Rev. sci. tech. Off. int. Epiz., 2006, **25** (2), 571-580

Listeria monocytogenes: food-borne pathogen and hygiene indicator

T. Jemmi [1] & R. Stephan [2]

(1) Swiss Federal Veterinary Office, Schwarzenburgstrasse 161, CH-3003 Bern, Switzerland
(2) Institute for Food Safety and Hygiene, Vetsuisse Faculty University of Zurich, CH-8057 Zurich, Switzerland

Summary

In the past 25 years, *Listeria monocytogenes* has become increasingly important as a food-associated pathogen. Most European Union countries have an annual incidence of human listeriosis of between two and ten reported cases per million. Because of its high case fatality rate, listeriosis ranks among the most frequent causes of death due to food-borne illness. *Listeria monocytogenes* infections are responsible for the highest hospitalisation rates (91%) amongst known food-borne pathogens and have been linked to sporadic episodes and large outbreaks of human illness worldwide.

The ability to persist in food-processing environments and multiply under refrigeration temperatures makes *L. monocytogenes* a significant threat to public health. *Listeria monocytogenes* contamination is one of the leading microbiological causes of food recalls, mainly of meat, poultry, seafood and dairy products.

Prevention and control measures are based on hazard analysis and critical control point programmes throughout the food industry, and on specific recommendations for high-risk groups.

Understanding how these micro-organisms adapt their cellular physiology to overcome stress is important in controlling *L. monocytogenes* in food environments.

Keywords

Ecology – Food safety – Food-borne disease – Listeria monocytogenes – Occurrence – Virulence.

Introduction

The genus *Listeria* is grouped with other Gram-positive non-spore forming bacilli. Members of the genus *Listeria* are generally aerobes or facultative anaerobes, catalase positive and oxidase negative. *Listeria* are motile via a few peritrichous flagella when grown at temperatures below 30°C. The genus includes six species:

– *L. monocytogenes*
– *L. innocua*
– *L. ivanovii*
– *L. seeligeri*
– *L. welshimeri*
– *L. grayi.*

Differentiation of the *Listeria* species can be made based on haemolytic activity and sugar fermentation (Table I).

The genomes of both *L. monocytogenes* and *L. innocua* have been sequenced. The genome of *L. monocytogenes* strain EGD (serotype 1/2a) is 2,944,528 base pairs (bp) long with 2,853 open reading frames and a guanine-cytosine (G + C) content of 39%. The genome of *L. innocua* is 3,011,209 bp long with 2,973 open reading frames and a G + C content of 37%. Surprisingly, many encoded proteins are similar to those of the soil bacterium *Bacillus subtilis*. *Listeria monocytogenes* has a single circular chromosome, while *L. innocua* also contains a plasmid of 81,905 bp.

Listeria monocytogenes is a food-borne pathogen that is distributed in a wide variety of environments. Human

Table I
Biochemical differentiation of *Listeria* species (25)

Listeria spp.	Haemolysis	CAMP	Acid production from:		
			D-Xylose	L-Rhamnose	Mannitol
L. monocytogenes	+	+	–	+	–
L. seeligeri	+	+/–	+	–	–
L. ivanovii	++	–	+	–	–
L. innocua	–	–	–	V	–
L. welshimeri	–	–	+	V	–
L. grayi	–	–	–	V	+

+: positive
+/–: weakly positive
–: negative
CAMP: is an acronym for Christie, Atkins, Munch, Petersen; the discoverers of this haemolysis phenomenon
V: variable

infection may lead to a serious and potentially life-threatening illness known as listeriosis (36). Reports from the United States of America (USA) show that *L. monocytogenes* infections are responsible for the highest hospitalisation rates (91%) amongst known food-borne pathogens (31). *Listeria monocytogenes* infections have been linked to both sporadic episodes and large outbreaks of human illness in various parts of the world (10). The general severity of the human clinical disease, coupled with the high case fatality rate associated with *L. monocytogenes* infections (10, 31), emphasise the critical importance of effective control measures against this food pathogen. However, the organism's ubiquity in food processing, distribution and storage environments, as well as its efficient stress adaptation capabilities make the control of this microbe in food a great challenge.

From the viewpoint of food safety, understanding how *Listeria* organisms are able to adapt their cellular physiology to overcome various forms of stress as well as current control measures is an important step in developing better methods of controlling *L. monocytogenes* in food-producing environments. Cold stress adaptation is one of the fundamental attributes of *L. monocytogenes* that is essential for its dissemination. The organism's robust cold adaptation capacity renders the use of low temperatures and refrigeration ineffective as control measures.

Ecology

All members of the genus *Listeria* are widely distributed in nature and have been isolated from soil, vegetation, sewage, water, animal feed, fresh and frozen meat, slaughterhouse wastes and the faeces of healthy animals. Thus, farm animals and their environment may present an important source of food contamination and infections for humans.

There have been suggestions that *L. monocytogenes* subtypes and lineages differ in their association with specific host and non-host environments. Epidemiological data from different countries show that the majority of human outbreaks are associated with three *L. monocytogenes* serotypes (1/2a, 1/2b and 4b), despite the fact that there are 13 serotypes potentially capable of infecting humans (59). This may reflect the greater adaptation of certain *L. monocytogenes* subtypes to food-associated environments and human infection.

Due to their ubiquitous presence, *Listeria* in general and *L. monocytogenes* in particular are also used as hygiene indicators in all stages of the food processing chain. Single *Listeria* strains can spread in manufacturing plants and even establish themselves as endemic organisms (45).

Pathogenesis and virulence factors

Listeria monocytogenes is pathogenic for animals and human beings without showing any significant host specificity. Infection occurs in several steps:

a) entry of the bacterium into the host

b) lysis of the phagosomal vacuole

c) multiplication in the cytosol

d) direct cell-to-cell spread using actin-based motility.

Each step requires expression of specific virulence factors. The major virulence genes are located in a cluster of genes on two different DNA loci and are mainly influenced by the positive regulatory factor A protein.

Several groups of virulence factors which are thought to be important in the pathogenicity of *Listeria monocytogenes* strains have recently been characterised:

a) the internalines, encoded by different internaline genes *(inl)*, which take part in the invasion of epithelial cells and seem to be jointly responsible for the tissue tropism of *L. monocytogenes* (6, 42)

b) listeriolysin O, encoded by the gene *hlyA*, and phosphatidylinositol-specific phospholipase C (PI-PLC), encoded by the gene *plcA*, which take part in lysis of the phagosomes of the host cell and thus make the intracellular growth of *Listeria* cells possible (29, 46)

c) act A-protein, which is involved in motility (4)

d) enzymes such as lecithinase, zinc metal protease and serine protease (15, 37, 54)

e) a fibronectin-binding protein, FbpA, has been recently described as a novel multifunctional *L. monocytogenes*

virulence factor which seems to be involved in intestinal and liver colonisation processes (7).

Listeriosis in animals

Many animal species can be infected with *L. monocytogenes*. Nevertheless, clinical disease is rare and mainly found in ruminants, in which it presents as meningoencephalitis, septicaemia, and abortions. Feeding of grass silage with high pH, which can be contaminated with large amounts of *Listeria*, is normally incriminated. Furthermore, *Listeria* spp. are a rare cause of mastitis in cattle and sheep (41, 48, 56). In these cases, contamination of milk can be due to direct shedding of *Listeria*.

Listeria spp. are shed in the faeces of asymptomatic animal carriers. Therefore, contamination of milk and meat is normally due to faecal contamination during the milking or slaughtering process.

Listeriosis in humans

Human infections primarily result from eating contaminated food and may lead to serious and potentially life-threatening listeriosis (36). Pregnant women, neonates, and elderly or immunocompromised adults are particularly susceptible to listeriosis, which typically presents as septicaemia, meningitis, or meningoencephalitis (36). In pregnant women, *Listeria monocytogenes* takes advantage of the natural localised immunosuppression at the maternal-fetal interface and causes abortions. A milder form of listeriosis that presents as febrile gastroenteritis was recognised in the 1990s (39). This disease state is induced when otherwise healthy hosts consume large numbers of *L. monocytogenes* organisms (14).

A minimal infective dose has not been determined in human infection studies and estimates vary from 10^2 colony-forming units (cfu) to 10^9 cfu, depending on the immunological status of the host. The incubation period for the disease varies from 11 to 70 days (median 21 days) in humans.

Most countries within the European Union have an annual incidence of listeriosis of between two and ten reported cases per million per year. Because of its high case fatality rate, listeriosis ranks among the most frequent causes of death due to food-borne illness. Reports from the USA show that *L. monocytogenes* infections are responsible for the highest hospitalisation rates (91%) amongst known food-borne pathogens (31). *Listeria monocytogenes* infections have been linked to both sporadic episodes and large outbreaks (Table II) of human illness in various parts of the world.

Table II
Examples of outbreaks of human food-borne listeriosis (28)

Country	Year	Food	Cases	Deaths	Serotype
United States of America	1976	Raw salad (?)	20	5	4b
New Zealand	1980	Fish (?)	22	7	1/2a
Canada	1981	Coleslaw	41	18	4b
United States of America	1983	Milk (?)	49	14	4b
United States of America	1985	Soft cheese	142	30	4b
Switzerland	1983-1987	Soft cheese	122	34	4b
United Kingdom	1987-1989	Pâté	355	94	4b
France	1993	Pork tongue in aspic	279	NK	4b
France	1993	Pork rillettes	38	10	4b
United States of America	1994	Milk	45	0	1/2b
Sweden	1994-1995	Fish	9	2	4b
France	1995	Soft cheese	17	4	4b
Canada	1996	Crab meat	2	0	1/2a
Italy	1997	Salad	1566	0	4b
United States of America	1998-1999	Hot dogs	50	>8	4b
Finland	1998-1999	Butter	25	6	3a
Finland	1999	Fish	5	NK	1/2a
France	1999-2000	Pork rillettes	10	2	4b
France	1999-2000	Pork tongue in jelly	32	10	4b
United States of America	2000	Turkey meat	29	7	NK
Switzerland	2005	Soft cheese	3	1	NK

NK: not known

Occurrence in foods, source and mode of transmission

Products such as raw milk, soft cheese produced from raw milk, raw meat products and salads are frequently implicated in the literature (23, 43, 44). Summarised data for the prevalence of *L. monocytogenes* in raw meat and raw milk are given in Tables III and IV. In this context, hygiene weak points during the slaughtering and milking processes are the main critical points for *Listeria* contamination.

Cross-contamination, which can occur within the environment of food-processing equipment, is considered to be a possible source of *Listeria* contamination in processed food. *Listeria monocytogenes* is able to attach to and survive on various working contact surfaces. One reason may be its ability to form biofilms (3, 60).

Table III
Prevalence of *Listeria monocytogenes* in raw meat

Country	Meat	Number of samples tested	Prevalence (%)	References
Italy	NK	113	8	27
Belgium	Poultry	772	38	52
United Kingdom	Poultry	100	60	35
Denmark	Minced meat	67	28	47
France	NK	112	17	40
Japan	Minced beef meat	41	12	19
	Minced pork meat	34	21	19
Switzerland	Minced meat	400	11	8

NK: species not known

Table IV
Prevalence of *Listeria monocytogenes* in raw milk

Country	Number	Prevalence (%)	References
Italy	40	0	30
Switzerland	310	0	49
Spain	95	45	5
Sweden	294	1	55
United States of America	861	7	53
	131	5	21
Canada	445	12	9
Brazil	440	13	32

Growth, survival and stress resistance

Generally, *Listeria* spp. strains grow between 1°C and 45°C under aerobic and facultative anaerobic conditions. Their optimal growth temperature is between 30°C and 37°C. *Listeria* spp. have the unusual ability to grow at refrigeration temperatures (57, 58). Minimal growth temperatures were determined for 100 strains of *Listeria* (24). The mean minimum temperature for *L. monocytogenes* growth was 1.7°C. No differences in growth temperature were observed among strains isolated from different sources.

Studies of growth models for *L. monocytogenes* have been published in recent years (e.g. 13, 26). These models include the effect of temperature, aqueous phase salt/water activity, pH, and other intrinsic or extrinsic factors on the growth of *L. monocytogenes*.

The thermotolerance of the organism has been examined in different studies in broth cultures and various food matrices. An overview of results obtained from broth cultures is given in Table V. The reported D-value (the decimal reduction time: the time interval required for one decimal reduction [90%] in the number of organisms surviving) at 64°C for *L. monocytogenes* is 2.1 min, with a z-value (the temperature difference required to change the D-value by a factor of 10) of 7.5°C. A more comprehensive overview for different food matrices is given in *Microorganisms in Foods 5: Characteristics of Microbial Pathogens* published by the International Commission on Microbiological Specifications for Foods (20).

Table V
Heat inactivation of *Listeria monocytogenes*

Temperature (°C)	Time for 6D reduction (min)
63	17
64	12.7
65	9.3
66	6.8
67	5.0
68	3.7
69	2.7
70	2.0
71	1.5
72	1.0
73	0.8
74	0.6
75	0.4

Source: Food and Drug Administration, Hazards and Controls Guidance (2001) (12)

Current transmission models assume that consumption of contaminated food products is the main route of human infection (38), and therefore that the epidemiological trends observed may reflect better adaptation of certain *L. monocytogenes* subtypes to food environments and subsequent human infection (17, 33). *Listeria monocytogenes* is endowed with numerous adaptive physiological traits that enable it to survive under a wide range of environmental conditions. It can overcome various types of stress, including the cold stress associated with the low temperatures of food production environments. The organism's robust cold adaptation capacities render the current use of low temperatures and refrigeration, which control most food-borne pathogens in food environments, ineffective.

The cold tolerance phenomenon in these microorganisms is a function of multiple genetic and physiological factors that sense the cold stress threat and efficiently induce appropriate cellular responses. The exact nature of molecular cold adaptation in *L. monocytogenes*, as in most psychotropic microbes, remains elusive, but certain molecular and physiological aspects of this phenomenon have been illuminated in model microorganisms (reviewed in 62). In the case of *L. monocytogenes*, research over the past few years has revealed various aspects of cold stress adaptation mechanisms in these organisms (for a review see 50). An improved understanding of how cold stress is sensed and adaptation measures implemented by *L. monocytogenes* may facilitate the development of better ways of controlling these pathogens in food and related environments.

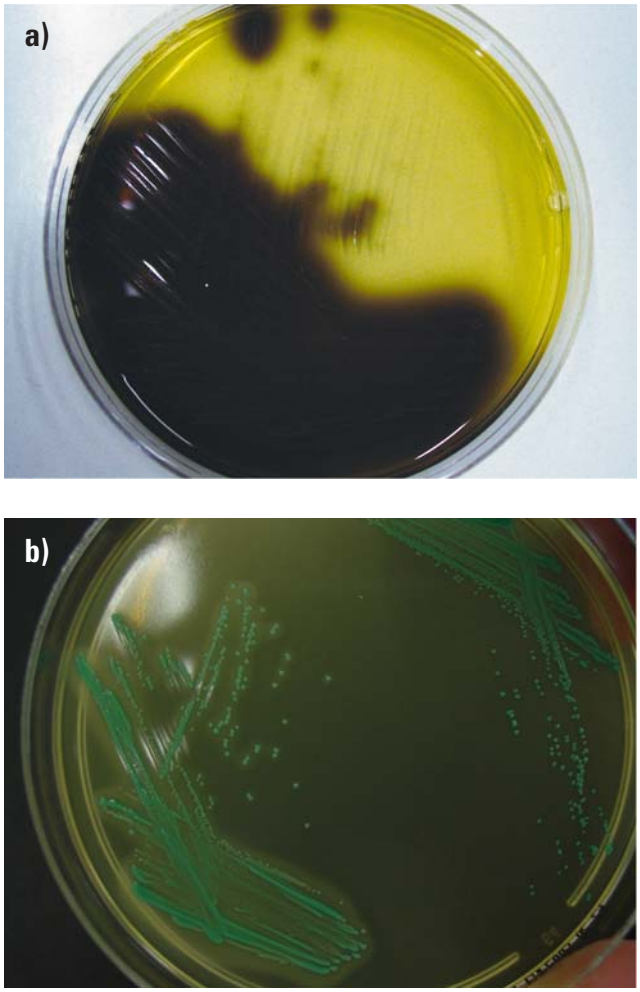

Fig. 1
Listeria monocytogenes
a) on Oxford medium
b) on Ottaviani Agosti medium

Diagnostics

A wide variety of methods are available for the detection of *L. monocytogenes*, either in animal feed, in human food, or in clinical specimens. Classical bacteriological techniques are still considered to be the 'gold standard'. In food, detection of *L. monocytogenes* is generally performed in a two-step cultural enrichment process and it takes on average one week for biochemical identification of a suspected *L. monocytogenes* colony. The bacteriological culture methods commonly used for detection and identification of *L. monocytogenes* include esculin and ferric iron in enrichment or plating media, which results, through the hydrolysation capacity of *Listeria*, in the formation of an intense black colour (Fig. 1a). More recently, chromogenic media have been developed that take advantage of the PI-PLC activity that is present in *L. monocytogenes* and *L. ivanovii*, but not the other *Listeria* spp. (Fig. 1b).

A variety of combinations of enrichment and plating media have been evaluated for the isolation of *L. monocytogenes*. Even if no single method is ideal for all types of food, regulatory agencies provide guidance through the publication of standardised methods for the isolation of *L. monocytogenes* (e.g. the Food and Drug Administration [FDA] method, the United States Department of Agriculture [USDA] method, and the International Organization for Standardization/Association Française de Normalisation [AFNOR] method).

Food-processing companies increasingly depend on rapid quality control tests, which deliver results within a shorter time and allow batches to be released following completion of the test. Advances in research have led to the development of more rapid *L. monocytogenes* detection assays that utilise immunological and nucleic acid-based techniques (61).

The use of serological tests for diagnostic purposes is largely considered to be unreliable (61). Nevertheless, serology can be used for epidemiological studies in defined populations (2).

A range of subtyping methods is available to distinguish between strains of *L. monocytogenes*. These methods include the more traditional techniques of serotyping, phage typing and isoenzyme analysis, as well as those based on the characterisation of DNA, such as pulsed field gel electrophoresis, ribotyping, and polymerase chain reaction-based methods. However, the differences in the potential of the various strains to cause disease are still, as yet, poorly understood.

Impact of *Listeria monocytogenes* on the food industry

A comprehensive assessment of the impact of *L. monocytogenes* on the food industry is not available from the literature. However, an initial approach was made in the Food and Agriculture Organization (FAO) expert consultation report on the trade impact of *Listeria* in fish products (11).

The impact on the food industry is partly due to recalls, which result in large economic losses. As an example, the FDA in the USA has taken firm action against many processors due to the presence of *L. monocytogenes* in their products. Since 1985, Class I recalls (i.e. those that could cause serious health problems or death) have been imposed on many ready-to-eat food products contaminated with *L. monocytogenes*, including cheeses, ice-cream, milk, fish, prepared salads, sandwiches, crab meat, smoked fish, and bakery products. From 1987 to 1992, there were recalls on 970 ready-to-eat products from 109 firms because of contamination with this organism. Between 1987 and August 1998 there were 112 Class I recalls for domestic or imported ready-to-eat seafood products (11). Examples of significant recent recalls are the *Listeria*-related recall of 4.2 million pounds (approximately 1.9 million kg) of egg salad on Vienna bread sandwiches (51), and a recall of a total of 2.8 million pounds (approximately 1.3 million kg) of various sausage, ham and turkey lunch products due to possible contamination with *L. monocytogenes* (1).

Furthermore, *L. monocytogenes* contamination may affect the food industry through rejection or detention of products. In addition to the direct costs of rejection and detention, there are economic costs that result from inspection/re-inspection, delays in distribution, transportation, expiry of shelf-life, and the opportunity cost of holding products.

Risk reduction strategies

Until recently, most available data derived from the results of prevalence studies based on the concept 'from farm to fork'. However, studies systematically analysing such data are rare. A recent approach aims to establish national and international studies to inform a comprehensive risk analysis. The purpose of one such study, carried out by Pak *et al.* (34), was to identify the main hazards associated with the spread of *L. monocytogenes* in dairy products in Switzerland and to determine the changes in the predominant serotypes of the isolates, using a database covering the years 1990 to 1999. Another study combined data on 2,053 imported and 164 exported meat and fish products from 425 production plants investigated for the presence of *L. monocytogenes* over a nine-year period (1992 to 2000) (22). The highest isolation risk was for marinated fish; the lowest was in cured and dried-meat products. Unconditional fixed-effects logistic regression analysis was used to identify the main hazards associated with the presence of *L. monocytogenes*. The production plant-level model considered potential risk factors for a positive culture by including a random effect for plant and year. Food category was the only significant factor; sampling site, country of origin and season were not significant. The authors concluded that control measures should be focused on specific food items in each production plant.

Risk management strategies are pursued at different levels along the food production chain. The faecal carriage of food-borne pathogens by livestock is strongly correlated with the hazard of milk and carcass contamination. In order to reduce the risk represented by *Listeria*, the maintenance of milking and slaughter hygiene is of central importance in food animal production (16, 18). Prevention and control measures are also based on the implementation of hazard analysis and critical control point (HACCP) programmes throughout the food industry, and specific recommendations to high-risk groups.

From the food safety point of view, understanding how *Listeria* organisms are able to successfully adapt their cellular physiology to overcome various forms of stress and current control measures is an important step in developing better ways of controlling *L. monocytogenes* in food environments. Cold stress adaptation is one of the fundamental attributes of *L. monocytogenes* that is essential for its dissemination in food environments.

Listeria monocytogenes – agent de toxi-infection alimentaire et indicateur d'hygiène

T. Jemmi & R. Stephan

Résumé

L'importance de *Listeria monocytogenes* en tant qu'agent de toxi-infection alimentaire n'a pas cessé de croître depuis vingt-cinq ans. Dans la plupart des États membres de l'Union européenne, l'incidence annuelle de la listériose humaine varie de deux à dix cas par million d'habitants. La listériose a un taux de létalité élevé qui en fait l'une des causes les plus fréquentes de décès parmi les maladies d'origine alimentaire. L'infection à *L. monocytogenes* est à l'origine de la plupart des hospitalisations (91 %) dues à des agents de toxi-infection alimentaire, et reste associée aussi bien à des cas isolés qu'à des épidémies qui surviennent partout dans le monde.

La capacité de *L. monocytogenes* à survivre aux conditions de transformation des denrées alimentaires et à résister aux basses températures en fait une menace de premier ordre pour la santé publique. La contamination par *L. monocytogenes* est l'une des principales causes de saisie de produits alimentaires, et concerne aussi bien la viande, la volaille, les fruits de mer que les produits laitiers.

La maîtrise des risques associés à *Listeria* passe par la surveillance de l'hygiène dans les laiteries et les abattoirs. Les mesures de prévention et de prophylaxie reposent sur l'application de programmes d'analyse des risques et de maîtrise des points critiques tout au long de la chaîne de production alimentaire et sur la prise en compte de recommandations spécifiques pour les groupes à haut risque.

Il importe de bien comprendre comment la physiologie cellulaire de ces micro-organismes s'adapte aux agressions, afin de pouvoir maîtriser le risque lié à *L. monocytogenes* dans les milieux où sont élaborées les denrées alimentaires.

Mots-clés

Cas – Écologie – Listeria monocytogenes – Sécurité sanitaire des aliments – Virulence.

La *Listeria monocytogenes*, un agente patógeno transmitido por los alimentos que también sirve de indicador de higiene

T. Jemmi & R. Stephan

Resumen

Desde hace 25 años, la *Listeria monocytogenes* se ha convertido en uno de los principales agentes patógenos transmitidos por los alimentos. En la mayoría de los países de la Unión Europea, la incidencia anual de la listeriosis en seres humanos oscila entre dos y diez casos notificados por millón. Debido a su elevada tasa de letalidad, la listeriosis es una de las enfermedades de transmisión alimentaria que provoca mayor número de muertes. Asimismo, de los agentes patógenos transmitidos por alimentos conocidos, es el que ocasiona

mayores porcentajes de hospitalización (91%). Esta bacteria ha sido asociada tanto a brotes esporádicos, como a grandes focos de listeriosis humana, en todas partes del mundo.
La capacidad para resistir a las condiciones existentes en las plantas de producción de alimentos y multiplicarse a temperaturas de refrigeración convierte a la *L. monocytogenes* en una grave amenaza para la salud pública. La contaminación con *L. monocytogenes* es una de las principales causas microbiológicas de devolución de alimentos, en particular de carne, aves de corral, mariscos y productos lácteos.
El mantenimiento de la higiene en las granjas lecheras y mataderos es capital para reducir el riesgo de presencia de la *L. monocytogenes*. Las medidas de prevención y control se basan en análisis de riesgos y programas de control de puntos críticos en todas las etapas de la industria alimentaria y, también, en la formulación de recomendaciones específicas para grupos de riesgo elevado.
Para luchar contra estos microorganismos en las plantas de producción alimentaria es preciso comprender cómo adaptan su fisiología celular para resistir a las agresiones.

Palabras clave
Ecología – Enfermedad transmitida por alimentos – Frecuencia – Inocuidad de los alimentos – Listeria monocytogenes – Virulencia.

References

1. Anon (2005). – Missouri firm expands recall of meat lunch maker's products for possible *Listeria* contamination. Available at: http://forums.chef2chef.net/showflat.php?Cat=0 &Number=382583&an=0&page=0#382583 (accessed on 5 December 2005).

2. Boerlin P., Boerlin-Petzold F. & Jemmi T. (2003). – Use of listeriolysin O and internalin A in a seroepidemiological study of listeriosis in Swiss dairy cows. *J. clin. Microbiol.*, **41**, 1055-1061.

3. Borucki M.K., Peppin J.D., White D., Loge F. & Call D.R. (2003). – Variation in biofilm formation among strains of *Listeria monocytogenes*. *Appl. environ. Microbiol.*, **69**, 7336-7342.

4. Domann E., Wehland J., Rohde M., Pistor S., Hartl M., Goebel W., Leimeister-Wachter M., Wuenscher M., Chakraborty T. (1992). – A novel bacterial virulence gene in *Listeria monocytogenes* required for host cell microfilament interaction with homology to the proline-rich region of vinculin. *EMBO J.* **11**, 1981-90.

5. Dominguez Rodriguez L., Fernández Garayzabal J.F., Vazquez Boland J.A., Rodriguez Ferri E. & Suárez Fernández G. (1985). – Isolation of microorganisms of the species *Listeria* from raw milk intended for human consumption. *Can. J. Microbiol.*, **31**, 938-941.

6. Dramsi S., Dehoux P., Lebrun M., Goossens P.L. & Cossart P. (1997). – Identification of four new members of the internalin multigene family of *Listeria monocytogenes* EGD. *Infect. Immunol.*, **65**, 1615-1625.

7. Dramsi S., Bourdichon F., Cabanes D., Lecuit M., Fsihi H. & Cossart P. (2004). – FbpA, a novel multifunctional *Listeria monocytogenes* virulence factor. *Molec. Microbiol.*, **53**, 639-649.

8. Fantelli K. & Stephan R. (2001). – Prevalence and characteristics of Shigatoxin-producing *E. coli* (STEC) and *Listeria monocytogenes* strains isolated from minced meat in Switzerland. *Int. J. Food Microbiol.*, **70**, 63-69.

9. Farber J.M., Sanders G.W. & Malcolm S.A. (1988). – The presence of *Listeria* spp. in raw milk in Ontario. *Can. J. Microbiol.*, **34**, 95-100.

10. Farber J.M. & Peterkin P.I. (1991). – *Listeria monocytogenes*, a food-borne pathogen. *Microbiol. Rev.*, **55**, 476-511.

11. Food and Agriculture Organization (FAO) (1999). – Report of the FAO expert consultation on the trade impact of *Listeria* in fish products, 17-20 May, Amherst, Massachusetts. FAO Fisheries Report no. 604. Available at: http://www.fao.org/documents/show_cdr.asp?url_file=/DOCREP/003/X3018E/X3018E01.HTM (accessed on 12 March 2006).

12. Food and Drug Administration Center for Food Safety and Applied Nutrition (2001). – Fish and Fisheries Products Hazards and Controls Guidance. Third Ed. Available at: http://www.cfsan.fda.gov/~comm/haccp4x4.html (accessed on 12 March 2006).

13. Francois K., Devlieghere F., Smet K., Standaert A.R., Geeraerd A.H., Van Impe J.F. & Debevere J. (2005). – Modelling the individual cell lag phase: effect of temperature and pH on the individual cell lag distribution of *Listeria monocytogenes*. *Int. J. Food Microbiol.*, **100**, 41-53.

14. Frye D.M., Zweig R., Sturgeon J., Tormey M., LeCavalier M., Lee I., Lawani L. & Mascola L. (2002). – An outbreak of febrile gastroenteritis associated with delicatessen meat contaminated with *Listeria monocytogenes*. *Clin. infect. Dis.*, **35**, 943-949.

15. Gaillot O., Pellegrini E., Bregenholt S., Nair S. & Berche P. (2000). – The ClpP serine protease is essential for the intracellular parasitism and virulence of *Listeria monocytogenes*. *Molec. Microbiol.*, **35**, 1286-1294.

16. Gobat P.F. & Jemmi T. (1990). – Epidemiological studies on *Listeria* spp. in slaughterhouses. *Fleischwirtschaft*, **70**, 1448-1450.

17. Gray M.J., Zadoks R.N., Fortes E.D., Dogan B., Cai S., Chen Y., Scott V.N., Gombas D.E., Boor K.J. & Wiedmann M. (2004). – *Listeria monocytogenes* isolates from foods and humans form distinct but overlapping populations. *Appl. environ. Microbiol.*, **70**, 5833-5841.

18. Husu J.R., Seppanen J.T., Sivela S.K. & Rauramaa A.L. (1990). – Contamination of raw milk by *Listeria monocytogenes* on dairy farms. *Zentralbl. Veterinärmed., B*, **37**, 268-275.

19. Inoue S., Nakama A., Arai Y., Kokubo Y., Maruyama T., Saito A., Yoshida T., Terao M., Yamamoto S. & Kumagai S. (2000). – Prevalence and contamination levels of *Listeria monocytogenes* in retail foods in Japan. *Int. J. Food Microbiol.*, **59**, 73-77.

20. International Commission on Microbiological Specifications for Foods (1996). – Microorganisms in foods 5: characteristics of microbial pathogens. Blackie Academic & Professional, London.

21. Jayarao B.M. & Henning D.R. (2001). – Prevalence of foodborne pathogens in bulk tank milk. *J. Dairy Sci.*, **84**, 2157-2162.

22. Jemmi T., Pak S.I. & Salman M.D. (2002). – Prevalence and risk factors for contamination with *Listeria monocytogenes* of imported and exported meat and fish products in Switzerland, 1992-2000. *Prev. vet. Med.*, **54**, 25-36.

23. Johansson T., Rantala L., Palmu L. & Honkanen-Buzalski T. (1999). – Occurrence and typing of *Listeria monocytogenes* strains in retail vacuum-packed fish products and in a production plant. *Int. J. Food Microbiol.*, **47**, 111-119.

24. Junttila J.R., Niemela S.I. & Hirn J. (1988). – Minimum growth temperatures of *Listeria monocytogenes* and non-haemolytic *Listeria*. *J. appl. Bacteriol.*, **65**, 321-327.

25. Linnan M.J., Mascola L., Lou X.D., Goulet V., May S., Salminen C., Hird D.W., Yonekura M.L., Hayes P. & Weaver R. (1988). – Epidemic listeriosis associated with Mexican-style cheese. *N. Engl. J. Med.*, **319**, 823-828.

26. Lu Z., Sebranek J.G., Dickson J.S., Mendonca A.F. & Bailey T.B. (2005). – Application of predictive models to estimate *Listeria monocytogenes* growth on frankfurters treated with organic acid salts. *J. Food Protec.*, **68**, 2326-2332.

27. Luppi A., Bucci G., Maini P. & Rocourt J. (1988). – Ecological survey of *Listeria* in the Ferrara area (northern Italy). *Zentralbl. Bakteriol. Mikrobiol. Hyg., A*, **269**, 266-275.

28. McLauchlin J., Mitchell R.T., Smerdon W.J. & Jewell K. (2004). – *Listeria monocytogenes* and listeriosis: a review of hazard characterisation for use in microbiological risk assessment of foods. *Int. J. Food Microbiol.*, **92**, 15-33.

29. Marquis H., Doshi V. & Portnoy D.A. (1995). – The broad-range phospholipase C and a metalloprotease mediate listeriolysin O-independent escape of *Listeria monocytogenes* from a primary vacuole in human epithelial cells. *Infect. Immunol.*, **63**, 4531-4534.

30. Massa S., Cesaroni D., Poda G. & Trovatelli L.D. (1990). – The incidence of *Listeria* spp. in soft cheeses, butter and raw milk in the province of Bologna. *J. appl. Bacteriol.*, **68**, 153-156.

31. Mead P.S., Slutsker L., Dietz V., McCaig L.F., Bresee J.S., Shapiro C., Griffin P.M. & Tauxe R.V. (1999). – Food-related illness and death in the United States. *Emerg. infect. Dis.*, **5**, 607-625.

32. Moura S.M., Destro M.T. & Franco B.D. (1993). – Incidence of *Listeria* species in raw and pasteurized milk produced in São Paulo, Brazil. *Int. J. Food Microbiol.*, **19**, 229-237.

33. Norrung B. & Skovgaard N. (1993). – Application of multilocus enzyme electrophoresis in studies of the epidemiology of *Listeria monocytogenes* in Denmark. *Appl. environ. Microbiol.*, **59**, 2817-2822.

34. Pak S.I., Spahr U., Jemmi T. & Salman M.D. (2002). – Risk factors for *L. monocytogenes* contamination of dairy products in Switzerland, 1990-1999. *Prev. vet. Med.*, **1663**, 1-11.

35. Pini P.N. & Gilbert R.J. (1988). – The occurrence in the UK of *Listeria* species in raw chickens and soft cheeses. *Int. J. Food Microbiol.*, **7**, 331-337.

36. Posfay-Barbe K.M. & Wald E.R. (2004). – Listeriosis. *Pediatr. Res.*, **25**, 151-159.

37. Raveneau J., Geoffroy C., Beretti J.L., Gaillard J.L., Alouf J.E. & Berche P. (1992). – Reduced virulence of a *Listeria monocytogenes* phospholipase-deficient mutant obtained by transposon insertion into the zinc metalloprotease gene. *Infect. Immunol.*, **60**, 916-921.

38. Rocourt J. & Bille J. (1997). – Foodborne listeriosis. *World Hlth Stat. Q.*, **50**, 67-73.

39. Salamina G., Dalle Donne E., Niccolini A., Poda G., Cesaroni D., Bucci M., Fini R., Maldini M., Schuchat A., Swaminathan B., Bibb W., Rocourt J., Binkin N. & Salmaso S. (1996). – A foodborne outbreak of gastroenteritis involving *Listeria monocytogenes*. *Epidemiol. Infect.*, **117**, 429-436.

40. Salvat G., Toquin M.T., Michel Y. & Colin P. (1995). – Control of *Listeria monocytogenes* in the delicatessen industries: the lessons of a listeriosis outbreak in France. *Int. J. Food Microbiol.*, **25**, 75-81.

41. Schoder D., Winter P., Kareem A., Baumgartner W. & Wagner M. (2003). – A case of sporadic ovine mastitis caused by *Listeria monocytogenes* and its effect on contamination of raw milk and raw-milk cheeses produced in the on-farm dairy. *J. Dairy Res.*, **70**, 395-401.

42. Schubert W.D., Urbanke C., Ziehm T., Beier V., Machner M.P., Domann E., Wehland J., Chakraborty T. & Heinz D.W. (2002). – Structure of internalin, a major invasion protein of *Listeria monocytogenes*, in complex with its human receptor E-cadherin. *Cell*, **111**, 825-836.

43. Schuchat A., Swaminathan B. & Broome C.V. (1991). – Epidemiology of human listeriosis. *Clin. Microbiol. Rev.*, **4**, 169-183.

44. Schwarzkopf A. (1996). – *Listeria monocytogenes* – aspects of pathogenicity. *Pathol. biol. (Paris)*, **44**, 769-774.

45. Senczek D., Stephan R. & Untermann F. (2000). – Pulsed-field gel electrophoresis (PFGE) typing of *Listeria* strains isolated from a meat processing plant over a 2-year period. *Int. J. Food Microbiol.*, **62**, 155-159.

46. Sibelius U., Schulz E.C., Rose F., Hattar K., Jacobs T., Weiss S., Chakraborty T., Seeger W. & Grimminger F. (1999). – Role of *Listeria monocytogenes* exotoxins listerolysin and phosphatidylinositol-specific phospholipase C in activation of human neutrophils. *Infect. Immunol.*, **67**, 1125-1130.

47. Skovgaard N. & Morgen C.A. (1988). – Detection of *Listeria* spp. in faeces from animals, in feeds, and in raw foods of animal origin. *Int. J. Food Microbiol.*, **6**, 229-242.

48. Stephan R., Senczek D., Müller Ch. & Feusi Ch. (2000). – Isolierung von *Listeria* spp. und *Aspergillus fumigatus* – zwei Fallberichte aus der Mastitisdiagnostik. *Schweizer Arch. Tierheilkd.*, **142**, 387-390.

49. Stephan R. & Bühler K. (2002). – Prävalenz von *Campylobacter* spp., *Salmonella* spp. und *Listeria monocytogenes* in Bestandesmilchproben aus der Nordostschweiz. *Arch. Lebensmittelhyg.*, **53**, 62-65.

50. Tasara T. & Stephan R. (2006). – Cold stress tolerance of *Listeria monocytogenes*: a review of molecular adaptive mechanisms and food safety implications. *J. Food. Protec.*, **69** (6), 1473-1484.

51. United Sates Department of Agriculture (USDA) (2002). – New Jersey firm expands recall of poultry products for possible *Listeria* contamination. Food Safety and Inspection Service, USDA, Washington, DC. Available at: http://www.fsis.usda.gov/oa/recalls/prelease/pr098-2002.htm (accessed on 12 March 2006).

52. Uyttendaele M., De Troy P. & Debevere J. (1999). – Incidence of *Listeria monocytogenes* in different types of meat products on the Belgian retail market. *Int. J. Food Microbiol.*, **53**, 75-80.

53. Van Kessel J.S., Karns J.S., Gorski L., McCluskey B.J. & Perdue M.L. (2004). – Prevalence of Salmonellae, *Listeria monocytogenes*, and fecal coliforms in bulk tank milk on US dairies. *J. Dairy Sci.*, **87**, 2822-2830.

54. Vazquez-Boland J.-A., Kocks C., Dramsi S., Ohayon H., Geoffroy C., Mengaud J. & Cossart P. (1992). – Nucleotide sequence of the lecithinase operon of *Listeria monocytogenes* and possible role of lecithinase in cell-to-cell spread. *Infect. Immunol.*, **60**, 219-230.

55. Waak E., Tham W. & Danielsson-Tham M.L. (2002). – Prevalence and fingerprinting of *Listeria monocytogenes* strains isolated from raw whole milk in farm bulk tanks and in dairy plant receiving tanks. *Appl. environ. Microbiol.*, **68**, 3366-3370.

56. Wagner M., Melzner D., Bago Z., Winter P., Egerbacher M., Schilcher F., Zangana A. & Schoder D. (2005). – Outbreak of clinical listeriosis in sheep: evaluation from possible contamination routes from feed to raw produce and humans. *J. vet. Med. B, infect. Dis. vet. public Health.*, **52**, 278-283.

57. Walker S.J., Archer P. & Banks J.G. (1990). – Growth of *Listeria monocytogenes* at refrigeration temperatures. *J. appl. Bacteriol.*, **68**, 157-162.

58. Wemekamp-Kamphuis H.H., Sleator R.D., Wouters J.A., Hill C. & Abee T. (2004). – Molecular and physiological analysis of the role of osmolyte transporters BetL, Gbu, and OpuC in growth of *Listeria monocytogenes* at low temperatures. *Appl. environ. Microbiol.*, **70**, 2912-2918.

59. Wiedmann M. (2002). – Molecular subtyping methods for *Listeria monocytogenes*. *J. AOAC int.*, **85**, 524-531.

60. Wong A.C. (1998). – Biofilms in food processing environments. *J. Dairy Sci.*, **81**, 2765-2770.

61. World Organisation for Animal Health (OIE) (2004). – *Listeria monocytogenes*. *In* Manual of Diagnostic Tests and Vaccines for Terrestrial Animals, 5th Ed. OIE, Paris, 1138-1152.

62. Wouters J.A., Rombouts F.M., Kuipers O.P., de Vos W.M. & Abee T. (2000). – The role of cold-shock proteins in low-temperature adaptation of food-related bacteria. *Syst. appl. Microbiol.*, **23**, 165-173.