



# *Listeria monocytogenes*

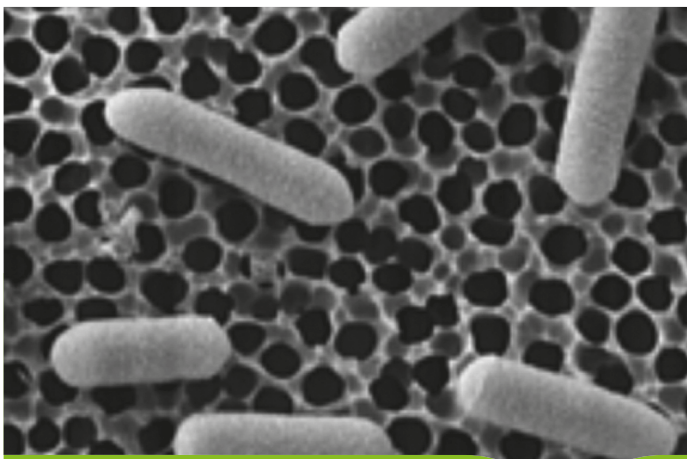
Family: *Listeriaceae*  
Genus: *Listeria*  
Bacterium

## Characteristics et sources of *Listeria monocytogenes*

### Main microbial characteristics

The genus *Listeria* contains eight species including *monocytogenes*, which is pathogenic for humans and animals, and the species *ivanovii*, which is pathogenic for animals but rarely for humans. *Listeria monocytogenes* causes a disease called listeriosis that affects humans and animals (zoonosis).

*Listeria* 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 and non-spore-forming. It is facultatively aerobic and anaerobic, 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, these serovars have been replaced for the French reference method by 5 serogroups determined by 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 frequently implicated in human cases. Reference molecular typing



*Listeria monocytogenes* (SEM)  
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is achieved by pulsed field gel electrophoresis (PFGE) using the restriction enzymes *Ascl* and *Apal* to obtain pulsotypes. Although studies mention the classification of virulence from avirulent strains to virulent epidemic strains, legislation currently considers all strains of *Listeria monocytogenes* as pathogenic.

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

**Table 1. Growth characteristics of *L. monocytogenes* (variable depending on the strains and the food matrix)**

| Parameters*      | Growth                       |       |      |
|------------------|------------------------------|-------|------|
|                  | Min.                         | Opt.  | Max. |
| Temperature (°C) | - 2                          | 30-37 | 45   |
| pH               | 4.0 - 4.3                    | ≈ 7   | 9.6  |
| a <sub>w</sub>   | 0.92 (0.90 avec du glycerol) | 0,99  | /    |

\* These parameters are interdependent and should not be considered separately.

### Hazard sources

*L. monocytogenes* is a ubiquitous soil bacterium, very widespread and resistant in the environment (soil, lakes, rivers, sewage or bay water, mainly decaying vegetation, etc.). Poorly produced silage (insufficient acidification) may contain *L. monocytogenes* in large quantities and can cause contamination in ruminants. Environmental contamination is mainly due to the excreta of both healthy and diseased animals: 6 to 30% of cattle, sheep, pigs, goats and chickens naturally harbour the bacteria in their digestive tract. These animals are the main source of contamination for humans.

### Transmission routes

Foodborne transmission is by far the most frequent route of transmission (99% of cases). Direct transmission is possible but rare. A pregnant woman can transmit the infection to her foetus in utero by transplacental passage of bacteria or during delivery when the foetus passes through the infected genital tract. Direct transmission has also been observed in veterinarians and farmers during parturition of an infected animal or from abortions associated with animal listeriosis. Nosocomial transmission, in gynaecology and obstetrics departments, or in nurseries, is rare.

### Recommendations for primary production

- Ensure the health quality of silage by controlling acidification and limiting contamination from soil.
- An essential prerequisite is strict compliance with general hygiene rules limiting faecal contamination in primary production of foodstuffs.
- Isolate sick animals, especially in dairy herds.
- Ensure hygiene during milking and cool milk rapidly.

## Human foodborne illness

### Nature of the disease

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

Listeriosis occurs in two forms: invasive (see Table 2) and non-invasive. Non-invasive forms are rare: they are essentially febrile gastroenteritis, for which some outbreaks have been recorded.

**Susceptible population groups<sup>(1)</sup>:** The people most likely to develop a severe form of listeriosis are pregnant women, people aged over 80 years or those with cancer or blood disorders, dialysis patients, insulin-dependent diabetics, organ transplant patients, people receiving chemotherapy, or corticosteroid or immunosuppressive therapy, people with liver disease (mainly cirrhosis) or an autoimmune disease, and people infected with HIV.

### Dose-effect<sup>(2)</sup> and dose-response<sup>(3)</sup> relationships

The dose-effect relationship is unknown. Regarding the relationship linking the probability of severe listeriosis to the dose ingested, this depends on the immune status of the host or the virulence of the strain. Currently, only the host's immune status is taken into account in published dose-response relationships. The exponential relationships proposed in 2004 by the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) predicted severe listeriosis in 1% of consumers after ingestion of a median dose of  $4 \cdot 10^{11}$  cells for the general population and  $10^{10}$  cells for susceptible population groups. Studies are underway to add to the knowledge on dose-effect and dose-response relationships, using data from recent outbreaks and animal models.

## Epidemiology

Listeriosis monitoring in France is conducted by the French Institute for Public Health Surveillance (InVS) through mandatory reporting since 1998, and by the National Reference Centre (NRC) for *Listeria*. Although rare, listeriosis is a foodborne infection with extremely high lethality (25 to 30%) and hospitalisation rates (>92%), resulting in significant costs from treating patients. There are about 300 cases of listeriosis in France every year, which have more recently all been sporadic cases, as no outbreak has been identified in France since 2003. Its incidence increased in 2006, for no identified reason, then stabilised in 2008. In 2010 there were 4.9 cases of listeriosis/million inhabitants and 5.2 cases per 100,000 births. Since 2006, while bacteriemic forms have been in the majority (about 53%) and are increasing, as are neuromeningeal forms (25%), the number of cases of maternal-neonatal (15%) and localised forms (about 7%) has been stable. According to the literature, about 70 outbreaks have been identified worldwide to date, including seven in France, where the implicated foods were pork tongue in jelly (1992 and 2000), rillettes (1993 and 2000), brie (1995), pont l'évêque (1997), époisses (1999), processed spreadable cheese (2002) and mortadella (2003). The 2011 outbreak in the United States linked to contaminated cantaloupe melons highlights the importance of continued surveillance of human cases to find the foods responsible and vice versa, to rapidly detect any foods not previously listed as a source of human contamination or responsible for outbreaks.

## Role of foods

### 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 grow are potential causes of listeriosis, when the storage (temperature/time) or preparation instructions described on their labels are not followed.

(1) Susceptible population group: 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].

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

(3) For a given effect, the relationship between the dose and the response, i.e., the probability of this effect appearing in the population.

Table 2. Disease characteristics

| Mean incubation period  | Target population                             | Main symptoms  | Duration of symptoms | Duration of the shedding period | Complications  |
|---|---|--|----------------------|---------------------------------|--|
| All forms combined:<br>2 to 88 days, median 17 days<br>Maternal-neonatal forms:<br>14 to 88 days, median: 28 days<br>Neuromeningeal forms:<br>2 to 19 days, median: 10 days | Entire population,<br>all age groups combined | Septicaemia/bacteraemia<br>Meningitis, meningococcal meningitis,<br>rhombencephalitis, brain abscess<br>Local infections             | Several days         | Unknown                         | Neurological sequelae<br>Lethality rate of 20 to 30% depending<br>on the study<br>Local infections |
|   | Pregnant women                                | Flu-like symptoms<br>(fever, chills, back pain)<br>Spontaneous abortion<br>Death <i>in utero</i> , prematurity<br>Neonatal infection | Several days         | Unknown                         | 20% lethality rate among newborns  |

# Inactivation treatments in industrial environments

**Table 3. Inactivation treatments for *L. monocytogenes* in foods**

| Disinfectants  | Effects of temperature  |                   |              |   |
|--|---|-------------------|--------------|---|
| Sensitive to all disinfectants* authorised in the food-processing sector, on condition that the recommended procedures for use are followed. | Values of D** and z***  |                   |              |   |
|  | <table border="1"> <tr> <td>D<sub>65°C</sub></td> <td>0.2 to 2 min</td> </tr> <tr> <td>z</td> <td>7.5°C (4 to 11°C)</td> </tr> </table>   | D <sub>65°C</sub> | 0.2 to 2 min | z |
| D <sub>65°C</sub>  | 0.2 to 2 min  |                   |              |   |
| z  | 7.5°C (4 to 11°C)   |                   |              |   |
| Ionisation   | High pressure   |                   |              |   |
| D <sub>10</sub> - value**** = 0.56 kGy (0.25 – 0.77)   | Resistance depends on the strain and the medium.<br>400 MPa for 10 min at 20°C<br>→ 2 log <sub>10</sub> reductions in phosphate buffer (pH 7).<br>400 MPa for 10 min at 20°C<br>→ 8 log <sub>10</sub> reductions in citrate buffer (pH 5.6).<br>400 to 500 MPa for 5 to 10 min at 20°C<br>→ 3 to 5 log <sub>10</sub> reductions in meat products.<br>350 MPa for 5 to 10 min at 20°C<br>→ 3 to 5 log <sub>10</sub> reductions in acidic products (e.g. fruit juices, jams). |                   |              |   |

\* Some strains are resistant to quaternary ammonium compounds.

\*\* D is the time needed to divide by 10 the initial population of a microbiological hazard.

\*\*\* z is the change in temperature (°C) that corresponds to a reduction by a factor of 10 in the decimal reduction time.

\*\*\*\* D<sub>10</sub> is the dose (in kGy) needed to reduce a population to 10% of its initial strength.

*L. monocytogenes* is rapidly destroyed above pH 10. The minimum pH for survival depends on the mineral and/or organic acids used. At an equivalent pH, acetic acid is more inhibitory than lactic acid, which is more inhibitory than citric acid.

## Monitoring in foods

*L. monocytogenes* is included in the 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 chain to which the criterion applies, the microbiological safety criteria can be "absence in 25 g" or "less than or equal to 100 cfu/g".

Food monitoring is carried out by the National Reference Laboratory (NRL), which receives and studies strains from the monitoring and control plans of the DGAL and the DGCCRF, as well as the National Reference Centre (NRC), which receives and studies strains from food alerts and investigations into human cases. Food and human strains are compared each week by the NRC, which reports the results of this comparison to a group of experts that include risk managers, analytical laboratory specialists and epidemiologists, to enable them to undertake any necessary investigations of food processing operators in order to prevent outbreaks spreading.

For animal feed, operators must comply with Regulation (EC) No. 183/2005, which contains no criteria for *L. monocytogenes*.

There are standardised reference methods for screening (NF EN ISO 11290-1<sup>(4)</sup>) and enumeration of *L. monocytogenes* (NF EN ISO 11290-2<sup>(5)</sup>) in products intended for human and/or animal consumption. Alongside these reference methods, there are also validated alternative methods (AFNOR, NordVal, MicroVal, AOAC): immunological methods, PCRs and molecular hybridisation.

## Recommendations to operators

- Observe good hygiene practices, especially regarding management of the production environment in sensitive sectors. Special attention should be paid to ensuring that food-processing premises and equipment can be thoroughly cleaned and dried.
- Implement a monitoring plan for any contamination of the production environment in sensitive sectors.
- Maintain the cold chain.
- Determine the use-by date (UBD) of marketed products by durability tests (according to the NF V01-003<sup>(6)</sup> standard), challenge tests (according to the NF V01-009<sup>(7)</sup> standard) and/or the application of predictive microbiology, combined with the history of the company's results and the processing method<sup>(8)</sup>.

## Domestic hygiene

### Recommendations for consumers

- For foods that must be kept cold, the refrigerator should be set to +4°C maximum. Whenever food has soiled surfaces, they should be cleaned immediately. Do not place unwrapped food directly on the shelves.
- Observe domestic hygiene: 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 to an internal temperature of more than 70°C.
- Adhere to use-by dates (UBD) for packaged foods and consume foods cut to order as quickly as possible.
- Pregnant women and those most at risk are advised to avoid the foods most commonly contaminated with *L. monocytogenes*, such as raw milk cheeses (especially soft cheese), cheese sold grated, the rind of cheeses, smoked fish, raw shellfish, taramasalata, raw sprouted seeds and cooked delicatessen products.

(4) Horizontal method for the detection and enumeration of *L. monocytogenes*. Part 1: detection method (+ amendment of 1 February 2005: modification of the isolation media).

(5) Horizontal method for the detection and enumeration of *L. monocytogenes*. Part 2: enumeration method (+ amendment of 1 February 2005: modification of the isolation media, of the haemolysis test, and inclusion of precision data).

(6) Food safety - Guidelines for implementing microbiological durability tests – Chilled perishable and highly perishable foodstuffs (June 2010).

(7) Hygiene and safety of foodstuffs – Guidelines for the implementation of microbiological challenge tests.

(8) SANCO/1628/2008. 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 foodstuffs.

# References and links

## General references

- AFSSA (2005). Avis n°2003-SA-0362 sur la révision de l'avis 2000-SA-0094 sur la classification des aliments au regard du risque représenté par *Listeria monocytogenes* et les protocoles de tests de croissance. [Opinion No. 2003-SA-0362 on the revision of Opinion 2000-SA-0094 on the classification of foods with regard to the risk represented by *Listeria monocytogenes* and challenge test protocols].  
<http://www.anses.fr/Documents/MIC2003sa0362.pdf>
- AFSSA (2009). Avis n°2008-SA-0174 sur l'augmentation des cas de listériose et le lien éventuel avec l'évolution des modes de production, de préparation et de consommation des aliments [Opinion No. 2008-SA-0174 on the increase in listeriosis cases and the possible link with changing methods of food production, preparation and consumption].  
<http://www.afssa.fr/Documents/MIC-Ra-ListerioseAliments.pdf>
- Goulet V., Leclercq A., Vaillant V., Le Monnier A., Laurent E., Thierry-Bled F., Pihier N. and De Valk H. (2008). Recrudescence récente des cas de listériose en France [Recent increase of listeriosis in France]. BEH 30-31: 268-272.  
[http://www.invs.sante.fr/beh/2008/30\\_31/beh\\_30\\_31\\_2008.pdf](http://www.invs.sante.fr/beh/2008/30_31/beh_30_31_2008.pdf)
- World Health Organization (2004). Risk Assessment of *Listeria monocytogenes* in ready-to-eat Foods: Interpretative summary.  
[ftp://ftp.fao.org/esn/jemra/mra4\\_en.pdf](ftp://ftp.fao.org/esn/jemra/mra4_en.pdf)
- World Organisation for Animal Health (2008). *Listeria monocytogenes* in Terrestrial Manual, Chapter 2.9.7.  
[http://www.oie.int/fileadmin/Home/eng/Health\\_standards/tahm/2.09.07\\_LISTERIA\\_MONO.pdf](http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/2.09.07_LISTERIA_MONO.pdf)
- Ryser E.T., Marth E.H., (eds). (1999). *Listeria*, listeriosis and food safety. 2<sup>nd</sup> edition, New York, NY: Marcel Dekker.

## Useful links

- WHO Collaborating Centre (WHOCC) and National Reference Centre (NRC) for *Listeria*: Microbes and Host Barriers Group, Paris - Institut Pasteur: <http://www.pasteur.fr/cnr/listeria>
- French Institute for Public Health Surveillance (InVS):  
<http://www.invs.sante.fr/surveillance/listeriose/index.htm>
- European Union Reference Laboratory (<http://www.ansespro.fr/eurl-listeria/>) and National Reference Laboratory (NRL) for *Listeria monocytogenes*: Maisons-Alfort Laboratory for Food Safety – ANSES
- World Health Organization (WHO): <http://www.who.int/foodsafety/en/>