

# The risk to vulnerable consumers from *Listeria monocytogenes* in blue cheese

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# **Executive summary**

This report was commissioned to assess the risk to vulnerable groups in the UK from blue cheese contaminated with *Listeria monocytogenes*. Blue cheese is defined as cheese internally ripened with Penicillium mould.

*L. monocytogenes* could be present in the raw milk, in the brine used in cheesemaking, or it may contaminate the cheese during processing, such as handling, cutting and packaging. Penicillium mould is introduced by piercing the cheese, which could also result in contamination with *L. monocytogenes*. Data from the UK suggest that the prevalence of *Listeria* spp. in blue cheese is around 1-3% of samples at retail, with enumeration levels varying from below the limit of detection (generally < 10 cfu/g) to 10<sup>3</sup> cfu/g. Prevalence studies from other European countries report low *Listeria* spp. prevalence in the cheese paste (interior) and higher prevalence in the rind, particularly of Gorgonzola.

A combination of factors, such as high salt, low pH and low water activity will inhibit *L. monocytogenes* growth, but this is part of a complex set of interactions which also includes storage temperature, initial pathogen levels, the presence of amino acids, free fatty acids, and peptides, which will affect the pathogen levels. There is also much variability in physiological factors such as pH and water activity, as observed by various surveys, even in the same type of cheese.

Blue cheese tends to be occasionally consumed in small portions of around 30 g. The infectious dose for vulnerable groups is considered to be low. Outbreaks of listeriosis linked to consumption of blue cheese are rare, with none reported in the UK.

The risk assessment concludes that the severity of of *L. monocytogenes* infection in vulnerable groups is **high** (severe illness: causing life-threatening or substantial sequelae or illness of long duration), with **low** uncertainty. The **frequency of occurrence** of listeriosis in the vulnerable population from consumption of blue cheese is considered **very low** (very rare but cannot be excluded), with **medium** uncertainty.

# Lay summary

#### Statement of purpose

This report assesses the risk to vulnerable people from *Listeria monocytogenes* in blue cheese. *L. monocytogenes* are bacteria that cause listeriosis, a disease which is very severe in vulnerable people. Vulnerable people include pregnant women, people over 65 years of age, infants, and those with a weakened immune system. The law states that cheese should not be contaminated with more than 100 colony forming units per gram of these bacteria at the end of shelf life.

Previous risk assessments concluded that while most semi-soft cheeses do not let *L. monocytogenes* grow, blue cheeses may be an exception. This is because the mould in blue cheese makes the interior less acidic, which helps *L. monocytogenes* grow.

#### **Hazard Identification**

*L. monocytogenes* is widespread in the environment and can grow at refrigeration temperatures. This makes it a particular problem in ready-to-eat foods such as cheese. It can grow at pH levels from 4.4 to 9.4. It can also remain in the environment in food factories for several years as it can be difficult to remove.

#### Hazard characterisation

Foodborne listeriosis is a relatively rare illness in comparison to other foodborne diseases. Listeriosis can be invasive or non-invasive. People with non-invasive listeriosis have mild symptoms that do not usually need medical attention. Invasive listeriosis typically affects vulnerable people, and can cause severe illness such as blood poisoning, inflammation of the brain and miscarriage or still-birth. It can also cause death.

A search found two potential listeriosis outbreaks and one individual case may have been caused by blue cheese worldwide. No listeriosis illnesses due to blue cheese were identified in the UK. Outbreaks caused by other cheeses with similar production methods were used to understand why contamination with the bacteria can happen.

#### **Exposure assessment**

Cheesemaking is complex, with many variable steps depending on what cheese is produced. *L. monocytogenes* can contaminate the blue cheese from unpasteurised milk, brine or the environment. Blue cheese, unlike other cheeses, is needled to allow air into the centre of the cheese for growth of the mould. This can also introduce the bacteria into the cheese. Blue cheese has very variable characteristics, so the same

type of cheese can have different acidity, moisture content and salt levels. These characteristics affect the ability of *L. monocytogenes* to survive and grow in the cheese.

Blue cheese is not frequently consumed by vulnerable consumers. When consumed, it is usually in low amounts.

The report looked at published data, for instance by Scottish local authorities and the Food Standards Agency (FSA), to understand how much contamination there is in blue cheese. These data suggest that overall percentage of blue cheeses contaminated with *L. monocytogenes* in the UK is low.

A search of the scientific literature on contamination in blue cheese from European countries found that most of these studies examined Gorgonzola cheese. The rinds of Gorgonzola were much more likely to be contaminated than the centre of the cheese, and it is possible for cutting of the cheese to contaminate the centre. Other cheeses were unlikely to be contaminated with the bacteria, and if they were, it was usually at very low levels, below 100 colony forming units per gram.

Research also shows that the acidic levels and levels of moisture in blue cheese can support *L. monocytogenes* growth. Most of these studies showed only a small amount of bacterial growth in the centre of the cheese.

#### **Risk characterisation**

The risk assessment concludes that the **severity** of *L. monocytogenes* infection in vulnerable people is **high** (severe illness: causing life-threatening or substantial chronic complications or illness of long duration), with a significant mortality rate. There is a **low** level of uncertainty due to the strong evidence that *L. monocytogenes* causes severe illness in vulnerable groups.

The **frequency of occurrence** of listeriosis in vulnerable people from consumption of blue cheese is considered **very low** (very rare but cannot be excluded), based on the evidence gathered for blue cheese including outbreaks, typical *L. monocytogenes* contamination levels, typical consumption levels and typical *L. monocytogenes growth*. The **uncertainty of occurrence** is considered to be **medium**. This is because there is variability in cheese production methods, many factors affecting bacterial growth that interact in complex ways and difficulty in tracing listeriosis cases to the food source.

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# **Abbreviations**

Term	Definition
a <sub>w</sub>	water activity - how much free water is in a product that is
	unbound, so available for microorganisms to grow
cfu	colony forming unit
EFSA	European Food Safety Authority
FDA	Food and Drug Administration (USA)
FSA	Food Standards Agency
FSS	Food Standards Scotland
L. monocytogenes	Listeria monocytogenes
NDNS	National Diet and Nutrition Survey
RTE	ready-to-eat
spp.	species

# 1. Statement of purpose

#### 1.1 Risk question

What is the risk to all vulnerable consumers from blue cheeses (pasteurised and unpasteurised) from *Listeria monocytogenes*?

This assessment will primarily inform a revision of FSA and FSS guidance on foods to avoid in pregnancy, to ensure that advice to pregnant consumers is based on the best available evidence. The assessors were also asked to consider the risk to vulnerable groups as well as pregnant consumers.

#### **1.2 Advice to consumers**

The <u>NHS website</u> (NHS, 2020) and <u>Northern Ireland official government website</u> (NIdirect, 2022) provide a section on foods to avoid eating in pregnancy. The cheeses that can be eaten are:

- pasteurised or unpasteurised hard cheeses, such as cheddar, Gruyere and parmesan
- pasteurised semi-hard cheeses, such as Edam and Stilton
- pasteurised soft cheeses, such as cottage cheese, mozzarella, feta, cream cheese, paneer, ricotta, halloumi, goats' cheese without a white coating on the outside (rind) and processed cheese spreads
- soft or blue cheese (pasteurised or unpasteurised) that has been cooked until steaming hot
- pasteurised milk, yoghurt, cream and ice cream

The cheeses that should be avoided are:

- any other foods made from unpasteurised milk, such as soft ripened goats' cheese
- pasteurised or unpasteurised mould-ripened soft cheeses with a white coating on the outside, such as Brie, Camembert and chèvre (unless cooked until steaming hot)
- pasteurised or unpasteurised soft blue cheeses, such as Danish blue, Gorgonzola and Roquefort (unless cooked until steaming hot)
- unpasteurised cows' milk, goats' milk, sheep's milk or cream

The advice on the <u>NHS Wales website</u> includes Stilton in the list of hard pasteurised cheeses that can be eaten (NHS Wales, 2022). The Welsh website holds similar advice about hard cheeses, cooked cheeses and soft cheeses made from pasteurised milk being safe to consume. The <u>NHS Scotland</u> website advises pregnant women to avoid unpasteurised semi-hard and soft cheeses, mould-ripened soft cheeses with a white

coating (such as brie, camembert, chèvre), and soft blue cheeses (such as Danish Blue, Gorgonzola, Roquefort), unless cooked (NHS Scotland, 2022).

## **1.3 Legislation**

Annex I of Retained Commission Regulation (EC) No 2073/2005 (for England, Wales and Scotland) (*Commission Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs (Text with EEA relevance)*, 2020) and <u>Commission</u> <u>Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for</u> <u>foodstuffs</u> (Northern Ireland) (*Commission Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs (Text with EEA relevance)*, 2014) have criteria for *L. monocytogenes* in food (Table 1).

Table 1 – Legislative criteria for *L. monocytogenes* in food. \* If the producing food business operator is not able to demonstrate that the product will not exceed the limit of 100 cfu/g throughout the shelf-life, the limit is Absence in 25 g.

Food category	Limits	Stage where the criterion applies
Ready-to-eat foods intended for infants and ready-to-eat foods for special medical purposes	Absence in 25 g	Products placed on the market during their shelf-life
Ready-to-eat foods able to support the growth of <i>L.</i> <i>monocytogenes</i> , other than those intended for infants and for special medical purposes	100 cfu/g*	Products placed on the market during their shelf-life
Ready-to-eat foods unable to support the growth of <i>L.</i> <i>monocytogenes</i> , other than those intended for infants and for special medical purposes	100 cfu/g	Products placed on the market during their shelf-life

The regulation lists products unable to support the growth of *L. monocytogenes* as including foods that fall into one of these categories:

- a pH ≤ 4.4
- an a<sub>w</sub> ≤ 0.92

- a shelf life of less than 5 days
- a pH  $\leq$  5.0 and an a<sub>w</sub>  $\leq$  0.94

#### **1.4 Previous risk assessments**

#### 1.4.1 Risk assessment of L. monocytogenes in UK retailed cheese

In 2006, the FSA commissioned a risk assessment for *L. monocytogenes* in UK retail cheese(Banks, 2006). This risk assessment took a deeper look at some specific cheeses as representative of the category including Gorgonzola, a soft blue veined pasteurised cheese with veins of blue green *Penicillium roqueforti* throughout the entire cheese structure. It described the physicochemical characteristics of the cheese and summarised data on the levels of contamination within the cheese, which are discussed later in the text. The moisture content of Gorgonzola at the point of consumption is approximately 50%, with an average water activity (a<sub>w</sub>) of 0.968 and 3.5 - 5.5% sodium chloride content. The report described that cheese rind was more likely to be contaminated with *L. monocytogenes* compared to the interior of the cheese (the cheese paste).

The report rated Gorgonzola and Stilton as low risk to the vulnerable population, whereas Roquefort was rated as moderate risk. Banks' conclusion is that blue cheese offers up a dilemma. Although the majority of blue cheese consumed in the UK is made from pasteurised milk, the needling of the rind and significant changes to the acidity level introduce a potential risk, as shown by the occasional detection of low numbers of *L. monocytogenes* in blue-veined cheese, especially on the rind, in both pasteurised and raw milk types. Additionally, blue cheeses are microbiologically very active during their production, the complex microbial associations and successions are difficult to predict and manage, and post-processing contamination can be introduced during activities such as portioning the cheese (Banks, 2006).

# 1.4.2 Quantitative assessment of relative risk to public health from foodborne Listeria monocytogenes among selected categories of ready-to-eat foods

In a 2003 Food and Drug Administration (FDA) quantitative assessment on ready-to-eat foods, cheese was categorised into six types based on moisture content (FDA, 2003). Blue cheese was included in the category of 'Semi-soft Cheese', 39-50% moisture. It is speculated that the FDA used semi-soft as a category due to the different consumption preferences and/or habits of the consumers in the USA. The risk assessment literature review found that semi-soft cheeses do not generally permit the growth of *L*.

*monocytogenes*. Of the 10 datasets found in the literature, levels of *L. monocytogenes* declined in eight studies, giving an overall mean decreasing growth rate at 5°C. The storage times had a maximum of 15 to 45 days.

The risk characterisation combined the exposure and dose-response models to predict the relative risk of illness attributable to each food category. The relative risk ranking of semi-soft cheese to non-vulnerable groups was considered to be a low in both the per serving category (< 1 case per billion servings) and per annum (< 1 case per annum). This is due to small serving sizes, the annual number of servings, a moderate contamination frequency, and the low levels of *L. monocytogenes* at retail.

The risk assessment suggests that the risk associated with these products mostly comes from recontamination, though it is important to note that this category is for all semi-soft cheese, not exclusively blue cheese.

The risk assessment stated that the work done reinforces past epidemiological conclusions that foodborne listeriosis is a moderately rare although severe disease and that vulnerable groups are more likely to contract listeriosis than the general population.

#### 1.4.3 Risk profile: Listeria monocytogenes in low moisture cheeses

This 2005 risk profile for the New Zealand Food Safety Authority provides a summary of the food/hazard combination and the risk posed to the New Zealand vulnerable population and considers three types of low moisture cheeses (Table 2) (Lake *et al.*, 2005).

Category	Moisture content
semi-soft	39 – 50%
hard	< 39%
very hard	< 34%

Table 2 - Three types of low moisture cheeses categorised in Lake et al., 2005.

The semi-soft category includes blue cheeses such as Stilton, Roquefort, Gorgonzola, Danish Blue). The report found that *L. monocytogenes* is usually inactivated during the ripening of low moisture cheeses due to a combination of physiological characteristics including lowering of the pH and salt content. However, blue cheese is an exception to this due to its mould-induced rise in pH during ripening which may allow survival. Pasteurisation and appropriate environmental hygiene measures were identified as

important measures for reducing the risks from these types of cheeses. The report concluded that, overall, *L. monocytogenes* in low moisture cheese did not represent a significant risk to human health.

A 2015 update to the risk profile did not find any substantial changes to the risk (Paulin *et al.*, 2015).

# 2. Hazard identification

Blue cheeses are blue-veined cheese or blue mould-ripened cheese which are ripened with *Penicillium roqueforti* or *Penicillium glaucum*. These give its colour and flavour (Almena-Aliste and Mietton, 2014). The *Penicillium* mould is usually present internally in blue cheeses rather than on the surface, which occurs in cheeses such as Brie and Camembert.

*Listeria monocytogenes* is a species of Gram positive, facultatively anaerobic, rodshaped bacteria, which is non-spore forming. There are many reservoirs of *L. monocytogenes*, as it can infect and cause listeriosis in ruminants (Walland *et al.*, 2015) and unlike other foodborne pathogens can live and grow in the natural environment without the need to grow within an animal host (Chasseignaux *et al.*, 2001). Environmental cross-contamination is a major issue with respect to *L. monocytogenes*. It can occur through direct contact with raw materials, personnel, aerosols and contaminated utensils, equipment, etc. Cross-contamination can occur at any step where the product is exposed to the environment, including processing, transportation, retail, catering and in the home. *L. monocytogenes* can form biofilms on foodprocessing equipment, food-contact surfaces and drains, and is able to persist for years in food-processing environments, leading to re-contamination of food or food contact surfaces. Cooking at temperatures higher than <u>70°C for 2 minutes</u> kills *Listeria* spp. (FSA, 2018a), however *Listeria* spp. can contaminate foods after production, for instance in sliced, cooked meats (Little *et al.*, 2009).

*L. monocytogenes* can grow over a wide range of temperatures, including refrigeration and thawing temperatures ( $-0.4 - 50^{\circ}$ C) (Farber and Peterkin, 1991). It is resistant to various environmental conditions, such as low oxygen, refrigeration temperatures, high salt or acidity which results in survival for long periods on foods, and for years in the environment, in the processing plant, and in household refrigerators (Miller, 1992). *L. monocytogenes* can survive freezing for several weeks at -18°C in food substrates. Minimum, optimum and maximum conditions for growth are given in Table 3.

The growth of *L. monocytogenes* at low temperatures is significant when taken into account with the recognition that domestic refrigerators run at higher than the

recommended temperature of between 1 and 5 °C. Mean temperatures exceeding 5 °C were recorded in 91% of refrigerators of UK consumers (Evans and Redmond, 2016). A similar study of domestic cold appliances in England found that the average temperature over seven days of 671 refrigerators was 5.3 °C (Biglia *et al.*, 2018).

*Listeria* spp. other than *L. monocytogenes* are not pathogenic to humans, with rare exceptions. Their presence can be used as an indicator to assess if *L. monocytogenes* can be found or grow in a food product. *Listeria* spp. are also environmental contaminants that can survive in both food processing premises and on equipment if inappropriate hygiene measures are used. If the conditions allow for *Listeria* spp. to survive then it is also possible for *L. monocytogenes* to survive.

Cases and outbreaks of listeriosis are predominantly associated with chilled ready-toeat foods such as pre-packed sandwiches, sliced and cooked meats, cheese and smoked fish. A large factor for this is due to *L. monocytogenes* being able to grow at refrigeration temperatures.

-	Minimum	Optimum	Maximum
Temperature (°C)	-0.4	37	45
рН	4.4	7.0	9.4
Water activity (a <sub>w</sub> )	0.92	-	-

Table 3 - Based on Table C (ICMSF, 1996) showing the minimum, optimum and maximum growth conditions for L. monocytogenes.

This assessment evaluates the risk to all vulnerable groups, including pregnant women, from *Listeria monocytogenes* in blue cheese. Vulnerable groups are defined according to the <u>FSA Guidance for healthcare and social care organisations (FSA, 2018b)</u>:

"Those individuals whose immune system is weakened in some way and who are therefore both more susceptible to developing listeriosis and likely to develop more severe symptoms as a result of the infection. This includes but is not limited to: cancer patients, patients undergoing immunosuppressive or cytotoxic treatment, unborn and newly delivered infants, pregnant women, people with diabetes, alcoholics (including those with alcoholic liver disease) and a variety of other conditions. Immune system capacity decreases progressively in the elderly, so elderly individuals are also included in this group. Rarely, infection can occur in patients without any known risk factors."

The "elderly" are those over 65 for the purpose of this risk assessment.

# 3. Hazard characterisation

*L. monocytogenes* is a human foodborne pathogen, with infection by *L. monocytogenes* known as listeriosis. Foodborne listeriosis is a relatively rare illness in comparison to other foodborne diseases (in 2019, 142 cases of listeriosis were reported in England and Wales, 6 were reported in Scotland), however the outcomes of illness can be serious with high fatality rates reported (PHE, 2018 and PHS, 2020). Listeriosis mainly affects vulnerable groups such as immunosuppressed people, infants and pregnant women (and their unborn children). Levels of miscarriage are around 30%, but a *L. monocytogenes* infection can be asymptomatic in the pregnant woman.

The development of listeriosis is typically initiated by ingestion of the organism, followed by its survival against the non-specific immune system defences of the gastrointestinal tract. *L. monocytogenes* is able to cross the gastrointestinal, placental, and blood–brain protective barriers.

#### 3.1 Symptoms

Various clinical manifestations are associated with *L. monocytogenes* infection, and these can be grouped in two categories: invasive and non-invasive listeriosis. Non-invasive listeriosis typically occurs in immunocompetent individuals, it has been observed during a number of outbreaks where the majority of cases developed symptoms of gastroenteritis, such as diarrhoea, fever and headache, after a short period of incubation (Aureli *et al.*, 2000). Non-invasive listeriosis has not been well studied as the clinical presentations do not typically warrant medical intervention and are therefore not identified and reported. Non-invasive listeriosis is typically self-limiting and symptoms only last a few days (Aureli *et al.*, 2000).

Invasive listeriosis typically occurs in vulnerable or immunocompromised individuals. The symptoms of invasive listeriosis are severe, and include fever, myalgia (muscle pain), septicaemia, and meningitis. The incubation period is usually one to two weeks but can vary from between a few days to 90 days (WHO, 2018) (Johnsen *et al.*, 2010). The long incubation period creates difficulty in identifying outbreaks and implicating food vehicles of infection. Invasive listeriosis presents a particularly serious risk to pregnant women including unborn babies, people with weakened immune systems and elderly people, and can cause very severe illness or even death (De Luca *et al.*, 2015). The case fatality rate of invasive listeriosis is high, ranging from 20 - 30% (Mead *et al.*, 1999). Pregnant women infected with *L. monocytogenes* can experience miscarriage, stillbirth and premature birth, which while not typically fatal for the mother can be fatal for the foetus (Pezdirc *et al.*, 2012). *L. monocytogenes* is a significant cause of septicaemia and meningitis in neonates. Overall, all people can become ill from *L.* 

*monocytogenes* infection, however elderly people, pregnant women, new-born babies and immunocompromised persons are high-risk groups.

#### Chronic and acute infections of L. monocytogenes

*L. monocytogenes* is established as a cause of acute, self-limited, febrile gastroenteritis in healthy persons. Invasive listeriosis causes severe acute (and sometimes fatal) illness, however post-listeriosis sequelae, such as neurological symptoms can persist chronically (Drevets and Bronze, 2008). In addition, there are findings that some bacteria are able to colonise and persist in the gallbladder, which suggests the occurrence of long-term and chronic infections and demonstrates the ability of pathogenic *Listeria* spp. to survive within the various microenvironments of the gastrointestinal tract for a long period (Gahan and Hill, 2005). Although rare, *L. monocytogenes* infections can also affect bone, joints and sites in the chest which could develop into chronic disease (Bader, Al-Tarawneh and Myers, 2016).

#### 3.2 Dose Response

Quantitative assessments indicate that among healthy adults, exposure to high doses  $(10^5 \text{ cfu/g or greater})$  of *L. monocytogenes* in foods is required to cause febrile gastroenteritis (Smith *et al.*, 2008). Levels of < 100 cfu/g in food at point of consumption are regarded as safe, meaning that people consuming foods with low levels of *L. monocytogenes* have an extremely low risk of contracting listeriosis (EFSA, 2014, EFSA, 2013 and EFSA et al., 2018). Nevertheless, as with all disease-causing microorganisms, there is no threshold below which there is a true "zero" risk for human illness. In ready-to eat (RTE) products intended for infants and for special medical purposes, or in RTE foods that are able to support the growth of the bacterium (such as milk, cheese, salmon etc), there must be absence of *L. monocytogenes* in 25 g of sample, unless the food business operator can demonstrate that levels will not exceed 100 cfu/g (EFSA *et al.*, 2018).

Dose-response data from human volunteer studies with *L. monocytogenes* or from volunteer studies with a surrogate pathogen do not exist. Existing dose-response relationships have been developed based on expert elicitation, epidemiological or animal data. The dose-response has been found to vary as a function of host susceptibility, the virulence of the strain and also the attributes of the food matrix the pathogen has contaminated (WHO, 2004).

In 2018, the European Food Safety Authority (EFSA) conducted a risk assessment on *"Listeria monocytogenes contamination in RTE food and the risk of human health"* (EFSA *et al.*, 2018). This risk assessment states that the average probability of a single *L. monocytogenes* cfu to cause illness in a specific host (the r value), reflects the strain

virulence and host susceptibility, and ranges three orders of magnitude, from the least (i.e., under 65 years old without underlying condition) to the most susceptible (i.e. immunocompromised) populations. Reported r values for specific outbreaks with highly susceptible populations increased the range by another five orders of magnitude. Thus, the probability of a single cfu to cause illness may range 100 million times depending on variability in host susceptibility and *L. monocytogenes* virulence (EFSA *et al.*, 2018). As a result, there is no single value for infectious dose.

In earlier dose response modelling work, Pouillot et al., 2015, reviewed available literature and estimated that the relative risk of listeriosis for pregnant women was 100 times higher than for non-pregnant women, and that relative risk levels more than 1000 times higher than that for the < 65-year-old general population have been reported for individuals with chronic lymphocytic leukaemia (Pouillot *et al.*, 2015). These data indicate the uncertainty in the understanding of infectious dose. For example, Pouillot et al., 2009 reported that the mean risk of contracting invasive listeriosis for susceptible (immunocompromised) individuals is 54 times that of the general population, but Pouillot et al., 2015, reported a risk level of more than x1000 for a specific medical condition.

#### 3.3 L. monocytogenes outbreaks linked to cheese

#### 3.3.1 Listeriosis cases in the UK

In 2020 in the EU (note - also includes UK data), L. monocytogenes was identified in 16 outbreaks in seven member states (Austria, Denmark, Finland, France, Germany, Italy, Netherlands) and in four outbreaks reported by two non-member states (Switzerland, United Kingdom) (EFSA and ECDC, 2021). The above-mentioned outbreaks were responsible for 163 cases, 126 hospitalisations and 30 deaths. In EU member states, L. monocytogenes was associated with the highest case fatality rate among outbreak cases (14.2%). The fatality rate of listeriosis outbreaks was also significant in nonmember states with 13 fatalities among a total of 43 cases caused by the outbreaks (30.2%). An L. monocytogenes outbreak reported by Switzerland deserves particular attention, due to its impact. The Swiss outbreak was caused by L. monocytogenes serovar 4b, resulting in 34 cases including 10 deaths (EFSA and ECDC, 2021) and the implicated food was a pasteurised soft brie (Food Safety News, 2020). The source of the outbreak was identified as persistent environmental contamination. In total (including the sporadic cases) in 2020, there were 1,876 confirmed invasive human cases of L. monocytogenes that caused 780 hospitalisations and 167 deaths in the EU (EFSA and ECDC, 2021).

According to annual Listeria surveillance reports by Public Health Scotland, there have been 17 cases of listeriosis reported in Scotland in 2017,12 cases in 2018 and 6 cases

in 2019. Overall cases reported in Scotland from 2007 to 2019 vary from year to year (without significantly increasing or decreasing) with the highest number of cases (22-23) reported in 2007 and lowest number of cases (6) reported in 2019 (HPS 2019, HPS 2020).

A study of human foodborne listeriosis in England and Wales between 1981 to 2015 was published in 2020 (McLauchlin, Grant and Amar, 2020). Between 1981 and 2015, 5252 human listeriosis cases were reported in England and Wales, broken down into community and hospital incidents. These cases were attributed to 17 outbreaks and 11 sporadic cases. The majority of human listeriosis cases were not attributed to a specific food exposure. The study notes that, although almost all cases of human listeriosis are foodborne, the proportion of cases where specific exposures were identified is small. This is due to the long incubation period of listeriosis, which creates difficulty in associating cases with specific commodities (McLauchlin, Grant and Amar, 2020). The study does not specifically comment on how many of the listeriosis cases were in vulnerable groups, however for the purposes of this risk assessment, it is assumed that the affected cases from hospital related outbreaks were in vulnerable groups.

#### 3.3.2 Listeriosis outbreaks in published studies

The above-mentioned studies are informative of the overall prevalence of listeriosis in the UK and EU, as a rare disease with a high mortality rare. Additionally, peer reviewed literature was searched for appropriate articles, where cheese products were identified as the source of foodborne listeriosis outbreaks.

Outbreaks of listeriosis due to cheese products were identified from supplementary tables from two papers (Wemmenhove et al., 2021 and FDA and Health Canada, 2015), and are provided in the Appendix (Section 8.2).

Additionally, a PubMed search was carried out to identify recent listeriosis outbreaks with information on the cause of the outbreak, the vulnerable groups affected, the product type, and which were supported with laboratory as well as epidemiological data. These nine recent outbreaks are chosen as representative for listeriosis incidents in cheese and studied in more detail. Details of the PubMed search terms used, the results and a summary of nine relevant articles found are provided in Annex 1.

The literature search resulted in two potential listeriosis outbreaks and one case caused by blue cheese, and these are summarised in Table 4.

Table 4 – Listeriosis outbreaks thought to be caused by blue cheese.

Year, Location	Cases (Deaths)	Detail	Reference
2011, United States	15 (1)	The illnesses were reported at wedding banquets and the blue-veined cheese in question was made from unpasteurised milk and aged for 60 days.	CDC, 2013 FDA and Health Canada, 2015
1989-1990, Denmark	26 (6)	Epidemiological/culture data indicate that the source of the outbreak was either a blue-mould cheese or a hard cheese.	Jensen et al., 1994
2003, Italy	1 (0)	Case was a 63-year-old immunocompromised man. <i>L.</i> <i>monocytogenes</i> from Gorgonzola in the patient's refrigerator, unopened Gorgonzola cheese of the same brand, and the cheese production plant matched samples from patient's blood. The two cheese packets from the patient's fridge had 150 cfu/g and 1,200 cfu/g and a pH of 7.4 Samples from the production plant and food store had levels <100 cfu/g and a pH of 6.2 – 7.0.	Gianfranceschi et al., 2006

Table 5 provides a brief summary of the nine outbreak studies, including the most relevant information for this risk assessment. While these are not blue cheeses, the source of *L. monocytogenes* contamination will be similar to what might occur during blue cheese production. For more information for each reported outbreak refer to Annex 1. The number of affected individuals varies between the outbreaks, with the smallest being two individuals in a Spanish incident and the highest being 389 people in a German incident. It is of note that in these published outbreak studies, vulnerable individuals were affected including the elderly (defined as individuals 65 and over years of age), pregnant women, new-borns, and children (under nine years of age). All nine outbreaks resulted in hospitalisation, while four resulted in deaths, seven resulted in pregnancy-associated illness and three resulted in foetal loss or miscarriage. Where information was available, it was noted if the product was made from pasteurised milk – in five outbreaks the product was made from pasteurised milk and in four outbreaks the

pasteurisation status of the cheese was not available. Where information on the type of cheese was available, this was noted.

Table 5 - Listeriosis outbreaks linked to cheese: year, location, number of cases, type of vulnerable groups affected, product type, and whether the implicated product made from pasteurised/non pasteurised milk. L. monocytogenes was isolated from the cheese and/or the premises in each case. Description and summary of findings for each outbreak study listed is available in Annex 1.

Year, Location (Reference)	Cases (deaths)	Vulnerable groups affected	Product type	Pasteurised?	Notes
2018-2020, Switzerland (Nüesch- Inderbinen <i>et al.</i> , 2021)	34 (10)	Elderly	Soft brie cheese	From pasteurised milk	Persistent environmental contamination of cheese dairy with outbreak strain. <i>L. monocytogenes</i> identified in 11 of 50 environmental samples; all five sequenced isolates matched outbreak strain. Recall of multiple cheeses. Brie is made from whole or semi-skimmed milk. The curd is obtained by adding rennet to raw milk. Brie can be inoculated with <i>Penicillium</i> cultures.
2013, United States (Choi <i>et al.</i> , 2014)	5 (1)	Elderly, pregnant, new-borns	Soft- ripened cheese	From pasteurised milk	Environmental isolates from plant and two cheeses from two different grocery stores matched outbreak strain. Sanitation deficiencies at plant – contamination thought to have occurred after pasteurisation. Three types of cheeses recalled. Crave Brothers Les Frères is a semi-soft, washed rind cheese similar to Reblochon.

Year,	Cases	Vulnerable	Product	Pasteurised?	Notes
Location	(deaths)	groups	type		
(Reference)		affected			
2012, Unites Stated (Heiman <i>et</i> <i>al.</i> , 2016)	22 (5)	Elderly, pregnant, new-borns	Ricotta salata/ other cheeses	Not reported	Samples from cut and repackaged cheeses matched outbreak strain. Some patients reported eating ricotta salata, which contained the outbreak strain; other patients reported eating cheese likely cut with same equipment or cross-contaminated in another way. Cheeses recalled. Ricotta salata is an Italian cheese made from sheep milk whey, which is pressed, salted and aged. It is milky white in colour with a firm texture.
2012, Spain (de Castro <i>et al.</i> , 2012)	2 (0)	Pregnant, new-borns	Fresh/soft cheese	From pasteurised milk	Six out of six samples from different batches of the same cheese contaminated with <i>L. monocytogenes</i> , five were above 100 cfu/g and one was $3.2 \times 10^4$ cfu/g. Product withdrawn.
2009-2012, Portugal (Magalhaes <i>et al.</i> , 2015)	30 (11)	Elderly, pregnant, new-borns	Queijo fresco	Not reported	Outbreak strain detected in cheese samples from plant. Cross-contamination between products or environment is thought to have contaminated brand eaten by cases. Queijo fresco is a white cheese, made from cow's milk and goat milk, acidified with rennet, lemon juice, or vinegar.

Year,	Cases	Vulnerable	Product	Pasteurised?	Notes
Location	(deaths)	groups	type		
(Reference)		affected			
2009-2011, Italy (Amato <i>et al.</i> , 2017)	43 (NA)	Pregnant, elderly	Soft cheese, Taleggio cheese	Not reported. Suspect smear- ripened cheese – likely not pasteurised	Cheese sample and environmental sampling in plant found <i>L. monocytogenes</i> with same PFGE profile. Not recalled. Taleggio is a semisoft, washed-rind, smear-ripened Italian cheese.
2009, United States (Jackson <i>et</i> <i>al.</i> , 2011)	13 (1)	Pregnant, new-borns	Asadero cheese	From pasteurised milk	Pasteurised milk from manufacturing facility contained the outbreak strain. Recall of cheese. Asadero is white, semi-hard Mexican cheese.
2009, Austria and Germany (Fretz <i>et al.</i> , 2010)	46 in Austria, 389 in Germany (NA)	Elderly	Acid curd cheese 'Quargel'	Not reported. Cheese was made from curdled milk, which ripens after addition of starter cultures – likely not pasteurised	Cases were ill with different <i>L. monocytogenes</i> isolates. Environmental isolates at plant matched case isolates; cheese sampled at plant had 3 <i>L. monocytogenes</i> strains at less than 100 cfu/g. Other Quargel cheese sampled had levels > 100 cfu/g. Cheese recalled. Quargel is an acid curd cheese with a red smear made from skimmed pasteurised milk. It is a soft yellow cheese.

Year, Location (Reference)	Cases (deaths)	Vulnerable groups affected	Product type	Pasteurised?	Notes
2002, Canada (McIntyre, Wilcott and Naus, 2015)	134 (NA)	Elderly, pregnant, children	Soft ripened cheese	From pasteurised milk	Two outbreaks – both from cheeses sold at two separate farmer's markets. Environmental transmission from farm animals to personnel to culture solutions used during cheese production for one outbreak. Wild birds are the likely source for second outbreak – swallows defecating in plant's open cistern water reservoir & failure in water disinfection system.

# 4. Exposure assessment

## 4.1 Risk pathway



Figure 1 - Stages of blue cheese production adapted from Banks 2006.

Cheesemaking is a complex process, with the basic steps outlined in Figure 1. Cheese can be made by small-scale artisans to large-scale producers. Variation can be found in all stages of the process, such whether the starting milk, is pasteurised or thermised, the length of maturation, whether the cheese is being produced at a small or large scale.

In Needling (Stage 5 of Figure 1) is included in blue cheese processing, as this allows air into the cheese which, in turn, encourages the growth of the mould that had been introduced at curd salting (Stage 4 of Figure 1) but was unable to grow in the condition until after needling. The level and prevalence of *L. monocytogenes* in raw milk is low in the pooled milk, however, individual cows can excrete high numbers of *L. monocytogenes*, so if milk is used without dilution, these levels could still be high (Hunt et al. 2012, Banks 2006). Commercial pasteurisation, if carried out correctly, will eliminate this risk. Initial levels of *Listeria monocytogenes* present in raw milk cheese, or cheese which undergoes incomplete pasteurisation, may be reduced as a result of competition from lactic acid bacteria and the effects of the low pH (high acidity) at the end of fermentation. *L. monocytogenes* may also be present under common brining conditions or the surrounding food processing areas and may contaminate the cheese during processing where the rind is penetrated, such as needling, cutting or packaging. Hard cheeses made of raw milk are generally considered safe, as their low aw (water available for growth of microorganisms) does not allow for growth of *Listeria* spp.

The main controlling factors for *L. monocytogenes* are temperature (during pasteurisation or thermisation - heat treatment applied at a lower temperature and shorter time than pasteurisation), pH, a<sub>w</sub> and interactions with other microorganisms. It is important to note that cheese is not necessarily a homogenous environment as the

rind and the core have different conditions and there is likely to be a gradient in these characteristics, that additionally change over time. This variation within the cheese as well as the changes to the physiological conditions over time raises uncertainties about *L. monocytogenes* growth and survival within the different areas of cheese. Additional areas of variability are within the cheese manufacturing process, as some dairies may have more consistent implementation of controls than others (particularly small artisanal cheesemakers) such as pasteurisation of the raw milk, or the use of starter culture or acids to coagulate the milk, additionally some products of protected designation of origin such as Stilton are required to be pasteurised.

#### 4.2 Blue cheese characteristics

Blue cheese, blue-veined cheese or blue mould-ripened cheese is a type of cheese ripened with *Penicillium roqueforti* or *Penicillium glaucum*, giving it its characteristic colour and flavour (Almena-Aliste and Mietton, 2014). The Penicillium mould is usually present internally within the cheese compared to surface mould-ripened cheeses such as Brie and Camembert. Cheese rind from cheeses such as Gorgonzola and Stilton are more likely to be contaminated with *L. monocytogenes* due to handing (Banks 2006, Specialist Cheesemakers Association 2023, Stilton Cheese Makers Association 2023). The Gorgonzola rind has been specifically designated as non-edible (Official Journal of EU, 2008).

Categorising cheese for food safety purposes is very challenging as there are many different varieties with different milks, pasteurisation steps, ageing length, fat, salt and moisture content etc. The Codex General Standard for Cheese classifies cheese according to firmness, fat content and principal curing characteristics, although there are multiple ways to categorise cheese (Almena-Aliste and Mietton, 2014).

Common types of blue cheese and their properties described in a previous risk assessment are presented in Table 6 below. In this classification, soft cheese has a water content (the amount of water in a product by weight) of 55% and semi hard internal mould cheese has a water content of 44-55% (Banks, 2006). Blue cheese can have salt levels varying from 2% to 7%, with Gorgonzola ranging from 3.5 - 5.5% (Banks, 2006).

Cheese physicochemical characteristics can be very different between core and rind. The microbiota of the rind contributes to its deacidification, so while Stilton curd is quite acidic at moulding (around pH 4.8, depending on the recipe), the rind of the cheese sees a rise in pH up to neutrality over the 3-6+ months over which it ages (Specialist Cheesemakers Association, 2023). Data from Stilton produced by 3 companies vary in pH from 4.60 - 6.87 and in a<sub>w</sub> from 0.922 to 0.939 (Dairy UK, 2023).

Table 6 - Description of different blue cheese styles found on the UK market and factors contributing to microbiological stability (adapted from Banks 2006). These data are in agreement with Trmčić et al., 2017 on  $a_w$  of Gorgonzola, Roquefort and Cambozola and Fernández-Salguero et al., 1986 on Danish Blue, Gorgonzola and Roquefort.

Designation	Cheese Type	a <sub>w</sub> , average	a <sub>w</sub> , range	pH Point of Make	pH Point of Sale	Pasteurised milk?
		0.045	0.000 0.055			
Semi hard internal mould blue	Danish Blue (Danablu)	0.945	0.926 - 0.955	4.6	6.1	Yes
	Stilton	0.964	0.961 – 0.966	4.6	6.8	Yes
	Roquefort	0.930	0.927 – 0.936	4.6	6.8	No
	Gorgonzola	0.940	0.932 – 0.952	4.7	6.9	Yes
	Shropshire Blue	0.966	-	4.6	6.5	Yes
	St. Agur	0.985	0.983 – 0.985	4.8	6.4	Yes
	Yorkshire Blue	0.968	0.967 – 0.968	4.9	6.2	Yes
Soft internal mould blue	Dolcelatte	0.975	0.973-0.982	4.8	6.6	Yes
	Cambozola	0.972	-	4.7	6.5	Yes

It is possible for cheeses to have characteristics outside of the ranges presented in Table 6, due to variations in recipes or the cheesemaking process. For instance, a study sampling cheeses at retail in 2010/2011 in the UK (HPA, 2013) found large variation in pH and a<sub>w</sub> values (see Table 7). The average pH and a<sub>w</sub> of all blue cheeses sampled was 6.35 and 0.938. Full data are available in Annex 2.

Table 7 – pH and  $a_w$  measurements of blue cheeses found on the UK market in 2010 and 2011 (HPA, 2013). Physicochemical characteristics of cheeses with 3 or more samples are reported here. Full data are available in Annex 2.

Cheese type	a <sub>w</sub> , average	a <sub>w</sub> , range	pH, average	pH, range
Blacksticks	0.937	0.913-0.960	6.82	5.69-7.83

Cheese type	a <sub>w</sub> , average	a <sub>w</sub> , range	pH, average	pH, range
Bleu	0.934	0.903-0.968	6.37	5.89-8.39
d'Auvergne				
Cambozola	0.959	0.937-0.977	7.32	6.93-7.85
Cornish Blue	0.926	0.917-0.947	6.76	6.15-7.75
Danish Blue	0.922	0.913-0.943	5.14	4.76-5.33
Dolcelatte	0.922	0.863-0.957	6.05	5.72-6.31
Gorgonzola	0.938	0.923-0.968	6.24	5.63-6.86
Roquefort	0.916	0.895-0.972	6.26	5.70-7.70
St. Agur	0.947	0.935-0.954	5.97	5.82-6.10
Yorkshire Blue	0.955	0.925-0.984	6.18	5.31-7.43

Blue cheese has an additional step during maturation where it is pierced to allow oxygen into the cheese for mould growth. This process could introduce *Listeria* spp. into the internal cheese. During maturation, the pH approaches neutrality, which would slow the inhibition of *Listeria* spp., but this can be affected by a combination of pH, salt level (e.g., relatively high in Roquefort; relatively low in other blue cheeses), length and temperature of maturation and competitive microflora. In addition, blue cheese is not a homogenous matrix so different areas may have different pH, salt and competitive microflora levels.

#### 4.3 Blue cheese consumption

The National Diet and Nutrition Survey (NDNS) data (DHSC, 2013, PHE and FSA, 2014, PHE and FSA, 2016, PHE and FSA, 2018 and PHE and FSA, 2020) were used to estimate the quantity of blue cheese that vulnerable consumers eat uncooked. The NDNS is a dietary survey covering a representative sample of around 1000 people per year in the UK, beginning in 2008. It is a snapshot over 4 days, so food that is regularly but infrequently consumed may not be reported.

The NDNS does not have details on if the participants fall within a vulnerable group other than due to age, so the data has been split into the general population (19 to

under 65-year-olds), and the over 65-year-olds. The consumption habits of women aged 16 to 49 years old were used as a proxy for pregnant women.

Table 8 shows the amounts of blue cheese consumed by age group, based on data from 2008 to 2019.

Table 8 - blue cheese consumed by age group, according to NDNS data. The table does not include blue cheese, which is included in recipes, which means that it only shows the consumption of uncooked blue cheese.

Age group	Number of consumers of blue cheese	Number of respondents in Population group	Mean g/person/day consumption (Acute – eaten in one sitting)
19 - 64 yrs. (General population)	88	5094	30
Women aged 16 -49 yrs. (Proxy for pregnant women)	33	2556	23
65 + yrs.	45	1538	31

The general population and the over 65-year-olds categories ate a mean amount of 30g in a sitting, with the proxy for pregnant women slightly less in a sitting at 23g.

#### 4.4 *L. monocytogenes* contamination in blue cheese

To estimate the exposure of vulnerable groups to *L. monocytogenes* in blue cheese, information was gathered on the frequency of contamination and contamination levels in blue cheese from official surveillance and literature data, as well as the growth characteristics of the pathogen in blue cheese.

#### 4.4.1 Scottish Food Sampling database

Microbiological testing data were extracted from the Scottish Food Sampling Database specifically looking for failures for *L. monocytogenes* and *Listeria* spp. in examined cheese products. Scottish Food Sampling Database data are produced by Local

Authority, enforcement and FSS-targeted sampling from a variety of retail and non-retail premises, including and not limited to manufacturers, processors, packers, restaurants and other caterers. Due to the nature of the dataset, it is not possible to only extract information for "blue cheese", therefore data for "cheese" products were extracted.

Data were extracted for the time period 2014-2021. The datasheet for unsatisfactory results (including enumeration data, where it was available) is provided in Annex 2. Between 2014 - 2021, 1085 tests for *L. monocytogenes* were performed on cheese products, out of which 13 were deemed unsatisfactory due to the presence of *L. monocytogenes* or high levels of the pathogen. This comprises a percentage rate of unsatisfactory results of 1.20% from all tests conducted. Between 2014–2021, 1192 tests for generic *Listeria* spp. were carried out on cheese products. 10 of these were deemed unsatisfactory results of 0.84% from all tests conducted. According to the microbiological testing data, the prevalence of *Listeria* spp. in cheese products in Scotland is low. However, some of the products which were unsatisfactory for the presence of *L. monocytogenes* were two pasteurised blue cheeses. These results demonstrate that *L. monocytogenes* have been recently found in pasteurised blue cheese.

The non-blue cheeses, which were deemed unsatisfactory for the presence of *L. monocytogenes* were Griffel (a type of raw cow's milk cheese), unspecified grated cheese, unspecified soft cheese (both made from processed cow's milk) and Caerphilly cheese (made from processed cow's milk). Three Gorgonzola samples were considered unsatisfactory due to the presence of *Listeria* spp.

Due to the lack of similar datasets for the rest of the UK, the Scottish sampling dataset is taken as a representative sample for the purposes of this risk assessment. The data suggest that overall prevalence of *Listeria monocytogenes* and/or *Listeria* spp. in cheese products in the UK is low. However, it needs to be noted, that due to the limitations of the dataset (i.e., the inability to filter data by cheese type) it is possible for the overall trend of low prevalence of *L. monocytogenes* in cheese products to be slightly biased towards low detection. Because it was not possible to obtain results "by cheese type", the obtained results represent an average and may underestimate the prevalence of *L. monocytogenes* in blue cheeses.

#### 4.4.2 UK survey 2010-2011

An FSA/EU survey of *Listeria* spp. in UK ready-to-eat food was carried out in 2010 – 2011 (HPA, 2013). Of 400 soft and semi-soft cheese samples, 88 were blue cheese (Annex 2). All but one blue cheese sample tested negative for *Listeria* spp. The sample,

simply referred to as 'blue' had *L. innocua* at levels below the limit of detection (< 10 cfu/g), was made from pasteurised cow milk, was tested on the last day of its shelf life, and had a pH of 6.74 and  $a_w$  of 0.944. Three other cheeses had levels of *L. innocua* below the limit of detection: a semi-soft cheese, and two soft cheeses. *L. monocytogenes* was not detected in any sample.

#### 4.4.3 Other incidents

A search of the FSA incidents and alerts system from 2000 to the present day resulted in 17 notifications of *Listeria* spp. identified in blue cheese products for the UK market, 3 of which were Stilton cheeses. A search of international non-EU signals over 2019 – 2023 identified 4 recalls of blue cheese due to presence of *Listeria* spp. A search of EU signals over 2019 – 2023 identified 39 recalls of blue cheese, mostly Gorgonzola, due to presence of *Listeria* spp.

The specification for Stilton states that it must have a minimum moisture level of 42% at maturity, and values at moulding and during early stages of maturation may be in the 50-55% range, or higher (Specialist Cheesemakers Association, 2023). A producer that made a Stilton-style cheese using milk from a herd with one cow with *Listeria* mastitis found that *Listeria* was not detectable in the milk, there was moderate levels in the curd stage, but these were once again undetectable in the fully mature interior of the cheese. However, a combined core and rind sample was above the legal limit.

The number of samples positive for *Listeria* spp. in Stilton, as provided by Dairy UK 2023:

- 2021: 5/4140 (<10cfu/g)
- 2022: 1/4055 (<10cfu/g)
- 2023: 6/106

Both crust and core samples were used for testing.

# 4.4.4 Prevalence and growth of *L. monocytogenes* in blue cheese from published studies

To obtain information on the prevalence and growth of *Listeria* spp. in blue cheese for the current risk assessment, the PubMed database was searched. Information on search terms and search results are provided in Annex 1. Surveillance studies carried out outside of Europe were excluded as they were not thought to be representative. All papers which were identified to contain relevant information are summarised below.

Articles identified through the risk assessments in Section 1.4 are also summarised below.

The optimum growth temperature of *L. monocytogenes* is 30 - 37 °C, and unlike most foodborne pathogens, it can grow at standard refrigerator temperatures, below 4 °C, which makes it a particular problem in ready-to-eat foods that are not cooked before eating.

*L. monocytogenes* can grow at pH values of between pH 4.4 and pH 9.4 (ICMSF, 1996). Optimal growth occurs around pH 7.0 and at a minimum  $a_w$  of 0.92 (Walker, Archer and Banks, 1990). According to legislation, food products with a pH  $\leq$  4.4, an  $a_w \leq$  0.92 or a combined pH  $\leq$  5.0 and  $a_w \leq$  0.94 are unable to support the growth of the pathogen (Section 1.3).

#### **Prevalence studies**

A survey of unpasteurised milk cheeses at retail in England between 2019 - 2020 sampled 77 blue cheeses, of which two (2.6%) were positive for *L. monocytogenes* in 25 g at levels < 100 cfu/g (Willis *et al.*, 2022). Overall, the study sampled 629 cheeses and detected *L. monocytogenes* in 3 samples – including a hard goat's cheese with levels >  $10^2$  cfu/g, considered potentially injurious to health. The study also found that 16% of the blue cheeses were stored above 8 °C at the time of sampling. Samples stored at > 8 °C were found at manufacturers, catering premises, supermarkets and other retail outlets. It also found a significant correlation between storage at high temperatures and unsatisfactory microbiological results.

An Italian study by Bernini et al., 2013 sampled blue-veined cheese matured for 50 days (C1) or 80 days (C2) for the presence of *L. monocytogenes* (see Table 9 for the physiological properties of these cheeses).

-	C1	C2
Maturation	50 days	80 days
Aw	0.97	0.93
Salt level	1.75 %	2.20 %
рН	5.98	6.8

Table 9 - Physiological properties of the cheeses in Bernini et al., 2013.

*L. monocytogenes* was not detected in the interior of the cheeses, from 100 samples tested, but was present in 55 out of 100 rind samples. Sample results are summarised in Table 10. C2 had a higher proportion of contaminated samples – 61.5% compared to 47.9% for C1 and was responsible for all the samples with contamination levels higher than 4 log<sub>10</sub> cfu/g.

Table 10 – Results of L.	monocytogenes de	tection in 100 sar	mples from 2 cheeses
(Bernini et al., 2013).			

Contamination levels (cfu/g)	Number of samples
Below limit of detection (<1 log <sub>10</sub> )	12
1-2 log <sub>10</sub>	12
3-4 log <sub>10</sub>	13
>4 log <sub>10</sub>	8

Microbiological challenge testing was also performed with  $1 - 2 \log_{10} cfu/g L$ . monocytogenes. Levels of the pathogen increased in C1 held at 4 and 8 °C for 10 days and 30 days. After 55 days, samples stored at 8 °C reached almost 5 log<sub>10</sub> cfu/g and samples stored at 4 °C decreased to a level of 3 log<sub>10</sub> cfu/g. Levels of the pathogen in C2 did not significantly change, at both 4 and 8 °C within the first 30 days. On day 55, samples stored at 8 °C reached 4.63 log<sub>10</sub> cfu/g and those stored at 4 °C reached ~ 3 log<sub>10</sub> cfu/g. The authors note that it is unlikely for consumers to store cheese for this long (Lambertz *et al.*, 2012).

A study of retail cheese on sale in Abruzzi, Italy, tested 2132 samples of which 47 were returned as positive for *L. monocytogenes* (2.2%) (Prencipe *et al.*, 2010). Gorgonzola cheeses had 21 out of 444 samples positive for *L. monocytogenes* (4.7%). Both the cheese and the rind were tested together so it was not possible to see if there was a difference between the cheese matrix and the rind. The physiochemical properties of the Gorgonzola at retail were 0.964  $a_w$  and a pH of 6.629. The paper pointed out that the levels of *L. monocytogenes* in Gorgonzola were considerably higher than what is seen in general in Italian cheeses.

A study of prevalence of *L. monocytogenes* in RTE foods in Sweden examined samples of different products (smoked fish, cheese, meats products) for an EU level and national survey in 2010 (Lambertz *et al.*, 2012). They examined soft and semi-soft cheeses (mould- and smear-ripened). They tested 456 samples of mould–ripened cheese, which included white and green-blue mould cheese. Two of these samples tested positive for

*L. monocytogenes,* but only one of these was a blue cheese and had levels <10 cfu/g. From the description of the food samples preparation, it is not possible to determine if the cheese samples included or did not include the rind.

A different study looked at *L. monocytogenes* strains from Gorgonzola cheese from 22 production plants between 2004 to 2007 (Lomonaco *et al.*, 2009). 95 strains were examined, and the authors concluded that there was evidence of persistent contamination of homogenous strains within the plants. Although the total number of tests carried out is unknown, of the 95 positives within that period, only one came from the cheese paste, 45 from rinds and 49 from the processing environment.

A study of Gorgonzola rinds during the maturation period in cellars found *L. monocytogenes* in 2 out of 18 rind swabs (11%), in a cellar where 5 out of 5 shelf swabs were also positive for the pathogen (Cocolin *et al.*, 2009).

A similar Italian study collected 1656 Gorgonzola cheese samples from an industrial plant from 2003 – 2004 and measured *L. monocytogenes* after packaging and at the end of shelf life, which ranged from 30 to 60 days after packaging (Manfreda *et al.*, 2005). Detection rates were 2.1% (31/1489) and 4.8% (8/167), respectively. The authors have commented that this is lower than for other blue-veined cheeses and may be because the Gorgonzola rind does not allow for the spread of *Listeria* spp. from the exterior to the rest of the cheese.

A 1996 study of Italian soft cheeses at retail in Italy found 4.9% of samples positive for *L. monocytogenes* (8 out of 164) and 22% positive for other *Listeria* spp. (36 out of 164 samples) (Pinto and Reali, 1996). This included 58 Gorgonzola samples, out of which 3 (5%) were positive for *L. monocytogenes* and 21 (36%) were positive for other *Listeria* spp. The samples taken included the exterior and interior portion of the cheese.

Banks 2006, summarised multiple studies on *L. monocytogenes* in Gorgonzola cheese, reproduced in Table 11 below.

Reference	No. samples taken	Type of sample	<i>L. monocytogenes</i> detection rate
Bottarelli et al., (1999)	40	Pre-packed Gorgonzola	5%
Soncini et al., (2002)	73	Gorgonzola	0%

Table 11 - Contamination of Gorgonzola cheese from studies reported in Banks 2006.

Reference	No. samples taken	Type of sample	<i>L. monocytogenes</i> detection rate
Soncini et al., (2002)	39	Gorgonzola/Mascarpone preparation	0%
GOLIS, (2004)	-	Cheese rind	60%

Furthermore, the GOLIS study (GOLIS, 2004) observed no difference in the detection rate of *L. monocytogenes* in Gorgonzola matured for less than 60 days, 61-80 days and more than 80 days. Of the rind samples positive for *L. monocytogenes*, 19% had levels less than 10 cfu/g; 41% had levels of  $10 - 10^3$  cfu/g and 40% had levels greater than  $10^3$  cfu/g. Contamination of the rind was thought to lead to cross-contamination of the internal area of the cheese through piercing. Artificial inoculation of the interior of the cheese with  $10^4$  *L. monocytogenes* resulted in a 10-fold to 1000-fold increase after 35 days at 6 °C. Artificial inoculation of the rind under the same conditions resulted in a less than 10-fold increase. All the *L. monocytogenes* strains isolated from Gorgonzola were part of the 1/2a serotype, which is one of the major contributors of listeriosis cases (Datta and Burall, 2018).

A study of cheeses in France found no *Listeria* spp. in 18 samples of Bleu d'Auvergne, a pasteurised milk blue cheese, whereas 2 out of 9 samples (22%) of Fourme d'Ambert, a semi-hard blue cheese made from pasteurised milk, positive for <10 cfu/g *L. monocytogenes* serotype 1/2 (Kinderlerer, Matthias and Finner, 1996). The authors also found high concentrations of medium- and long-chain fatty acids, pH and fat content in the veins of the blue cheeses. They hypothesised that medium- and long-chain fatty acids in blue mould ripened cheeses acted as natural preservatives, inhibiting the growth of *L. monocytogenes* even in conditions of high pH where they would be expected to grow. Woo et.al., 1984 evaluated the free fatty acid content in a variety of cheeses and concluded that the two blue cheeses tested contained very high concentrations of free fatty acids compared to other soft cheeses like camembert, brie, Port Salut, Monterey Jack and Limburger, and these are likely important flavour compounds in blue cheese.

A survey of Spanish Valdeón blue cheese found none of the 11 cheeses sampled were positive for *L. monocytogenes* in 25 g (Lopez-Diaz *et al.*, 1996).

A survey of cheeses at retail in Sweden found no positives for *L. monocytogenes* among 95 green/blue mould cheeses and one positive for *L. monocytogenes* among 27 white-green/blue mould cheeses (3.7%) (Loncarevic, Danielsson-Tham and Tham,

1995). This cheese was made with heat-treated rather than raw milk and levels of the pathogens were < 100 cfu/g. Overall, this study found that cheeses made from raw milk were more likely to be positive for the pathogen than cheeses made from heat-treated milk (42% vs 2%).

#### **Growth studies**

A study on the growth of *L. monocytogenes* in Gorgonzola was carried out by first inoculating pasteurised milk with 4 to 5 log<sub>10</sub> cfu/g and preparing the cheese according to traditional methods (Dalzini *et al.*, 2017). The pH of the rind and core initially decreased and remained steady for 35 days which probably caused the 2-log<sub>10</sub> observed decrease of *L. monocytogenes*. A rapid increase in the pH followed, to final values of pH 6.8 in the core and pH 5.8 in the rind. The final a<sub>w</sub> was 0.962. The cheese was also subjected to thermal abuse at 8°C for 7 days to simulate retail storage and 12°C for 8 weeks to simulate consumer storage. The levels of *L. monocytogenes* on the rind did not significantly change during this time from an initial 4.8 log<sub>10</sub> cfu/g, but there was an increase from 5.4 to 7.1 log<sub>10</sub> cfu/g in the core. The authors also studied post-processing contamination on inoculated slices of Gorgonzola kept at 8 °C. These increased from  $1.5 - 2 \log_{10}$  cfu/g to a maximum concentration of about 6 log<sub>10</sub> cfu/g in about 14-21 days but there was no further growth in concentration up until day 100.

A study by Bernini et al., 2016, looked at whether cutting could be responsible for contaminating the Gorgonzola cheese paste with L. monocytogenes, by carrying out microbiological challenge tests with rind with different levels of contamination. Cutting of the whole cheese at the industrial level, as well as cutting at the point of sale/in the home were both carried out. There were two types of Gorgonzola – sweet (60-day maturation) and piquant (80-day maturation). The sweet Gorgonzola had 1.98 ± 0.38 % salt, a pH of 5.86  $\pm$  0.58 and an a<sub>w</sub> of 0.97  $\pm$  0.03. The piquant Gorgonzola had 2.31  $\pm$ 0.48 % salt, a pH of 6.92  $\pm$  0.46 and an a<sub>w</sub> of 0.92  $\pm$  0.01. The researchers found that cutting could contaminate the cheese paste, with higher rind contamination leading to a higher percentage of paste contamination. However, all paste samples that had L. monocytogenes were below the limit of detection, despite rind contamination varying from 1 to 3  $\log_{10}$  cfu/g. Sweet Gorgonzola contaminated with 0 to 3  $\log_{10}$  cfu/g of L. monocytogenes showed a significant increase when held at 4 °C by day 15. Piquant Gorgonzola contaminated with 0 to 3 log<sub>10</sub> cfu/g of L. monocytogenes did not show a significant difference after 15 days – other than the sample with 1 log<sub>10</sub> cfu/g, which increased by 0.5 cfu/g. The higher salt and lower a<sub>w</sub> may have contributed to this difference in growth.

A 2000 study measured the growth of *L. monocytogenes* in Stilton held in normal atmosphere and modified atmosphere packaging for 6 weeks at 2 - 8 °C. No significant

growth was observed in Stilton in normal atmosphere packaging (Whitley, Muir and Waites, 2000). The pH of the cheese varied from pH 5.9 to 6.7.

A 1996 study carried out in the United States examined the levels of *L. monocytogenes* in blue cheeses containing naturally modified milk fat (Schaffer, Tatini and Baer, 1995). Milk containing naturally modified fat was obtained from lactating dairy cows, which had been fed special diets (containing either soybeans or sunflower seeds). Each milk was pasteurized, standardised to 3.6% milk fat and inoculated with L. monocytogenes (laboratory strains Scott A and V7). The inoculated milks were then manufactured into blue cheeses. Levels of L. monocytogenes were monitored during manufacture and aging of the above-mentioned cheeses. The initial inoculum levels were 3.4 log<sub>10</sub> cfu/ml for all three types of milk. The numbers of *L. monocytogenes* after hooping reached a maximum of 4.5 log<sub>10</sub> cfu/g in all tested cheeses. During aging, L. monocytogenes followed the same pattern of decline in the modified fat cheeses to the one observed in the control cheeses. Despite an increase of pH in all cheeses from pH 4.9 to 6.8 between 60 and 120 days, levels of L. monocytogenes did not increase. Previous studies have shown that L. monocytogenes levels increase significantly in mould ripened soft cheeses such as Camembert and Brie, when the pH of these cheeses' increases. However, in this study of blue cheeses, a similar increase was not observed. This could be due to the effect of higher amounts of free fatty acids and/or higher salt concentrations in blue cheeses, compared to Camembert or Brie cheeses.

Back et al., 1993 inoculated commercial cheeses with *L. monocytogenes* strains, as shown in Table 12. Little or no growth was observed in Stilton and Mycella, which is a Danish cheese similar to Gorgonzola. Significant growth was observed in the Cambazola and Lymeswold, which are soft cheeses, especially at 10 °C. The authors commented that blue cheese ripening leads to proteolysis, releasing amino acids and peptides which may stimulate the growth of *L. monocytogenes*.

Table 12 - Growth study performed by Back et al., 1993. Cheeses were inoculated with L. monocytogenes strain BL90/15, except the second Blue Lymeswold\*, which was inoculated with strain BL87/9B. Where growth exceeded 1 log<sub>10</sub> cfu/g, the cells are highlighted.

Cheese	Storage	Storage	Initial count,	Final count,
	period, days	temperature, °C	log₁₀ cfu/g	log₁₀ cfu/g
Blue Stilton	14	5	2.8	2.8
Blue Stilton	14	10	2.8	2.8
Cambazola	18	5	2.5	5.1

Cheese	Storage	Storage	Initial count,	Final count,
	period, days	temperature, °C	log <sub>10</sub> cfu/g	log <sub>10</sub> cfu/g
Cambazola	14	10	2.5	7.3
Mycella	18	5	2.8	2.8
Blue Lymeswold	27	6	1.6	3.1
Blue Lymeswold	20	10	1.6	6.7
Blue Lymeswold*	27	6	1.5	4.7
Blue Lymeswold*	20	10	1.5	6.7

A study from 1991 by Genigeorgis et al., 1991, examined the growth and survival of *L. monocytogenes* in purchased retail cheeses stored at temperatures from 4 to 30°C. Forty-nine cheeses representing 24 types and 28 brands were purchased from supermarkets. Pieces of cheeses of approximately 1.5 x 0.5 cm were surface inoculated with log<sub>10</sub> 3.95 to 4.36 cells of a *L. monocytogenes* pool of five strains (Scott A, V7, RM-1, VPH1, VPH2). The blue cheese (type not specified) used had a pH value of 5.1 and salt in water percentage of 6.1%. The inoculated samples were stored at 4, 8, and 30°C for up to 36 days. *L. monocytogenes* at levels of log<sub>10</sub> 3.95 - 4.36 cfu/g was unable to initiate growth at 4 to 30°C in the blue cheese. The low pH was considered to be the inhibiting factor of *L. monocytogenes* growth in the cheese.

A 1989 study examined the growth of *L. monocytogenes* during the ripening and manufacture of blue cheese (Papageorgiou and Marth, 1989). Pasteurised milk was inoculated with two strains of *L. monocytogenes* (Scott A and CA) which was then made into blue cheese. *L. monocytogenes* was present in the curd sampled during cheese-making at approximately 1.0 log<sub>10</sub> cfu/g greater than in the inoculated milk. During the first 24 hours of production, the levels of *L. monocytogenes* increased by 0.58 - 1.22 log<sub>10</sub> cfu/g, though 2 lots with rapid acid production had no significant growth. Growth stopped when the pH of the cheese dropped below 5, and there was significant decrease of both strains during the first 50 days of ripening (2.65 and 2.73 log<sub>10</sub> cfu/g for Scott A and CA, respectively). The pH of the cheese began to increase after day 50 which allowed survival but not growth of *L. monocytogenes*. Scott A numbers remained static during days 50 to 120 of storage and CA number remained static until day 80 when the numbers started to decrease gradually. Survival on the surface of the cheese was similar to survival in the interior. The high salt content of 4.26 to 4.85% during ripening was assumed to be why the *L. monocytogenes* growth was static. Additionally,

the production of free fatty acids and their derivatives during the cheesemaking process was also thought to be unfavourable to *L. monocytogenes* growth (NIZO, 2022), (ComBase, 2023).

#### 4.4.5 Tools for modelling growth

Online models exist for predicting pathogen growth in food with various physicochemical properties.

ComBase is an online tool for quantitative food microbiology whose focus is describing and predicting how microorganisms survive and grow under a variety of primarily foodrelated conditions. The broth growth model can be adjusted to fit the bacteria, and physiochemical conditions of the cheese. It additionally has 3 entries of data for growth of *Listeria* spp. in blue cheese based on: Stilton, a blue cheese curd and blue cheese during ripening.

The <u>Listeria Calculator</u> is another online model that calculates the amount of undissociated lactic acid against the cut-off value for inhibition of *L. monocytogenes*.

# 5. Risk characterisation

Blue cheese can become contaminated with *L. monocytogenes* at different stages of the cheesemaking process. Cheesemaking is a complex process, involving many steps which change the physiological properties of the cheese over the time that it matures.

Blue cheese contamination with *L. monocytogenes* can originate from the milk. Pasteurisation of the milk used to make cheese would kill the pathogen and the level of *L. monocytogenes* in milk is usually low even before pasteurisation if the milk has been pooled as a result of dilution. A majority of blue cheeses for sale at retail in commercial outlets in the UK are made from pasteurised milk. Further reductions in levels are achieved due to the low pH in the initial maturation stages which, at least prevents growth. Introduction of *L. monocytogenes* is more likely to occur during production, from the environment, personnel or cross-contamination with other foods and tools. This is supported by data from listeriosis outbreaks linked to other cheeses, where cross-contamination during manufacturing and after pasteurisation is a common root cause. The pH of blue cheeses increases during maturation, to around pH 6 – 7, though the matrix is not homogenous. This pH is favourable for the survival and growth of *L. monocytogenes*. It is likely that the same cheese produced by different manufacturers, on different days, will have different physiological characteristics which may be more or less favourable for *L. monocytogenes* growth. While blue cheese can become internally contaminated with *L. monocytogenes* during production, it is much more common for *L. monocytogenes* to colonise the rind of blue cheese without affecting the cheese paste. Higher levels of *L. monocytogenes* have been detected in the rind of both Gorgonzola and Stilton, however, conditions in the Gorgonzola paste are more favourable to growth than in the rind.

Studies have shown that the external *L. monocytogenes* can then contaminate the internal paste during cutting and packaging. The resulting levels in the paste are likely not enough to cause infection, but storage at temperatures slightly above recommended temperatures (~ 8 °C) could increase pathogen levels sufficiently to cause illness in vulnerable groups. It is important to note that the studies looking at contamination from cutting were on Gorgonzola and while the pH of some blue cheese tested were similar, the aw and other physiological factors can vary, which will affect growth and survival.

Other than temperature (during pasteurisation or thermisation) and pH, other controlling factors for *L. monocytogenes* are a<sub>w</sub>, salt levels and interactions with other microorganisms. Low a<sub>w</sub> (0.92 - 0.93), with salt levels of around 2% were shown to be sufficient to prevent *L. monocytogenes* growth in blue cheese, despite a favourable near-neutral pH. The production of free fatty acids by the Penicillium moulds are also thought to inhibit the pathogen growth.

Scottish sampling data (used as a representative sample for the UK) found that the overall presence of *Listeria* spp. in cheese products is low, and a recent UK retail survey found around 3% of blue cheese samples were positive for *L. monocytogenes* at levels < 100 cfu/g. Most published prevalence studies at retail were of Gorgonzola cheeses and found *L. monocytogenes* to be common in the maturation environment of the cheese processing areas, as well as the rind but not the core of the cheese. Gorgonzola rind is labelled as not for consumption. Other prevalence studies from European countries, and additional data obtained from industry representatives on blue cheeses found similar results as the UK retail survey.

The available information about consumption of blue cheese by vulnerable groups suggests that it is occasionally consumed in small quantities. The infectious dose of *L. monocytogenes* needed to cause illness in vulnerable groups is not known but thought to be low.

Other risk assessments reviewed include a report commissioned by the FSA in 2006 that rated Gorgonzola and Stilton (pasteurised milk cheeses) to be low risk to vulnerable groups and Roquefort as moderate risk, as it is made from unpasteurised milk. A 2003 FDA report found semi-soft cheeses to be a moderate risk using a two-dimensional matrix of per serving vs. per annum rankings, though this included all semi soft cheese and not just blue cheese. A New Zealand report classed common blue cheeses (Stilton,

Roquefort, Gorgonzola, Danish Blue) as low moisture cheeses and found that these do not generally present a risk to human health.

The risk assessment concludes that the **severity** of *L. monocytogenes* infection in vulnerable groups is **high** (severe illness: causing life-threatening or substantial sequelae or illness of long duration), with a significant mortality rate. Outbreak data on listeriosis where cheese was the reported source of infection are limited, however, where data exists, severe outcomes are reported for vulnerable groups. The above characterised severity has **low uncertainty**, as there is strong evidence available that *L. monocytogenes* causes severe illness in vulnerable groups.

The frequency of occurrence of listeriosis in the vulnerable population from consumption of blue cheese is considered very low (very rare but cannot be excluded). Consumption of blue cheese in vulnerable groups occurs infrequently, and the quantities are small (around 30 g or less). Coupled with the low occurrence and levels of L. monocytogenes in blue cheese at retail in the UK, this leads to the estimated low frequency of occurrence of listeriosis. In addition, no outbreaks linked to blue cheese in the UK were found from a literature search; only 2 potential listeriosis outbreaks and one individual listeriosis case linked to blue cheese were identified in the rest of the world, for a total of 42 cases over a ~ 30-year period. Current molecular-based techniques (whole genome sequencing, pulsed-field gel electrophoresis, multilocus sequence typing) are necessary, along with epidemiological data, to trace the source of listeriosis outbreaks, given the pathogen's long incubation period. The UK Health Security Agency employs whole genome sequencing on all *L. monocytogenes* isolates from patients, but there often are not corresponding food samples to test. The long incubation period also makes it difficult for patients to recall their food consumption. As a result, it is unsurprising that a majority of listeriosis cases do not get traced back to the food source. For this reason, the uncertainty of occurrence is considered to be medium.

Tables from ACMSF (<u>ACM/1334</u>) adapted from EFSA 2006 modified from OIE 2004 are provided in the Appendix Section 8.1, describing the qualitative categories for the risk characterisation.

# 6. Uncertainties

- Levels of *L. monocytogenes* at retail in the UK of commonly consumed blue cheeses Stilton, Roquefort, Danish Blue, Gorgonzola
- If Stilton has the ability to support growth of *L. monocytogenes*, taking into account that it has been found within retail samples.

- More accurate consumption data for vulnerable groups and whether vulnerable groups are already avoiding consuming blue cheese, as this may explain the low number of outbreaks related to blue cheese.
- How representative consumption of blue cheese for women aged 16-49 is for pregnant women
- The infectious dose in different vulnerable groups, depending on the type of different vulnerabilities (I.e., the infectious dose could differ depending on the on pre-existing conditions for each vulnerable individual)
- Better source attribution (such as through sequencing) for *L. monocytogenes* infections. The long incubation period of *L. monocytogenes* can make it difficult to link cases to causes and the source of the *L. monocytogenes* in the environment/food ingredients may not be identified, which may have prevented historical outbreaks associated with blue cheese from being identified.
- Extent of variability in physiological characteristics throughout cheese, from different manufacturers at different times and areas of the cheese wheel.

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# 8. Appendix

#### 8.1 Risk characterisation categories

ACMSF (<u>ACM/1334</u>) adapted from EFSA 2006 modified from OIE 2004 definition of qualitative categories for probability of occurrence and uncertainty.

Frequency category	Interpretation
Negligible	So rare that it does not merit to be considered
Very Low	Very rare but cannot be excluded
Low	Rare but does occur
Medium	Occurs regularly
High	Occurs very often
Very High	Events occur almost certainly

Table 13 - definition of qualitative categories for probability of occurrence

Table 14 - definitions of qualitative categories for severity of consequence

Severity category	Interpretation		

Negligible	No effects, or so mild they do not merit to be considered
Low	Mild illness: not usually life-threatening, usually no sequelae, normally of short duration, symptoms are self-limiting (e.g., transient diarrhoea)
Medium	Moderate illness: incapacitating but not usually life- threatening, sequelae rare, moderate duration (e.g., diarrhoea requiring hospitalisation)
High	Severe illness: causing life-threatening or substantial sequelae or illness of long duration (e.g., chronic hepatitis)

Table 15 - definitions of qualitative categories for expressing uncertainty

Uncertainty category	Interpretation
Low	There are solid and complete data available; strong evidence is provided in multiple references; authors report similar conclusions
Medium	There are some but no complete data available; evidence is provided in small number of references; authors report conclusions that vary from one another
High	There are scarce or no data; evidence is not provided in references but rather in unpublished reports or based on observations, or personal communication; authors report conclusions that vary considerably between them

#### 8.2 Listeriosis outbreaks linked to cheese

Table 16 - Overview of listeriosis outbreaks in 1983–2019 that were related to consumption of cheese. Compiled from EFSA reports (Eurosurveillance), CDC reports,

Google and a general search in Web of Science and Scopus. Adapted from E. Wemmenhove et al.<sup>1</sup>

Year of outbreak	Cheese group	Cases	Case- fatality rate (%)	Reference
1983–1987	Soft cheese made from raw milk	122	27	<u>Bula et al. (1995)</u>
1985	Mexican-style soft cheeses	142	34	<u>Linnan et al. (1988)</u>
1989	Camembert	2	0	<u>Ries et al. (1990)</u>
1989/1990	Blue cheese	26	23	<u>Jensen et al. (1994)</u>
1995	Brie de Meaux	NR	NR	<u>Goulet et al. (1995)</u>
1995	Soft cheese	37	30	Vaillant et al. (1998)
1997	Soft cheese	14	0	<u>Jacquet et al.</u> ( <u>1999)</u>
1997	Soft cheese	NR	NR	<u>RNSP (1997)</u>
2000	Mexican-style cheese	13	0	<u>Cartwright et al.</u> (2013)

<sup>&</sup>lt;sup>1</sup> A model to predict the fate of Listeria monocytogenes in different cheese types – A major role for undissociated lactic acid in addition to pH, a<sub>w</sub>, and temperature. International Journal of Food Microbiology. Volume 357, 2 November 2021, 109350. <u>https://doi.org/10.1016/j.ijfoodmicro.2021.109350</u>

Year of outbreak	Cheese group	Cases	Case- fatality rate (%)	Reference
2001	Soft cheese	33	0	<u>Carrique-Mas et al.</u> (2003)
2001	Soft cheese	120	NR	<u>Danielsson-Tham et</u> <u>al. (2004)</u>
2001	Cheese	19	0	<u>Makino et al. (2005)</u>
2002	Cheese made from pasteurized milk	86	0	Pagotto et al. (2006)
2003	Soft cheese from raw milk	17	0	<u>Gaulin et al. (2003)</u>
2005	Mexican-style cheese	23	22	<u>MacDonald et al.</u> (2005)
2005	Queso fresco	9	NR	FIOD (2005)
2005	Tomme cheese	10	50	<u>Bille et al. (2006)</u>
2006	Soft cheese	189	14	<u>Koch et al. (2010)</u>
2006	Soft cheese	78	17	<u>EFSA (2007)</u>
2007	Camembert cheese	17	18	<u>Johnsen et al.</u> (2010)
2007	Mature cheese	NR	NR	<u>Vít et al. (2007)</u>
2008	Cheese	92	NR	<u>Taillefer et al.</u> (2010)

Year of outbreak	Cheese group	Cases	Case- fatality rate (%)	Reference
2008	Brie cheese	90	4	<u>NBC (2008)</u>
2008	Mexican-style cheese	8	0	<u>Cartwright et al.</u> (2013)
2009–2012	Queso fresco	30	37	<u>Magalhaes et al.</u> (2015)
2009–2011	Smear-ripened cheese (Tallegio)	NR	NR	<u>Amato et al. (2017)</u>
2010	Soft cheese (Panela, Queso fresco, Requeson)	5	0	FIOD (2010)
2010	Soft cheese	28	11	FIOD (2015)
2010	Acid-curd cheese (Quargel)	14	36	<u>Fretz et al. (2010)</u>
2011	Fresh cheese (chives)	2	NR	FIOD (2011)
2011	Mexican-style cheese	7	29	<u>Jackson et al.</u> ( <u>2011)</u>
2011	Pave de Nord (Mimolette- type cheese made from pasteurized milk)	12	33	<u>Yde et al. (2012)</u>
2012	Latin-style fresh cheese (pasteurized milk)	2	0	<u>De Castro et al.</u> (2012)
2012	Ricotta cheese	22	18	<u>CDC (2012)</u>

Year of outbreak	Cheese group	Cases	Case- fatality rate (%)	Reference
2013	Camembert / Brie	18	17	Newsdesk (2013)
2014	Fresh cheese	1	13	<u>CDC (2014a)</u>
2014	Soft cheese	5	20	<u>CDC (2014b)</u>
2015	Cheese made from unpasteurized milk	2	0	<u>Del-Valdivia-Tapia</u> et al. (2015)
2015	Fresh cheese	3	33	FIOD (2015)
2017	Smear cheese	8	25	<u>CDC (2017)</u>
2019	Deli sliced cheese (suspected)	10	10	<u>CDC (2019)</u>

NR: Not reported.

Table 17 - Cheese associated Listeria outbreaks. Adapted from "*Joint FDA / Health Canada Quantitative Assessment of the Risk of Listeriosis from Soft-Ripened Cheese Consumption in the United States and Canada*" Report<sup>2</sup>. \*Outbreaks not found in Table 16.

Year	Location	Implicated Cheese	No. of Illnesses (Deaths) <sup>1</sup>	Raw Milk	Reference
1983-1987	Switzerland	Vacherin Mont d'Or	122 (34)	No	(Bula et al. 1995; Norton and Braden 2007)
1985	U.S. (CA)	Queso fresco and queso cotija	142 (48)	Likely (or cross contaminatio n)	(CDC 1985; Norton and Braden 2007)
1989-1990	Denmark	Multiple	26 (6)	NS <sup>2</sup>	(Jensen et al. 1994; Norton and Braden 2007)
1995	France	Brie de Meaux	37 (11)	Yes	(Goulet et al. 1995; Norton and Braden 2007)
1997	France	Livarot	14	Yes	(Jacquet et al. 1998)
1999	France	"Epoisses" like	3	Yes	(AFSSA 2000, page 50) *

<sup>2</sup> Joint FDA / Health Canada Quantitative Assessment of the Risk of Listeriosis from Soft-Ripened Cheese Consumption in the United States and Canada: Report. Food Directorate, Health Canada. Center for Food Safety and Applied Nutrition, Food and Drug Administration. July 2015 https://www.fda.gov/media/90488/download

Year	Location	Implicated Cheese	No. of Illnesses (Deaths) <sup>1</sup>	Raw Milk	Reference
2000	U.S. (NC)	Queso fresco	13 (5 stillbirths)	Yes	(MacDonald et al. 2005; Norton and Braden 2007)
2001	Sweden	Fresh cheese	>120	Yes	(Danielsson- Tham et al. 2004)
2001	Japan	Washed cheese	86	No	(Makino et al. 2005)
2002	Canada (QC)	Multiple types	17	Y	(Gaulin et al. 2003; Norton and Braden 2007)
2003	U.S. (TX)	Queso fresco	13 (2)	Yes	(Norton and Braden 2007; Swaminatha n and Gerner- Smidt 2007) *
2005	U.S. (TX)	Queso fresco	12	Yes	(CDC 2005)
2005	Switzerland	Tomme	10 (3 + 2 miscarriages )	Yes	(Bille et al. 2006)
2006	U.S. (OR)	Unspecified	3	No	(CDC 2013a) *
2006-2007	Germany	Harzer Käse	189 (26)	No	(Koch et al. 2010)

Year	Location	Implicated Cheese	No. of Illnesses (Deaths) <sup>1</sup>	Raw Milk	Reference
2007	Norway	Camembert	17 (3)	No	(Johnsen et al. 2010)
2008	Canada (QC)	Multiple	38 (5)	No	(MAPAQ 2010; Gaulin et al. 2012) *
2008	Chile	Brie	91(5)	NS	(Promed 2008)
2008	US (multi- State)	Mexican style asadero cheese	8 (0)	No	(Jackson et al. 2011) (CDC 2013c)
2009	US (multi- state)	Mexican style	8 (3)	No	CDC, 2013 #1958 *
2009-2010	Austria, Germany, Czech Republic	Quargel acid- cured cheese	34 (8)	No	(Fretz et al. 2010a; Fretz et al. 2010b; Schoder et al. 2012) *
2009	US (multi- State)	Mexican- style cheese	18 (0)	No	(CDC 2013a; CDC 2013c) *
2010	US (multi- State)	Mexican- style cheese	6 (1)	No	(CDC 2013a; CDC 2013c) *
2011	Belgium	Hard cheese (Pave du Nord)	12 (4)	No	(Yde et al. 2012)
2011	US (MI)	Chive Cheese, Ackawi cheese	2 (1)	No	(CDC 2013a; CDC 2013c)

Year	Location	Implicated Cheese	No. of Illnesses (Deaths) <sup>1</sup>	Raw Milk	Reference
2011	US (NJ)	Mexican- style cheese	2 (0)	No	(CDC 2013a; CDC 2013c) *
2011	US (multi- State)	Blue veined aged cheese	15 (1)	Yes	(CDC 2013a; CDC 2013c) *
2012	US (multi- State)	Ricotta	22 (4)	No	(CDC 2012)
2012	Spain	Latin – style fresh cheese	2 (0)	Unclear	(de Castro et al. 2012)
2013	US (multi- State)	Soft-ripened cheese	6 (1)	No	(CDC 2013b) *

<sup>1</sup>. The number of cases associated with a particular food is not always clearly stated in the publications. <sup>2</sup>. NS – Not Stated





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