Chapter 1 Introduction

[1.1]. Listeria spp.

Listeria was isolated for the first time from rabbit liver, in which it caused tissue necrosis, by Prof. Hülphers. First publication describing *Listeria* was Murray and coworkers, in which a bacterium causing spontaneous lethal disease among rabbits in Cambridge University animal breeding was isolated (Murray 1926). Pirie (Pirie 1927)isolated a new bacterial species, named *Listerella hepatolytca* honouring Lord Lister, from some clinical cases among South Africa gerbils. After examination of both isolates at Lister Institute in London, high similarity between of these isolates was found and this bacterium was named *Listerella monocytogenes*, later corrected in *Listeria* because of former attribution of *Listerella* to another bacterium. Clinical history of *L. monocytogenes* included a case of sheep infection (Gill 1933) and then in a human patient in 1929 (Nyfeldt 1929).

[1.2]. Main genus characteristics

Listeria is a Gram positive rod-shaped, round-ended and non-sporing bacterium, which is motile at 20-25°C because of peritrichous flagella (flagella is not active at 37°C) and is facultative anaerobic. Cells can be found single or aggregated in short chains. On nutrient agar, this microbe forms smooth, puntiform and traslucent round colonies. After 24 hours of incubation colonies have a glistening surface (S-form), while longer incubation gives colonies with slight roughness (R-form). Exposure to light obliquely transmitted gives colonies a blue-green appearance, whereas they seem bluish-gray under normal condition of illumination. For its morpholocial appearance, *Listeria* has been long included in the bacterial group of coryneforms, even if molecular methods recently developed allowed to give it more clearly defined position. Within this genus it is possible distinguishing, from a biochemical point of view, six diverse bacterial species: *L. monocytogenes*, *L. innocua*, *L. ivanovii* (Dongyou 2008), *L. welshimeri*, *L. seeligeri* and *L. gray*. There is also a seven species mentioned within this genus, *Listeria murray*, which has been defined as a subgroup of *L. gray* through molecular investigations (DNA–DNA hybridization, multilocus enzyme electrophoresis, and rRNA restriction fragment length polymorphism) (Rocourt 1992). Phylogenetic analysis based on 16S and 23S rRNA and analysis of the genes *iap* have indicated that five of the six species within the genus *Listeria* can be divided into two main lineages: *L. monocytogenes* and *L. innocua* for a distinct group, while *L. welshimeri*, *L. ivanovii*

and *L. seeligeri* are grouped all together, although *L. welshimeri* seemed to be more distanced from the other two members. *L. gray* represent by itself the most distanced branch of this genus (Hain et al. 2006). Of all mentioned species only *L. monocytogenes* and *L. ivanovii* harbor virulence properties causing disease in human and animal hosts, respectively. Biochemical properties of all them are reported in Tab. 1.1. *Listeria monocytogenes* can grow in most of laboratory media with pH ranging from 4.3 until to 9.4 (Ottaviani, Ottaviani e Agosti 1997). Minimal a_w for growth has been reported to be 0.90. Growth temperature cover a quite wide range from -1,5 to 45°C, even if low temperature increase time of lag phase: this property is often exploited in cold enrichment isolation procedure (Hansen, Gerner-Smidt e Bruun 2005). Optimal temperature is considered to be 30-37°C. *L. monocytogenes* is able to grow in presence of CO_2 even at low temperature, but CO_2 concentration higher than 70% can inhibit its growth if temperature is less than 7°C (Wimpfheimer 1990). Because of its ability to grow in so wide-ranged parameters of growth, *L. monocytogenes* is considered widely and ubiquitary distributed in the environment, making difficult it to define its ecological niche. *Listeria* posseses survival capacity in presence of 10% NaCl and 200 ppm $NaNO_2$, as well as in moist and dry environments at specific sites within food manufacturing environments. Even when present at high levels in foods, spoilage or taints are not generally produced. *L. monocytogenes* does not show high thermal resistance and is not able to resist to milk pasteurization: its D values (decimal reduction times) range from 16.7 to 1.3 min at 60°C and 0.2 to 0.06 min at 70°C (Dongyou 2008).

	L. monocytogenes	*L. ivanovii*	*L.- seeligeri*	*L. innocua*	*L. welshimeri*	*L. gray*
Gram staining	+	+	+	+	+	+
Catalase test	+	+	±	+	+	+
β-hemolysis	+	+	+	-	-	-
CAMP test						
Staphylococcus aureus	+	-	+	-	-	-
Rhodococcus equi	-	+	-	-	-	-
Acid production starting from						
esculin	+	+	+	+	+	+
maltose	+	+	+	+	+	
mannitole	-	-	-	-	-	+
xylose	-	+	+	-	+	-
rhamnose	+	-	-	v	v	±
α.methyl-D-rhamnoside	+	-	±	+	+	±
Virulence in mice	+	+	-	-	-	-
Serotype	1/2a, 1/2b, 1/2c, 3a, 3b, 3c, 4a, 4ab, 4b, 4c, 4d, 4e, 7	5	1/2b, 4c, 4d, 6b	6a, 6b	6a, 6b	

Tab. 1.1: Bioichemical traits of interest of bacterial species within *Listeria* genus. +, positive reaction; –, negative reaction; v, variable or weak reaction (RYSER, 1999).

[1.3]. *Listeria* infections: clinical description

Listeria infections can occurs in almost all domestic animals, even if the most common host is the ruminant. Monogastric animals can be susceptible to the microorganism, even if it is a rare event mainly manifested as septicemia, while the main symptoms in animals can be summarized in encephalitis, septicemia

and abortion (3) with the possibility to excrete the pathogen through the milk, whose incidence has been associated with indoor housing and silage feeding, and poor hygiene procedure application.

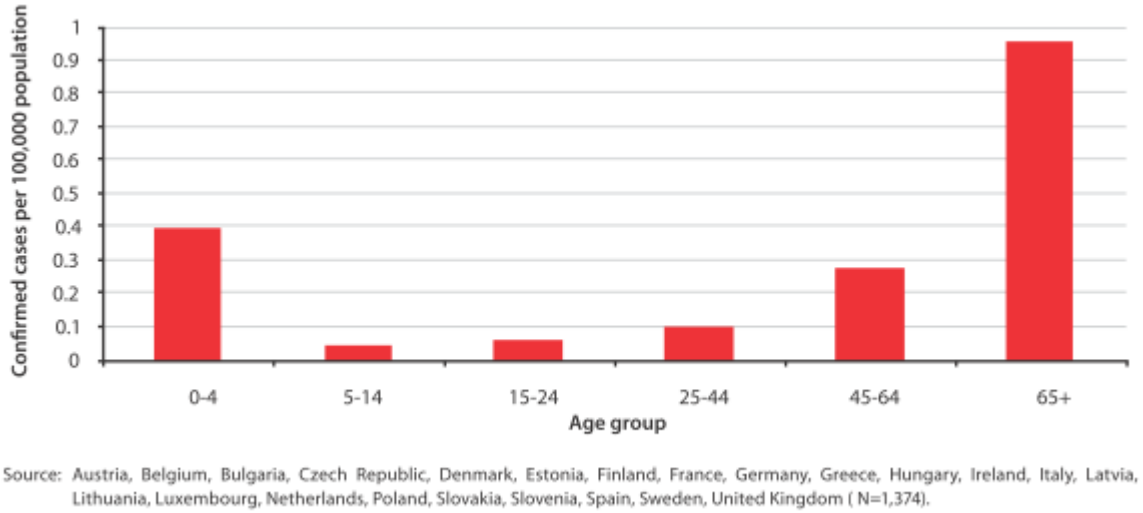
In humans *L. monocytogenes* can provoke both a noninvasive and invasive forms of illness. Non-invasive listeriosis can affect even healthy adult individuals, but there is still a lack of knowledge about infectious dose and the interactions of organisms and host remain unclear. Characteristic symptomatology involve gastroenteritis, fever, diarrhea and vomiting with an average incubation time of 18-20 hours. Related frequency of incidence is extremely variable and it is possible that the pathogen can be present in human host without any apparent symptoms, fact which make difficult to diagnosticate the disease in hospital. In its invasive form of disease, because of ability of microorganism to migrate through placenta, *Listeria monocytogenes* can lead in pregnant women to spontaneous abortion, stillbirth or severely ill baby. Further source of its diffusion could be attributed to acquisition from newborn due to post-natal infection of mother or other infected babies. Usually mothers rarely have severe symptoms, because the pathogen prefers focusing on fetus due to its higher sensitivity.

Listeriosis can affect also healthy and non-pregnant subjects, among whom the most sensitive group comprise of immunocompromised and elderly because of the reduced effectiveness of their immune system. Listeriosis, in this group of patients, manifests as meningitis and septicemia. Incubation time usually cover a time period of one day until several weeks. Generally there are defined three main possible routes of listerial contamination:

- *Contact with animals*: wild animals, as well as domestic ones, can carry *Listeria* and most of them can manifest symptoms of listeriosis. Septicemia and abortion are frequently encountered in sheep and other animals and disease can be transmitted to human hosts: in this case infection assumes the form of skin infection in people exposed to direct contact with animals (e.g. farmers and veterinarians) and express mild form of self-resolving symptoms, although it cannot be excluded the possibility of evolution in more severe forms.
- *Cross-infection of newborn in hospital*: listeriosis is reported as cause of 29 incidents in UK with a 25% of late onset of neonatal cases.
- *Foods*: majority of reported episodes, especially outbreak episodes, has been related to ingestion of food contaminated by *L. monocytogenes*, and will be more extensively discussed in further pages.

1,381 confirmed cases of listeriosis were reported in 2008 In Europe. The number of confirmed cases of listeriosis (Tab. 1.2) decreased slightly when compared with reported cases concerning 2007. Listeriosis mainly occurred among elderly people (55.2% of cases took place in individuals over 65 ages). The second highest notification rate was with regard to children under the age of five (0.4 cases per 100,000 population). The case fatality rate for human listeriosis was 20.5%, whose highest value in terms of incidence in selected

groups of population was found among the elderly (Fig. 1.1). The EU notification rate was 0.3 per 100,000 population with highest notification rates observed in Denmark, Finland and Sweden (EFSA 2010).



Source: Austria, Belgium, Bulgaria, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Luxembourg, Netherlands, Poland, Slovakia, Slovenia, Spain, Sweden, United Kingdom (N=1,374).

Fig. 1.1: Age-specific distribution of reported confirmed case s of huma n listerios (EFSA 2010).

Country	Report type[2]	2008			2007	2006	2005	2004
		Cases	Confirmed cases	Confirmed cases/ 100,000				
Austria	C	31	31	0.4	20	10	9	19
Belgium	C	64	64	0.6	57	67	62	89
Bulgaria[3]	A	5	5	0.1	11	6	-	-
Cyprus	U	0	0	0	0	1	-	-
Czech Republic	C	37	37	0.4	51	78	15	16
Denmark	C	51	51	0.9	58	56	46	41
Estonia	C	8	8	0.6	3	1	2	2
Finland	C	40	40	0.8	40	45	36	35
France	C	276	276	0.4	319	290	221	236
Germany	C	306	306	0.4	356	508	510	296
Greece	C	1	1	<0.1	10	7	8	3
Hungary	C	19	19	0.2	9	14	10	16
Ireland	C	13	13	0.3	21	7	11	11
Italy	C	75	75	0.1	65	51	51	25
Latvia	C	5	5	0.2	5	2	6	3
Lithuania	A	7	7	0.2	4	4	2	1
Luxembourg	C	1	1	0.2	3	4	0	-
Malta	U	0	0	0	0	0	0	-
Netherlands	C	52	44	0.3	68	64	96	55
Poland	C	33	33	0.1	43	28	22	10
Portugal	-[4]	-	-	-	-	-	-	38
Romania[3]	-	-	-	-	0	-	-	-
Slovakia	C	8	8	0.1	9	12	5	8
Slovenia	C	3	3	0.1	4	7	3	1
Spain	C	88	88	0.2	81	78	68	100
Sweden	C	60	60	0.7	56	42	35	44
United Kingdom	C	206	206	0.3	261	208	223	232
EU Total		**1,389**	**1,381**	**0.3**	**1,554**	**1,590**	**1,443**	**1,281**
Iceland	U	0	0	0	4	0	0	-
Liechtenstein	U	0	0	0	0	0	-	-
Norway	C	34	34	0.7	49	27	14	23
Switzerland	C	43	43	0.6	60	68	73	58

Tab. 1.2: Reported listeriosis cases in humans 2004-2008, and notification rates for confirmed cases in 2008 (EFSA 2010). Total number of cases are reported concerning 20004, while for 2005-2008 number of confirmed cases is reported. A: aggregated data report; C: case-based report; -: No report; U: unspecified.

[1.4]. *Listeria monocytogenes* infectious cycle

In order to ensure a more complete view, here is briefly summarized the whole process of host colonization. After ingestion of polluted food, *L. monocytogenes* is exposed to highly adverse environmental conditions (proteolytic enzymes, low pH of stomach, bile salts and inflammatory attacks), to which the

pathogen can withstand thanks to stress response-related genes and proteins (Hamon 2006). Subsequent steps are adhesion and internalization of host through internalins: this group is composed of surface proteins, among which InlA and InlB are worth of a special mention, facilitate invasion in host tissues evasion from host immune system during its surveillance activity.

At this point pathogen can be located in cell vacuole, from which mic robe can escape through lysis factors (listeriolysin O or LLO and phosphatidylinositol-phospholipase C or PI-PLC), and then in cellular cytosol, where it can grow and multiplicate. Further steps consist in propelling toward cytoplasmatic membrane (this action is mediated by ActA which lead to polarized actin tails enabling intracellular motility) and formation of envelopes for spreading infection in adjacent cells and beginning of a new infection cycle (Freitag 2009).

[1.5]. Molecular properties of interesting

L. monocytogenes has been subjected to notable evolutionary process leading to acquisition of a collection of molecular functions and determinants, which have played a contributory role in itsspreading and colonization as an intracellular pathogen. Virulence-associated genes previously mentioned can be found in a 9.6 kb single chromosomic location, pathogenicity island, which is regulated by suitable regulation factor, PrfA, the corresponding of which is collocated immediately downstream the virulence cluster and activates trascription of other molecular virulence-related determinants. Beside these genetic functions, other genes, like invasin associated protein or p60, are reported as associated to expression of potential pathogenicity. (Vàsquez-Boland, et al. 2001)

i. ***hly*** The *L. monocytogenes* hemolysin, listeriolysin 0 (LLO) is the best described protein related to *L. monocytogenes* virulence. It belongs to sulfhydryl-activated pore-forming cytolysins protein family. Its role is mediating lysis of bacterium-containing vacuoles, after that bacterium is able to grow and multiuply in host cytoplasm, using cytoplasm itself as growth medium.
ii. ***pleA*** Adjacent to *hly* and transcribed divergently, there is *pleA* which encodes a phosphatidylinositol-specific phospholipase C (PI-PLC) , whose function could be summarized in hydrolysis of both PI and PI-glycan. The plcA sequence shows 30% amino acid identity with *Bacillus thuringiensis* and *Bacillus cereus* PI-PLC. Interestingly other gram-positive bacteria such as *Staphylococcus aureus*, *Clostridium novyi*, and *Bacillus anthracis* also possess PI-PLC activity.
iii. ***prfA.*** This gene encodes for a protein that is responsible for regulating itself and other virulence-associated genes (i.e., *plcA*, *hlyA mpl*, *actA*, and *plcB*). *prfA* is the second gene of an operon and can be expressed either from its own promoter located in the *pkcA-prfA* intergenic region or from the

plcA promoter, suggesting that *prfA* regulates its own synthesis. Whether the *prfA* gene product acts directly on all of the genes under its control has not been demonstrated, but in *B. subtilis*, the *prfA*-encoded gene product directly activates the transcription of hly. In addition, it was hypothesized that PrfA may recognize a 14-bp palindromic sequence found in the -35 region of the promoters for *hly*, *picA*, and *mpl*, suggesting that this palindrome may be the target site for PrfA-mediated activation. *prfA* gene is present in all serovars of the pathogenic species *L. monocytogenes* and expression of *prfA*-regulated genes is thermoregulated (Vàsquez-Boland, et al. 2001).

[1.6]. Relevant aspects of *Listeria monocytogenes* concerning food processing environments

Tolerance to adverse conditions and antimicrobial resistance

Being a facultative anaerobe, *L. monocytogenes* can grow in vacuum or modified atmosphere packaged foodstuffs (Buchanan, et al. 1998). Temperature optimum determined through culture monitoring has been 30-37°C, but this microbe can multiply at refrigerated temperatures and survive during frozen storage. Temperature upper limit has been defined as 45°C and pasteurization at 71.6°C for 15s can reduce notably bacterial contamination. *L. monocytogenes* can cover a large pH range from 4.3 to 9.6 with optimum in neutral or moderately alkaline values. Growth Minimal a_w is at 0.90, even this bacterium has demonstrated high tolerance for high osmotic pressures (Farber and Perkin 1991, Ryser 1999, Dongyou 2008).

The ability of this bacterium to grow at refrigeration temperatures makes *L. monocytogenes* a post-processing contaminant in long-shelf-life refrigerated foods. The widespread distribution of *L. monocytogenes* and its capability to survive on dry and moist surfaces favors post-processing contamination of foods from both raw product and factory sites (McLauchlin 1996). In addition, *Listeria* spp. also show unusual tolerance to high salt concentrations (up to 10% NaCl and sodium nitrite). The capacity of *L. monocytogenes* to withstand severe environmental stresses depends on its efficient stress response mechanisms: various salt stress response genes (including *betL, gbuABC, opuC, opuB, lmo1421,* and *bsh*) have been characterized, a majority of which are regulated by an alternative sigma factor, σB (encoded by *sigB*)—a protein subunit of RNA polymerase (RNAP). Mutations in *sigB* and related genes result in lower acid and salt tolerance in *L. monocytogenes*. Further, σB also influences *L. monocytogenes* virulence gene expressions by co-regulating a pleiotropic virulence regulator gene, *prfA*. A number of *L. monocytogenes* genes expressed in response to growth at low temperature have also been identified. However, although *Listeria* spp. are known to tolerate alkali and pressure well, the underlying mechanisms against these stresses are poorly understood (Gandhi 2007, Dongyou 2008).

Sanitizers conventionally employed in food processing plants have shown good effectiveness in reducing population density, even if it possible establishment of adherent communities can lead in improved tolerance against biocide molecules. Exposure to antimicrobials could also induce adaptation and cross-resistance to other bactericidal agents (further disinfectants and/or antibiotics): antibiotic and biocide antibacterial actions reveal strong similarities concerning target action mechanism and/or clinical aspects (like uptake through passive diffusion, effect structural changes of membrane and/or effect on diverse key steps of bacterial metabolism). In presence of toxic agent or stress source, response/adaptation of bacterial cells take place activating some similar defence mechanisms to confer resistance against structurally non-related molecules (Poole, 2002; Cloete, et al. 2003). Mechanisms possibly involved could be generally grouped into two main categories:

- Intrinsic resistance represent an innate trait conferred by the bacterial genome and applied strategies include impermeability, efflux, biofilms and/or transformation of toxic compounds. In order to decrease intracellular levels of harmful molecules, Gram negative bacteria can modify permeability through limiting synthesis of porines, which are pore-forming proteins across the cell membrane and altering the lipopolysaccharide structure (Nikaido 2003, Poole et al. 2002). Another mechanism is a overexpression of efflux pumps (protein complexes capable to expel antibiotics) (Poole 2007).

- Acquired resistance occurs due to mutations and acquisitions of mobile genetic elements (transposons, plasmids) coding for resistance-related proteins (enzymes, transporters). Similarly, the acquired traits may protect against antibiotics and biocides (Maillard 2007). In addition, some of the mechanisms that play a major role in resistance are controlled by diverse genetic cascade regulations that share common gene regulators (sox*S*, *marA*) (SCENHIR 2009, Poole 2002).

Contamination of raw materials

Other causes of concern are related to the ubiquitous presence of *Listeria* in environment and the possibility of its isolation from a wide range of raw foods: Beuchat (1996) has demonstrated presence of this bacterium in sewage, soil, decaying vegetation, silage, plants in both cultivated and uncultivated areas, feces of wild and domestic animals, and wildlife feedings grounds.

Because of its widespread presence in natural environments and in animals, which can vary depending on species and country [as shown in Tab. 1.3 (EFSA 2010), *L. monocytogenes* could be introduced in initial phases of food processing. Highest levels are reported especially for sheep, even if similar incidence could be found in goats or in cattle. This could be translated in high probability of introduction of *L. monocytogenes* in initial phases of food supply chain. Milk and slaughterhouses offer a good example.

Despite low prevalence (Meyer-Broseta, et al. 2003), *L. monocytogenes* is often found in animals, both diseasedand healthy (Fthenakis, et al. 1998, Wagner, et al. 2000), although contamination from animals is not the main route: actually it would more appropriate consider *Listeria* finding in foods as the result of a multisource contamination due to the contribution from environment and poor hygiene practices applied (Hassan, Mohammed e McDonough 2001, Sanaa, et al. 1993). Prevalence in slaughterhouses has been associated to alive livestock due to its presence in faces, tonsils and hide (Buncic 1991), even if variation in strain predominance and prevalence in *Listeria* spp. and *Listeria monocytogenes* environmental isolates population has been shown related to diverse hygiene quality (Borch e Christensen 1996, Saide-Albornoz, et al. 1995). *Listeria* can be isolated also in acquatic environments, however water does not represent the natural ecological niche for this microorganism.

Country	*L. monocytogenes*		*Listeria* spp., unspecified	
	Units tested	% Pos	% Pos	Details
Gallus gallus				
Bulgaria	54	0	0	
Germany	170	1.2	0	Flock
Ireland	166	0	0	
Italy	47	0	0	Herd
Netherlands	1,587	0	0	Flock
Total (*Gallus gallus*, 5 MSs)	**2,024**	**<0.1**	**0**	
Turkeys				
Bulgaria	28	0	0	
Ireland	32	0	0	
Netherlands	63	0	0	
Total (turkeys, 3 MSs)	**123**	**0**	**0**	
Pigs				
Estonia	84	1.2	0	
Germany	443	0.7	0	Herd
Ireland	480	0	0	
Italy	46	6.5	0	Herd
Netherlands	3,659	0	0	
Slovakia	65	0	0	
Total (pigs, 6 MSs)	**4,777**	**0.1**	**0**	
Cattle (bovine animals)				
Bulgaria	30	0	0	
Estonia	80	21.3	0	
Germany	854	9.4	0	Herd
Ireland	8,666	0.5	0.3	
Italy	231	0	1.3	
	147	0.7	0	Herd
Netherlands	3,556	0	1.3	
Slovakia	463	0.6	0	
Total (cattle, 7 MSs)	**14,027**	**1.1**	**0.5**	
Switzerland	26	0	38.5	
Goats				
Germany	129	17.1	0	Herd
Ireland	106	0	0	
Italy	25	4.0	0	
	84	1.2	0	Herd
Netherlands	320	0	6.6	
Total (goats, 4 MSs)	**664**	**3.6**	**3.2**	
Sheep				
Austria	62	30.6	1.6	
Bulgaria	34	2.9	0	
Estonia	34	11.8	2.9	
Germany	367	15.8	0	Herd
Greece	34	26.5	0	
Ireland	1,065	0.8	0.3	
Italy	49	0	0	
	292	0	2.1	Herd
Netherlands	687	0	3.1	
Slovakia	429	2.8	0	
Total (sheep, 9 MSs)	**3,053**	**3.6**	**1.0**	
Switzerland	27	0	85.2	
Sheep and goats				
Italy	33	0	9.1	

Tab. 1.3: *Listeria* spp. and *L. monocytogenes* in animals, 2008 (EFSA 2010)

Food category	Sampling unit	Absence in 25g		≤100 cfu/g	
		Units tested	Non-compliant %	Units tested	Non-compliant %
1. RTE food intended for infants and for medical purposes					
1.1 Hospital or care home	Single	99	0	-	-
1.2 Processing plant	Batch	310	0	-	-
	Single	2	0	-	-
1.3 Retail[1]	Batch	426	0	-	-
	Single	53	0	-	-
2. RTE products of meat origin other than fermented sausage					
2.1 Processing plant	Batch	15,505	2.0	-	-
	Single	1,132	6.2	-	-
2.2 Retail[1]	Batch	-	-	1,290	0.9
	Single	-	-	16,653	0.2
3. RTE products of meat origin, fermented sausage					
3.1 Processing plant	Single	-	-	14	0
3.2 Retail[1]	Batch	-	-	24	0
	Single	-	-	1,828	0.5
4. Milk, RTE					
4.1 Farm	Batch	735	0	-	-
	Single	62	4.8	-	-
4.2 Processing plant	Batch	49	0	-	-
	Single	346	1.7	-	-
4.2 Retail[1]	Single	-	-	130	0
5. Soft and semi- soft cheese, RTE					
5.1 Farm	Single	39	0	-	-
5.2 Processing plant	Batch	4,552	2.4	-	-
	Single	1,708	1.0	-	-
5.3 Retail[1]	Batch	-	-	562	2.8
	Single	-	-	2,116	0.2
6. Hard cheese, RTE					
6.1 Processing plant	Batch	-	-	2,162	0
	Single	-	-	243	0
6.2 Retail[1]	Batch	-	-	260	0
	Single	-	-	1,762	0.2
7. Other dairy products, RTE					
7.1 Farm	Single	2	0	-	-
7.2 Processing plant	Batch	3,212	0	-	-
	Single	312	0.3	-	-
7.3 Retail[1]	Batch	-	-	235	0
	Single	-	-	1,522	0
8. Fishery products, RTE					
8.1 Processing plant	Batch	1,087	4.5	-	-
	Single	544	5.5	-	-
8.2 Retail[1]	Batch	-	-	182	0
	Single	-	-	7,174	0.4
9.Other RTE products					
9.1 Catering	Single	-	-	1	0
9.2 Hospital or care home	Batch	92	0	-	-
9.3 Processing plant	Batch	840	11.1	-	-
	Single	148	1.4	-	-
9.4 Retail[1]	-	-	-	109	0
	Batch	-	-	992	0.5
	Single	-	-	11,558	0.2

Tab. 1.4: Compliance with *L. monocytogenes* criteria laid down by Regulation (EC) No 2073/2005 in food categories in the EU, 2008 (EFSA 2010).

L. monocytogenes on food processing equipment

L. moncytogenes has been reported as a contaminant of food processing tools in different sectors of food supply chain (dairy, fishery, meat and poultry), but it is a more probable contaminants of several non-food contact surfaces, including walls, doors, trucks and/or shoes (Fonnesbech Vogel, et al. 2001, Miettinen, Aarnisalo e Sjöberg 2001, Norton, et al. 2001, Suihko, et al. 2002). Also contamination of processing equipment is notably diversified, ranging from tanks and conveyors until to slicing and packaging apparatus. Main common characteristic of mentioned surfaces is presence of narrow openings and hard-to-reach sites, which make difficult and inefficient sanitation procedures leading to presence of the pathogen even on treated surfaces. Temperature seems conditioning presence of this food-borne pathogen on the above mentioned facilities: *L. monoctogenes* is more abundant at low environmental temperature than high corresponding one, where competitive microflora grows at higher level and heterogeneity. Also kind and status of material could play an important role: smooth stainless steel surface are less easy to be colonized by *L. monocytogenes* than damaged and/or rough plains (Chasseignaux, et al. 2002).

Abundance in foods

There is a wide diversity in harboring Listeria and *L. monocytogenes* among foods with heterogeneous abundance within a certain tipology. In dairy products cheese, with special mention regarding soft cheeses, are the one with highest *Listeria* prevalence ranging between 0 and 30%, while fish and fishery derivatives are less frequently associated with this microorganism, compared to meat or dairy, but show an higher prevalence (0-50%): within this typology *Listeria* contamination interests particularly not termically treated such as cold smoked salmon, whereas heat-treated foodstuffs show listerial contamination less than 12%.

Also quantitative amount of contamination is worth of being mentioned: if admitted presence in foodstuffs at time consumption is put at 100 CFU/g, several reports throughout Europe have reported levels above this limit: Nørrung et al. Have reported level higher than 100 CFU/g in 1.3% of heat-treated meat products and in 0.3-0.6% of conserved meat and fish derivates in years 1994-1995 and1997-1998, respectively (Nørrung, Andersen e Schlundt 1999, Goulet, et al. 2001), while in other more limited studies 0-1% was reported as containing more than 100 CFU/g (Rørvik e Yndestad 1991, Harvey e Gilmour 1993, Jemmi, Pak e Salman 2002). Interesting aspects are also related to measurement of food safety control measures applied in European countries, which have allowed to decrease significantly listerial presence in foods. However, these procedures must be performed systematically and periodically in order to keep under control this food-related risk.

Tab. 1.5 shows that the highest levels of non-compliance at retail was observed in ready-to-eat (RTE) fermented sausage (0.5%) and RTE fishery products (0.4%) followed by cheeses, RTE meat products and other RTE products (0.2% non-compliance each). For the batch-based sampling at retail the highest non-compliance was reported for soft and semi-soft cheeses (2.8%), followed by products of meat origin other than fermented sausages (0.9%) and other RTE products (0.5%). There is also a slight decrease in incidence of *L. monocytogenes* in fisheries from 2006 to 2007 and even bigger decline was found in 2008 in comparison (EFSA 2010).

Food category	Sampling unit	Absence in 25g		≤100 cfu/g	
		Units tested	Non-compliant %	Units tested	Non-compliant %
1. RTE food intended for infants and for medical purposes					
1.1 Hospital or care home	Single	99	0	-	-
1.2 Processing plant	Batch	310	0	-	-
	Single	2	0	-	-
1.3 Retail[1]	Batch	426	0	-	-
	Single	53	0	-	-
2. RTE products of meat origin other than fermented sausage					
2.1 Processing plant	Batch	15,505	2.0	-	-
	Single	1,132	6.2	-	-
2.2 Retail[1]	Batch	-	-	1,290	0.9
	Single	-	-	16,653	0.2
3. RTE products of meat origin, fermented sausage					
3.1 Processing plant	Single	-	-	14	0
3.2 Retail[1]	Batch	-	-	24	0
	Single	-	-	1,828	0.5
4. Milk, RTE					
4.1 Farm	Batch	735	0	-	-
	Single	62	4.8	-	-
4.2 Processing plant	Batch	49	0	-	-
	Single	346	1.7	-	-
4.2 Retail[1]	Single	-	-	130	0
5. Soft and semi- soft cheese, RTE					
5.1 Farm	Single	39	0	-	-
5.2 Processing plant	Batch	4,552	2.4	-	-
	Single	1,708	1.0	-	-
5.3 Retail[1]	Batch	-	-	562	2.8
	Single	-	-	2,116	0.2
6. Hard cheese, RTE					
6.1 Processing plant	Batch	-	-	2,162	0
	Single	-	-	243	0
6.2 Retail[1]	Batch	-	-	260	0
	Single	-	-	1,762	0.2
7. Other dairy products, RTE					
7.1 Farm	Single	2	0	-	-
7.2 Processing plant	Batch	3,212	0	-	-
	Single	312	0.3	-	-
7.3 Retail[1]	Batch	-	-	235	0
	Single	-	-	1,522	0
8. Fishery products, RTE					
8.1 Processing plant	Batch	1,087	4.5	-	-
	Single	544	5.5	-	-
8.2 Retail[1]	Batch	-	-	182	0
	Single	-	-	7,174	0.4
9.Other RTE products					
9.1 Catering	Single	-	-	1	0
9.2 Hospital or care home	Batch	92	0	-	-
9.3 Processing plant	Batch	840	11.1	-	-
	Single	148	1.4	-	-
9.4 Retail[1]	-	-	-	109	0
	Batch	-	-	992	0.5
	Single	-	-	11,558	0.2

Tab. 1.5: Compliance with *L. monocytogenes* criteria laid down by Regulation (EC) No 2073/2005 in food categories in the EU, 2008 (EFSA 2010).

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