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Review. Dairy farm management and production practices associated with the presence of *Listeria monocytogenes* in raw milk and beef

P. Santorum[1], R. Garcia[1], V. Lopez[2] and J. V. Martinez-Suarez[3,]*

[1] *Centro de Investigación y Formación Agrarias (CIFA), Consejería de Desarrollo Rural, Ganadería, Pesca y Biodiversidad de Cantabria. C/ Héroes 2 Mayo, 27, 39600 Muriedas, Cantabria, Spain*
[2] *Departamento de Bioinformática y Salud Pública, Instituto de Salud Carlos III. 28220 Majadahonda, Madrid, Spain*
[3] *Departamento de Tecnología de Alimentos, Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA). Autopista A-6 km 7.5; 28040 Madrid, Spain*

Abstract

Human listeriosis is a severe foodborne disease caused by *Listeria monocytogenes*. It is a zoonosis that represents a significant concern for the food industry due to the high mortality rate it causes and the fact that the organism is capable of growing at refrigeration temperatures. Dairy products and ready-to-eat meats are among the foods most often involved in listeriosis outbreaks. *Listeria* is a common contaminant in the dairy environment, both on the farm and in the processing plant. The main sources of *L. monocytogenes* in dairy farms are manure and improperly fermented silage. If silage crops are grown on contaminated land, a new cycle of silage contamination and faecal shedding by ruminants that consume such silage may ensue. High loads of *L. monocytogenes* produced in farm environments may thus represent a primary source for the introduction of this pathogen into the human food supply chain; dairy cows would represent a reservoir for the bacterium, and raw milk and beef would represent the main vehicles for its transmission from dairy farms to humans. Even if contamination originates in post-processing environments, contaminated raw foods may still represent a vehicle for introducing *L. monocytogenes* into food processing plants. Molecular typing methods have confirmed that common strains of *L. monocytogenes* are present in dairy farm-associated isolates and isolates from both human epidemic and sporadic cases. Pre-harvest (on-farm) control of listeriosis should be based mainly on the control of manure, silage, herd health and milking practices.

Additional key words: animal reservoir; dairy primary production; environmental sources; food vehicles; listeriosis.

Resumen

Revisión. Tipos de gestión y producción de las granjas de ganado vacuno lechero relacionados con la presencia de *Listeria monocytogenes* en la leche y la carne

La listeriosis humana es una grave enfermedad transmitida por alimentos y causada por *Listeria monocytogenes*. Se trata de una zoonosis que supone una gran preocupación para la industria alimentaria debido a su alta tasa de mortalidad y al hecho de que el microorganismo es capaz de crecer a temperaturas de refrigeración. *Listeria* es un contaminante habitual en las granjas y plantas de productos lácteos. En las granjas las fuentes principales de *L. monocytogenes* son el estiércol y los ensilados mal fermentados. Si la cosecha utilizada para producir ensilados procede de campos contaminados, puede comenzar un nuevo ciclo de contaminación de los ensilados y liberación del patógeno en las heces de los animales. El ganado vacuno lechero representa uno de los principales reservorios de este microorganismo y la leche y la carne representarían los principales vehículos para su transmisión desde la granja de producción de leche al ser humano. Incluso cuando la contaminación del alimento procede de etapas posteriores al procesado, los alimentos crudos contaminados también representan uno de los vehículos de entrada de *L. monocytogenes* en las plan-

*Corresponding author: joaquin@inia.es
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Abbreviations used: CDC (Centers for Disease Control and Prevention); EFSA (European Food Safety Authority); EU (European Union); EUCAST (European Committee on Antimicrobial Susceptibility Testing); ILSI (International Life Sciences Institute); MLEE (multi locus enzyme electrophoresis); MLST (multi locus sequence typing); PCR (polymerase chain reaction); PFGE (pulsed-field gel electrophoresis); RTE (ready-to-eat); SNP (single nucleotide polymorphism); VTEC (verotoxigenic *Escherichia coli*).

tas de procesado de alimentos. Los métodos de tipificación molecular han confirmado que en las granjas de ganado vacuno lechero existen cepas de *L. monocytogenes* idénticas a las cepas responsables de casos esporádicos y epidémicos de listeriosis humana. El control de la listeriosis en las granjas de producción de leche ha de basarse fundamentalmente en el control del estiércol, los ensilados, la salud de los animales y las prácticas de ordeño.

Palabras clave adicionales: fuentes de contaminación ambientales; listeriosis; producción primaria lechera; reservorios animales; vehículos de transmisión alimentarios.

Introduction

Foodborne diseases cause a significant burden on public health and the economy. The World Health Organization estimates that unsafe food sickens one in three people every year worldwide, but the actual incidence of foodborne illness is probably much higher (Buckley & Reid, 2010). The majority of the pathogens causing microbial foodborne diseases are considered to be zoonotic. In addition, zoonoses include many diseases transmitted to humans by routes other than food (for example, by direct contact with animals), and are reported to affect over 380,000 European Union (EU) citizens each year (EFSA, 2011; Lahuerta *et al.,* 2011). In the EU, campylobacteriosis, salmonellosis and yersiniosis are the most commonly reported zoonotic infections in humans. Listeriosis and Verotoxigenic *Escherichia coli* (VTEC) infection are also important due to their severity. While some diseases have continued to decline (salmonellosis and yersiniosis) or remain stable (campylobacteriosis), others have increased considerably (listeriosis and VTEC infections) in the EU (Lahuerta *et al.,* 2011). The decline in foodborne diseases caused by particular pathogens can be related to both the introduction of and adherence to effective control measures to minimise the risk of infection.

European legislation establishes hygiene requirements for food producers and operators including farms, as part of a policy to introduce traceability throughout the food supply chain (EFSA, 2011). The absence of pathogens in livestock and the entire farm environment is not an achievable goal; however, risk reduction measures can be implemented on farms to minimise the spread of pathogens within a herd or flock (Duffy, 2009). Controlling zoonotic agents in animal reservoirs has the effect of reducing the challenges faced by food safety management systems during processing and, further along, minimising risks to the food supply chain. This especially relates to zoonotic hazards transmitted to humans through food consumption, such as *Salmonella* spp., *Listeria monocytogenes*, thermophilic *Campylobacter* spp. and enterohaemorrhagic *Escherichia coli*. All these pathogens constitute problems for the modern dairy farm. Although pasteurisation has significantly reduced the zoonotic risks from contaminated dairy products, outbreaks of illness continue to result from post-processing contamination and consumption of raw milk products (Van Kessel *et al.,* 2011).

Among these biological hazards, *L. monocytogenes* is particularly important because it causes a serious invasive illness (Swaminathan *et al.*, 2007). In the EU, human listeriosis was the first cause of death due to foodborne illness in 2009 (EFSA, 2011). Unfortunately, the decrease in the incidence of human listeriosis observed in 2007 and 2008 in the EU did not continue in 2009. In that year, the EU reported an increase of 19% in human cases of listeriosis compared to 2008 (EFSA, 2011; Lahuerta *et al.,* 2011). The increase primarily reflected a higher rate of listeriosis among elderly people (Allerberger & Wagner, 2010). The cause of this selective increased incidence was unknown. Many of these same epidemiologic features may also be occurring in the US (CDC, 2011).

The main route of transmission of human listeriosis is associated to consumption of contaminated food (ILSI Res Foundation-Risk Sci Inst, 2005; Swaminathan *et al.*, 2007), although other modes of transmission can occur, including transplacental mother-to-child transmission. Infection can also be transmitted, albeit very rarely, directly from infected animals to humans (*e.g.,* occupational infections), as well as between humans (Fig. 1) (Allerberger & Wagner, 2010). Due to transmission via contaminated food, *L. monocytogenes* is a major cause of massive product recalls worldwide. The bacterium is known to multiply at temperatures down to +2-4°C. The foods known to be associated with transmission of listeriosis are mostly ready-to-eat (RTE) foods that support the growth of *L. monocytogenes* (ILSI Res Foundation-Risk Sci Inst, 2005; EFSA, 2007). Dairy products and RTE meats are among the foods most often involved in listeriosis outbreaks. Although these foods are extensively tested for *L. monocytogenes* contamination, it is very difficult to eliminate completely this bacterium from the

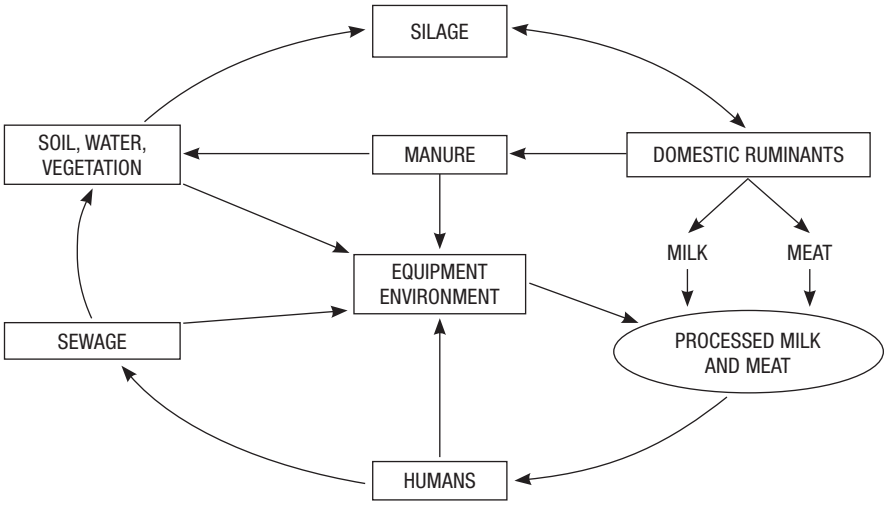

Figure 1. Potential routes of transmission of *Listeria monocytogenes* among habitats and host populations along the dairy food supply chain. Adapted from Ryser (2007), Swaminathan *et al.* (2007), and Allerberger & Wagner (2010). Oval indicates the area of greatest risk of *L. monocytogenes* multiplication associated to human exposure.

processing plant environment. Consequently, there is an increased risk of human illness if post-processing contamination occurs, because bacterial growth can occur between processing and consumption, even under refrigerated conditions.

Listeria monocytogenes in the dairy farm environment

Listeria monocytogenes is a ubiquitous organism that can be isolated from virtually all the environments along the food supply chain. In dairy farms, the principal places where *Listeria* can be found are soil, water and forage (Sauders & Wiedmann, 2007). Fox *et al.* (2009) determined the occurrence of *L. monocytogenes* in the dairy farm environment, and particularly in milking facilities, showing a relatively high prevalence (19%) of *L. monocytogenes* in 298 environmental samples from 16 farms in Ireland. They also detected a correlation between the level of hygiene standards on the farm and the occurrence of the pathogen in the environment (water, soil, silage, cow faeces, milk, etc.).

The high prevalence of *L. monocytogenes* in farm environments may favour the emergence of new more virulent strains. Transmission of these strains to both humans (Lopez *et al.*, 2006) and animals (Bundrant *et al.*, 2011) may occur through various routes, mainly through food or feed, but also directly from other infected humans or animals, as well as a consequence of the greater dissemination in the environment due to the spread of organic wastes/effluents from dairy farms (Swaminathan *et al.,* 2007; Van Kessel *et al.*, 2011) (Fig. 1). Using real-time polymerase chain reaction (PCR), Korthals *et al.* (2008) found that 28% of dust specimens from the rural environment were contaminated by *L. monocytogenes*, demonstrating its potential transfer by air, as well.

Water and soil contamination

Lyautey *et al.* (2007) reported a statistically significant link between the occurrence of *L. monocytogenes* in surface water samples and their proximity to an upstream dairy farm and degree of cropped land. The prevalence and level of *Listeria* spp. in livestock waste samples may be related to livestock husbandry and farm waste management practices (Hutchison *et al.*, 2005a). Sewage sludge is often used for land fertilisation, and if sludge and manure from farm animals contain *L. monocytogenes*, it can be spread to the soil and vegetation. In addition, soil can become contaminated through faecal shedding of infected domestic and wild animals.

Survival of *Listeria monocytogenes* in manure

Some pathogens that are shed in faeces may persist in the farm environment for extended periods. Aging

of manure tend to inactivate contaminating pathogens. Spreading fresh or minimally treated manure straight onto cropland increases the risk of *L. monocytogenes* transfer into the feed and food supply chains (Santorum *et al.*, 2007).

Although other zoonotic agents show higher prevalence when livestock groups include calves under 3 months of age, this is not the case for *L. monocytogenes* in which no differences in prevalence are found between waste samples from adult and younger calves that were still receiving milk (Hutchison *et al.*, 2005a).

Listeria can survive for up to six months in dairy slurry (Nicholson *et al.*, 2005; EFSA, 2009a), whereas it does not survive for more than one month in solid manure kept in dung heaps, in which temperatures rise to above 55°C (EFSA, 2009a; Kim & Jiang, 2010). A study by Jiang *et al.* (2004) reported the survival of *L. monocytogenes* in manure-amended soil for up to seven weeks.

Listeria monocytogenes can multiply in fresh dairy manure, but different factors affect growth potential and survival, including temperature, light intensity and moisture content (Kim & Jiang, 2010). In feedlot cattle manures, *L. monocytogenes* was found to be one of the most predominant pathogens with 10^2 to 10^7 genome copies per gram (dry weight) of manure recovered as assessed by quantitative PCR (Klein *et al.*, 2010).

High stocking density is usually important for some zoonotic agents, but it did not appear to influence either *L. monocytogenes* levels or prevalence in manure (Hutchison *et al.*, 2005a). However, spring cattle waste was more likely to contain *Listeria* spp., and special precautions should be taken before this manure is spread directly as an organic amendment in grasslands or croplands.

Animal wastes containing any form of bedding have lower prevalences and content of pathogenic *Listeria* spp. (Hutchison *et al.*, 2005a). Therefore, the use of bedding as manure treatment is a simple control measure that could be recommended to *L. monocytogenes* control at the farm level.

Ecology of *Listeria monocytogenes* and molecular subtyping methods

Source tracking of *L. monocytogenes* has proved to be difficult due to its ubiquitous nature and also because cases of listeriosis are generally sporadic. Nevertheless, substantial genotypic diversity appears to exist, which allows for precise source tracking in cases of clinical disease epidemics (Swaminathan *et al.*, 2007).

Listeria monocytogenes isolates are divided into 13 serotypes (Farber & Peterkin, 1991) but over 95% of isolates found either in human listeriosis cases or in foods belong to serotypes 1/2a, 1/2b and 4b (McLauchlin, 1990). Additionally, serotype 4b isolates have been implicated in the majority of listeriosis outbreaks worldwide (Kathariou, 2002). This fact limits the usefulness of serotyping in epidemiological investigations.

Genomic macrorestriction based on rare-cutting endonucleases followed by pulsed-field gel electrophoresis (PFGE) remains as the gold standard test for routine molecular subtyping of most clinically important foodborne pathogens, including *L. monocytogenes* (Brosch *et al.*, 1996; Graves & Swaminathan, 2001; Gerner-Smidt *et al.*, 2006; Fugett *et al.*, 2007; Ortiz *et al.*, 2010). Analysis of data using bioinformatics software clusters isolates according to banding pattern similarity in a genetic distance tree known as a dendrogram (Nightingale, 2010) (Fig. 2). PFGE is a highly standardised but labour-intensive method.

Multi-Locus Sequence Typing (MLST) is a DNA sequence-based evolution of Multi-Locus Enzyme Electrophoresis (MLEE). MLST is just as applicable to global epidemiology as MLEE, while providing better discriminatory power and ensuring inter-laboratory data-sharing (Chan *et al.*, 2001). Unfortunately, it is time-consuming and expensive, and a common MLST test suitable for *L. monocytogenes* does not exist for epidemiological studies (Revazishvili *et al.*, 2004; Nightingale *et al.*, 2010).

For molecular subtyping of *L. monocytogenes*, real-time PCR technology has been applied to single nucleotide polymorphism (SNP) typing based on MLST (Honjoh *et al.*, 2008). However, rapid and reliable methods for SNP typing of *L. monocytogenes* are not available (Nightingale, 2010).

Molecular subtyping work has indicated that subpopulations within *L. monocytogenes* may have adapted to (i) colonise specific environmental niches, (ii) infect specific mammalian hosts, or (iii) possess different virulence characteristics (Nightingale, 2010).

In spite of its ubiquity, some subtypes of *L. monocytogenes* can be unique to specific niches such as specific foods (Fugett *et al.*, 2007), and some can persist in particular processing environments for extended periods (Ortiz *et al.*, 2010). The processing environ-

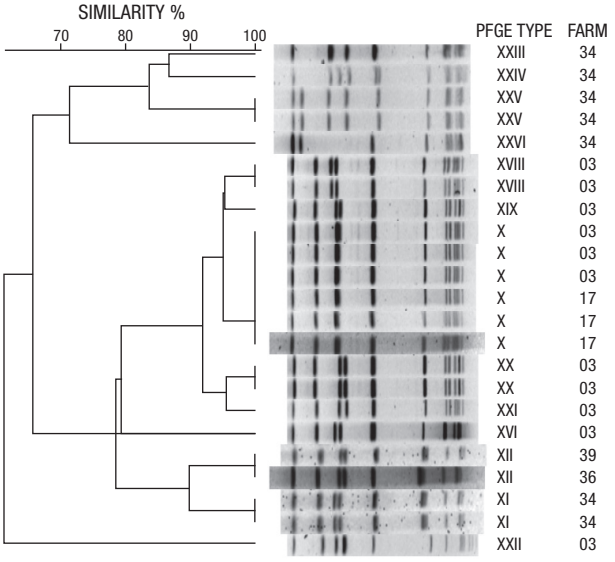

Figure 2. *Listeria monocytogenes* diversity in five unrelated dairy farms in the Cantabria region of Northern Spain. This example of a dendrogram of the *Asc*I PFGE (pulsed-field gel electrophoresis) patterns of 23 isolates shows two farms (03 and 34) with a highly polymorphic *L. monocytogenes* population (five and seven different pulsotypes, respectively), while other farms (17, 36, and 39) contained a single pulsotype. Pulsotypes X and XII were shared between some farms (03 and 17, or 36 and 39, respectively) unrelated epidemiologically and placed at very distant locations (López V. *et al.,* unpublished results).

ment is the main origin of contamination of processed food with *Listeria*. Contamination of the processing environment results from on-farm *Listeria* contamination from silage, dairy cattle and raw milk (Arimi *et al.*, 1997). Processors must specifically enforce restrictions and procedures that prevent cross-contamination between the dairy farm environment and the processing environment (Ho *et al.*, 2007b) as well as between raw and processed food.

Listeria monocytogenes is a genetically diverse species containing four genetic lineages or divisions (I-IV). Two of these lineages (I and II) cause predominantly human infections, whereas lineages III and IV have never been involved in human disease, being responsible of clinical listeriosis in animals (Jeffers *et al.*, 2001; Nightingale, 2010). The explanation of this finding is unknown, since both lineages share most of known virulence factors.

Most *Listeria monocytogenes* human isolates correspond to lineage I, despite the more frequent exposure to lineage II isolates, which are commonly isolated from environmental sources. Epidemiological studies have also identified three groups of highly clonal lineage I, serotype 4b strains, termed epidemic clones, which have been associated to listeriosis outbreaks worldwide (Kathariou, 2002). Molecular subtyping has allowed the identification of these human strains among dairy farm-associated isolates (Ho *et al.*, 2007a). Borucki *et al.* (2004) found that 23% of human strains isolated sporadically were identical to one or more farm isolates. Dairy farms thus appear to be a significant source of human strains of *L. monocytogenes* (Nightingale *et al.*, 2004), although the degree of diversity of *L. monocytogenes* can be very different among different farms (Esteban *et al.*, 2009; Fox *et al.*, 2011; Latorre *et al.*, 2011). Figure 2 shows PFGE characterization of a collection of *L. monocytogenes* isolates originating from different farms located in the Cantabria region of Northern Spain. As it can be seen, some PFGE patterns were common to multiple farms located in different geographical areas, while some farms had unique PFGE patterns. This variability could be related to differences in farm management practices (Nightingale *et al.*, 2005).

Listeria monocytogenes in dairy cow feed

Diet and faecal shedding of *Listeria monocytogenes*

Various reports support the hypothesis that the diet of healthy animals may affect excretion of *L. monocytogenes* considerably. Animals fed entirely on hay or manufactured feedstuffs usually excrete low or undetectable levels of *Listeria*, while animals fed on silage tend to excrete more frequently the microorganism (Fenlon *et al.*, 1996). Nevertheless, hay and concentrates can also be sources of *L. monocytogenes* in barns (Husu *et al.*, 1990). For instance, Mohammed *et al.* (2009) found twice the incidence of *L. monocytogenes* in drinking troughs, feeders and cow beds (66%, 65%, and 55%, respectively) than in silage (30%). Although pathogen control procedures such as heat treatment or the addition of chemicals can be used in the feed manufacturing process, Molla *et al.* (2010) strongly question the conventional wisdom that processed feed is not a source of bacterial contamination.

Grazing ruminants also results in a lower prevalence of *L. monocytogenes* in faecal samples in comparison

to silage-fed animals (Sauders & Wiedmann, 2007; Wesley, 2007). Consumption of contaminated feed can lead to contamination in the herd, but different husbandry practices may account for reported variations in the incidence of healthy carriers (Van Kessel *et al.*, 2011).

Diet and animal listeriosis

Listeriosis in cattle is closely linked to consumption of contaminated silage (Fenlon *et al.*, 1996), but the pervasive nature of the bacterium can make it difficult to identify the source of infection. Animal listeriosis cases sometimes occur in animals not fed silage, and environmental sources have been speculated to be responsible for at least some of these cases (Sauders & Wiedmann, 2007). The potential for contamination at the feed mill exists through a variety of means such as contaminated ingredients and the presence of birds and rodents; consequently, contamination of processed animal feed may also be a source of animal infection (Van Kessel *et al.*, 2011).

Contamination of crops used in the production of animal feed

Forage crops can become contaminated through the practice of fertilising land with manure and sewage sludge or through animal faecal shedding (Sauders & Wiedmann, 2007). *L. monocytogenes* can persist in grasslands, which points to animal feeding as the way of recirculation in farms (Jiang *et al.*, 2004) (Fig. 1). Good management of cropland with respect to the spread of animal manure is critical in maintaining control of *L. monocytogenes*.

The presence of this pathogen in grasses for grazing or harvesting for use as fresh fodder has not been thoroughly investigated. Hutchison *et al.* (2005b) studied the decline of pathogens in fescue plots, finding that 128 days were required for the complete decline of *L. monocytogenes* levels.

Contamination within silage bales

Feeding dairy cattle with improperly fermented silage was found to be significantly associated with contamination of bulk milk with *L. monocytogenes* (Sanaa *et al.*, 1993). *Listeria* spp. have frequently been isolated from poorly fermented silages (*i.e.,* pH > 4) (Sauders & Wiedmann, 2007; Vilar *et al.,* 2007). If silage is contaminated, *L. monocytogenes* can survive for a long period of time. For example, Dijkstra (1971) reported that *L. monocytogenes* could survive for 4-6 years in naturally contaminated silage. Additionally, post-harvest processing of grass can markedly affect the quantity of *Listeria* present (Sauders & Wiedmann, 2007).

The most practical way to decrease the survival rate of *L. monocytogenes* in grass silage is producing intensively fermented silages and storing these for more than 30 days (Pauly & Tham, 2003). However, under aerobic conditions, sufficient fermentation to drop the pH to below 4.5 may not occur, or growth of aerobic microorganisms such as yeasts and moulds may be initiated; this tends to increase the silage pH to favourable levels for the growth of *L. monocytogenes* (Ivanek *et al.*, 2006).

Listeria monocytogenes in dairy cows

Healthy animals

The ways that dairy cattle are raised vary greatly, and variations in scale of production and management systems explain that many different factors can impact the carriage and faecal shedding of *L. monocytogenes* (Duffy, 2009). Differences in sampling and analytical methods can also give rise to variability in *L. monocytogenes* prevalence. Overall prevalence below 10% is commonly reported when individual animals are sampled from several (Husu, 1990) or only one herd (Vilar *et al.*, 2007). However, prevalence values above 20% for faecal shedding of *L. monocytogenes* have been reported in healthy cattle from several herds (Nightingale *et al.,* 2004; Esteban *et al.,* 2009). Within-herd prevalence can vary from 0 to 100% (Ho *et al.*, 2007a; Esteban *et al.*, 2009). In a longitudinal study of dairy herds, *L. monocytogenes* was isolated regularly from faecal samples in one out of three herds, with a within-herd prevalence ranging from 0% to 25.5% on a farm that was regularly found to be positive for *L. monocytogenes* (Pradhan *et al.*, 2009).

The duration of faecal shedding is not well understood, but it could be dependant on both silage contamination and exposure to stress (Fenlon *et al.*, 1996;

Ivanek *et al.*, 2006). Seasonal variations in faecal shedding could be associated with increased silage feeding during the cold season (Nightingale *et al.*, 2005; Ryser, 2007). Dairy farming related stress factors may lead to increased occurrence of *L. monocytogenes* in cows. For example, it has been reported that transporting live animals over long distances (> 100 km) significantly increased the level of *Listeria* excretion (Fenlon *et al.*, 1996). In addition, the stress caused increased peristaltic movements of contents along the bowel, resulting in a higher rate of *L. monocytogenes* excretion, which could increase the spread of listeriosis and/or cross-contamination among the animals (EFSA, 2009a).

Infected animals

Husbandry management practices, animal health and hygiene, and quality and storage conditions of feedstuffs are associated with ruminant listeriosis and faecal shedding of *L monocytogenes* (Nightingale *et al.*, 2005; EFSA, 2009a). Clinical disease is rare; the overall incidence of clinical listeriosis in dairy cattle is 4 per 1,000 animals and year (Erdogan *et al.*, 2001; Wesley, 2007). However, in spite of antibiotic treatment and correction of acid-base alteration, case fatality is about 30 to 50% (EFSA, 2009b).

Zoonotic pathogens may contaminate bulk milk if milk is obtained from cows with udder infections (mastitis). Some mastitis cases have been associated with infection by *L. monocytogenes* (Winter *et al.*, 2004; Wesley, 2007). Nevertheless, the primary source of *Listeria* contamination in bulk tanks is faecal matter that enters the milking system during the milking process.

In general, the great majority of strains of *L. monocytogenes* are susceptible to the majority of the antimicrobials used currently in veterinary and human listeriosis therapy (Morvan *et al.*, 2010; EUCAST, 2011). The low incidence of antibiotic resistance in *L. monocytogenes* is a distinctive feature of the microorganism, which is also observed in the food production chain (Lungu *et al.*, 2011). Nevertheless, there still exists the risk of foodborne infections due to resistant *L. monocytogenes* strains isolated from livestock products and their related environments. For instance, a recent analysis of 202 food and environmental isolates revealed 4 strains with acquired resistance to erythromycin, tetracycline-minocycline or trimethoprim (Granier *et al.,* 2011).

Listeria monocytogenes in dairy cow-derived foods

Both raw milk and raw meat can be contaminated with *L. monocytogenes*. Consequently, *L. monocytogenes* can be found in contaminated dairy cow-derived foods which were not subjected to a process that would kill the organism. Such products include raw fermented meats and cheeses made from raw milk. The bacterium has also been found in processed foods, such as dairy products made from pasteurised milk and lunch meat that were contaminated after processing.

Raw milk

In most surveys of bulk tank raw milk, *L. monocytogenes* was detected in 1-16% of samples (Ryser, 2007; Vilar *et al.,* 2007; Mohammed *et al.,* 2009; Fox *et al.*, 2011; Van Kessel *et al.*, 2011). This variability might reflect differences in farm management practices, geographical locations, yearly seasons, sampling or analytical methods, etc. Antognoli *et al.* (2009) reported that geographical location and herd size affected rates of *L. monocytogenes* contamination in bulk milk, with the risk of contamination increasing according the herd size. Cleanliness in the animal exercise area was also related to low levels of bulk milk contamination (Sanaa *et al.*, 1993).

Infected cows may shed *L. monocytogenes* in milk (Winter *et al.*, 2004), being poor milking and barn hygiene important risk factors for raw milk contamination (Sanaa *et al.*, 1993; Van Kessel *et al.*, 2011). Hygienic milking practices involving udder and teat cleaning and disinfection can help reduce milk contamination.

Once introduced into the milking parlour or equipment, *L. monocytogenes* can readily colonise these moist environments. The capacity of this bacterium to grow at low temperatures explain that refrigerated storage, while useful in decreasing the growth of the microorganism, does not completely avoid bacterial multiplication.

Farms that use a milking parlour have significantly lower odds of *L. monocytogenes* contamination of milk compared to farms that use tie-stall systems (Hassan *et al.*, 2001; Vilar *et al.*, 2007). According to Hassan *et al.* (2001), *L. monocytogenes* can be present in milk filters on dairy farms, as they found that 12.6% of the in-line milk filters tested positive. In a different report,

Latorre *et al.* (2011) presented evidence that a source of *L. monocytogenes* contamination was milking equipment, since 67.6% of in-line milk filter samples and 19.7% of bulk tank milk samples were positive for *L. monocytogenes*. Predominant and persistent strains of *Listeria* might be more adapted to the specific ecological environment of the milking system than strains that appear only sporadically (Vazquez-Villanueva *et al.,* 2010; Latorre *et al.,* 2011).

Processing environment

In all food supply chains a contaminated processing environment is the main source of foodborne *L. monocytogenes* (Swaminathan *et al.,* 2007). Some specific subtypes of *L. monocytogenes* can also persist for extended periods in many different food processing environments (Ortiz *et al.,* 2010), including dairy food processing plants (Vazquez-Villanueva *et al.*, 2010; Latorre *et al.,* 2011). In the dairy plant, *Listeria* can persist in a variety of sites, although it is most frequently found in moist environments or areas with condensed or standing water or milk, including drains, floors, coolers, conveyors and case washing areas (Arimi *et al.,* 1997; Ho *et al.,* 2007b; Fox *et al.,* 2009).

Dairy products

Contamination of milk or dairy products with *L. monocytogenes* is a cause for great concern because multiple human listeriosis outbreaks have been linked to contaminated milk or dairy products since the early 1980s (Swaminathan & Gerner-Smidt, 2007). Recent listeriosis outbreaks linked to dairy products (Fretz *et al.*, 2010; Johnsen *et al.*, 2010; Koch *et al.*, 2010) serve as a reminder of the importance of controlling this pathogen in the dairy industry.

Milk used in the production of raw milk cheeses must be subjected to routine monitoring for *Listeria* spp. However, contamination seems in most cases to come from the production environment (cross-contamination), rather than from the raw milk itself; consequently, producing safe raw milk cheese depends upon the conditions and practices under which it is produced (Ho *et al.*, 2007b; Fox *et al.*, 2011). Soft cheeses made from raw milk under uncontrolled conditions are considered to be high-risk foods for susceptible individuals. Nevertheless, EU data for 2009 showed that *L. monocytogenes* was most often detected in soft and semi-soft cheeses made from pasteurised milk (EFSA, 2011). In addition, it has been demonstrated in recent outbreaks due to *Listeria*-contaminated cheeses, that products made from pasteurised or heat-treated milk were more affected than those made with raw milk (Johnsen *et al.*, 2010; Koch *et al.*, 2010).

Beef

A significant portion of cows on a dairy farm are culled; culled dairy animals thus represent an important proportion of the beef market. Culled animals harbouring zoonotic pathogens or raised on infected farms can therefore pose a risk for introducing infected products into the food supply (Troutt & Osburn, 1997). *L. monocytogenes* is a potential microbiological hazard for foodborne illness from culled dairy cows. However, the prevalence of the pathogen in cattle and beef varies considerably from survey to survey.

Transmission of *L. monocytogenes* and other pathogens through the consumption of beef can result from microbial cross-contamination from gastrointestinal sources during slaughter, dressing and further processing. Obtaining comprehensive information on carcass contamination levels is an essential first step in establishing food safety objectives for a particular beef production system (Hathaway, 1997). In general, contamination of whole carcasses with *L. monocytogenes* does not occur at a high rate. According to a literature survey carried out by Rhoades *et al.* (2009), the mean prevalence of *L. monocytogenes* on hides was 12% (10-13%). Hide data may be an indication of the regional prevalence, as different results have been found for two distantly located beef processing plants (0.8 versus 18.7%) (Rivera-Betancourt *et al.*, 2004). Low rates of contamination of pre-evisceration carcasses reported for the same two plants (0.0 versus 1.1%) are more likely related to their harvest practices and procedures (Rivera-Betancourt *et al.*, 2004). Other authors have reported seasonal variations in the *Listeria* prevalence data collected from pre-evisceration carcasses of four beef processing plants, ranging from undetectable in warm season to as high as 71% during cooler weather conditions (Guerini *et al.,* 2007).

In spite of this, samples of minced beef have shown high *L. monocytogenes* rates, demonstrating that processing can significantly increase the level of con-

tamination (Fenlon *et al.,* 1996). According to Rhoades *et al.* (2009), the mean prevalence of *L. monocytogenes* in raw beef products is 10% (1.6-24%). Thus, *Listeria* spp. are frequently present in all types of raw meat. Raw meats and meat products may therefore constitute a risk of cross-contamination to the food processing environments and other foods processed in the same environment. Controls within the slaughterhouses and meat processing rooms are not usually targeted at controlling *L. monocytogenes* specifically, but raw meat destined for products such as fermented meat where no cooking process takes place, should be monitored for avoiding *L. monocytogenes* contamination.

Reducing all potential sources for faecal contamination of products to the maximum extent is the most important factor in achieving desired food safety objectives for fresh beef. Even the presence of *L. monocytogenes* in the gastrointestinal tract contents is likely to have a significant effect on subsequent contamination levels of beef carcasses (Hathaway, 1997). Dairy cows represent a suitable reservoir for *L. monocytogenes*; hence a reduction of the intestinal carriage rate in livestock herds would contribute to reducing the contamination risk at the slaughterhouse (Esteban *et al.*, 2009; Adam & Brülisauer, 2010).

Conclusions and recommendations

The main sources of *L. monocytogenes* present on dairy farms are soil, effluents, water, manure, and improperly fermented silage. Dairy cows represent a suitable reservoir for the bacterium, and raw milk and beef are the main vehicles for its transmission from farms to humans (Fig. 1). The combination of a high rate of bacterial survival in the environment and the asymptomatic course of infection in animals often leads to problems with long-term contamination of animals and the farm premises. Cycling of the pathogen between animals and their surrounding environment is essentially continuous and difficult to break (Van Kessel *et al.,* 2011). However, the *L. monocytogenes* load within habitats and host populations as well as the rate of transmission among them could be reduced (Ivanek *et al.*, 2006). Pre-harvest (on-farm) control of listeriosis should be based on dairy farming practices that are beneficial for both the welfare of the dairy cow and food safety for both milk and beef products (EFSA, 2009a; Buckley & Reid, 2010), including the following:

I. Manure treatment to inactivate *L. monocytogenes* and grassland or cropland management with respect to the spread of animal manure as an organic amendment.

II. Control of silage fermentation and feed quality in regards to *L. monocytogenes*. The most practical way to decrease the survival rate of *L. monocytogenes* in grass silage is to produce silages intensively fermented and store these for more than 30 days.

III. Hygienic husbandry practices, effective herd health management, maintaining low livestock densities, utilizing confined animal feeding operations and management for preventing animal stress. Reducing the intestinal carriage rate would contribute to reducing the contamination risk along the milk and beef chains.

IV. Hygienic preparation of animals for slaughter, hygienic milking, and preventive monitoring of bulk milk for *L. monocytogenes*. Reducing all potential pathways for faecal contamination of products to the maximum extent practicable is the most important factor in achieving desired food safety objectives for raw milk and beef.

Adequate studies on the effects of these recommended dairy farming practices should fill the lack of knowledge on the efforts needed to control the bacterium on farms.

Further insight into specific practices to reduce the *L. monocytogenes* load on the dairy farm and its impacts upon production will be essential to provide consumers with greater assurances of the safety of their food.

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