	One to several days	- Bacteraemia: rare (2-10%) - Non-maternal-neonatal forms - Maternal-neonatal forms

² People with a higher than average probability of developing symptoms of the disease, or severe forms of the disease, after exposure to a foodborne hazard [definition used in ANSES data sheets].

³ Relationship between the dose (the number of microbial cells ingested during a meal) and the effect on an individual.

⁴ For a given effect, the relationship between dose and response, i.e. the probability of this effect occurring in the population.

Table 3: Epidemiological data on listeriosis in France between 2009 and 2019 (*Santé Publique France* data; updated in April 2020)

Year	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019
Number of cases	328	312	282	346	369	374	413	375	371	339	373
Maternal-neonatal forms (% of number of cases)	50 (15%)	43 (14%)	35 (12%)	38 (11%)	41 (11%)	49 (13%)	37 (9%)	33 (9%)	32 (9%)	29 (9%)	32 (9%)
Deaths (<i>Foetal-neonatal mortality*</i>)	14 (28%)	13 (30%)	9 (26%)	11 (29%)	6 (15%)	9 (18%)	10 (27%)	9 (27%)	6 (19%)	6 (21%)	11 (34%)
Non-maternal-neonatal forms (% of number of cases)	278 (85%)	269 (86%)	247 (88%)	308 (89%)	328 (89%)	324 (87%)	376 (91%)	342 (91%)	339 (91%)	310 (91%)	341 (91%)
Deaths (<i>Mortality</i>)	68 (24%)	53 (20%)	50 (20%)	60 (19%)	64 (20%)	51 (16%)	75 (20%)	53 (15%)	58 (17%)	41 (13%)	59 (17%)

* stillbirths and miscarriages

Role of food

Main foods to consider

Food contamination by *L. monocytogenes* can occur at all stages of the food chain (e.g. cooked food can become contaminated during handling after cooking). Most ready-to-eat foods can potentially be contaminated, but the level and frequency of contamination are variable and generally low. Only those foods in which *L. monocytogenes* can survive and/or grow are potential vectors of listeriosis when the storage (temperature/time) or preparation instructions described on their labels are not followed.

The foods incriminated in outbreaks in France belong to the following categories: cooked delicatessen meat products, soft cheeses, especially those made from raw milk, and ready-to-eat meals. Other foods that have been implicated in outbreaks in other countries include smoked fish, fresh produce (melons, sprouted seeds, ready-to-eat salads, etc.), and ice cream.

Inactivation treatments in industrial environments (Table 4)

Monitoring in food

L. monocytogenes is included in the microbiological safety criteria of Regulation (EC) No 2073/2005 as amended. Depending on the food's characteristics, the possible growth of *L. monocytogenes*, and the stage of the food production chain to which the criterion applies, the microbiological limits can be "absence in 25 g" or "less than 100 *L. monocytogenes* per gram". A guide published by the European Union Reference Laboratory (EURL) describes the different methods for classifying foods and identifying the corresponding microbiological criterion⁸.

The NRC receives and studies strains from food alerts and results of investigations into human cases. The National Reference Laboratory (NRL) receives and studies strains from the surveillance and control plans of the DGAL and DGCCRF. There are standardised reference methods for screening (NF EN ISO 11290-1⁹) and enumeration of *L. monocytogenes* (NF EN ISO 11290-2¹⁰) in products intended for human and/or animal consumption. Alongside these, there are validated alternative methods (AFNOR, NordVAL, MicroVal, AOAC) based on the use of chromogenic agars, immunological methods, PCR, and molecular hybridisation.

Table 4: Impact of treatments in industrial environments

Treatment	Conditions	Impact	Matrix
Temperature	D ⁵ and z ⁶ values: D _{65°C} = 0.2 to 2 min.; z = 7.5°C (4 to 11°C)		Skimmed milk
Disinfectants	Disinfectants authorised in the food industry	Sensitive provided that the recommended procedures for use are followed. Some clones have increased tolerance to quaternary ammoniums	
High pressures	500 to 600 MPa for 5 to 10 min at 20°C	3 to 5 log ₁₀ reductions	Meat products
	350 MPa for 5 to 10 min at 20°C	3 to 5 log ₁₀ reductions	Acidic products (e.g. fruit juices, jams)
Ionising radiation	D ₁₀ ⁷ (depending on T°C) = 0.56 (0.25 – 0.77) kGy		

⁵ D is the time required to obtain a tenfold reduction in the population of the microbiological hazard initially present.⁶ z is the temperature increase (°C) corresponding to a tenfold reduction in the decimal reduction time D.⁷ D₁₀ is the dose of ionising radiation (in kGy) required to obtain a tenfold reduction in the population of the microbiological hazard initially present.⁸ SANCO/11510/2013. GUIDANCE DOCUMENT on *Listeria monocytogenes* shelf-life studies for ready-to-eat foods, under Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs⁹ Horizontal method for the detection and enumeration of *L. monocytogenes*. Part 1: detection method (modification of the isolation media).¹⁰ Horizontal method for the detection and enumeration of *L. monocytogenes*. Part 2: enumeration method (modification of the isolation media, of the haemolysis test, and inclusion of precision data).

Domestic hygiene

Recommendations to operators

- Observe good hygiene practices, especially regarding control of the processing environment. Special attention should be paid to ensuring that food processing equipment can be cleaned and to the effectiveness of cleaning-disinfection and drying procedures.
- Implement a monitoring plan for any contamination of the processing environment.
- Respect the cold chain.
- Apply appropriate inactivation treatments.
- Determine the use-by date (UBD) of marketed products by durability tests, challenge tests, and/or the application of predictive microbiology, combined with the history of the company's results and the processing method.
- Particular attention should be paid to foods intended for certain susceptible population groups.

Recommendations to consumers

- For foods that must be kept cold, the refrigerator should be set to +4°C maximum. Whenever food has soiled surfaces, these should be cleaned immediately. Do not place unwrapped food directly on shelves.
 - Observe domestic hygiene rules: clean utensils and work surfaces before and after use, wash hands after handling raw products.
 - Wash vegetables and herbs thoroughly before eating or cooking.
- Store leftovers for no more than 3 days, and for foods to be consumed hot, heat them to an internal temperature of more than +70°C.
- Adhere to use-by dates (UBD) for packaged foods and consume non-prepacked foods as quickly as possible.
 - Pregnant women and other sensitive populations are advised to avoid foods such as certain cooked delicatessen meat products, soft cheeses with bloomy (such as camembert or brie) or washed rind (such as munster or pont l'évêque), especially if they are made from raw milk, cheeses sold grated, raw or undercooked meat, raw shellfish, raw fish (sushi, sashimi, taramasalata), smoked fish and shelled crustaceans sold cooked.

Links

General references

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Pouillot, R. et al. Infectious Dose of *Listeria monocytogenes* in Outbreak Linked to Ice Cream, United States, 2015—Volume 22, Number 12—December 2016—Emerging Infectious Disease journal—CDC.

Ryser E.T., Marth E.H., (eds). (2007). *Listeria*, listeriosis and food safety. Third edition, New-York NY: CRC Press

Useful links

National Reference Centre (NRC) and WHO Collaborating Centre (WHOCC) for *Listeria*: Institut Pasteur: <https://www.pasteur.fr/fr/sante-publique/centres-nationaux-reference/cnr/listeria>

Santé Publique France: <https://www.santepubliquefrance.fr/maladies-et-traumatismes/maladies-infectieuses-d-origine-alimentaire/listeriose>

European Union Reference Laboratory (<https://eurl-listeria.anses.fr/en/minisite/listeria-monocytogenes/eurl-listeria-monocytogenes>) and National Reference Laboratory (NRL) for *Listeria monocytogenes*: ANSES Laboratory for Food Safety.