Prevalence and survival of *Listeria monocytogenes* in various types of cheese – A review

Review of *Listeria monocytogenes* in cheese

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ABSTRACT

Since the publication of Regulation (EC) N°2073/2005, RTE food allowing the development of *Listeria monocytogenes*, including cheese, have to be exempt of the pathogen in 25g. This review was carried out in order to gather studies on the prevalence of the pathogen in various types of cheese in Europe, while also including data about the situation in other continents. Given that Regulation (EC) N°2073/2005 distinguishes cheeses allowing or not the survival of *L. monocytogenes* based on pH and water activity (a_w), the review also focuses on the determinants of this growth/no growth in the same types of cheese.

Keywords: *Listeria monocytogenes*; Cheese; Product safety.

INTRODUCTION

Although listeriosis is not one of the most commonly occurring foodborne diseases, the increasing number of reported cases has led to a growing interest from scientists and authorities (Cabedo *et al.* 2008). Listeriosis, caused by the pathogenic bacterium *Listeria monocytogenes*, is generally a benign disease for immunocompetent people. Nevertheless, it can be deleterious for some of the population, including neonates, elderly people, pregnant women and immunocompromised patients, as well as people suffering from diabetes or liver and renal diseases (Doorduyn *et al.* 2006; Buchanan *et al.* 2017). Individuals aged over 65 years represent the majority of the reported cases in Europe (EFSA 2016). For this age group, occurrence of listeriosis is two times higher for males than for females (Takkinen 2017). Death linked with listeriosis occurs in around 20.0-30.0% of cases for patients from vulnerable groups (Sanaa *et al.* 2004). In 2015, 2206 cases of listeriosis were monitored in the European Union, causing 270 deaths. Long-term data highlight an increase in reported cases during the last decades (EFSA – ECDC 2016).

A huge part (99%) of human listeriosis is attributable to food consumption (Takkinen 2017). Various types of food that already caused listeriosis outbreaks have clearly been identified, including cheese.

L. monocytogenes represents a noticeable threat in food because of its ability to survive under an impressive diversity of conditions. On the one hand, the bacterium is known to be psychrotrophic, i.e. able to grow below 7°C. Some strains of *L. monocytogenes* could be able to survive at temperatures a few degrees under the freezing point, but without proliferation (Carpentier and Cerf 2011). On the other hand, the pathogen is also able to multiply at temperatures up to 45°C, with optimal growth between 30 and 37°C (Saltijeral *et al.* 1999). *L. monocytogenes* also tolerates a wide pH range. For instance, Carpentier and Cerf (2011) reported that the bacterium can grow in environments with a pH between 4.6 and 9.5. Therefore, with respect to pH level, there are many foods that seem susceptible to the multiplication of *L. monocytogenes*. Tolerance of *L. monocytogenes* to pH is also linked with the water activity (a_w). It is commonly admitted that the bacterium is not capable of growth at a a_w lower than 0.92 (Nolan *et al.* 1992).

In addition, *L. monocytogenes* is also halotolerant, being able to grow in concentration of salt up to 10.0% (Ferreira *et al.* 2014). Bacteria of the genus *Listeria* are also facultative anaerobic. They are thus able to grow under low levels of oxygen and under high carbon dioxide conditions (Gandhi and Chikindas 2007; Lungu *et al.* 2009). Obviously, tolerance to salt, temperature, low oxygen concentrations, pH and a_w varies among the strains

(Gandhi and Chikindas 2007).

With respect to these parameters, the European Commission (EC) has established criteria in order to define the acceptability of a ready-to-eat (RTE) food. The latter are based on available data on presence/absence or enumeration of *L. monocytogenes* throughout the food supply chain for a given type of food.

Regulation (EC) N°2073/2005 mentions that the bacterium cannot grow in food under a pH of 4.4 or an aw of 0.92. Moreover, a combination of a pH under 5.0 and a a_w lower than 0.94 could also be inhibitory. If these criteria are not met, the food is considered susceptible to the multiplication of *L. monocytogenes*. In this case, European Commission demands a total absence of *L. monocytogenes* in 25 g of the product when food leaves the producer’s control. An alternative criterion can be applied when the producer can demonstrate that during the whole shelf-life, the contamination will never be higher than a threshold value of 100 cfu/g of product (EC 2005).

As a RTE-food, cheese has to comply with Regulation (EC) N°2073/2005. This review will focus on the occurrence of the pathogen in various types of cheese worldwide since the publication of Regulation (EC) N°2073/2005. The paper will try to put this prevalence in relation with the physico-chemical conditions (pH and a_w) met in these cheeses and with the survival of the pathogen during process, ripening and storage. Articles on the occurrence of *L. monocytogenes* published within the period 2005-2018 were thus gathered using Google Scholar, with English and French keywords.

OCCURENCE OF *LISTERIA MONOCYTOGENES* IN CHEESE

A diverse variety of cheeses is now available on the market (Little *et al.* 2008). Therefore, classification of these products is extremely difficult. No consensus has been established yet, and authors are inclined to use different vocabulary and criteria to describe thecheeses, including maturation characteristics or moisture content (Martinez-Rios and Dalgaard 2018). Several parameters must be taken into account to define a cheese, including the origin of the milk (bovine, caprine, ovine, etc.), milk treatment (raw, thermized, pasteurized or microfiltered milk), milk homogenization, the use of a microbial starter and/or rennet to obtain the curd, cooking of the curd, moulding, pressing, method for salting, addition of spices or other specific ingredients and conditions of ripening (relative moisture, temperature, time, maturing medium, rind washing, etc.). All these factors have an impact on the final properties of the cheese. According to the Codex Alimentarius, a texture-based classification should be established following the percentage of moisture on a fat-free basis (MFFB). A decrease in MFFB results in a distinction between soft, semi-soft, semi-hard and hard cheeses (CAC 2013). This review will consider three main categories, namely fresh cheeses, which should be classified apart from other soft cheeses due to important manufacturing differences, soft and semi-soft cheeses, and semi-hard and hard cheeses.

Two types of analyses are generally performed in order to investigate the occurrence of *L. monocytogenes* in cheese: presence/absence studies in 25g of product (qualitative data) and enumeration of the bacterium (quantitative data).

Fresh cheeses

Following the definition of Martinez-Rios and Dalgaard (2018), fresh cheeses are "curd-style cheeses which do not undergo any ripening". Manufacture generally involves lactic curdling and only a small concentration of rennet. Fresh cheeses can be moulded or not moulded. Fresh cheeses are really popular in Latin America and in the south of the United States (Soto Beltran *et al.* 2015). Table 1 summarises the studies on the presence of *L. monocytogenes* in 25g of various fresh cheeses. The prevalence of contaminated samples substantially varies among studies and countries. Many of the published articles deal with Hispanic-style fresh cheese (or Latin-style fresh cheese), such as Minas Frescal in Brazil or Queso Fresco in Mexico. The occurrence of contamination of Latin-style fresh cheese ranges from 0.0 to 37.5% (Kinde *et al.* 2007; Moreno-Enriquez *et al.* 2007; Brito *et al.* 2008; Cabedo *et al.* 2008; Torres-Vitela *et al.* 2012; Soto Beltran *et al.* 2015; Reda *et al.* 2016). *L. monocytogenes* can reach levels higher than 10^4 cfu/g in Minas Frescal (Brito *et al.* 2008). In Europe, the bacterium has also been isolated from unspecified fresh cheeses from Italy(Rantsiou *et al.* 2008; Parisi *et al.* 2013). In Austrian fresh cheeses collected from retail stores, a percentage of contamination comparable to Latin-style fresh cheese has been observed (Wagner *et al.* 2007). Similar findings have also been reported for white cheese from Turkish bazaars (Arslan and Özdemir 2008).

The use of raw milk is often cited as a major factor for the contamination with *L. monocytogenes* in dairy products. According to Federal Agency for the Safety of the Food Chain (FASFC) (2011), the bacterium was present in 2.2-10.2% of raw milk samples in Europe. However, milk heat treatment was sometimes insufficient to guarantee the absence of *L. monocytogenes* in cheese. Indeed, at least one study reported that fresh cheeses made from pasteurized milk carried the pathogen (Rosas-Barbosa *et al.* 2014). Parisi *et al.* (2013) found that the totality of 20 raw milk samples were free of the pathogen, but cheeses processed with milk from the same dairies were contaminated. This can be attributed to post-processing contamination, which represents the major cause of cheese contamination with *L. monocytogenes* (Schvartzman *et al.* 2011; Ibarra-Sànchez *et al.* 2017). In factories, the pathogen has already been isolated from floors, drains, conveyor belts, crates, brine and workers equipment (Larson *et al.* 1999; Gudbjörnsdóttir *et al.* 2004; Pintado *et al.* 2005; Fox *et al.* 2011; Osaili *et al.* 2012; Parisi *et al.* 2013; Ferreira *et al.* 2014; Rosas-Barbosa *et al.* 2014, Ibarra-Sànchez *et al.* 2017).As highlighted in Table 1, *L. monocytogenes* can be isolated from cheeses taken at various points of distribution.

Handcrafted fresh cheeses seemed to be more frequently contaminated than cheeses from larger factories (Ibarra-Sànchez *et al.* 2017). Globally, an improved hygiene quality in relation with the level of industrialization can be observed.

Generally, samples with contamination higher than 100 cfu/g are scarce (Rantsiou *et al.* 2008). From Table 1, it can be observed that studies enumerating the pathogen are really scarce. It would however be highly interesting to focus on the levels of the pathogen to know the potential risk related to the consumption of such products.

The presence of *L. monocytogenes* in some fresh cheeses is not surprising. Unfortunately, only few studies evaluated a_w and pH of the analysed samples. Nevertheless, physico-chemical properties of fresh cheese are generally ideal for the growth of the bacterium: high moisture content (>50%), average pH higher than 6 and relatively low salt content (0.85%) (Olarte *et al.* 1999; USDA-FSIS 2003; Brito *et al.* 2008, Ibarra-Sànchez *et al.* 2017). Aside from a Swedish study, all cheeses from Table 1 with an average pH value higher than 4.4 were found to be contaminated with *L. monocytogenes* (Rosengren *et al.* 2010; Torres-Vitela *et al.* 2012; Soto Beltran *et al.* 2015). As a consequence, several large scale listeriosis outbreaks due to the consumption of fresh cheese have been reported in the literature. Indeed, 12 outbreaks linked with fresh cheese have been identified since 2005, for a total of 139 cases, and causing at least 25 deaths (Martinez-Rios and Dalgaard 2018) Due to these outbreaks, it is recommended in the United States that pregnant women avoid the consumption of fresh cheese (Torres-Vitela *et al.* 2012). As highlighted by Martinez-Rios and Dalgaard (2018), EFSA should analyse more fresh cheese samples while determining the prevalence of *L. monocytogenes* in European cheeses. Indeed, only 2% of fresh cheeses are included in their panel.

However, some fresh cheeses are less susceptible to *L. monocytogenes* survival. Indeed, some exceptions are reported, such as Ayib, a cottage cheese from Ethiopia. Ayib is much more acidic than the fresh cheeses previously discussed, with an average pH of 4. A study on Ayib reported only 1.0% of contaminated samples (Gebretsadik *et al.* 2011). A Cottage cheese from Egypt, with a pH around 4.2, was free of *L. monocytogenes*, as well as Kareesh cheese, another Egyptian fresh cheese (Ismaiel *et al.* 2014; Reda *et al.* 2016). Further, it can be expected that Walloon Maquee, a high moisture acidic fresh cheese from Belgium with a low pH, is less susceptible to *L. monocytogenes* contamination and growth. Studies on these acidic cheeses are rarer because it is expected that their pH prevents survival of the bacterium. Nevertheless, data from Table 1 demonstrate that a pH under the key value of 4.4 can sometimes be insufficient to prevent survival of the bacterium (El Marrakchi *et al.* 1993; El Marnissi *et al.* 2013).

Although they require a heat treatment during processing, Burrata, Cream cheese, Ricotta and Mozzarella comply with the definition of fresh cheeses. These product present physico-chemical conditions favourable for the multiplication of *L. monocytogenes*. In two studies performed by Di Pinto *et al.* (2010) and Dambrosio *et al.* (2013), respectively none of 186 Mozzarella and of 404 Burrata samples were contaminated. During Burrata and Mozzarella manufacture, the curd is dipped in hot water (80-90°C) before the *pasta filata* process. This step is called thermoplastification (Ibarra-Sànchez et al 2017). This treatment is sufficient to kill pathogens originating from milk, but the subsequent steps present possibilities for exogenous contamination to occur. Cream cheese was more susceptible to listerial contamination; nearly 2.0% of the 108 samples being contaminated (Di Pinto *et al*. 2010). This type of cheese also undergoes a heat treatment after curdling, but at a lower temperature, around 55°C. This seems to be insufficient to kill all the *L. monocytogenes* bacteria. In addition to that, probability of post-processing contamination is again well real. Requeson, a whey cheese from Mexico, presented a prevalence of 6.7% (Rosas-Barbosa *et al*. 2014). On the opposite, 30 samples of Ricotta, another whey cheese, were free of *L. monocytogenes* (Parisi *et al*. 2013). Requeson and Ricotta are however cooked up to 80-90°C during process. Again, post-processing steps play thus a major role in the contamination with *L. monocytogenes* in the product (Santorum *et al*., 2012).

Soft and semi-soft cheeses

Soft cheese is manufactured without pressing, with a relatively short ripening time, and has a creamy texture. In contrast to fresh cheese, soft cheese can be manufactured from enzymatic or lactic curd. Soft cheeses can be divided into two main categories. On the one hand mold-ripened soft cheeses have a typical white rind, composed of *Penicillium camemberti* and/or *Geotrichum candidum*. Camembert and Brie are well known mold-ripened soft cheeses. On the other hand, smear-ripened soft cheeses, i.e. washed rind or bacterium-ripened soft cheeses, generally present red rinds. During ripening, they are brushed or washed with salted water containing or not specific starters. The rind is generally composed of coryneform bacteria, now presented as *Actinobacteria* (Rea *et al.* 2007). Pressing is part of the production process of semi-soft cheese, but due to a limited ripening time, it remains creamy and foldable. A wide variety of semi-soft cheeses can be found in European countries, including Saint-Paulin and Reblochon. Blue-veined cheeses, containing *Penicillium roqueforti* in their core, are considered as soft or semi-soft cheeses in this review.

The diversity of soft and semi-soft products and processes is much greater than for fresh cheeses. In a study conducted in Belgium, soft and semi-soft cheeses had a pH range from 4.16 to 7.47, and an a_w from 0.93 to 0.99 (Lahou and Uyttendaele 2017). However, the majority of soft and semi-soft cheeses presents physico-chemical conditions that are favourable for the survival and growth of *L. monocytogenes*, in terms of both pH and a_w.

Table 2 presents studies published since 2005 on the occurrence of the bacterium in soft and semi-soft cheeses. Presence was always determined in 25g of sample. Several studies have revealed that soft cheeses, mainly mold- and smear-ripened cheeses, are the most problematic in terms of *L. monocytogenes* contamination (Choi *et al.* 2016; EFSA– ECDPC 2016; Lahou and Uyttendaele 2017). Smear-ripened soft cheese is more likely to be contaminated with the pathogen, due to the high amount of post-processing handling, including rind washing and cheese turning (Izquierdo *et al.* 2009). In Germany in 2000, 20 tons of red smear cheese were recalled (Rudolf and Scherer 2001). In 2015, such a recall also occurred in Belgium with Herve cheese, another smear-ripened soft cheese (Lahou and Uyttendaele 2017). Finally, contaminated Taleggio, an Italian smear-ripened soft cheese, was responsible for an outbreak in Italy in 2011 (Amato *et al.* 2017).

Like for fresh cheeses, it appears that the occurrence of *L. monocytogenes* in soft and semi-soft cheeses is quite variable. Globally, the majority of the studies reported percentages of incidence between 0.0 and 14.0% (Vitas *et al.* 2004; Manfreda *et al.* 2005; Colak *et al.* 2007; Wagner *et al.* 2007; Cabedo *et al.* 2008; Prencipe *et al.* 2010; Angelidis *et al.* 2012; Osaili *et al.* 2012; Rakhmawati *et al.* 2013; Iannetti *et al.* 2016; Ahmed *et al.* 2017; Gelbíčová *et al.* 2017; Lahou and Uyttendaele 2017). However, some of them reported extremely high proportions of contamination among samples. The highest proportion of contaminated samples was 46.0% in Portuguese Castelo Branco (Pintado *et al.* 2015). Filiousis *et al.* (2009) focused on soft and semi-soft cheeses obtained from Greek markets, and reported that 40.0% of samples were contaminated. Among dairy product panels, soft and semi-soft cheeses are often the most contaminated (Martinez-Rios and Dalgaard 2018). Unfortunately, physico-chemical data are not available for the two surveys reporting the highest occurrence. Some studies reporting high prevalence of *L. monocytogenes* are nevertheless biased due to too small sample sizes. In these cases, a single contamination has a huge impact on the final prevalence (Filiousis *et al.* 2009; Rosas-Barbosa *et al.* 2014).

While some soft cheeses present unfavourable conditions for the survival of *L. monocytogenes*, like those with a low pH, most of them generally present favourable conditions for its survival. For instance, the pH of Castelo Branco rind and core were reported to be around 6 and 5.4, respectively, after 15 days of ripening (Pintado *et al.* 2005). No further evolution in pH was observed during ripening and storage. Worse, pH may increase in the rind during ripening of some red smear cheeses (Rudolf and Scherer 2001). Ripening and storage are thus critical stages. For instance, Manfreda *et al.* (2005) compared the occurrence of *L. monocytogenes* in Gorgonzola just before packaging and at the end of shelf life. The number of contaminated samples reaching the limit of detection grew from 2.1 to 4.8%. Regarding the type of milk used, an older study from Rudolf and Scherer (2001) found no significant difference in contamination between cheeses made from ovine, bovine or caprine milk.

Although *L. monocytogenes* is not present in a cheese sample, other *Listeria* species can sometimes be isolated, such as *L. innoccua* (Angelidis *et al.* 2012). The presence of other species of the genus suggests that the conditions could be suitable for the growth of *L. monocytogenes* as well, and that specific measures should be implemented (Pintado *et al.* 2005).

It is important to distinguish cheese rinds and cores. Rinds are much less acidic, and thus more favourable for the multiplication of the pathogen. For instance, Camembert or Brie rinds can have a pH higher than 7 (Prencipe *et al.* 2010). In a blue-veined cheese from Italy, 55.0% of the 120 samples presented a contamination in their rind, but not in their paste (Bernini *et al.* 2013). Similar findings have been reported for Taleggio (Iannetti *et al.* 2016). Given that post-processing contamination is the most occurring transmission route, more attention should be paid to cheese surface. *L. monocytogenes* was isolated on the surface of Prato cheese, a Brazilian semi-soft cheese, as a results of contaminated food contact surfaces (Barancelli *et al.*, 2014). As a consequence, it is sometimes advised to remove rinds before consumption (Prencipe *et al.* 2010). In addition, risk of transmission of the pathogen from rinds to pastes during cutting procedures should be more considered (Bernini *et al.* 2016; Iannetti *et al.* 2016).

Recent studies in Europe are encouraging. Of 3452 samples of soft cheese from retail stores all over the European Union, only 0.5% were contaminated with *L. monocytogenes* (Rakhmawati *et al.* 2013). Lahou and Uyttendaele (2017) isolated the bacterium from 3.1% of 32 soft cheeses in Belgium, while only 0.4% of 525 samples were contaminated in Sweden (Lambertz *et al.* 2012).

Differences in occurrence for a given kind of cheese could be explained by the level of modernization of the process. Indeed, in small traditional dairies, automation and sanitary quality of the equipment are limited (Colak *et al.* 2007). Like for fresh cheese, the use of raw milk is not a key factor for the growth of *L. monocytogenes*. In the EFSA report on zoonoses for the year 2015, non-compliances associated with cheeses made with pasteurized milk (1.3%) were just a little bit smaller than non-compliance associated with cheeses made from raw milk (1.4%) (EFSA- ECDC 2016). Based on 7 EFSA reports covering the period 2005-2015, Martinez-Rios and Dalgaard (2018) found no significant difference of prevalence between raw-milk and pasteurised-milk soft/semi-soft cheeses.

Hard and semi-hard cheeses

Hard and semi-hard cheeses are characterized by a lower a_w compared to fresh, soft and semi-soft cheeses. This decrease is obtained by fast curdling, eventual cooking and intensive pressing of the curd, combined with an extended ripening period. The pH of hard cheeses is rather variable, with values ranging from 4.9 to 8.0 (Saltijeral *et al.* 1999; Almeida *et al.* 2007). Hard cheeses present a_w values ranging from 0.91 to 0.97 (Smukowski, 2013).

Currently, no listeriosis outbreaks linked with hard cheeses are referenced (Martinez-Rios and Dalgaard 2018). Table 3 summarises studies on the proportion of hard and semi-hard cheeses in which *L. monocytogenes* was detected(in 25g of sample). Globally, the number of contaminated samples is close to 0.0 (Alcazar Montanez *et al.* 2006; Kongo *et al.* 2006; Gil *et al.* 2007; Cabedo *et al.* 2008; Little *et al.* 2008; Filiousis *et al.* 2009; Prencipe *et al.* 2010; Arrese and Arroyo-Izaga 2012; Almeida *et al.* 2013). The low prevalence of the bacterium is explained by the lower a_w of hard and semi-hard cheeses, creating unfavourable conditions for survival and growth of *L. monocytogenes* (Kongo *et al.* 2006; Abrahão *et al.* 2008). According to Rudolf and Scherer (2001), hard cheeses made in the same dairies as contaminated soft cheeses, and with the same ripening flora, were not contaminated at the end of the ripening period, confirming that the physico-chemical conditions met in hard cheese do not allow the survival of the pathogen. Nevertheless, Arrese and Arroyo-Izaga (2012) detected other species of the genus *Listeria* in Idiazabal cheese, an ovine milk hard cheese from Basque Country.

One study detected higher occurrence of the pathogen than aforementioned researches. Almeida *et al.* (2007) observed an occurrence of 5.5%, but with a very limited sample size (18 cheeses). In fact, only one sample was contaminated in that study. In fact, Almeida *et al.* (2013) observed an increase in the number of contaminated samples in relation with the decrease in the size of the dairies and the level of industrialization. Dalmasso and Jordan (2014) reported a percentage of 7.0% of contaminated samples of cheddar cheese sampled before ripening. This last study highlights the role of the ripening period in the decrease in *L. monocytogenes* levels in hard and semi-hard cheeses. This role will be described in the next part of this review.

Survival OF *L. MONOCYTOGENES* IN CHEESE

In order to understand the survival of *L. monocytogenes* in cheese during process, ripening, packaging and storage, challenge-tests can be performed. These consist of an inoculation of the pathogen during process. According to Bernini *et al.* (2013), "challenge testing evaluates if an inoculated organism can grow in a specific product and determines the point at which the growth reaches unacceptable levels in a specific product". The pathogen can also be directly injected into the final product. Alternatively, studies can focus on natural contamination of the product. This approach is called a "durability study". Both types of investigation have advantages and disadvantages. On the one hand, durability studies seem to be more realistic because contamination is natural. Indeed, it is difficult to mimic a correct level of contamination when challenge-testing a product. On the other hand, it is sometimes very hard to perform a durability study because of the low occurrence or low level of contamination of the concerned product (EURL *Lm* 2014).

A wide variety of inoculation tests have recently been performed. These investigations focused on the influence of several parameters, including ripening duration, storage temperature and level of initial contamination. Inoculation can occur at different steps of the process, such as cheese processing, ripening, packaging or storage.

Some authors also opted for a use of *L. innocua* for these experiments, due to its safety. However, in the latter case, researchers should choose a strain that behaves as similar as possible to *L. monocytogenes* in order to mimic its growth Samelis *et al.* 2009; Pinto *et al.* 2009).

Table 4 hereafter summarizes the main conclusions of papers focusing on the survival of *L. monocytogenes* in various types of cheese.

Fresh cheese

The a_w of fresh cheese cannot prevent the survival and, in some cases, the growth of *L. monocytogenes.* Regarding low pH fresh cheese, such as Katiki (pH 4.3-4.5) or Galotyri (pH 3.8-4.4), a decrease is generally observed during storage at all temperatures (Rogga *et al.* 2005; Kagkli *et al.* 2009). A longer persistence is frequently observed at lower temperatures. However, Schoder *et al.* (2003) demonstrated that seven days of storage at 7°C was unable to cause a decrease in the levels of *L. monocytogenes* in a cheese with a pH of 4.3. Fresh cheeses with a lower acidity are not able to reduce the contamination. Kapetanakou *et al.* (2017) reported constant levels of *L. monocytogenes* (100 cfu/g) in a Cottage cheese with a pH of 5.0 during shelf-life. In Queso Blanco (pH 6.8), *L. monocytogenes* was able to grow, irrespective of the storage temperature (Uhlich *et al.* 2006). In addition to pH, level of initial inoculum also had an influence (Schoder *et al.* 2003). Coating of spices around fresh cheeses was not found to prevent listerial growth (Lobacz *et al.* 2016).

Soft and semi-soft cheese

Soft cheeses represent the most risky category of cheeses regarding *L. monocytogenes*, due to favourable pH and a_w. In terms of temperature, it is observed that the multiplication of *L. monocytogenes* is also slower at lower temperatures in soft and semi-soft cheeses (Back *et al.* 1993; Lahou and Uyttendaele 2017). Camembert is the most common soft cheese studied regarding the growth of *L. monocytogenes* (Back *et al.* 1993; Gay and Amgar 2005; Linton *et al.* 2008; Kapetanakou *et al.* 2017). All studies on Camembert reported the same observations: it is susceptible to the multiplication of *L. monocytogenes*. For soft cheeses, it is important to focus on the distinction between core and rind. Indeed, for mold- and smear-ripened cheeses, microflora on the rind and in the core are different. Cheese pastes are indeed rich in lactic acid bacteria (LAB), while the rind is mainly composed of white moulds and yeasts (Back *et al.* 1993; Kapetanakou *et al.* 2017). In blue-veined cheeses, moulds are also observed in the core. In smear-ripened cheeses, no moulds are observed, but yeasts are found on the surface, predominantly from the genus *Debaryomyces* (Mounier *et al.* 2005; Irlinger *et al.* 2015). Mounier *et al.* (2005) reported that these yeasts produce alkaline compounds leading to an increasing pH. As a result, some less acid-tolerant bacterium can grow, including *Brevibacterium linens* or species from the genus *Corynebacterium*.

Evolution of pH during cheese processing, ripening and storage is highly associated with this microflora (Dalzini *et al.* 2017). During the first hours after processing, LAB grow rapidly and their metabolism produces organic acids from carbohydrates, resulting in a decrease of 1.5 to 2 pH units (Prieto *et al.* 2000; Flórez *et al.* 2006; Dalzini *et al.* 2017). After a few days, molds start to grow on the rind or in the paste respectively for mold-ripened cheeses and blue-veined cheeses (Prieto *et al.* 2000). Due to the proteolytic activity of molds, an increase in pH is generally observed in the concerned cheese part, associated with an increased concentration of free amino acids (Prieto *et al.* 2000; Flórez *et al.* 2006; Dalzini *et al.* 2017). Alkaline compounds resulting from lactate metabolization are also responsible for this increased pH (Dalzini *et al.* 2017).

As a consequence, a much higher pH is observed in the rind than in the core of mold- and smear-ripened cheeses, sometimes increasing up to 7.0 during ripening of Camembert or Brie (Back *et al.* 1993; Millet *et al.* 2006; Schvartzman *et al.* 2014; Bernini *et al.* 2016; Kapetanakou *et al.* 2017). In blue-veined cheese pastes, pH grew up to values higher than 6 (Prieto *et al.* 2000; Flórez *et al.* 2006; Dalzini *et al.* 2017). The behaviour of *L. monocytogenes* in soft cheese is highly correlated with pH evolution. While no increase in *L. monocytogenes* contamination in the core of Camembert was observed at refrigeration temperature, Back *et al.* (1993) observed an increase of 2 log cfu/g on the rind, where pH is increased, during 40 days of storage. This dominant localization of *L. monocytogenes* on the surface was also observed with the use of bioluminescent strains (Dalzini *et al.* 2017). Furthermore, similar results have been reported for Saint-Nectaire, Greek Halloumi and Gorgonzola cheeses (Millet *et al.* 2006; Bernini *et al.* 2016; Kapetanakou *et al.* 2017). On the opposite, Dalzini *et al.* (2017) observed a growth of inoculated *L. monocytogenes* higher than the limit of 2 log cfu/g in the core of Gorgonzola, while population of the pathogen remained stable on the rind. According to Corsetti *et al.* (2001), yeasts that develop in mold-ripened and blue-veined soft cheeses could sometimes enhance the ability of *L. monocytogenes* to grow, by producing growth factors.

The type of milk also has an influence. Pasteurized milk cheeses generally seem more susceptible to the multiplication of the pathogen in soft cheese, if post-pasteurization contamination. The endogenous microflora of raw milk, composed among others of LAB, could play an inhibitive role on *L. monocytogenes* due to increased competition (Schvartzman *et al.* 2011; Tiwari *et al.* 2014). In soft cheese manufactured by direct acidification, i.e., by adding lactic acid, the population of *L. monocytogenes* was increased by 2 to 3 log cfu/g in comparison with cheese including lactic starter (Naldini *et al.* 2009). Some enzymes found in raw milk, for instance lactoferrin and lactoperoxydase, which are compounds with bacteriostatic properties, can also prevent *L. monocytogenes* growth (Food and Agriculture Organization of the United Nation/ World Health Organization 2005; Gay and Amgar 2005; Tiwari *et al.* 2014; Lahou and Uyttendaele 2017).

Ripening duration also plays a role. Indeed, a_w progressively diminishes during ripening and cheese becomes harder. As a consequence, smaller growth was observed during storage of Gorgonzola within 80 days of ripening ($a_w = 0.92$) in comparison with Gorgonzola aged for 50 days ($a_w = 0.97$). Growth was also delayed by 30 days in 80-day ripened cheese (Bernini *et al.* 2013). In a further study performed by Bernini *et al.* (2016), piquant Gorgonzola ripened for 80 and 120 days did not permit the growth of the bacterium, while it was possible in sweet Gorgonzola with a shorter ripening duration.

Regarding semi-soft cheese, studies suggest that it is more difficult for the pathogen to grow in this type of cheeses. Condoleo *et al.* (2016) found no growth of the bacterium during storage of an Italian raw ovine milk semi-soft cheese. Pinto *et al.* (2009) observed a decrease in the levels of *L. monocytogenes* in Minas traditional

Serro cheese with inoculum levels ranging from 10 to 1000 cfu/g. Overall, studies suggest that it is possible to detect *L. monocytogenes* in semi-soft cheese, but that its growth is limited.

Hard and semi-hard cheese

Studies on the occurrence of *L. monocytogenes* in hard and semi-hard cheese indicate that it is difficult for the bacterium to grow in this type of cheeses. Inoculation studies confirmed these findings. Although a growth of the bacterium was observed during manufacture of Swiss hard cheese, it was no longer detectable after ripening (Buazzi *et al.* 1992; Bachmann and Spahr 1995). No growth was observed in Gouda, Parmesan,Cheddar, Cantal, Edam and Pecorino (Ryser and Marth 1987; Northolt *et al.* 1988; Yousef and Marth 1990; Chatelard-Chauvin *et al.* 2015; Ortenzi *et al.* 2015; Kapetanakou *et al.* 2017).

Bachmann and Spahr (1995) reported that the pH of Swiss hard and semi-hard cheeses increased by 0.3 to 0.9 units during ripening. Thus, a_w is generally the most limiting factor for *L. monocytogenes* in hard or semi-hard cheese. For instance, a_w lower than 0.90 in Cantal or lower than 0.92 in Gouda rinds have been reported (Wemmenhove *et al.* 2013; Chatelard-Chauvin *et al.* 2015). In naturally contaminated Cheddar (pH 5.5), the bacterium never reached the threshold value of 100 cfu/g, and disappeared during the storage period (Dalmasso and Jordan 2014). For Chihuahua and Manchego, two Mexican cheeses, levels of the bacterium remained at the level of initial inoculum (10^6 cfu/g) during storage (Solano-López and Hernández-Sánchez 2000). Both natural and artificial contaminations lead thus to the same observations for hard and semi-hard cheeses.In Cheddar, Pecorino and Parmesan, pH could be the limiting factor. Specifically, pH values were found to decrease to 5.0 during ripening and storage, while a_w remained above of 0.94 (Ryser and Marth 1987; Yousef and Marth 1990; Ortenzi *et al.* 2015). The NaCl percentage in these types of cheese seems to have no influence on the behaviour of the pathogen, while decreasing the salt content of Cheddar cheese did not change the survival of *L. monocytogenes* (Hystead *et al.* 2013).

Contrary to soft cheeses, surveys report that hard cheeses made from pasteurized or thermized milk are not more likely to support listerial growth than raw milk cheese (Ryser and Marth 1987; Solano-López and Hernández-Sánchez 2000; Samelis *et al.* 2009). If the starter culture probably plays a role in the inhibition of *L. monocytogenes*, the key step explaining this is the duration of the ripening (Kandarakis *et al.* 1998, Çetinkaya and Soyutemiz 2004). Indeed, ripening period for hard cheeses is generally six months up to several years.

The effect of storage temperature on the behaviour of *L. monocytogenes* in hard cheese is complex. Overall, it appeared that storage at room temperature could favour a decrease in the population of *L. monocytogenes*

(Valero *et al.* 2014). According to Giannou *et al.* (2009), “the lower the storage temperature, the higher and longer the survival of *L. monocytogenes* was”. Refrigerated storage could even permit the levels of contamination to be maintained or grown (Bellio *et al.* 2016; Moosavy *et al.* 2017). However, scientists expect negative effects of an increased storage temperature on appearance and physico-chemical characteristics of the cheeses (Moosavy *et al.* 2017).

Surprisingly, *L. monocytogenes* was found to disappear during storage of Graviera, a cheese with an average pH of 5.6 and an a_w of 0.95. These physico-chemical values are usually considered as insufficient to prevent the multiplication of the pathogen (Giannou *et al.* 2009). LAB seems to play a major role in this inhibition (Kagkli *et al.* 2009). It is well established that LAB are more active when the temperature is higher, i.e. at room temperature (Valero *et al.* 2014). Samelis *et al.* (2009) observed that a decrease in *L. monocytogenes* contamination was linked with an increase in LAB populations during the early stages of ripening and storage. These raw milk endogenous bacteria are responsible for increased competition for nutrients. They can also produce bacteriocins (Reis *et al.* 2012; Kapetanakou *et al.* 2017). Brining time could also be of interest in the prevention of *L. monocytogenes* contamination. Wemmenhove *et al.* (2016) showed indeed that a_w of Gouda cheeses decreased with brining time (0.96, 0.93 and 0.90 for 0.33, 2.1 and 8.9 days of brining respectively).

Regarding cheese weight, no influence on the behaviour of the bacterium has been reported (Chatelard-Chauvin *et al.* 2015). Finally, according to Wemmenhove *et al.* (2018), the behaviour of *L. monocytogenes* in hard cheese could also be influenced by the concentration of undissociated lactic acid. They showed that *L. monocytogenes* was unable to grow in Gouda cheeses when undissociated lactic acid concentration are higher than 6.35 mM.

To our knowledge, a single study reported the growth of *L. monocytogenes* in a hard cheese, Gruyere, made from pasteurised milk (Leong *et al.* 2014). The fact that this cheese was stored at an abusive temperature of 25°C could explain the growth of *L. monocytogenes*.

CONCLUSION

Occurrence and survival of *L. monocytogenes* in cheese are important research topics, listeriosis being the only foodborne disease for which an increase is observed for the period 2012-2016. Globally, it seems well established in the literature that some categories of cheese are more susceptible to allow the growth of *L. monocytogenes* . For instance, soft, semi-soft cheeses and non-acidic fresh cheeses are the riskiest regarding the presence of *L. monocytogenes*. If the pathogen can sometimes be found in hard, semi-hard and acidic fresh cheeses, its growth is generally not possible, due to lower pH or moisture. The trend that favours the use of pasteurised milk for cheese production does not seem to be backed by the available literature. It indeed seems that no obvious difference can be observed in the prevalence of *L. monocytogenes* in raw compared to pasteurized milk cheese. Worse, pasteurized milk could favour the survival of the pathogen, the cheese being free of competitive natural lactic microflora. Moreover, most of cheese contamination are not linked to the microbial quality of the milk but to a lack of hygiene during the post-pasteurization or post-processing steps. Another important factor to take into account while talking about prevalence and survival of *L. monocytogenes* is the physico-chemical differences between cheese surface and cores. Indeed, pH is generally more favourable on the surface. Another factor to take into account while studying the prevalence of *L. monocytogenes* is its heterogeneous distribution in a single batch, but also in a single piece.

FUTURE RESEARCH AND RECOMMENDATIONS

This review revealed that most studies focus on cheese from Hispanic countries or from France. Data from other European countries, like Belgium are currently scarce However, there is a wide diversity of typical cheeses in these regions Therefore, it would be of interest to perform a large-scale investigation on the occurrence of *L. monocytogenes* in these countries, for instance in Belgium. This study should be followed by inoculation and shelf-life studies for a panel of Belgian cheeses. In these studies, the pathogen should be inoculated either in the core or on the surface, depending on the physico-chemical conditions. Furthermore, a lot of studies presented in this review used to high initial contaminations, which did not reflect the reality. It is indeed suggested by EURL *Lm* to target an initial inoculum of 2 log cfu/g. In addition, EURL *Lm* also advises that the temperature should vary during storage of inoculated cheeses during shelf-life studies, in order to mimic the different steps of the food supply chain. Very few papers have considered these changes in storage temperatures. The purpose of such a large-scale investigation would be to extrapolate the results to all cheeses presenting the same properties. Afterwards, producers could take advantage of the conclusions without being forced to perform their own challenge-tests. In addition to physico-chemical parameters, the microbial richness of cheeses can also play an important role in the survival *L. monocytogenes*. Combining investigation of these factors together within a single survey could provide interesting and important information.

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