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Published in:
Food Control

Link to article, DOI:
10.1016/j.foodcont.2017.07.020

Publication date:
2018

Document Version
Peer reviewed version

Citation (APA):
Martinez Rios, V., & Dalgaard, P. (2018). Prevalence of *Listeria monocytogenes* in European cheeses - A systematic review and meta-analysis. *Food Control*, *84*, 205-214. https://doi.org/10.1016/j.foodcont.2017.07.020

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

(EFSA, 2013a). 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 $\left(p_i = {}^{s_i}/_{n_i}\right)$ data was studied as observed effect size (θ_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 (θ_i) is a common metric that permits direct comparison and summation of primary studies (Borestein et al., 2009).

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

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):

$$T_i = \theta_i + \varepsilon_i = \mu + u_i + \varepsilon_i \quad (2)$$

where T_i is the true effect size for each primary study (i = 1, 2, …), ε_i is the sampling error and μ is the mean true effect size. u_i represents the true variation in effect sizes being compose of within-study (σ^2) and between-study variance (τ^2).

The between-study variance (τ^2) is estimated from the Q-statistic (DerSimonian & Laird 1986),

$$\hat{\tau}^2 = \begin{cases} \frac{Q-(k-1)}{\sum w_i - \frac{\sum w_i^2}{\sum w_i}}, & for\ Q > (k-1) \\ 0, & for\ Q \leq (k-1) \end{cases} \quad (3)$$

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).

$$Q = \sum w_i (T_i - \mu)^2 \quad (4)$$

$$\mu = \frac{\sum_i w_i T_i}{\sum_i w_i} \quad (5)$$

$$w_i = \frac{1}{\sigma_i^2 + \tau_i^2} \quad (6)$$

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 τ^2 and I^2 indices are related and higher τ^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:

$$T_i = \beta_0 + \beta_1 X_{1i} + \cdots + \beta_k X_{ki} + u_i + \varepsilon_i \quad (7)$$

with $(X_1 to X_k)$ being study characteristics and β_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, $r^2 = 0.86$, Fig. 2).

4. Discussion

It is critical to understand and quantified 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 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 compare 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 analysed 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-Izaga (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.*

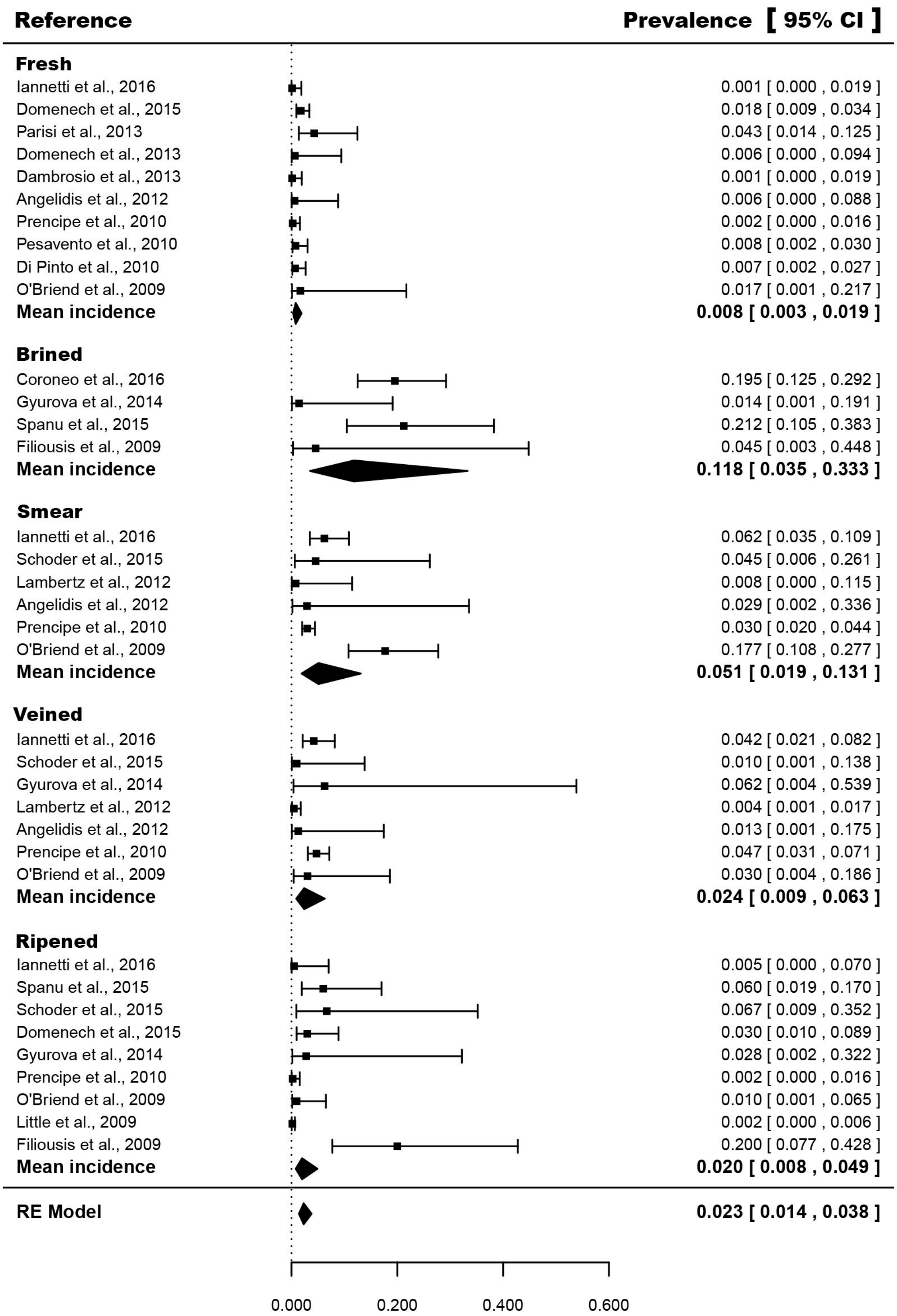

Fig. 1. Forest plot of the multilevel model based on scientific literature reporting prevalence of *L. monocytogenes* in different types of cheeses

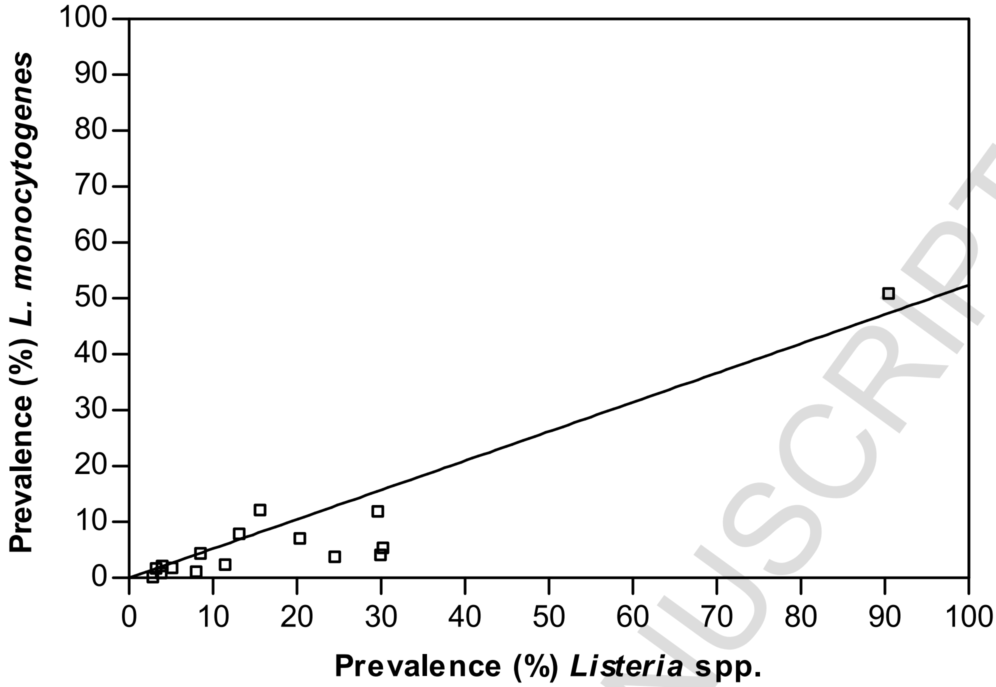

Fig. 2. Comparison of observed prevalence (%) for *Listeria* spp. and *L. monocytogenes* in European cheeses.

Table 1
Overview of listeriosis outbreaks caused by cheese during the period from 1983 to 2016.

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

USA	2011	NR[b]	2(?[c])	Fresh cheese (Chives cheese)	FIOD, 2011
Austria-Germany	2011-2013	1/2b	7(?[c])	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(?[c])	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

[a] Number of listeriosis cases
[b] Serotype not reported (NR)
[c] Fatalities uncertain
[d] Septic abortion i.e. fatality

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

		Number of *L. monocytogenes* positive (s) /total number of cheese samples (*n*)				
References	Survey year	Fresh	Ripened	Veined	Smear	Brined
Filiousis et al., 2009	2005-2006		4/20			0/10
Little et al., 2009	2006-2007		2/1240			
O'Brien et al., 2009	2007	0/29	1/104	1/33	14/79	
Di Pinto et al., 2010	2007-2009	2/294				
Pesavento et al., 2010	2008	2/258				
Prencipe et al., 2010	2005-2006	1/437	1/449	21/444	24/802	
Angelidis et al., 2012	2010	0/83		0/38	0/16	
Lambertz et al., 2012	2006-2012			2/465	0/62	
Dambrosio et al., 2013	2009-2010	0/404				
Doménech et al., 2013	2005-2009	0/77				
Parisi et al., 2013	2008-2010	3/70				
Gyurova et al., 2014	2011-2012		0/17	0/7		0/34
Doménech et al., 2015	2006-2012	9/507	3/100			
Schoder et al., 2015	NS[a]		1/15	0/50	1/22	
Spanu et al., 2015	2011-2013		3/50			7/33
Iannetti et al., 2016	2011-2012	0/421	0/106	8/190	11/177	
Coroneo et al., 2016	NS[a]					15/87

Total	17/2,580	15/2,101	32/1,218	50/1,158	24/164

[a] Not specified; but assumed within the period 2005-2015.

Table 3

Prevalence data (s/n) from EFSA reports.

Type of cheese		Number of *L. monocytogenes* positive (s) /total number of cheese samples (*n*)						
		EFSA, 2006[a]	EFSA, 2007[a]	EFSA, 2009[a]	EFSA, 2010[a]	EFSA, 2011[a]	EFSA, 2015[a]	EFSA, 2016[a]
		2005[b]	2006[b]	2007[b]	2008[b]	2009[b]	2013[b]	2015[b]
Hard	Un-pasteurized	0/969	38/718	16/3,242	2/1,606	2/1,001	15/1,618	11/858
	Pasteurized	0/1,367	5/3,284	68/9,449	85/10,877	15/7,246	77/8,288	19/2,384
Soft/ Semi-soft	Un-pasteurized	29/1,505	13/1,959	16/5,943	5/4,203	6/774	155/2,880	10/707
	Pasteurized	25/5,973	22/4,736	853/16,333	70/5,585	41/4,087	49/10,668	67/5,123

[a] References
[b] Survey year

Table 4

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

Type of cheeses		Cheese samples (n) with > 100 *L. monocytogenes*/g /Total number of samples (n)						
		EFSA, 2006[a]	EFSA, 2007[a]	EFSA, 2009[a]	EFSA, 2010[a]	EFSA, 2011[a]	EFSA, 2015[a]	EFSA, 2016[a]
		2005[b]	2006[b]	2007[b]	2008[b]	2009[b]	2013[b]	2015[b]
Hard	Un-pasteurized	?[c]	?[c]	2/1,569	0/133	2/940	1/2,854	0/880
	Pasteurized	0/672	7/1,701	14/2,292	3/4,005	1/9,894	10/3,041	0/141
Soft/ Semi-soft	Un-pasteurized	1/1,174	0/64	2/1,008	17/484	0/775	3/2,718	10/809
	Pasteurized	0/3,231	3/1,093	1/2,727	10/3,230	12/4,702	9/1,351	5/1,209

[a] References
[b] Survey year
[c] Not reported

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

References	Country	Sample size	Number of samples positive for different *Listeria* species					
			L. monocytogenes	*L. innocua*	*L. grayi*	*L. welshimer*	*L. ivanovii*	*L. seeligeri*
Comi et al., 1990	Italy	1740	65	145	15	185	18	0
Massa et al., 1990	Italy	121	2	2	0	0	0	0
Quagilo et al., 1992	Italy	246	29	42	0	0	0	2
Rota et al., 1992	Spain	58	1	2	0	0	0	0
Pinto & Reali, 1996	Italy	132	7	30	0	2	0	1
Theodoridis et al., 1998	Greece	334	26	8	0	0	0	10
Bottarelli et al., 1999	Italy	100	2	2	0	0	0	0
Rudolf & Scherer, 2000	Germany	50	2	13	0	0	0	0
Rudolf & Scherer, 2001	Austria	274	19	33	0	0	0	4
Vitas et al., 2004	Spain	99	1	6	1	0	0	0
Pintado et al., 2005	Portugal	63	32	23	0	0	0	2
Pesavento et al., 2010	Italy	258	2	6	1	1	0	0
Angelidis et al., 2012	Greece	137	0	1	2	0	0	1
Parisi et al., 2013	Italy	70	3	3	0	0	0	0
Schoder et al., 2015	Europe	87	2	8	0	0	0	0
Spanu et al., 2015	Italy	83	10	3	0	0	0	0
Total		3,852	203	327	19	188	18	20

Table 6

Meta-analysis results for prevalence of *L. monocytogenes* from scientific literature

Meta-analysis type	Prevalence (CI)[a]	τ^2 [b]	I^2(%)[c]	Q[d]
Random-effects	0.023 (0.014-0.038)	1.72	86	197***[e] (df = 35)
Multilevel		1.12	75	108***[e]
Fresh cheese	0.008 (0.003-0.019)[Af]			(df = 31)
Ripened cheese	0.020 (0.008-0.049)[ABf]			
Veined cheese	0.024 (0.009-0.063)[Bf]			
Smear cheese	0.051 (0.019-0.131)[Bf]			
Brined cheese	0.118 (0.035-0.333)[Bf]			

[a] 95% confidence interval.
[b] Between-study variance.
[c] Between-study variance index proposed by Higgins & Thompson (2002).
[d] Q-statistic proposed by DerSimonian & Laird (1986).
[e] P-value < 0.001.
[f] 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

Meta-analysis type	Prevalence (CI)[a]	τ^2 [b]	I^2(%)[c]	Q[d]
Random-effects	0.007 (0.005-0.011)	1.09	98	1712***[e] (df = 27)
Multilevel		1.17	88	1174***[e] (df = 24)
Hard and un-pasteurized	0.006 (0.003-0.015) [f]			
Hard and pasteurized	0.012 (0.002-0.010)[f]			
Soft/semi-soft and un-pasteurized	0.009 (0.004-0.019)[g]			
Soft/semi-soft and pasteurized	0.010 (0.004-0.022)[g]			

[a] 95% confidence interval.
[b] Between-study variance.
[c] Between-study variance index proposed by Higgins & Thompson (2002).
[d] Q-statistic proposed by DerSimonian & Laird (1986).
[e] P-value < 0.001.
[f] Mean values within hard cheeses do not differ significantly ($p > 0.05$).
[g] 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.

Meta-analysis type	Prevalence (CI)[a]	τ^2 [b]	I^2(%)[c]	Q[d]
Random-effects	0.002 (0.001-0.004)	1.22	84	154***[e] (df = 25)
Multilevel		1.18	82	95***[e] (df = 22)
Hard and un-pasteurized	0.001(0.000-0.004)[f]			
Hard and pasteurized	0.002 (0.001-0.005)[f]			
Soft/semi-soft and un-pasteurized	0.004 (0.002-0.012)[g]			
Soft/semi-soft and pasteurized	0.002 (0.001-0.006)[g]			

[a] 95% confidence interval.
[b] Between-study variance.
[c] Between-study variance index proposed by Higgins & Thompson (2002).
[d] Q-statistic proposed by DerSimonian & Laird (1986).
[e] P-value < 0.001.
[f] Mean values within hard cheeses do not differ significantly ($p > 0.05$).
[g] 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

Meta-analysis type	Prevalence (CI)[a]	τ^2 [b]	I^2(%)[c]	Q[d]
Random-effects	0.012 (0.008-0.018)	1.78	97	1961***[e] (df = 63)
Multilevel		1.38	97	1909***[e] (df= 62)
Scientific literature	0.007 (0.004-0.011)[Af]			
EFSA reports	0.024 (0.015-0.038)[Bf]			

[a] 95% confidence interval.
[b] Between-study variance.
[c] Between-study variance index proposed by Higgins & Thompson (2002).
[d] Q-statistic proposed by DerSimonian & Laird (1986).
[e] P-value < 0.001.
[f] 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**