Prevalence of *Listeria monocytogenes* in European cheeses: A systematic review and meta-analysis

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ABSTRACT

Both in Europe and worldwide cheese has caused important outbreaks of listeriosis and can be a vehicle for transmission of *Listeria monocytogenes* to consumers. A systematic review and meta-analysis were conducted using scientific literature and European Food Safety Authority (EFSA) reports to summarize available data on the prevalence of *L. monocytogenes* in different types of cheeses produced in Europe. Meta-analysis models were used to estimate mean prevalence of the pathogen and to compare prevalence among types of cheeses (fresh, ripened, veined, smear and brined) and cheeses produced using, respectively, pasteurized or un-pasteurized milk. Data from a total of 130,604 samples were analysed. Mean prevalence for presence during 2005-2015 estimated from scientific literature (2.3% with confidence interval (CI): 1.4-3.8%) was more than three times higher than results from EFSA reports (0.7%; CI: 0.5-1.1%). The prevalence differed among types of cheeses including fresh (0.8%; CI: 0.3-1.9%), ripened (2.0%; CI: 0.8-4.9%), veined (2.4%; CI: 0.9-6.3%), smear (5.1%; CI: 1.9-13.1%) and brined (11.8%; CI: 3.5-33.3%). Mean prevalence of *L. monocytogenes* in soft/semi-soft cheeses were not significantly different (P > 0.05) for cheeses produced from pasteurized (0.9%; CI: 0.4-1.9%) or un-pasteurized (1.0%; CI: 0.4-2.2%) milk. For cheese samples reported by EFSA 0.2% CI: 0.1-0.4% had concentration of *L. monocytogenes* above the critical European limits of 100 cfu/g. In addition, this systematic review focused on groups/species of microorganisms suitable as indicator organisms for *L. monocytogenes* in cheeses to reflect the level of production hygiene or as index organisms to assess the prevalence of *L. monocytogenes* in cheeses. However, no suitable indicator or index organisms were identified. The performed meta-analyses improved our understanding of *L. monocytogenes* prevalence in different types of cheeses and provided results that can be useful as input for quantitative microbiological risk assessment modelling.

Keywords: Occurrence, fresh cheese, soft and semi-soft cheeses, risk assessment
1. Introduction

The genus *Listeria* includes more than 20 species that can be divided into three clades (Weller et al. 2015). Two *Listeria* species belonging to the same clade are generally considered to be pathogenic, *L. monocytogenes* in humans and *L. ivanovii* in other mammals. Nevertheless, there have been some reports of *L. seeligeri* and *L. ivanovii* causing illness in humans (Cummins et al., 1994; Rocourt et al., 1986). The likelihood of *L. monocytogenes* infection leading to listeriosis is greatest among certain groups; including pregnant woman, neonates, immunocompromised adults and the elderly (Ryser & Marth, 2007). Within the European Union (EU) there has been a statistically significant increasing trend of listeriosis over the period 2009-2015. Specifically, the numbers of confirmed human cases of listeriosis were 1,331 and 2,206 in 2009 and 2015, respectively (EFSA, 2016). A total of 270 deaths due to listeriosis were reported within nineteen EU member states. The overall EU notification rate of listeriosis was 0.46 cases per 100,000 population with a case-fatality rate of 17.7% (EFSA, 2016). Seven EU Member States and Norway provided information from conventional serotyping of *L. monocytogenes* (accounting for 23.3% of all confirmed cases). The most common serotypes in 2013 were 1/2a (57.5%) and 4b (34.3%), followed by 1/2b (6.4%), 1/2c (1.4%), 3a and 3b (both 0.2%) (EFSA, 2015).

In 2010-2011 an EU baseline survey (EFSA, 2013a) collected data about presence of *L. monocytogenes* and the non-compliance for different ready-to-eat (RTE) food categories at retail. The proportion of *L. monocytogenes* positive samples at retail was highest in fish products (mainly smoked fish), followed by soft and semi-soft cheeses and RTE meat products. Specifically, the EU prevalence of *L. monocytogenes* in cheeses at retail was 0.47% (CI: 0.29-0.77%) determined as 16 positive samples out of 3393 at the end of shelf-life. For these 2010-2011 samples 0.06% (CI: 0.02-0.24%) determined as two samples out of 3393 exceeded the critical concentration of 100 cfu/g.
In 2015 fifteen samples out of 3039 exceeded the critical concentration of 100 cfu/g (EFSA, 2016).

The first reported outbreak of human listeriosis associated with consumption of cheese occurred in the USA during 1985 (Linnan et al., 1988) and was caused by a fresh cheese. Since then, several outbreaks associated with consumption of cheese have occurred worldwide and fatalities continue to be reported (Table 1). Clearly, it is important to collect information and to analyse data in an attempt to improve our understanding and options to better manage this risk.

Meta-analysis is a statistical approach that can be used to analyse, for example, prevalence data (effect size) originating from various sources (primary studies) and in this way provide an overview of effects and variability (Glass, 1976; Sutton, et al., 2001). Lately, meta-analysis has been used to study several food safety issues and the quantitative results obtained can been used as inputs in risk assessment models (Baron et al., 2009).

Fortunately, prevalence and concentrations of *L. monocytogenes* in cheeses and cheese processing environments are low. Therefore, to evaluate its potential presence other index or indicator microorganisms that are easier to determine or quantify can be relevant to analyse. Index organisms can be used to assess likelihood of the presence of a pathogen whereas indicator organisms demonstrate a failure in Good Hygiene Practices (GHP) (Brodsky, 1995; Mossel, 1978). EU Regulation (EC) No 2073/2005 use coagulase-positive staphylococci as index organisms to assess the likelihood of staphylococcal enterotoxins in cheese made from raw or pasteurized milk and *E. coli* is used as an indicator for the level of production hygiene in cheese made from milk that has undergone heat treatment. Furthermore, *Listeria* spp. has been used as index organisms for the likely presence of *L. monocytogenes* in food (FSIS, 2014; Gilbert et al., 2000).
The objective of the present study was to perform a systematic review and a meta-analysis of the prevalence of *L. monocytogenes* in different types of European cheeses and study potential indicator organisms for assessment of production hygiene or index organisms for implementation in the assessment of product safety.

2. Materials and methods

2.1. Literature search and inclusion criteria

A systematic review was performed following the protocol presented by Sargeant et al., 2005. Literature searches were carried out to identify suitable scientific literature using Web of Science (2017) or DTU Findit (2017) databases for papers indexed since 1985 as well as Google searches using English, French, Italian, and Spanish terms for combinations of *Listeria* spp., *L. monocytogenes*, cheese, dairy, prevalence, incidence and occurrence. Electronic searches were carried out to identify reports of the prevalence for *Listeria* spp. in cheese. This included reports by national and international organizations such as World Health Organization (WHO), EFSA and the International Commission for Microbiological Specification in Foods (ICMSF).

For inclusion in the meta-analysis results had to meet three requirements: (i) to come from original studies, (ii) to be obtained by using approved (FDA/FIL-IDF or ISO) microbiological methods for detection of *Listeria* spp. and (iii) originate from cheeses produced in Europe during the period of 2005 to 2015.

2.2. Data and definitions

Cheese-type definitions were necessary in order to categorize studies from scientific literature. Available information allowed for a classification based in maturation characteristics. For
the purpose of this paper, the following definitions apply. Fresh cheeses are curd-style cheeses which do not undergo any ripening (CAC, 2013), for example, queso fresco, cottage cheese, Mozzarella or Ricotta. Ripened cheeses are not ready for consumption shortly after manufacture and maturation is needed for development of specific cheese characteristics (CAC, 2013), for example, Gouda, Edam, Cheddar or Parmesan. Veined cheeses are ripened cheeses in which ripening has been accomplished primarily by the development of the mould Penicillum roqueforti throughout the interior and/or on the surface, for example, Roquefort, Gorgonzola, Cabrales, Stilton or Danablu. Smear cheeses are ripened cheeses where the surface is treated with Penicillum candidum, Penicillum camemberti or Brevibacterium linens, for example, Brie, Camembert, Limburger or Taleggio. Brined cheeses are ripened and stored in brine until they are sold or packed, for example, Feta or Ricotta salata (Fox et al., 2000).

Classification of cheese in EFSA reports are based on cheese moisture content. Soft-cheeses have a percentage of moisture, on a fat-free basis, higher than 67 %. Semi-soft cheeses have 62 to 67 % fat-free moisture and are characterized by their firm but elastic feel. Hard cheeses have 49 to 56 % fat-free moisture (CAC, 2013; EFSA, 2013b).

2.3. Problem statement

To estimate prevalence of L. monocytogenes in cheese during the period 2005-2015 (i) from scientific literature data, (ii) from data in EFSA reports, (iii) from scientific literature and data in EFSA reports when combined and (iv) to study groups/species of microorganisms suitable as indicator or index organisms to assess prevalence of L. monocytogenes in cheeses.

2.4. Description of data sets for meta-analysis and regression modelling

From each primary study the number of samples positive for L. monocytogenes (s) and the total number of samples (n) were extracted. Information about year of survey, country, sample
weight and information on sampling at production site or at retail were also collected from each primary study. Meta-analysis for prevalence of *L. monocytogenes* in cheese as reported in the scientific literature was based on 17 primary studies including a total of 7,221 samples (Table 2), while data from seven EFSA reports with a total of 123,383 samples were included (Table 3 and Table 4). The regression model used to evaluate indicator/index organisms for *L. monocytogenes* in European cheeses was based in 16 primary studies all from the scientific literature and including a total of 3,852 samples (Table 5).

2.5. Meta-analysis

Prevalence data was studied as observed effect size ($\theta_i$) and they were logit transformed in order to restrict values to a range between 0-1 and to stabilize variance (Eq. 1; Viechtbauer, 2010). The parameter measuring effect size ($\theta_i$) is a common metric that permits direct comparison and summation of primary studies (Borestein et al., 2009).

$$\theta_i = \text{logit } p_i = \ln \left( \frac{p_i}{1-p_i} \right) = \ln \left( \frac{s_i}{n_i-s_i} \right)$$

(1)

Models with random-effects were used to calculate prevalence values (mean and 95% CI) of *L. monocytogenes* across primary studies (Eq. 2; Borestein et al., 2009):

$$T_i = \theta_i + \varepsilon_i = \mu + u_i + \varepsilon_i$$

(2)

where $T_i$ is the true effect size for each primary study ($i = 1, 2, \ldots$), $\varepsilon_i$ is the sampling error and $\mu$ is the mean true effect size. $u_i$ represents the true variation in effect sizes being compose of within-study ($\sigma^2$) and between-study variance ($\tau^2$).

The between-study variance ($\tau^2$) is estimated from the Q-statistic (DerSimonian & Laird 1986),
\[ \tau^2 = \begin{cases} 
\frac{Q - (k - 1)}{\sum w_i - \frac{\sum w_i}{\sum w_i}} & \text{for } Q > (k - 1) \\
0 & \text{for } Q \leq (k - 1) 
\end{cases} \]  

(3)

where \( Q \) is calculated by Eq. 4 and 5, \( k \) is the number of studies and \( w_i \) the weight assigned to each study (Eq.5).

\[ Q = \sum w_i (T_i - \mu)^2 \]  

(4)

\[ \mu = \frac{\sum w_i T_i}{\sum w_i} \]  

(5)

\[ w_i = \frac{1}{\sigma_i^2 + \tau_i^2} \]  

(6)

A significant value of the Q-statistic indicates a real effect difference between primary studies and suggests the use of a multilevel model (Xabier et al., 2014). The \( I^2 \) index was used to measure the extent of between-study variance dividing the difference between the result of the Q-statistic and its degrees of freedom \((k - 1)\) by the \( Q \) value itself, and then multiply by 100. Higgins & Thompson (2002) proposed a classification of \( I^2 \) values with percentages of around 25\% \((I^2 = 25)\), 50\% \((I^2 = 50)\) and 75\% \((I^2 = 75)\) corresponding to low, medium and high between-study variance, respectively. The \( \tau^2 \) and \( I^2 \) indices are related and higher \( \tau^2 \) values corresponds to higher \( I^2 \) index values.

Multilevel meta-analysis including type of cheese and pasteurized or unpasteurized milk were used to account for some of the observed between-study variance in prevalence data.

The multilevel models used were formulated as:

\[ T_i = \beta_0 + \beta_1 X_{1i} + \cdots + \beta_k X_{ki} + u_i + \epsilon_i \]  

(7)
with \((X_1 \text{ to } X_k)\) being study characteristics and \(\beta_k\) the moderator effects.

Meta-analysis modelling was performed by using R version 3.1.3 (R Development Core Team) and the “metafor” package (Viechtbauer, 2010), which provides functions for fitting of random-effects and multilevel models as well as meta-analytical graphs including forest plots.

### 2.6. Regression modelling

A linear regression model \((y = a + bx)\) was used to evaluate the relation between prevalence of \(Listeria\) spp. (\(x\)) and prevalence of \(L.\ monocytogenes\) (\(y\)). Regression modelling was performed with R and an F-test was used to evaluate if the linear model could be reduced to \(y = bx\).

### 3. Results

#### 3.1. Meta-analysis of prevalence data from scientific literature

The overall prevalence for presence of \(L.\ monocytogenes\) in cheese was 2.3% (CI: 1.4-3.8%). Variability in reported prevalence among studies was high (Table 6 and Fig.1) and the between-study variance slightly decrease from \(\tau^2 = 1.72\) to 1.12 when cheeses were grouped in categories by the multilevel model. Nevertheless, unexplained variability remained high (\(I^2 = 75\%\); \(p\)-value < 0.001 in Table 6).

Fresh cheese had the lowest mean prevalence of 0.8% (CI: 0.3-1.9%), followed by ripened cheese 2.0% (CI: 0.8-4.9%), veined cheese 2.4% (CI: 0.9-6.3%) and smear cheese 5.1% (CI: 1.9-13.1%). Brined cheese had the highest \(L.\ monocytogenes\) prevalence of 11.8% (CI: 3.5-33.3%) (Table 6 and Fig. 1).

#### 3.2. Meta-analysis of prevalence data from EFSA reports
The overall prevalence for presence of *L. monocytogenes* in cheese was 0.7% (CI: 0.5 – 1.1%) with high between-studies variance (Table 7). A multilevel model determined the prevalence of *L. monocytogenes* in hard and soft/semi-soft cheeses produced from un-pasteurized or pasteurized milk. No significant effect of pasteurization (p > 0.05) was observed within hard or soft/semi-soft cheeses (Table 7).

A second random-effects meta-analysis was performed to assess non-compliance with the criterion of 100 cfu/g for *L. monocytogenes* in ready-to-eat (RTE) foods. 0.2% (CI: 0.1-0.4) of the cheese samples had more than 100 *L. monocytogenes*/g and high between-study variance was observed (Table 8). Prevalence of *L. monocytogenes* in hard and soft/semi-soft cheese produced with un-pasteurized or pasteurized milk was estimated. Pasteurization of milk had no significant effect (p > 0.05) within hard or soft/semi-soft cheeses (Table 8).

### 3.3. Meta-analysis of combined prevalence data from scientific literature and EFSA reports

The overall prevalence of *L. monocytogenes* in European cheeses was 1.2% (CI: 0.8-1.8%). High between-study variance was observed and a significant difference (p < 0.001) was determined between data from the scientific literature and from EFSA reports data (Table 9).

### 3.4. Evaluation of index organisms for prevalence of *L. monocytogenes* in European cheeses

Of 3852 samples reporting presence of *Listeria* spp., 203 (5.3%) were positive for *L. monocytogenes*, 327 (8.5%) *L. innocua*, 19 (0.5%) *L. grayi*, 188 (4.9%) *L. welshimer*, 18 (0.5%) *L. ivanovii* and 20 (0.5%) *L. seeligeri*. The correlation factor was sufficient to describe the relation between prevalence of *Listeria* spp. (x) and prevalence of *L. monocytogenes* (y) in cheeses (y = 0.52 x, r² = 0.86, Fig. 2).

### 4. Discussion
It is critical to understand and quantify the prevalence of *L. monocytogenes* in cheeses since they are an important vehicle for transmission of the pathogen and infection causes the highest fatality case rate among zoonotic diseases (EFSA, 2016).

EU mean prevalence of *L. monocytogenes* in cheese from scientific literature exceeded what was reported by EFSA for the same period. This may result from a focus on problematic cheese products in scientific studies whereas EFSA reports include a larger number of samples from hard cheeses where *L. monocytogenes* can be inactivated and prevalence therefore is lower. The data from scientific studies corresponded to previous studies reporting prevalence between 0 and 4.8% (Esho et al., 2013; Manfreda et al., 2005; Rosengren et al., 2010), but some other studies reported more than 40% prevalence (Loncarevic et al., 1995; Pintado et al., 2005).

Mean prevalence of *L. monocytogenes* in fresh cheese was similar to the overall prevalence obtained from EFSA data. In 1985 consumption of contaminated fresh cheese (queso blanco) was directly linked to more than 142 cases of listeriosis, including 48 deaths (Linnan et al., 1988). From 2009 to 2012 there was an outbreak in Portugal linked to 30 cases of listeriosis, including 11 deaths and related to consumption of fresh cheeses (curded cheese and queijo fresco) (Magalhães et al., 2015). Furthermore, Greco et al., (2014) for example demonstrated how prevalence of *L. monocytogenes* can be high (24.4%) in mozzarella cheese as a result of cross-contamination.

Fresh cheeses were excluded from the EFSA baseline survey on prevalence of *L. monocytogenes* in certain RTE foods within EU during 2010-2011 (EFSA, 2013a). Interestingly, EFSA (2015) started to differentiate between fresh and soft/semi-soft cheeses but included only 2.1% fresh cheese samples compared to 80.1% hard cheese samples from a total of 13,718 cheese samples. Hard cheese have never been linked to a listeriosis outbreak (Table 1) and as it does not support growth of *L. monocytogenes* (Dalmasso & Jordan, 2014; Wemmenhove et al., 2013; Yousef
& Marth, 1990) the large number of these samples does not correspond to a risk-based sampling approach.

It is important to note that mean prevalence for brined cheese was estimated from only four studies with smaller sample sizes compared with other types of cheese. Consequently, there is a high level of uncertainty and results may be biased by results from a single study (Fig. 1; Table 6). In 2012, Ricotta salata imported from Italy and contaminated with *L. monocytogenes* was involved in a listeriosis outbreak in the USA with 22 hospitalizations and 4 deaths (CDC, 2012). Furthermore, ricotta salata supports growth of *L. monocytogenes* (Coroneo et al., 2016; Spanu et al., 2012) and production of this cheese includes manual processing of the curd and exposure to processing environments that increase the risk of *L. monocytogenes* contamination (Spanu et al., 2013). Our findings suggest that prevalence of *L. monocytogenes* in fresh and brined cheese are not negligible; therefore we encourage EFSA to increase and independently report sampling of fresh and brined cheeses since they have been related with listeriosis outbreaks recurrently (Table 1).

As shown by EFSA reports, contamination of cheese by *L. monocytogenes* is not specific to un-pasteurized milk cheeses since cheeses made from pasteurized milk can be contaminated due to inadequate pasteurization or post-pasteurization contamination (De Buyser et al., 2001; Donnelly, 2001). Our report is the first of our knowledge to analyse EFSA prevalence data of cheeses made from un-pasteurized and pasteurized milk. There was no significant difference in prevalence between cheeses produced with un-pasteurized or pasteurized milk; either for hard or soft/semi-soft cheeses (Table 7 and 8). This may be due to requirements leading to the use of milk of high microbiological quality for the production of un-pasteurized milk cheese and to post-pasteurization contamination of pasteurized milk cheese. Tiwari et al., (2015) compared the risk of soft/semi-soft cheese made from un-pasteurized or pasteurized milk and estimated a higher risk for un-pasteurized milk cheese as a consequence of the higher contamination rate of milk due to the lack of
pasteurization and growth of *L. monocytogenes* in un-pasteurized milk cheese but inactivation in the
same pasteurized milk cheese. But this study observed no significant effect of pasteurization in
prevalence of *L. monocytogenes* in soft/semi-soft cheese. We provide mean prevalence and
distributions for *L. monocytogenes* in soft/semi-soft cheese that can be combined with concentration
data of *L. monocytogenes* (cfu/g) for the same period in un-pasteurized and pasteurized milk cheese
to perform a quantitative risk assessment of the end product (Crépet et al., 2007) and results from
both studies could be compared.

Prevalence and concentration of *L. monocytogenes* in cheeses are low, hence evaluation of
potential presence of other index or indicator microorganisms easier to determine or quantify was
considered. *Listeria* spp. has been proposed as index organisms for presence of *L. monocytogenes* in
RTE foods and as indicator of inadequate hygiene conditions in food production practices and
environment (FSAI, 2011; Gilbert et al., 2000; McLauchlin, 1997). These findings were confirmed
by the present study and we found prevalence of *L. monocytogenes* corresponded to prevalence of
*Listeria* spp. when multiplied by a factor of 0.52. This was further supported by Trmčić et al.,
(2016) where 273 cheese samples had 12 positive for *Listeria* spp. and five of these positive for *L.
monocytogenes*. Silva et al., (2003) also found 33% of *Listeria* spp. positive samples from cheese
and dairy processing plants to be *L. monocytogenes* positive. However, Arrese & Arroyo-Izagag
(2012) found no *L. monocytogenes* positive amongst 51 cheese samples with five samples positive
for other *Listeria* spp. Microbiological methods for detection and quantification of *Listeria* spp. are
not more performant than available methods for *L. monocytogenes* (Gasanov et al., 2005).
Therefore, we do not consider *Listeria* spp. a useful index- or indicator-organism *L. monocytogenes*
despite the relation reported in the present study (Fig. 2).

5. Conclusions
Meta-analysis provided pooled prevalence estimates for *L. monocytogenes* in specific types of cheeses, however, significant between-study variance was observed. Overall prevalence of *L. monocytogenes* in cheese as estimated from scientific literature data was higher than reported by data from EFSA during the same period 2005-2015. Considering prevalence of *L. monocytogenes* in cheeses produced with un-pasteurized or pasteurized milk no significant difference in prevalence was observed. The results obtained provided a broad picture of *L. monocytogenes* prevalence in cheeses and can be used as an important input in quantitative microbial risk assessments. *Listeria* spp. was not a useful index- or indicator-organism for *L. monocytogenes* in cheeses although prevalence of *Listeria* spp. was related to prevalence of *L. monocytogenes*. 
Fig. 1. Forest plot of the multilevel model based on scientific literature reporting prevalence of \textit{L. monocytogenes} in different types of cheeses.
Fig. 2. Comparison of observed prevalence (%) for *Listeria* spp. and *L. monocytogenes* in European cheeses.
Table 1
Overview of listeriosis outbreaks caused by cheese during the period from 1983 to 2016.

<table>
<thead>
<tr>
<th>Country</th>
<th>Year</th>
<th>Serotype</th>
<th>No. of cases (fatalities)</th>
<th>Implicated food</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Switzerland</td>
<td>1983-1987</td>
<td>4b</td>
<td>122(31)</td>
<td>Smear cheese (Vacherin Mont d’Or)</td>
<td>Büla et al., 1995; Bille et al., 2006</td>
</tr>
<tr>
<td></td>
<td>1985</td>
<td>4b</td>
<td>142(48)</td>
<td>Fresh cheese (Queso Fresco)</td>
<td>Linnan et al., 1988</td>
</tr>
<tr>
<td>USA</td>
<td>1989</td>
<td>NR(^b)</td>
<td>2(0)</td>
<td>Smear cheese (Camembert)</td>
<td>Ries et al., 1990</td>
</tr>
<tr>
<td>Luxembourg</td>
<td>1989-1990</td>
<td>4b</td>
<td>26(6)</td>
<td>Veined or ripened cheese</td>
<td>Jensen et al., 1994</td>
</tr>
<tr>
<td>Denmark</td>
<td>1995</td>
<td>4b</td>
<td>37(11)</td>
<td>Smear cheese (Brie de Meaux)</td>
<td>Goulet et al., 1995; Arnold &amp; Coble, 1995</td>
</tr>
<tr>
<td></td>
<td>1997</td>
<td>4b</td>
<td>14(?)</td>
<td>Smear cheese (Pont l’Evêque)</td>
<td>Ryser &amp; Marth, 2007; Goulet et al., 2013</td>
</tr>
<tr>
<td>USA</td>
<td>2000</td>
<td>4b</td>
<td>13(5)</td>
<td>Non-commercial fresh cheese (Queso Fresco)</td>
<td>MacDonald et al., 2005</td>
</tr>
<tr>
<td>Sweden</td>
<td>2001</td>
<td>1/2a</td>
<td>≥120(0)</td>
<td>Fresh cheese</td>
<td>Carrique-Mas et al., 2003; Danielsson-Tham et al, 2004</td>
</tr>
<tr>
<td>Japan</td>
<td>2001</td>
<td>1/2b</td>
<td>38(0)</td>
<td>Smear cheese</td>
<td>Makino et al., 2005</td>
</tr>
<tr>
<td>Canada</td>
<td>2002</td>
<td>4b</td>
<td>47(0)</td>
<td>Soft and semi-soft cheese</td>
<td>Gaulin et al., 2003</td>
</tr>
<tr>
<td>Canada</td>
<td>2002</td>
<td>4b</td>
<td>86(0)</td>
<td>Cheese made from pasteurized milk</td>
<td>Pagotto et al., 2006</td>
</tr>
<tr>
<td>Switzerland</td>
<td>2005</td>
<td>1/2a</td>
<td>10 (3+2(^c))</td>
<td>Smear cheese (Soft “Tomme”)</td>
<td>Bille et al., 2006</td>
</tr>
<tr>
<td>USA</td>
<td>2005</td>
<td>NR(^b)</td>
<td>9(?)</td>
<td>Fresh cheese (Queso fresco)</td>
<td>FIOD, 2005</td>
</tr>
<tr>
<td>Czech Republic</td>
<td>2006</td>
<td></td>
<td>78(13)</td>
<td>Soft cheese</td>
<td>EFSA, 2007</td>
</tr>
<tr>
<td>Germany</td>
<td>2006-2007</td>
<td>4b</td>
<td>189(26)</td>
<td>Acid curd cheese</td>
<td>Koch et al., 2010</td>
</tr>
<tr>
<td>Norway</td>
<td>2007</td>
<td>NR(^b)</td>
<td>17(3)</td>
<td>Smear cheese (Camembert)</td>
<td>Johnsen et al., 2010</td>
</tr>
<tr>
<td>Chile</td>
<td>2008</td>
<td>NR(^b)</td>
<td>91(5)</td>
<td>Smear cheese (Brie)</td>
<td>Promed, 2008</td>
</tr>
<tr>
<td>Canada</td>
<td>2008</td>
<td>NR(^b)</td>
<td>38(5)</td>
<td>Cheeses</td>
<td>Gaulin &amp; Ramsay, 2010</td>
</tr>
<tr>
<td>USA</td>
<td>2008</td>
<td>1/2a</td>
<td>8(0)</td>
<td>Fresh cheese (Oaxaca cheese)</td>
<td>Jackson et al., 2011</td>
</tr>
<tr>
<td>Austria-Germany-Czech Republic</td>
<td>2009-2010</td>
<td>1/2a</td>
<td>34 (8)</td>
<td>Fresh cheese (Quargel)</td>
<td>Fretz et al., 2010; Rychli et al., 2014</td>
</tr>
<tr>
<td>Portugal</td>
<td>2009-2012</td>
<td>4b</td>
<td>30 (11)</td>
<td>Fresh cheese (Cured cheese and queijo fresco)</td>
<td>Magalhães et al., 2015</td>
</tr>
<tr>
<td>USA</td>
<td>2010</td>
<td>NR(^b)</td>
<td>5(0)</td>
<td>Fresh cheese (Panela, queso fresco, Requeson)</td>
<td>FIOD, 2010</td>
</tr>
<tr>
<td>USA</td>
<td>2010-2015</td>
<td>NR(^b)</td>
<td>28(3)</td>
<td>Fresh cheeses</td>
<td>FIOD, 2015b</td>
</tr>
</tbody>
</table>
USA 2011 NR b 2(?) Fresh cheese (Chives cheese) FIOD, 2011
Austria-Germany 2011-2013 1/2b 7(?) Fresh cheese Schmid et al., 2014
Spain 2012 1/2a 2(0) Fresh cheese (Queso fresco) De Castro et al., 2012
USA 2012 NR b 22(4) Brined cheese (Ricotta salatta) CDC, 2012; Coroneo et al., 2016
USA 2013 NR b 5(1) Smear cheese (Les Freres) FIOD, 2013
Australia 2013 NR b 18(?) Smear cheese NSW, 2013
USA 2013-2014 NR b 4(1) Fresh cheese FIOD, 2014a
USA 2014 NR b 7(1) Fresh cheese FIOD, 2014b
USA 2015 NR b 3(1) Fresh cheese (Panela, Queso Fresco, Requeson, Cotija) FIOD, 2015b

Table 2
Prevalence data (s/n) from the scientific literature.

<table>
<thead>
<tr>
<th>References</th>
<th>Survey year</th>
<th>Fresh / n</th>
<th>Ripened</th>
<th>Veined / n</th>
<th>Smear / n</th>
<th>Brined / n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Filiousis et al., 2009</td>
<td>2005-2006</td>
<td>4/20</td>
<td></td>
<td></td>
<td>0/10</td>
<td></td>
</tr>
<tr>
<td>Little et al., 2009</td>
<td>2006-2007</td>
<td>2/140</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>O’Brien et al., 2009</td>
<td>2007</td>
<td>0/29</td>
<td>1/104</td>
<td>1/33</td>
<td>14/79</td>
<td></td>
</tr>
<tr>
<td>Di Pinto et al., 2010</td>
<td>2007-2009</td>
<td>2/294</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pesavento et al., 2010</td>
<td>2008</td>
<td>2/258</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prencipe et al., 2010</td>
<td>2005-2006</td>
<td>1/437</td>
<td>1/449</td>
<td>21/444</td>
<td>24/802</td>
<td></td>
</tr>
<tr>
<td>Angelidis et al., 2012</td>
<td>2010</td>
<td>0/83</td>
<td></td>
<td>0/38</td>
<td>0/16</td>
<td></td>
</tr>
<tr>
<td>Lambertz et al., 2012</td>
<td>2006-2012</td>
<td>2/465</td>
<td></td>
<td>0/62</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dambrosio et al., 2013</td>
<td>2009-2010</td>
<td>0/404</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Doménech et al., 2013</td>
<td>2005-2009</td>
<td>0/77</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parisi et al., 2013</td>
<td>2008-2010</td>
<td>3/70</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gyurova et al., 2014</td>
<td>2011-2012</td>
<td>0/17</td>
<td>0/7</td>
<td></td>
<td>0/34</td>
<td></td>
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<tr>
<td>Doménech et al., 2015</td>
<td>2006-2012</td>
<td>9/507</td>
<td>3/100</td>
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<td></td>
<td></td>
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<tr>
<td>Schoder et al., 2015</td>
<td>NS a</td>
<td>1/15</td>
<td>0/50</td>
<td>1/22</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spanu et al., 2015</td>
<td>2011-2013</td>
<td>3/50</td>
<td></td>
<td></td>
<td>7/33</td>
<td></td>
</tr>
<tr>
<td>Iannetti et al., 2016</td>
<td>2011-2012</td>
<td>0/421</td>
<td>0/106</td>
<td>8/190</td>
<td>11/177</td>
<td></td>
</tr>
<tr>
<td>Coroneo et al., 2016</td>
<td>NS a</td>
<td></td>
<td></td>
<td></td>
<td>15/87</td>
<td></td>
</tr>
</tbody>
</table>

a Number of listeriosis cases
b Serotype not reported (NR)
c Fatalities uncertain
d Septic abortion i.e. fatality
<table>
<thead>
<tr>
<th>Type of cheese</th>
<th>Number of <em>L. monocytogenes</em> positive (s) / total number of cheese samples (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>EFSA, 2006&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>2005&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2006&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Hard</td>
<td>Unpasteurized</td>
</tr>
<tr>
<td></td>
<td>Pasteurized</td>
</tr>
<tr>
<td>Soft/Semi-soft</td>
<td>Unpasteurized</td>
</tr>
<tr>
<td></td>
<td>Pasteurized</td>
</tr>
</tbody>
</table>

<sup>a</sup> Not specified; but assumed within the period 2005-2015.

### Table 3
Prevalence data (s/n) from EFSA reports.

### Table 4
Cheese samples in non-compliance with EU food safety limits for *L. monocytogenes* in RTE foods.


### Table 5
European studies reporting the prevalence of *Listeria* species in cheeses.

<table>
<thead>
<tr>
<th>References</th>
<th>Country</th>
<th>Sample size</th>
<th>Number of samples positive for different <em>Listeria</em> species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Massa et al., 1990</td>
<td>Italy</td>
<td>121</td>
<td>L. monocytogenes: 2, L. innocua: 2, L. grayi: 0, L. welshimer: 0, L. ivanovii: 0, L. seeligeri: 0</td>
</tr>
<tr>
<td>Quagilo et al., 1992</td>
<td>Italy</td>
<td>246</td>
<td>L. monocytogenes: 29, L. innocua: 42, L. grayi: 0, L. welshimer: 0, L. ivanovii: 0, L. seeligeri: 2</td>
</tr>
<tr>
<td>Rota et al., 1992</td>
<td>Spain</td>
<td>58</td>
<td>L. monocytogenes: 1, L. innocua: 2, L. grayi: 0, L. welshimer: 0, L. ivanovii: 0, L. seeligeri: 0</td>
</tr>
<tr>
<td>Pinto &amp; Reali, 1996</td>
<td>Italy</td>
<td>132</td>
<td>L. monocytogenes: 7, L. innocua: 30, L. grayi: 0, L. welshimer: 2, L. ivanovii: 0, L. seeligeri: 1</td>
</tr>
<tr>
<td>Theodoridis et al., 1998</td>
<td>Greece</td>
<td>334</td>
<td>L. monocytogenes: 26, L. innocua: 8, L. grayi: 0, L. welshimer: 0, L. ivanovii: 0, L. seeligeri: 10</td>
</tr>
<tr>
<td>Bottarelli et al., 1999</td>
<td>Italy</td>
<td>100</td>
<td>L. monocytogenes: 2, L. innocua: 2, L. grayi: 0, L. welshimer: 0, L. ivanovii: 0, L. seeligeri: 0</td>
</tr>
<tr>
<td>Rudolf &amp; Scherer, 2000</td>
<td>Germany</td>
<td>50</td>
<td>L. monocytogenes: 2, L. innocua: 13, L. grayi: 0, L. welshimer: 0, L. ivanovii: 0, L. seeligeri: 0</td>
</tr>
<tr>
<td>Rudolf &amp; Scherer, 2001</td>
<td>Austria</td>
<td>274</td>
<td>L. monocytogenes: 19, L. innocua: 33, L. grayi: 0, L. welshimer: 0, L. ivanovii: 0, L. seeligeri: 4</td>
</tr>
<tr>
<td>Vitas et al., 2004</td>
<td>Spain</td>
<td>99</td>
<td>L. monocytogenes: 1, L. innocua: 6, L. grayi: 1, L. welshimer: 0, L. ivanovii: 0, L. seeligeri: 0</td>
</tr>
<tr>
<td>Pintado et al., 2005</td>
<td>Portugal</td>
<td>63</td>
<td>L. monocytogenes: 32, L. innocua: 23, L. grayi: 0, L. welshimer: 0, L. ivanovii: 0, L. seeligeri: 2</td>
</tr>
<tr>
<td>Pesavento et al., 2010</td>
<td>Italy</td>
<td>258</td>
<td>L. monocytogenes: 2, L. innocua: 6, L. grayi: 1, L. welshimer: 1, L. ivanovii: 0, L. seeligeri: 0</td>
</tr>
<tr>
<td>Angelidis et al., 2012</td>
<td>Greece</td>
<td>137</td>
<td>L. monocytogenes: 0, L. innocua: 1, L. grayi: 2, L. welshimer: 0, L. ivanovii: 0, L. seeligeri: 1</td>
</tr>
<tr>
<td>Parisi et al., 2013</td>
<td>Italy</td>
<td>70</td>
<td>L. monocytogenes: 3, L. innocua: 3, L. grayi: 0, L. welshimer: 0, L. ivanovii: 0, L. seeligeri: 0</td>
</tr>
<tr>
<td>Schoder et al., 2015</td>
<td>Europe</td>
<td>87</td>
<td>L. monocytogenes: 2, L. innocua: 8, L. grayi: 0, L. welshimer: 0, L. ivanovii: 0, L. seeligeri: 0</td>
</tr>
<tr>
<td>Spanu et al., 2015</td>
<td>Italy</td>
<td>83</td>
<td>L. monocytogenes: 10, L. innocua: 3, L. grayi: 0, L. welshimer: 0, L. ivanovii: 0, L. seeligeri: 0</td>
</tr>
</tbody>
</table>
### Table 6
Meta-analysis results for prevalence of *L. monocytogenes* from scientific literature

<table>
<thead>
<tr>
<th>Meta-analysis type</th>
<th>Prevalence (CI)</th>
<th>(\tau^2)</th>
<th>(I^2(%))</th>
<th>(Q^d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Random-effects</td>
<td>0.023 (0.014-0.038)</td>
<td>1.72</td>
<td>86</td>
<td>197***&lt;sup&gt;e&lt;/sup&gt; (df = 35)</td>
</tr>
<tr>
<td>Multilevel</td>
<td>0.008 (0.003-0.019)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.12</td>
<td>75</td>
<td>108***&lt;sup&gt;e&lt;/sup&gt; (df = 31)</td>
</tr>
<tr>
<td>Fresh cheese</td>
<td>0.020 (0.008-0.049)&lt;sup&gt;ABf&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ripened cheese</td>
<td>0.024 (0.009-0.063)&lt;sup&gt;BF&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Veined cheese</td>
<td>0.051 (0.019-0.131)&lt;sup&gt;BF&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smear cheese</td>
<td>0.118 (0.035-0.333)&lt;sup&gt;BFJ&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> 95% confidence interval.
<sup>b</sup> Between-study variance.
<sup>c</sup> Between-study variance index proposed by Higgins & Thompson (2002).
<sup>d</sup> Q-statistic proposed by DerSimonian & Laird (1986).
<sup>e</sup> P-value < 0.001.
<sup>f</sup> Mean values for classes with the same capital letter do not differ significantly (p > 0.05).

### Table 7
Meta-analysis results for prevalence of *L. monocytogenes* from EFSA reports

<table>
<thead>
<tr>
<th>Meta-analysis type</th>
<th>Prevalence (CI)</th>
<th>(\tau^2)</th>
<th>(I^2(%))</th>
<th>(Q^d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Random-effects</td>
<td>0.007 (0.005-0.011)</td>
<td>1.09</td>
<td>98</td>
<td>1712***&lt;sup&gt;c&lt;/sup&gt; (df = 27)</td>
</tr>
<tr>
<td>Multilevel</td>
<td>0.006 (0.003-0.015)&lt;sup&gt;f&lt;/sup&gt;</td>
<td>1.17</td>
<td>88</td>
<td>1174***&lt;sup&gt;c&lt;/sup&gt; (df = 24)</td>
</tr>
<tr>
<td>Hard and un-pasteurized</td>
<td>0.012 (0.002-0.010)&lt;sup&gt;f&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soft/semi-soft and un-pasteurized</td>
<td>0.009 (0.004-0.019)&lt;sup&gt;g&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soft/semi-soft and pasteurized</td>
<td>0.010 (0.004-0.022)&lt;sup&gt;g&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> 95% confidence interval.
<sup>b</sup> Between-study variance.
<sup>c</sup> Between-study variance index proposed by Higgins & Thompson (2002).
<sup>d</sup> Q-statistic proposed by DerSimonian & Laird (1986).
<sup>e</sup> P-value < 0.001.
<sup>f</sup> Mean values within hard cheeses do not differ significantly (p > 0.05).
<sup>g</sup> Mean values within soft/semi-soft cheeses do not differ significantly (p > 0.05).
Table 8
Meta-analysis results assessing non-compliance with the criterion of “> 100 cfu/g” for L. monocytogenes in cheeses as reported by EFSA.

<table>
<thead>
<tr>
<th>Meta-analysis type</th>
<th>Prevalence (CI)</th>
<th>τ²</th>
<th>I²(%)</th>
<th>Qd</th>
</tr>
</thead>
<tbody>
<tr>
<td>Random-effects</td>
<td>0.002 (0.001-0.004)</td>
<td>1.22</td>
<td>84</td>
<td>154***c</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(df = 25)</td>
</tr>
<tr>
<td>Multilevel</td>
<td>1.18</td>
<td>82</td>
<td>95***c</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(df = 22)</td>
</tr>
<tr>
<td>Hard and un-pasteurized</td>
<td>0.001(0.000-0.004)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hard and pasteurized</td>
<td>0.002 (0.001-0.005)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soft/semi-soft and un-pasteurized</td>
<td>0.004 (0.002-0.012)</td>
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<td></td>
</tr>
<tr>
<td>Soft/semi-soft and pasteurized</td>
<td>0.002 (0.001-0.006)</td>
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<td></td>
<td></td>
</tr>
</tbody>
</table>

- 95% confidence interval.
- Between-study variance.
- Between-study variance index proposed by Higgins & Thompson (2002).
- Q-statistic proposed by DerSimonian & Laird (1986).
- P-value < 0.001.
- Mean values within hard cheeses do not differ significantly (p > 0.05).
- Mean values within soft/semi-soft cheeses do not differ significantly (p > 0.05).

Table 9
Meta-analysis results for prevalence of L. monocytogenes from combined data

<table>
<thead>
<tr>
<th>Meta-analysis type</th>
<th>Prevalence (CI)</th>
<th>τ²</th>
<th>I²(%)</th>
<th>Qd</th>
</tr>
</thead>
<tbody>
<tr>
<td>Random-effects</td>
<td>0.012 (0.008-0.018)</td>
<td>1.78</td>
<td>97</td>
<td>1961***c</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(df = 63)</td>
</tr>
<tr>
<td>Multilevel</td>
<td>1.38</td>
<td>97</td>
<td>1909***c</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(df = 62)</td>
</tr>
<tr>
<td>Scientific literature</td>
<td>0.007 (0.004-0.011)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EFSA reports</td>
<td>0.024 (0.015-0.038)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- 95% confidence interval.
- Between-study variance.
- Between-study variance index proposed by Higgins & Thompson (2002).
- Q-statistic proposed by DerSimonian & Laird (1986).
- P-value < 0.001.
- Mean values for classes with different capital letters differed significantly (p < 0.001).
Acknowledgements

The present study was supported by DTU Food and by Danish Veterinary and Food Administration. We thank Dr. Ursula Gonzales-Barron from Instituto Politécnico de Bragança, Portugal for advice on R code to performed forest plot.
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Highlights:

- Overview of listeriosis outbreaks caused by cheese 1983-2016

- Overall prevalence of *L. monocytogenes* in European cheese 2005 - 2015

- Prevalence of *L. monocytogenes* in different types of cheese

- No indicator or index organism identified for *L. monocytogenes* in cheese