Listeria monocytogenes as a microorganism of public health significance

Author: Russell Ramage

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‘I declare that confidentiality of people discussed in this work is maintained; there is no identifiable information of these individuals.’

For my parents

Marshall (1930 – 1976) and Annie (1936 – 2009) Ramage

My dad for his quiet resolve and the time we never got to share.

My mum for her tenacious resilience and for never giving up in the face of the greatest adversity.

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Abstract

Listeriosis is a foodborne illness of significant public health concern exhibiting high mortality rates particularly among vulnerable groups. Regulation European Commission (EC) 2073/2005 requires ready to eat food business operators to take preventative control measures in their production environments and to sample these environments and associated food products to ensure they are free from listeriosis' causative organism, *Listeria monocytogenes*. Ready to eat food manufacturers in Northern Ireland were surveyed anonymously to determine their level of compliance with and understanding of this regulation's Chapter 1 Annex 1 food safety criteria. Respondents were also surveyed regarding compliance with the regulation's *Listeria monocytogenes* contamination preventative control measures of HACCP, environmental monitoring, results trending and product recall. 49 responses from a possible total of 128 originated from ready to eat food sectors which had previously been indicated in listeriosis outbreaks. These included cooked meats, sandwiches, ready meals, dairy and salads/vegetables. Responses indicated statistically significant ($p<0.05$) non-compliance with and unawareness of Chapter 1 Annex 1's food safety criteria. However, survey responses indicated industry compliance with preventative Listeria control measures particularly that of product recall. This study realises the potential for creation of educational or training awareness to help guide industry through the requirements of Regulation (EC) 2073/2005 to enhance product safety and protect public health.

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Abbreviations

Abbreviation	Definition
ACMSF	Advisory Committee on the Microbiological Safety of Food
BFFF	British Frozen Food Federation
BRC	British Retail Consortium
BRCGS	British Retail Consortium Global Standard
CAFRE	College of Agriculture, Food and Rural Enterprise
CDC	Centres for Disease Control and Prevention
CFA	Chilled Food Association
Cfu/g	Colony forming units per gram
EC	European Commission
EFSA	European Food Safety Authority
EMS	Environmental Monitoring Systems
EPS	Extracellular polymeric substances
EU	European Union
FAO	Food and Agriculture Organisation of the United Nations
FSA	Food Standards Agency
FSAI	Food Safety Authority of Ireland
FSC	Food safety criteria
FSMS	Food safety management systems
FSN	Food Safety News
FSS	Food Standards Scotland
FTB	Food Technology Branch
GFSI	Global Food Safety Initiative
Gov	Government
GPU	Gastrointestinal Pathogens Unit
HACCP	Hazard Analysis Critical Control Point
HSA	Health Security Agency
NI	Northern Ireland
PAA	Peracetic acid-based compounds
PHA	Public Health Agency
PHE	Public Health England
PHS	Public Health Scotland
QAC	Quaternary ammonium compounds

RSPH	Royal Society for Public Health
RTE	Ready to eat
RVH	Royal Victoria Hospital
Salads/veg	Salads/vegetables
spp	Species
The regulation	Regulation (EC) 2073/2005
UK	United Kingdom
USA	United States of America
WGS	Whole genome sequencing
WHO	World Health Organisation

Symbols

Symbol	Name
χ^2	chi-squared test
°C	degrees centigrade
>	greater than
<	less than
≤	less than or equal to
-	minus
%	per cent
™	trademark

Chapter 1

1.1 Introduction

With more than 200 known food transmitted diseases currently representing a significant public health threat, research suggests that foodborne disease mortality from contaminated foods represents the main public health concern for almost 1 quarter of the world's population (Silk et al., 2013; World Health Organisation (WHO), 2021). One such disease, listeriosis, was first recognised as a foodborne illness of public health significance after a large Canadian outbreak involving high fatalities in 1981 (Schlech et al., 1983).

Linked epidemiologically to the consumption of contaminated foods, human listeriosis' causative agent, *Listeria monocytogenes* is an opportunistic foodborne pathogen which can infect different cell types and cross intestinal, placental and blood-brain barriers (Jalali and Abedi, 2008; Todd and Notermans, 2011; Macleod, Beeton and Blaxland, 2022). Relatively rare compared to other foodborne illnesses, listeriosis symptoms range from mild disease when non-invasive, to severe infection when invasive, especially among pregnant individuals, the elderly, the young and those with a compromised immune system. Complications include septicaemia, miscarriage and meningitis with United Kingdom (UK) listeriosis presenting hospitalisation and mortality rates of over 90 per cent (%) and 25% respectively (Radoshevich and Cossart, 2017; Ranjbar and Halaji, 2018).

The European Union's (EU) most common cause of foodborne illness deaths, other research reinforces listeriosis' position worldwide as a major foodborne illness with high mortality rates (European Food Safety Authority (EFSA), 2018; Shamloo et al., 2019). However, although nearly all human cases are foodborne, only a small number of cases can be linked to a specific food, an anomaly supported by other more systematic research identifying a lack of analytical epidemiology for small cluster cases for example (Gray, 2006; McLauchlin, Grant and Amar, 2020). Listeriosis' outbreak history presents both a realistic UK public health and food industry concern (Macleod, Beeton and Blaxland, 2022). Outbreaks occur when listeriosis affects large population numbers within a particular area within a short timeframe and are recognised when the same strain of *Listeria monocytogenes* is identified in 2 or more clinical cases (Ranasinghe et al., 2020; Macleod Beeton and Blaxland, 2022). Although 13 different known *Listeria monocytogenes* serotypes cause human disease,

serotype 4b has been responsible for most listeriosis outbreaks (British Frozen Food Federation (BFFF), 2004). Additionally, research suggests most outbreaks involve eating contaminated ready to eat (RTE) foods such as pre-packed sandwiches and cooked sliced meats where high prevalence rates of *Listeria monocytogenes* occur (Little et al., 2009; Scobie et al., 2019).

Many historical listeriosis incidents have involved hospital outbreaks. Between 1999 and 2014, 10 English and Welsh hospital outbreaks involved 37 cases with those affected either immunocompromised adults or pregnant women and their unborn infants (Coetzee et al., 2011). Only 2 of the outbreaks involved contamination levels in excess of 10^2 colony forming units per gram (cfu/g) with the others less than (<) 10^2 cfu/g (McLauchlin et al., 2020). Sandwiches collected in these outbreaks contained fillings including cooked meats, egg, cheese, salad or cooked fish/shellfish. One major drawback of these cases' epidemiology however arises from attributing root cause to sandwich fillings alone. In 8 of the outbreaks, the implicated *Listeria monocytogenes* strain was recovered from associated production environments highlighting cross-contamination's potential in listeriosis outbreaks (Little et al., 2012).

RTE food processing environments are constantly at risk from *Listeria monocytogenes* colonisation constituting potential public health concerns and economic losses for manufacturers when products are recalled (Strydom et al., 2016). The RTE food sector exercises many *Listeria monocytogenes* controls. Tompkin et al. (1999) suggests these can reduce contamination but critics argue that the microorganism and its potential for contaminating RTE foods can never be completely eliminated from processing environments and that effective control proves both expensive and resource intensive (Malley et al., 2015).

Supporting this, recent research demonstrates that despite cleaning and disinfection (sanitisation), *Listeria monocytogenes* can persist in RTE food manufacturing environments (Madden et al., 2018; O'Grady, 2024;). However, cleaning and disinfection are still regarded as crucial *Listeria monocytogenes* control methodologies in RTE processing facilities to reduce cross-contamination of the environment and foods (Ohman et al., 2024). Furthermore, evidence demonstrates that effective cleaning and disinfection in these types of food environments can help control *Listeria monocytogenes'* multiplication and product contamination (Tompkin, 2002).

Current UK food safety legislation has sought to increase public health protection using harmonised quality systems management approaches within RTE processing environments. Regulation European Commission (EC) 2073/2005 (the regulation) is one such piece of legislation aiming to enhance food safety in the interests of public health. It does so by regulating various *Listeria monocytogenes* controls for high-risk RTE food product manufacturers including environmental monitoring, Hazard Analysis Critical Control Point (HACCP) and trend analysis to identify potential food safety threats. It also mandates microbiological criterion allowing food manufacturers to assess a food's acceptability, identify contaminated food batches and facilitate listeriosis preventative controls at industry level (Pérez-Lavalle, Carrasco and Valero Diaz, 2020). Manufacturers must assess against these food safety criteria (FSC) to verify their HACCP based food safety procedures (Food Safety Authority of Ireland (FSAI), 2024). For *Listeria monocytogenes* in RTE foods, these criteria are laid out in Chapter 1 Annex 1. Whilst this section contains criteria for foods for special medical purposes and infants, these do not fall within the scope of this study. However, sections 1.2 and 1.3 mandate criterion for *Listeria monocytogenes* in RTE foods able or unable to support the growth of the pathogen and at which point in the product lifecycle they apply. Failure to meet these criterion might consequence removal from the market or product recall.

Current proposals recommend a regulation amendment extending the criterion for foods able to support the growth of the pathogen where a proper shelf-life assessment had not been conducted (Food Standards Scotland (FSS), 2016). However, sceptics are critical and argue that the current legislation has robust public health effectiveness and that the proposed changes will not improve food safety (Chilled Food Association (CFA), 2021; Ridler, 2021).

This study aims to critically evaluate *Listeria monocytogenes* as a microorganism of public health concern. Its objectives are:-

1. A critical evaluation of current literature regarding *Listeria monocytogenes* as a microorganism of public health significance and its role in UK foodborne disease (Chapter 2).

2. A critical evaluation of cleaning and disinfection as a control measure for *Listeria monocytogenes* in the RTE food industry (Chapter 3).

3. A critical assessment of the level of compliance/non-compliance with and awareness/non-awareness of the regulation regarding *Listeria monocytogenes* within the RTE food industry in Northern Ireland (NI) (Chapter 4).

Chapter 2

2.1 Listeria

The genus listeria currently comprises 28 species (spp) (Siriken, Ayaz and Erol, 2014; Kaszoni-Rückerl et al., 2020; Orsi et al., 2023). Of these, *Listeria monocytogenes* singularly causes human listeriosis (Gasanov, Hughes and Hansbro, 2005). Kathariou (2002) supports this analysis although it is possible that *Listeria seeligeri* and *Listeria ivanovii* have also caused human infections (Government (Gov).UK, 2020). Ranasinghe et al. (2020) support this alternative theory in a wide-ranging study suggesting several human listeriosis cases have involved *Listeria ivanovii*.

Ubiquitous in nature, *Listeria monocytogenes*' human transmission is usually through contaminated foods particularly those indicated later in section 2.4 (Ricci et al., 2018). *Listeria monocytogenes* presents a concern for food manufacturers by exhibiting environmental tolerances restrictive for other foodborne pathogens. Psychrotrophic and able to survive and grow between 0 degrees centigrade (°C) and 45°C, it can exist in refrigerated food production areas where other microorganisms cannot, resulting in bacterial reservoirs (Chan et al., 2008). Killed by cooking above 65°C, the bacterium tolerates traditional food preservation techniques including salt curing and fermentation and demonstrates biocide resistance usually effective against other environmental microorganisms (Sleator, Gahan and Hill, 2003; Martínez-Suárez, Ortiz and López-Alonso, 2016; EFSA, 2024). McAuliffe (2023) supports these findings indicating tolerance properties including resistance to commonly used preservatives and sanitisers. Table 2.1 demonstrates environmental tolerances of concern to food manufacturers.

Table 2.1 Growth and survival limits of *Listeria monocytogenes* (FSAI, 2011a)

Parameter	Range	Optimal	Can survive (but no growth)
Temperature (°C)	-1.5 – 45	30 – 37	minus (-) 18
pH	4.2 – 9.5	7	3.3 – 4.2
Water activity	0.90 – 0.99	0.97	<0.90
Salt (%)	<0.5 - 12	Not applicable	greater than (>) 20

2.2 Listeriosis

UK listeriosis cases are outlined by Public Health England (PHE) in their Gastrointestinal Pathogens Unit (GPU) surveillance reports (Gillespie et al., 2006; Gov.UK, 2024b). Whilst most cases are asymptomatic, invasive listeriosis can result in a 20% to 30% fatality rate (Ramaswamy et al., 2007). UK listeriosis incidences are higher now than since the 1960s but why is this the case? Lamont et al. (2011) suggest UK food culture changes have resulted in increased availability and consumption of foods more likely to be contaminated with *Listeria monocytogenes*. However, although outcomes are often severe, reports suggest UK listeriosis is rarely reported and that less than half of historic listeriosis incidents have been described in relevant literature (McLauchlin, Grant and Amar, 2020; Food Safety News (FSN), 2024a). Additionally, it has been suggested that lengthy incubation periods, lengthy food recall periods and the wide variety of foods that can be contaminated with *Listeria monocytogenes*, mean that causative foods can only be attributed accurately to 10% of reported cases in some UK regions (Goulet et al., 2013).

2.2.1 Listeriosis symptoms

Madden et al. (2018) suggest that in healthy individuals, listeriosis is rare resulting usually in a mild infection with fever and diarrhoea (Health Protection Surveillance Centre, 2017). Contrastingly, there are established cases where invasive listeriosis has occurred in healthy populations (Shamloo et al., 2019). In at-risk groups, more severe symptoms are experienced together with a high mortality rate of 20 – 30% (WHO, 2018). Mimicking its environmental tolerances, the microorganism can adapt to the gastrointestinal tract's acidic, low oxygen environment then manifest clinically and once consumed, contaminated foods can give rise to listeriosis after 1 to 90 days incubation (Ferreira et al., 2014). Table 2.2 demonstrates typical listeriosis symptoms.

Table 2.2 Typical clinical listeriosis symptoms

Listeriosis symptoms	Reference
Diarrhoea, mild fever, nausea and vomiting, pregnancy abortion, septicaemia and meningitis particularly in immunocompromised patients	Shamloo et al. (2019)
Mild, flu-like sickness in healthy people may be replaced by severe, systemic infections including meningitis, septicaemia and abortion in high-risk groups including pregnant women, unborn children, the elderly, immunocompromised people and infants	Mateus et al. (2013)
Septicaemia, meningitis or some other types of central nervous system infections	Ranasinghe et al. (2020)
Invasive listeriosis infection in pregnancy can lead to fever, chills, headache and haemocytosis in pregnant mothers resulting in stillbirth	Mylonakis et al. (2002)
For infants surviving pregnancy complications can present including pneumonia and bacterial meningitis. In nonperinatal listeriosis complications can include central nervous system infections including meningitis and brain abscess	Drevets and Bronze (2008).

2.2.2 Listeriosis infective dose

One question that needs to be asked though is what exactly constitutes a *Listeria monocytogenes* infective dose? Due to its long onset time, uncertainty exists around exact numbers but research indicates that less than or equal to (≤) 100 cfu/g are low risk for healthy individuals but high risk for the immunocompromised. In contrast, risk increases with individual vulnerability and strain virulence but for healthy individuals it has been suggested as greater than (>) 1000 cfu/g of food (EFSA, 2008; Pouillot et al., 2016; FSN, 2024b). This analysis is supported by other evidence including an EU baseline listeriosis survey where contaminated samples contained >100 cfu/g (UK Health Security Agency (HSA), 2023). Pouillot et al. (2016), indicated an infective dose of 8.2×10^3 cfu/g resulting from a community-based ice cream listeriosis outbreak. However, one of this study's main weaknesses was that the contaminated product contained variable *Listeria monocytogenes* levels and some immune-compromised study participants may have received a smaller infective dose than those within the main study body.

2.3 Epidemiology

Epidemiology allows public health officials to count compatible laboratory-confirmed listeriosis cases consistently regardless of jurisdiction (Centres for Disease Control

and Prevention (CDC), 2022). Confirmed listeriosis cases are defined as “a person with a clinically compatible illness and isolation of *Listeria monocytogenes* from a normally sterile site” (UKHSA, 2021).

2.3.1 England and Wales

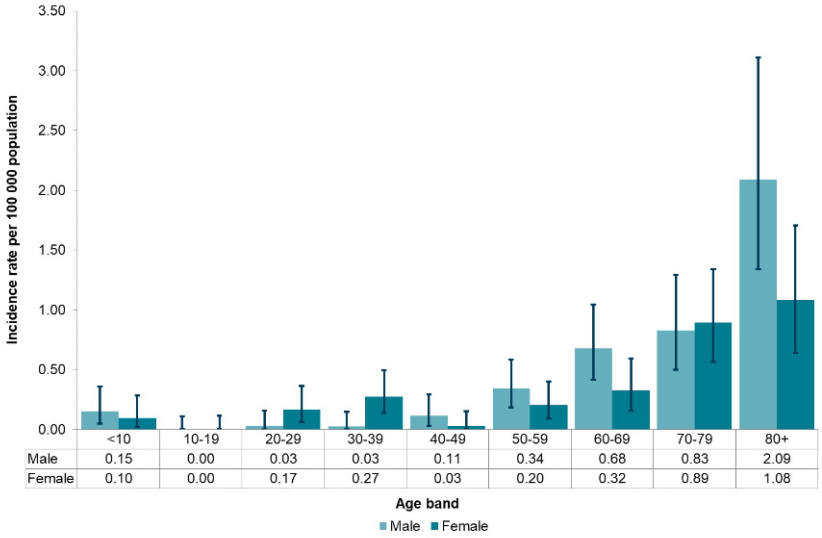
From a total of 160 cases in England and Wales in 2021, 20% were pregnancy related of which 20.7% resulted in stillbirth or miscarriage. Non-pregnancy case mortality rate was 17.5% (Gov.UK, 2024a). Table 2.3 demonstrates English and Welsh annual case numbers within the period indicated. The case numbers increase has been attributed to Lamont’s previously suggested changes however lower-case numbers had been potentially reported in the previous few years due to interannual variation and the effects of the COVID-19 pandemic (Gov.UK, 2024b).

Table 2.3 English and Welsh case numbers 1990 to 2022

Period	Case numbers	Details	References
1990 - 2000	114 - 136	Not applicable.	Macleod, Beeton and Blaxland (2022)
2006 - 2019	135 – 226	Not applicable.	Macleod, Beeton and Blaxland (2022)
2022	167 a 6.4% increase on previous 5 years	Increase possibly due to 4 major outbreaks including a national outbreak involving smoked fish. Listeriosis during pregnancy responsible for 14.4% of all cases.	Gov.UK (2024b)

Listeriosis links with age have already previously been indicated and figure 2.1 demonstrates higher 2022 listeria incidence rates in the elderly population particularly those 80 years plus. Why is this the case though? Whilst evidence suggests this is not just a UK phenomenon, the Advisory Committee on the Microbiological Safety of Food (ACMSF) (2009) suggest a range of contributory factors including underlying conditions, ignoring use-by dates, using dirty dishcloths and too-high fridge temperatures.

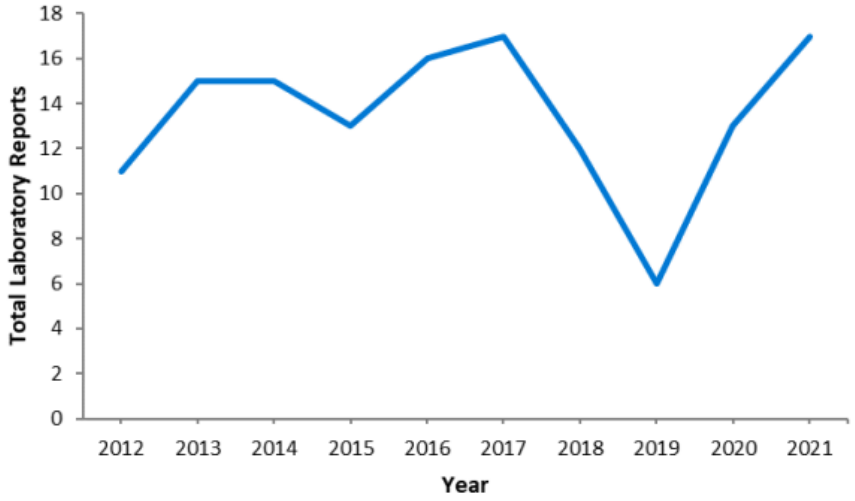
Figure 2.1 Listeriosis in England and Wales in 2022 dependant on age and sex (Gov.UK, 2024b)



2.3.2 Scotland

Public Health Scotland (PHS) notified 13 laboratory reported listeriosis cases in 2020, increasing to 17 in 2021, both an increase on 2019's 7 reported cases (PHS, 2023). Figure 2.2 demonstrates Scottish case number fluctuations between 2012 and 2021.

Figure 2.2 Listeriosis reported cases 2012 – 2021 (PHS, 2023)



Several drawbacks exist with Scottish listeriosis data. Firstly, there is a small number of annually reported cases and secondly data is gathered for clinical rather than disease surveillance reasons. Therefore, existing data might bias groups more likely to be tested including the very young and very old and only represent a proportion of the listeriosis cases rather than the true picture. Furthermore, 2020 and 2021 reporting restrictions in the wake of the COVID-19 pandemic may also have resulted in

underreported results as did changes in behaviours leading to health-care treatments (PHS, 2023). However, having said that, the 2012 to 2021 aggregate data shows a pattern emulating that of England and Wales, namely that the majority of cases reported are from the elderly population, those 65 years or older.

2.3.3 Northern Ireland

Northern Ireland's Public Health Agency (PHA) publish gastrointestinal infections epidemiological data annually, the most recent of which indicates 6 listeriosis cases in 2022, down from 8 in 2021 but an increase from the lowest recorded number of cases of 1 in 2017 (PHA, 2022). Table 2.4 demonstrates NI listeriosis infections between 2013 and 2022 compared with those of other reported gastrointestinal infections.

Table 2.4 NI laboratory reported gastrointestinal infections 2013 – 2022 (Adapted from PHA, 2022)

Organism	**2013**	**2014**	**2015**	**2016**	**2017**	**2018**	**2019**	**2020**	**2021**	**2022**
Campylobacter spp	1355	1414	1320	1258	1421	1475	1350	1237	1655	1698
Norovirus	386	272	335	618	299	250	335	51	120	305
Salmonella spp	155	111	124	141	128	155	153	66	94	171
Escherichia coli O157	72	54	33	81	57	85	43	57	48	77
Clostridium perfringens	24	23	34	24	25	20	20	25	23	31
Listeriosis	2	4	6	4	1	3	5	7	8	6

Clearly, listeriosis cases represent the lowest numbers reported but what could be the reason for this? It might be because *Listeria monocytogenes* is not routinely tested for in cases of gastroenteritis due in part to the range of listeriosis symptoms outlined previously. It could also be due to surveillance data interpretation challenges created by different inter-laboratory testing policies (PHA, 2018).

How does the UK compare with elsewhere? Table 2.5 compares UK confirmed listeriosis case numbers with some European countries between 2018 and 2022.

Table 2.5 Listeriosis cases in UK and Europe 2018 – 2022 (Adapted from EFSA, 2023)

Country	2022	2021	2020	2019	2018
France	451	435	334	373	338
Germany	548	562	546	571	678
Ireland	17	14	6	17	21
Italy	345	230	155	202	178
Spain	437	355	191	504	370
UK	173	185	144	154	168

In 2022 listeriosis was Europe's fourth highest reported zoonosis after *Salmonella*, *Campylobacter* and *Yersinia enterocolitica* (EFSA, 2023). The table clearly demonstrates that the UK compares favourably with EU countries. Critics suggest this is attributable to current, effective UK hygiene practices including chilled food shelf-life a third to a half of those in Europe (Goodburn, 2023). However, research suggests that UK listeriosis whilst less common than *Escherichia coli* and *Campylobacter* for example, still causes more deaths and requires more hospitalisation than both combined (BFFF, 2024).

2.4 Outbreaks and foods involved

Global listeriosis outbreaks are increasing with most of those recently reported originating from Europe (Ranasinghe et al., 2020). Causative food vehicles have included pasteurised and non-pasteurised milks, cheese and butter, fresh fruit and vegetables, salads, RTE cooked meats, processed meats and smoked meats (McLauchlin et al., 2004; MacDonald et al., 2005; Swaminathan and Gerner-Smidt, 2007; Thomas et al., 2020). Table 2.6 summarises some larger global listeriosis outbreaks and food vehicles involved.

Table 2.6 Global listeriosis outbreaks

Year	Country	Food	Cases	Death rate	References
1981	Canada	Coleslaw	41	9 (27%)	Schlech et al. (1983)
1987 - 1989	UK	Pâté	355	94 (27%)	McLauchlin et al. (2004)
1993	France	Pork rillettes	38	10 (26%)	Goulet et al. (1998)
2002	America	RTE meats	54	8 (14.8%)	Swaminathan and Gerner-Smidt (2007)
2013 - 2015	Denmark	Smoked fish	20	7 (35%)	Gillesberg Lassen et al. (2016)
2017 - 2018	South Africa	Polony	937	193 (27%)	Thomas et al. (2020)

Historically, UK listeriosis outbreaks in the late 1980s involved pâté, soft cheese, cooked chicken and vegetables (Bannister, 1987; Kerr et al., 1990; McLauchlin et al., 1991; Gilbert, McLauchlin and Velani, 1993). The pâté outbreak in table 2.6 was singularly responsible for a near doubling of cases in England, Wales and NI between 1985 and 1989 (McLauchlin et al., 1991).

Between 1990 and 2000 up to 136 listeriosis cases were reported annually (ACMSF, 2003). Although fewer outbreak studies exist from this period, 4 cases representing a potential listeriosis cluster involving sandwiches and immuno-compromised patients were identified in North-East England (Graham et al., 2002). Deemed a notifiable disease in 2010, 12 outbreaks were reported between 1999 and 2019 primarily involving pre-prepared sandwiches or RTE salads (PHE, 2010; Macleod Beeton and Blaxland, 2022). However, McLauchlin et al. (2020) suggest that other unpublished sporadic and community-acquired listeriosis outbreaks existed within this period and that only 0.8% of reported listeriosis cases could be linked to a specific food (McLauchlin, Grant and Amar, 2020). Having said that, several of these outbreaks have been individually studied including those involving pork pies, crab meat, frozen sweetcorn and ox tongue (Lamden et al., 2013; Awofisayo-Okuyelu et al., 2016; Elson et al., 2018; McLauchlin et al., 2021a).

2.4.1 Hospital listeriosis outbreaks

Hospital acquired listeriosis from RTE foods continues to contribute to UK outbreak statistics. Some of these are summarised in table 2.7.

Table 2.7 UK hospital acquired listeriosis

Year	Location	Cases	Deaths	Contributory factors	Reference
2008	Belfast Royal Victoria Hospital (RVH)	7	3	Immuno-compromised patients over 60 years old served with contaminated sandwiches Patients were storing contaminated cooked meats from supermarkets in bedside lockers Possible community exposure	PHA (2008)
2012	Antrim Area Hospital, Causeway Hospital Coleraine	4	1	Patients provided with sandwiches from outside caterers contaminated with *Listeria monocytogenes* at <100 cfu/g	Smyth (2012)
2017	Yorkshire and Humber	1	0	53-year-old male colitis patient served contaminated sandwiches from a hospital supplier on 12 occasions	McLauchlin, Grant and Amar (2020); McLauchlin et al. (2021b)
2019	Manchester Royal Infirmary	9	2	Immuno-compromised patients served sandwiches from an outside caterer who had used contaminated chicken from a cooked meat company	PHE (2020)

Although appearing simplistic, the Belfast outbreak was complex in nature due to additional patient contributory factors and exacerbated further by *Listeria monocytogenes*' long incubation period meaning community exposure could not be ruled out. The most recent hospital based listeriosis outbreak when writing this research was that of the Manchester Royal Infirmary (PHE, 2020). However, whilst this outbreak affected 9 patients in other hospitals around Liverpool, Leicester and Derby, the deaths were 57- and 84-year-old female patients with a history of health problems who died very shortly after eating contaminated chicken and mayonnaise sandwiches.

2.4.2 Community listeriosis outbreaks

RTE foods have also been responsible for community based listeriosis outbreaks. Table 2.8 summarises some of these outbreaks that occurred nationally between 1987 and 2013.

Table 2.8 Community based listeriosis outbreaks 1987 - 2013

Outbreak year	Cases	Region	Food	Epidemiology	Serotype	Microbiological evidence	References
1987-1989	378	UK wide	Pâté	Association between pâté from 1 manufacturer and infected cases	4b	Samples from a Belgian pâté manufacturer contaminated with >10^3 cfu/g *Listeria monocytogenes* same strain	McLauchlin et al. (1991)
2009	14	England regional	Sliced cooked meats	Patient history revealed consumption of contaminated sliced cooked meats	4	Outbreak strain of *Listeria monocytogenes* up to 10^4 cfu/g recovered from a sliced meat manufacturer	McLauchlin, Grant and Amar (2020)
2009 – 2010	10	London, Yorkshire and Humber, North West	Sliced cooked meats	All cases ate contaminated sliced ham or tongue	1/2a	Same *Listeria monocytogenes* strain up to 10^3 cfu/g recovered from a single manufacturer supplying regional outlets	McLauchlin, Grant and Amar (2020)
2010 – 2012	13	Yorkshire and Humber	Pork pies	Consumption of pies contaminated with outbreak strain significantly increased listeriosis likelihood	4	Outbreak strain recovered at <20 cfu/g from multiple retailers supplied by same manufacturer	Awofisayo-Okuyelu et al. (2016)
2012 – 2013	5	North West	Cooked pressed beef in gelatin	Link established between cases and pressed beef from a single producer	1/2a	Outbreak strain recovered at up to 10^2 cfu/g from a single manufacturer supplying butchers and market stalls	McLauchlin, Grant and Amar (2020)
2013 (August)	3	West Midlands, Yorkshire and Humber	Crab meat	All 3 cases linked to eating crab meat prior to illness	4	A continuation of a 2011 - 2013 outbreak with final numbers of outbreak strain recovered up to 10^5 cfu/g	Elson et al. (2018)

Table 2.8 clearly demonstrates a range of RTE foods responsible for these historic outbreaks. More recently, between 2015 and 2023, 8 outbreaks involving contaminated smoked fish were epidemiologically linked using surveillance

questionnaires and in 2021, 3 English and Welsh outbreaks involved cooked beef tongue, corned beef and smoked fish (Whitworth, 2023). Between 2018 and 2019 a Europe-wide listeriosis outbreak involving contaminated frozen sweetcorn traced to a Hungarian processor affected 12 people in the UK (FSN, 2021; McLauchlin et al., 2021a). 2022 saw 4 English and Welsh outbreaks with a further 12 people affected by the contaminated fish issue carried over from 2021 (FSN, 2024a). In 2023 an outbreak involving soft cheese involved 1 death in the London, South England area.

However, whilst these outbreak's details appear well-established, evidence suggests inherent difficulties when identifying community based listeriosis outbreaks (EFSA, 2013). Although epidemiology or microbiology can identify an outbreak's causative food, food exposure patterns are often separated geographically by large distances as in the Hungarian sweetcorn case. Furthermore, it has been well-established that *Listeria monocytogenes* has a long incubation period and complex attributes including a low infective dose and prolonged resistance to traditional cleaning and disinfection methods within food processing environments. Even when *Listeria monocytogenes* is recovered from an affected patient and food that they have been in direct contact with, without whole genome sequencing (WGS), cross contamination of the suspect food in domestic refrigerators cannot be ruled out. Critics argue that the recovery of the same strain from patient and causative food needs to be indefatigable (McLauchlin, Grant and Amar, 2020). Consequentially, public health investigations of human listeriosis outbreaks require epidemiology, microbiology and data from the food chain regarding the causative agent for a full and complete investigation.

Chapter 3

3.1 RTE foods and *Listeria monocytogenes* contamination

Studies suggest that RTE food's greatest contamination risk is from recontamination post listericidal treatment (Tompkin et al., 1999). Table 3.1 summarises a range of listericidal treatments designed to manufacture products containing undetectable numbers of *Listeria monocytogenes.*

Table 3.1 Example listericidal treatments producing RTE foods

Food type	Listericidal treatment	References
RTE salads and other fresh produce	Free chlorine at defined concentrations or alternatives	Holah (2022)
Cooked, baked, roasted meats and fish	Cooking to a core temperature of 75°C for 30 seconds or equivalent to achieve a 6 logarithm *Listeria monocytogenes* reduction	FSAI (2020)
Liquid milk	Pasteurisation at 71.7°C for 15 seconds or equivalent	CampdenBri (2022)
Various	Irradiation, high pressure processing or listericidal product formulations including pH <4.4 or water availability <0.92	FSAI (2020)

Furthermore, post-listericide contamination in RTE processing environments has previously been indicated as the root cause of many listeriosis outbreaks, some of which are presented in table 3.2 below.

Table 3.2 Listeriosis outbreaks and root causes (Adapted from Goodburn, 2023)

Country	Year	Cases	Fatalities	Product	Root Cause
UK	1989	>200	>17	Pâté	Post-process hygiene
France	1992	272	92	Jellied pork tongue	Post-process hygiene
United States of America (USA)	1999	101	17	Cooked meat	Air filtration unit contamination
Canada	2008	57	22	Cooked sliced meat	Post-process contamination
Denmark	2014	41	17	Cooked meat	Post-process contamination
South Africa	2018	1060	216	Cooked RTE meats	Post-process contamination
Spain	2019	>200	3	Cooked meats	Post-process contamination

3.2 Cleaning and disinfection (sanitisation)

The regulation establishes RTE food businesses' statutory requirements to prevent the contamination of food, keep their premises clean and disinfected and to environmentally monitor these premises.

Goodburn's (2020) extensive analytical research suggests that effective cleaning and disinfection is among the most important RTE processing environmental controls to prevent such re/cross-contamination by *Listeria monocytogenes*. Other evidence supports this suggesting its importance is such that it merits adoption by the Codex Alimentarius Commission into their HACCP food safety management system (Tompkin, 2002; Food and Agriculture Organisation of the United Nations (FAO), 2023). This importance is further substantiated in specific outbreaks including that from the Blue Bell Creameries where cleaning and disinfection failures resulted in profound consequences (FSN, 2019; Lee et al., 2021).

Whilst other extensive research reinforces these findings, critics argue that one drawback is the consideration that cleaning and disinfection efficiency lapses potentially result in product contamination (Zottola, 1994; Murugesan et al., 2015; Leong et al., 2017). Furthermore, other evidence supports that ineffective cleaning and disinfection can permit significant levels of *Listeria monocytogenes* contamination particularly in difficult to clean areas and *Listeria monocytogenes'* ability to persist in RTE premises and even recolonise equipment is well established (Carpentier and Cerf, 2011; Conficoni et al., 2016; FSS, 2024).

Additionally, other evidence suggests that ineffective cleaning and disinfection can lead to harbourage sites existing within RTE food premises allowing *Listeria monocytogenes* to persist then subsequently contaminate/recontaminate RTE food post-listericidal treatment (Zhang et al., 2021). Although other areas can function as indirect sources of *Listeria monocytogenes* contamination, some of these key harbourage sites are identified in table 3.3.

Table 3.3 *Listeria monocytogenes* harbourage sites with contamination potential (Adapted from Tompkin et al., 1999)

RTE food premises area
Filling or packaging equipment
Conveyor belts
Slicers, dicers, shredders, blenders and other size-reducing equipment
Hoppers and collators
Storage racks and ingredient containers
Hand tools, gloves, aprons
Food containers, baskets, tote-bins
Floors, footwear, drains

Effective cleaning and disinfection programmes should target these areas together with other food and hand contact surfaces. Typically, they initially employ a grease-removing detergent followed by a listericidal disinfectant or sanitiser or alternatively a 1-stage sanitiser may be used (Macleod, Beeton and Blaxland, 2022). Sodium hypochlorite-based compounds, peracetic acid-based compounds (PAA) or quaternary ammonium compounds (QAC) are traditionally used in manual application operations (Duze, Marimani and Patel, 2021). Typically applied physically as liquids or foams at the end of a production shift, one major drawback of this application is that some sanitisation procedures fail to acknowledge the presence of elevated levels of significant organic residues left behind on many food processing surfaces. These can potentially reduce the procedure's effectiveness (Ruiz-Llacsahuanga et al., 2022). Gram et al. (2007) also supports these findings with research that demonstrated up to a 2 logarithmic difference in *Listeria monocytogenes* removal efficacy dependant on the type of food matrix and residual soiling left behind on food production surfaces. However, this study's main weakness was that it was laboratory based and may have failed to consider contributory factors that exist in real food production environments that would have potentially increased its relevance. To target *Listeria monocytogenes*, Tompkin et al. (2019) suggest the application of these sanitisers in the steps outlined in table 3.4.

Table 3.4 Cleaning and disinfection procedure to target *Listeria monocytogenes* (Tompkin et al., 2019)

Cleaning and disinfection procedure including application of sanitiser (sodium hypochlorite or QAC)	
Step 1	Dry clean
Step 2	Equipment pre-rinse
Step 3	Equipment visual inspection
Step 4	Equipment foam and scrub
Step 5	Equipment rinse
Step 6	Equipment visual inspection
Step 7	Floor clean
Step 8	Sanitise floors and equipment
Step 9	Post sanitisation verification
Step 10	Dry floors

Ohman et al. (2024) observed a >5 logarithmic *Listeria monocytogenes* reduction on equipment and surfaces using this multistep cleaning and disinfection compared with other sanitisation procedures. However, Aase et al. (2000) demonstrated that occasional microorganism strain resistance required the use of increased minimum inhibitory listericide concentrations. Whilst critics contradicted this idea, other research supports these findings suggesting that incomplete listericide removal from surfaces may result in sub-lethal levels remaining post cleaning and disinfection. This can induce selection pressure on *Listeria monocytogenes* facilitating the rise of tolerant strains (Heir et al., 2004; Duze, Marimani and Patel, 2021). Consequently, maintaining the same disinfectant concentration constantly may have no listericidal effect and a wealth of research demonstrates the microorganism's presence in premises even though critical contact points are regularly sanitised (Carpentier and Cerf, 2011).

Having said that, one food industry strategy is to disinfect harbourage sites like floors first and research suggests that cleaning and disinfection should concentrate on these areas (Berrang et al., 2013; Lønnerup Bislev, 2024). For example, although effective drain cleaning can be complex and unpleasant, it is essential to reduce the risk of *Listeria monocytogenes* aerosol contamination from pooled water collected in drains. Consequently, potential food contact surface contamination from floors can then be targeted in a second sanitisation further reducing contamination risk, a method previously substantiated by research (Carpentier, 2010).

Such is the potential for drains to contaminate RTE production areas that for some high-risk foods the procedure should happen daily. Other evidence also suggests that

floors and waste containers in high-risk RTE food production environments should be sanitised daily (Tompkin et al., 2019). *Listeria monocytogenes* cleaning and disinfection frequency is usually controlled via schedules. A simplified example schedule is presented in table 3.5 which includes cleaning and disinfection frequency, potential cleaning and disinfection agent, application and application concentration.

Table 3.5 *Listeria monocytogenes* cleaning and disinfection application summary (Adapted from Diversey, 2018)

Step	Surface	Product	Application	Concentration percentage (%)
Daily cleaning	Food contact surface, equipment, floors	Chlorinated Alkaline detergent	As per table 2.3	2 – 5%
	Small parts, removable surfaces, tools	Chlorinated Alkaline detergent	Static bath soak or machine wash	0.5 – 4%
Terminal or pre-operation	Food contact surface, equipment, floors	QAC or PAA	Spray or clean in place (CIP)	1 – 4%
Mid shift	Food contact surface, equipment, floors	QAC	Spray	1 – 4%
Hand hygiene	Hands	Hand soap and disinfectant	Soap dispenser	1%

Although table 3.5 indicates the inclusion of a mid-shift sanitisation, only dry clean-ups are recommended during food production since wet procedures can introduce the potential for *Listeria monocytogenes* aerosol onto clean surfaces. (Conference for Food Protection, 2016).

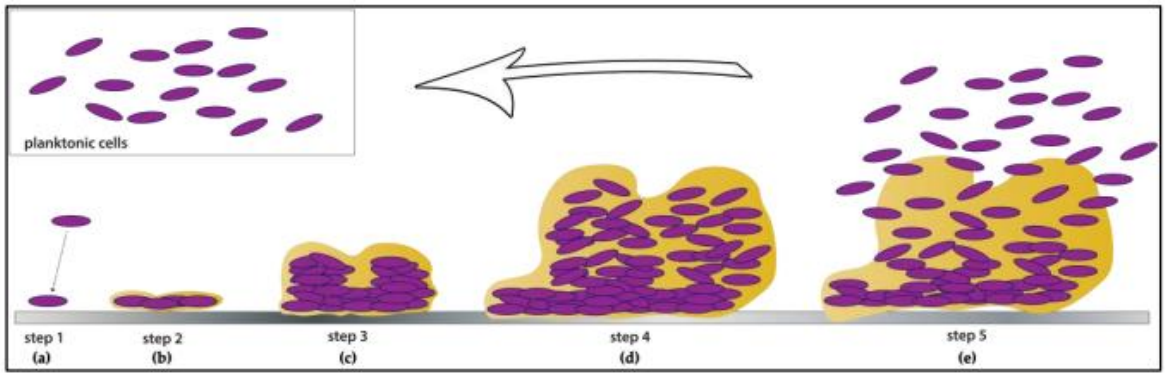
Traditional processes using water can also be time consuming requiring equipment dismantling to facilitate manual sanitisation and equipment with electrical parts might not be cleaned thoroughly (Ferreira et al., 2014). Here, hydrogen peroxide mist can effect a 2 logarithmic *Listeria monocytogenes* reduction on some surfaces (Møretrø et al., 2019). However, one of this application technique's main weaknesses is its ineffectiveness on some types of plastic conveyor belts commonly used in the food industry when particularly heavily soiled. Other novel cleaning and disinfection methods explored in recent years include ozone, ultraviolet light and fogging (BFFF, 2024).

3.3 Biofilms

One key tolerance property encountered by cleaning and disinfection is the microorganism's ability to form biofilms. Robbins et al. (2005) demonstrated enhanced listericidal tolerance properties to ozone, chlorine and hydrogen peroxide in RTE food manufacturing environments through the formation of biofilms which form when *Listeria monocytogenes* cells on production surfaces arrange in complex structures and embed in a matrix of extracellular polymeric substances (EPS) produced by the organism (Fleming et al., 2007). These EPS then confer properties on the microorganism that it lacks when not in biofilm form (planktonic form) namely better surface adhesion, higher surface removal resistance and increased listericide tolerance (Colagiorgi et al., 2017).

Reis-Teixeira, Alves and de Martinis (2017) identified that in the final biofilm development stage, *Listeria monocytogenes* planktonic cells can detach from the biofilm and disperse into the RTE production environment representing a potential source of contamination and figure 3.1 below summarises biofilm formation from the stage where *Listeria monocytogenes* planktonic cells become attached to a surface.

Figure 3.1 The biofilm development stages (Adapted from Vasudevan, 2014)



(a) planktonic cells reversibly attach to surfaces; (b) the adhered cells form a monolayer and produce extracellular matrix; (c) the cells within the self-produced extrapolymeric matrix continue to grow and form multilayered microcolonies; (d) cells are irreversibly attached to the surface and embedded in the matrix: the biofilm is mature; (e) last stage of biofilm formation – planktonic cells can detach from the biofilm and colonize new surfaces

One question that needs to be asked is whether other RTE production environment microorganisms assist *Listeria monocytogenes* in biofilm formation? Carpentier and Cerf's (2011) research suggests this was unlikely. However, other microorganisms such as *Pseudomonas* spp have similar growth properties to those of *Listeria monocytogenes* in cold, wet, RTE food processing environments. Contradicting

Carpentier and Cerf's findings, wide ranging research demonstrated the ability of *Pseudomonas* spp biofilms to assist *Listeria monocytogenes'* survival post cleaning and disinfection (Thomassen et al., 2023).

Numerous studies have demonstrated *Listeria monocytogenes'* increased sanitiser resistance the longer the biofilm remains in the RTE food processing environment (Fagerlund et al., 2017; Mazaheri et al., 2022;). Biofilm formation prevention control via early cleaning and disinfection would seem critical and this has previously been demonstrated with similar conclusions also proposed by other researchers (Zhao et al., 2013; Rodríguez-López et al., 2018). Regarding biofilm listericides, peracid sanitisers work effectively against planktonic *Listeria monocytogenes* achieving considerable biofilm reduction (Fatemi and Frank, 1999). However, this laboratory-based study may have been more useful if conducted in a RTE food processing environment. Having said that, this research demonstrated promise in that mixed *Pseudomonas* spp/*Listeria monocytogenes* biofilms were used and the PAA employed demonstrated more effective results than traditional chorine-based disinfectants. Furthermore, several researchers have indicated the effective use of novel biofilm biocontrol methods using lactic acid bacteria, bacteriophages, enzymes and bacteriocins (Gray et al., 2018; Rodríguez-López et al., 2018). However, this area of work still needs some development since any microorganism presence in RTE food processing environments is considered undesirable because of their potential food spoilage or food safety risks (Rodríguez-López et al., 2018).

3.4 Environmental Monitoring Systems (EMS)

Niches within the harbourage sites discussed earlier may allow establishment of *Listeria monocytogenes* resulting in ineffective cleaning and disinfection. Some of these niches are summarised in table 3.6.

Table 3.6 Niches where routine cleaning and disinfection may be ineffective (Adapted from Tompkin et al., 1999)

Niches where routine sanitisation may be ineffective
Hollow rollers on conveyor belts
Slicers, dicers
Rubber seals on equipment and doors
Porous conveyor belts
Cleaning equipment such as mops and brushes
Pooled water in production areas
Personal protective equipment such as aprons and gloves

Here, *Listeria monocytogenes* specific EMS are crucial to verify cleaning and disinfection's effectiveness within the RTE processing environment (Tompkin et al., 1999). Niche areas identified by an EMS can be risk-categorised into zones then targeted by more specific or frequent sanitisation to eradicate their *Listeria monocytogenes* contamination potential. This protocol has previously been effectively demonstrated and supported by other research underlining the importance of an EMS in helping reduce the potential risk of RTE food contamination (Henriques, Gama and Fraqueza, 2017; Shimojima et al., 2023).

Where the microorganism might pose a public health risk, article 5 of the regulation requires RTE manufacturers to monitor their processing areas and equipment for *Listeria monocytogenes*. Furthermore, article 9 of the regulation mandates that food business operators should analyse unsatisfactory results trends and take appropriate action to prevent the occurrence of *Listeria monocytogenes* risks. Many modern food safety management systems (FSMS) recognise the importance of cleaning and disinfection in controlling *Listeria monocytogenes*' risks. The British Retail Consortium Global Standard (BRCGS) for example, supports manufacturers by building stringent and specific controls based on the regulation into their FSMS to support enhanced product safety and public health (British Retail Consortium (BRC), 2022; BRC, 2023). This is done by requiring holders of the standard in RTE food premises to demonstrate compliance with its requirements for specific *Listeria monocytogenes* cleaning and disinfection protocols and adoption of an EMS whose results are trended to demonstrate that the system is working effectively.

Chapter 4

4.1 Materials and Methods

4.1.1 Survey background and rationale

Listeria monocytogenes is one of several microorganisms specifically controlled by the regulation but despite this, remains responsible for significant outbreaks of foodborne illness and product recalls throughout the UK. Experience established working within the Northern Irish RTE food industry over many years indicated that the regulation could be subject to misinterpretation and its Chapter 1 Annex 1 food safety criteria (FSC) often lacked compliance with regard to control of *Listeria monocytogenes*. The main aim of this research objective was to survey Northern Ireland's RTE food manufacturers regarding their levels of compliance/non-compliance with the regulation's Chapter 1 Annex 1 *Listeria monocytogenes* FSC and their level of awareness/non-awareness of its contents relevant to their product's food safety.

4.1.2 Pilot study

Before data collection commenced, the research protocol was approved by the University of Derby's formal ethics application procedure. To test the survey, an initial pilot questionnaire consisting of 22 questions was designed using Microsoft Forms™ (Appendix 1). These included background questions regarding respondent's job roles, type of RTE manufacturing facility and then more specific questions directly related to the regulation that would collect data regarding respondent's level of compliance or non-compliance with Chapter 1 Annex 1 of the regulation and their level of awareness or non-awareness of the legislation's *Listeria monocytogenes* specific content.

The regulation contains statutory duties regarding a manufacturer's *Listeria monocytogenes* food safety responsibilities. To reduce the effects of bias, questions regarding these duties were set to require a yes or no response and where appropriate, respondents were also offered an 'other' option. Ambiguity and leading questions were also avoided. To reduce limitations in response accuracy the questionnaire was kept short and easy to answer with an indication at the beginning of the questionnaire regarding how long it should take to complete.

To test the survey, on 22nd February 2024, the pilot questionnaire was emailed (Appendix 2) to 20 staff members of the Food Technology Branch (FTB) of the College of Agriculture, Food and Rural Enterprise (CAFRE) in NI. All pilot test subjects had an

industrial food technology background and some were also experienced in the use of questionnaires and surveys. Respondents were asked to attempt the questionnaire and provide verbal/email feedback regarding its ease of use.

The pilot study responses (Appendix 3) revealed some wording issues, some complexities around how the initial questions had been set up and alternative ideas around ease of use. Taking these comments on board, the initial survey questionnaire was redesigned and improved before the actual data collection commenced.

4.1.3 Final questionnaire question types

The redesigned survey (Appendix 5) contained 26 questions in total. Generalised questions allowed respondents to offer a range of information regarding their business type, their role in the business, business size, type of RTE food manufactured and whether or not they conducted cleaning and disinfection programmes specifically for *Listeria monocytogenes* biofilms. Other questions required respondents to indicate their compliance/non-compliance with or awareness/non-awareness of more specific duties required by the regulation's Chapter 1 Annex 1 FSC. These are summarised in table 4.1.

Table 4.1 Survey questions based on statutory requirements of the regulation

Question number	Regulation	Statutory requirement
4	Article 3(1)	HACCP
9	Article 5(2)	RTE manufacturers will sample the process area and equipment for *Listeria monocytogenes*
10	Article 9	Analyse trends in results to identify unsatisfactory results
15 and 16	Article 4(2), Article 5(5) and Chapter 1 Annex 1 food safety criteria category 1.2 or 1.3 sampling plan	Compliance with Chapter 1 Annex 1 food safety criteria sampling frequencies. Alternatives agreed with local authority
14, 17 and 18	Chapter 1 Annex 1 food safety criteria category 1.2 or 1.3 limits and stage where criterion applies	Compliance with Chapter 1 Annex 1 food safety criteria
17 and 19	Chapter 1 Annex 1 food safety criteria category 1.2 or 1.3 limits and sampling plan	Compliance with Chapter 1 Annex 1 food safety criteria identification of unsatisfactory results within sampling plan
20	Article 7(2)	Product recall for product tested against Chapter 1 Annex 1 food safety criteria achieving unsatisfactory results
22 - 25	Chapter 1 Annex 1 food category 1.1, 1.2 and 1.3	Awareness of statutory requirements within Chapter 1 Annex 1 food safety criteria

4.1.4 Participants

The population chosen for the study was the Northern Irish RTE food manufacturing industry. Participants were selected using CAFRE's database of Northern Irish food industry clients and permission had been sought and granted from CAFRE's FTB Head of Branch (Appendix 4) to access the database for the purpose of this study.

4.1.5 Data analysis

Descriptive and statistical analysis of the data was undertaken using Microsoft Excel (Microsoft, Redmond Washington, USA) and R version 4.4.0 (R Foundation for Statistical Computing, Vienna, Austria). Respondent's awareness of (or non-awareness of) and compliance with (or non-compliance with) the regulation's Chapter 1 Annex 1 FSC was assessed using the chi-squared test (χ^2) with Yate's correction and the chi-squared test (χ^2) without Yate's correction to assess if observations were significant ($p<0.05$) by comparing them to expected counts (50/50 in this case). A comparison of respondent's awareness and compliance levels was assessed using the chi-squared test (χ^2) with Yate's correction and Fisher's exact test to test for Independence ($p<0.05$) between the 2 sets of data.

Chapter 5

5.1 Results

5.1.1 Survey size and respondent's business size

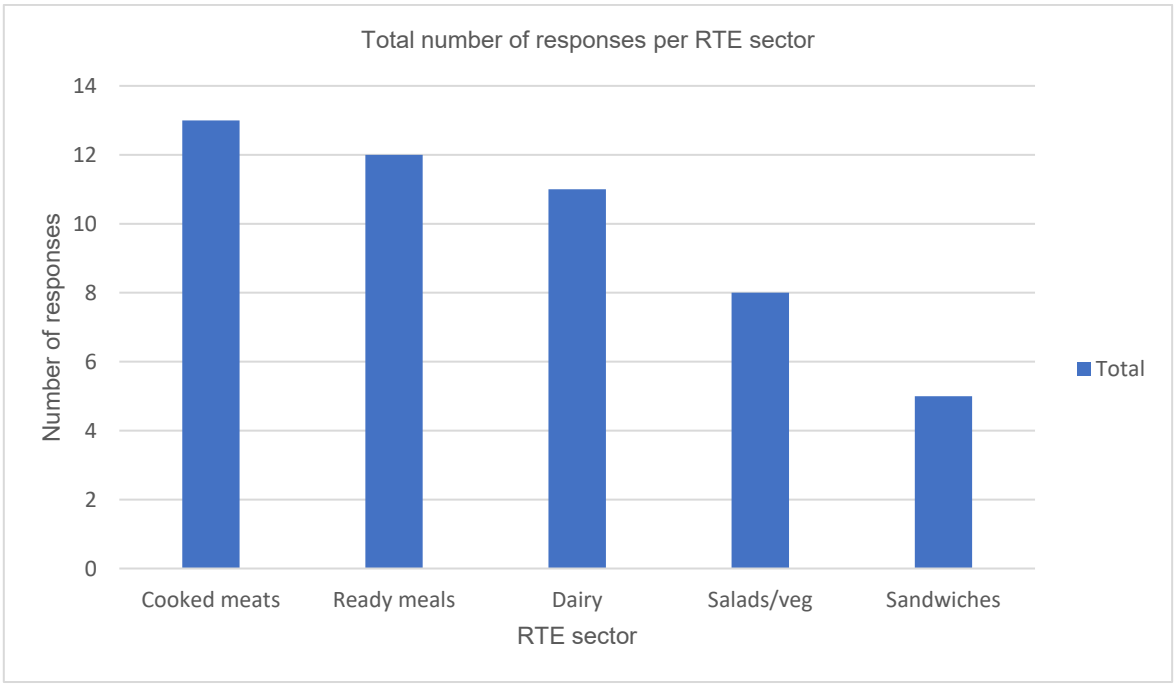
In total, 49 responses were received from a study population of 128 Northern Irish RTE food businesses. Categorised by product type into 5 main RTE food sectors, table 5.1 summarises the types of food products reported by survey respondents within each of these RTE sectors.

Table 5.1 Product types within each RTE sector of the survey responses

RTE Sector	Example foods reported by survey respondents
Cooked meats	Cooked deli-meats, smoked deli-meats, cured deli-meats, cooked fish, cooked ham, cooked chicken, cooked pork, cooked beef
Ready meals	Coleslaws, potato salads, cooked pies, sausage rolls, quiches, RTE savoury foods, ready meals (ambient, chilled or frozen), RTE savoury foods, ambient deserts, sushi, soups, processed egg (pasteurised liquid egg)
Dairy	Fresh cheese, RTE cold blended dairy spreads, pasteurised, yoghurt, cream, cottage cheese and other soft cheeses, hard cheese, RTE processed cheese,
Salads/vegetables	Sliced fresh apple, washed and cut fruit, vegetables and salad crudités, washed and cut vegetable packs,
Sandwiches	Sandwiches, wraps, paninis,

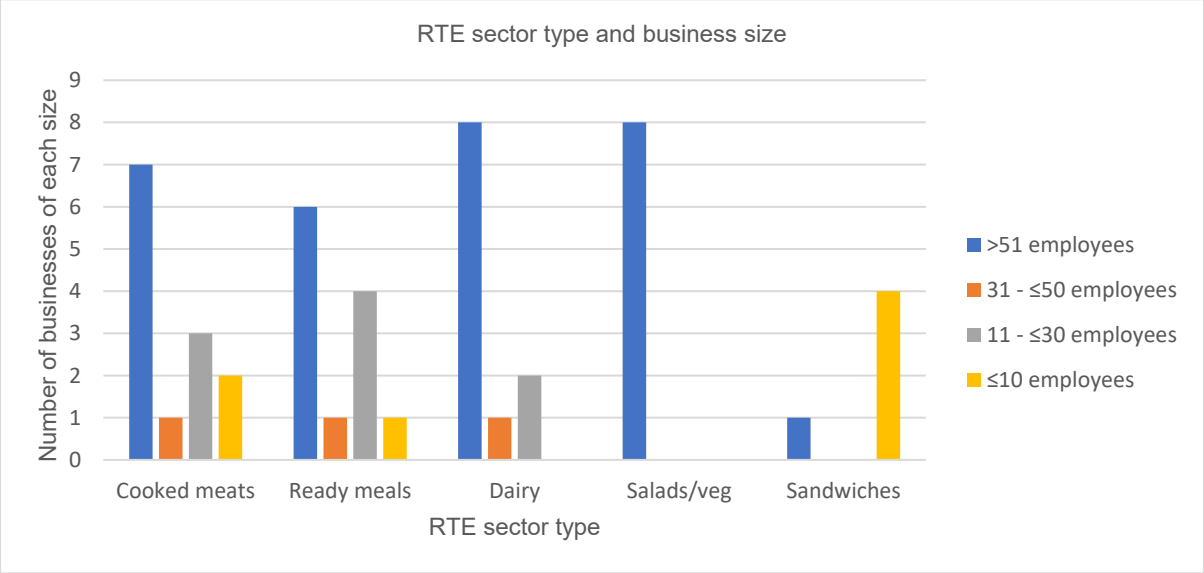
Figure 5.1 indicates the number of survey responses from each RTE sector.

Figure 5.1 Numbers of survey responses across each RTE sector from 49 respondents



The cooked meats sector had the largest number of responses at 26.53% followed by ready meals (24.5%), dairy (22.45%), salads/vegetables (salads/veg) (16.33%) and sandwiches (10.20%). Figure 5.2 indicates respondent's business sizes by employee numbers across the RTE sectors.

Figure 5.2 Business sizes within each RTE sector



53.85% of cooked meat businesses, 50% of ready meals, 72.73% of dairy, 100% of salads/veg and 20% of sandwiches all had >51 employees. The cooked meat sector also had 15.38% of responses from the smallest business size of <10 employees with 8.33% ready meal respondents also from this business size. However, most sandwiches sector businesses (80%) reported <10 employees.

5.1.2 Respondent's job title/role

Within RTE food businesses, technical and quality managers are those expected in theory, to have the most extensive knowledge of the regulation's mandatory requirements. Table 5.2 summarises survey question 2 responses where respondents identified their job role/title.

Table 5.2 Survey job role/title responses by sector with indicated HACCP team membership

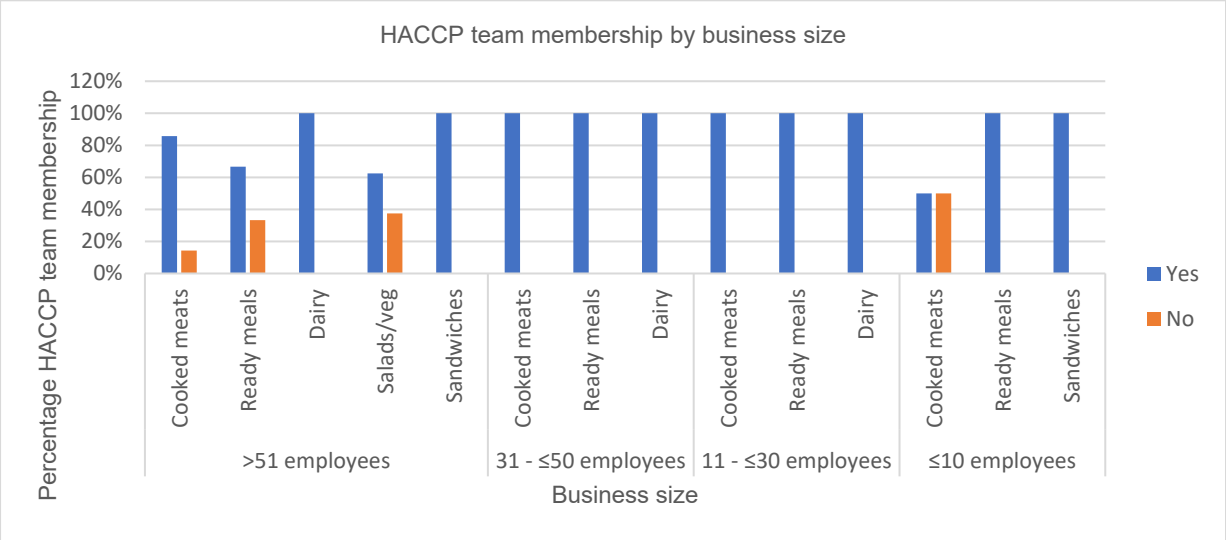
Job Title/Role	Sector					Total	Number of HACCP Team Members
	Cooked Meats	Ready Meals	Dairy	Salads/Vegetables	Sandwiches		
Technical Manager	3	6	3	3	1	**16**	**16**
Quality Manager	3	1	4	0	2	**10**	**10**
Other							
Assistant Quality Manager	1	0	0	0	0	**1**	**1**
Chef	1	0	0	0	0	**1**	**0**
Director	1	1	0	0	0	**2**	**2**
Ex Technical Management	1	0	0	0	0	**1**	**1**
Food Safety Consultant	0	0	0	0	1	**1**	**1**
Food Technologist	2	0	1	0	0	**3**	**2**
Food Technology Development Advisor	0	0	1	0	0	**1**	**1**
Head of Supply Chain	0	0	1	0	0	**1**	**1**
Head of Sustainability and Innovation	0	0	0	1	0	**1**	**1**
Head of Technical	0	1	0	0	0	**1**	**1**
Laboratory Manager	0	0	0	2	0	**2**	**0**
Owner	1	0	0	0	0	**1**	**1**
Project Manager	0	1	0	0	0	**1**	**0**
Compliance Auditor	0	0	1	0	0	**1**	**1**
Quality Auditor	0	1	0	0	0	**1**	**1**
Sales	0	0	0	1	0	**1**	**1**
Senior Quality Assistant	0	0	0	1	0	**1**	**0**
Supervisor	0	0	0	0	1	**1**	**1**
Supplier Auditor	0	1	0	0	0	**1**	**0**
Total	**13**	**12**	**11**	**8**	**5**	**49**	**42**

With 16 (32.65%) technical managers and 10 (20.41%) quality managers, the 23 other responses indicated various job titles/roles from chef through heads of departments to other senior staff.

5.1.3 HACCP team membership.

Survey question 4 related to regulation Article 3(1) and asked respondents to indicate if they were a member of the HACCP team. Table 5.2 indicates that all technical and quality managers were HACCP team members. 16 of 23 other responses were also included in this category giving a total of 42 (85.71%) HACCP team membership. Figure 5.3 demonstrates respondent's HACCP team membership status by business sizes and RTE sector.

Figure 5.3 HACCP team membership per business size across the RTE sectors

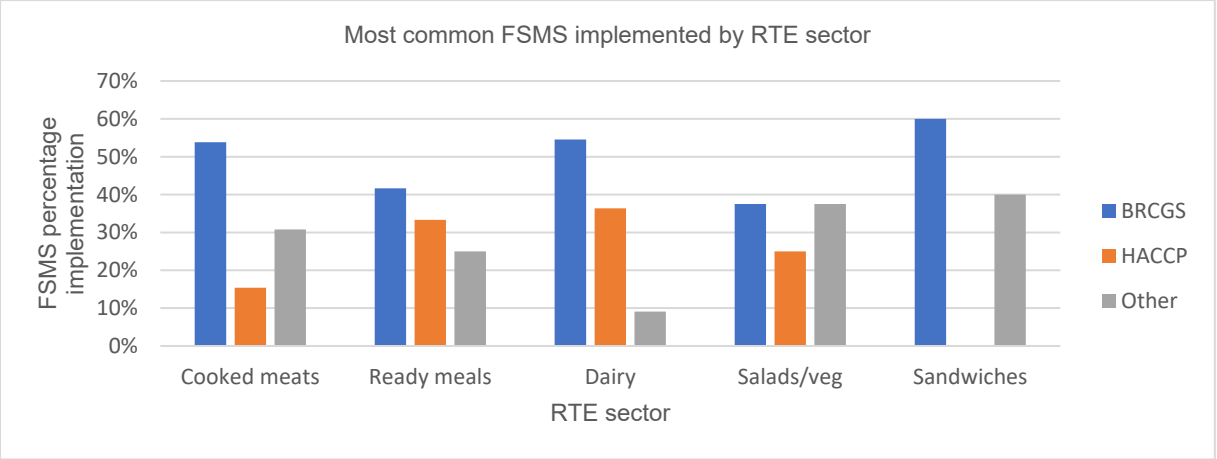


Irrespective of business size, 100% of sandwich and dairy sector respondents were HACCP team members. In the ready meals RTE sector, HACCP team membership increased with decreasing business size from 66.67% in businesses with >51 employees to 100% in all 3 smaller business size categories. This pattern was also observed in the RTE cooked meats sector where HACCP team membership increased from 85.71% in businesses with >51 employees to 100% in businesses with 31 - ≤50 and 11 - ≤30 employees respectively. However, unlike the ready meals sector, in cooked meat businesses with ≤10 employees, HACCP team membership levels dropped to only 50%. Responses were only received in the RTE salads/veg sector from respondents in businesses with >51 employees where 62.5% of respondents were HACCP team members.

5.1.4 An implemented FSMS

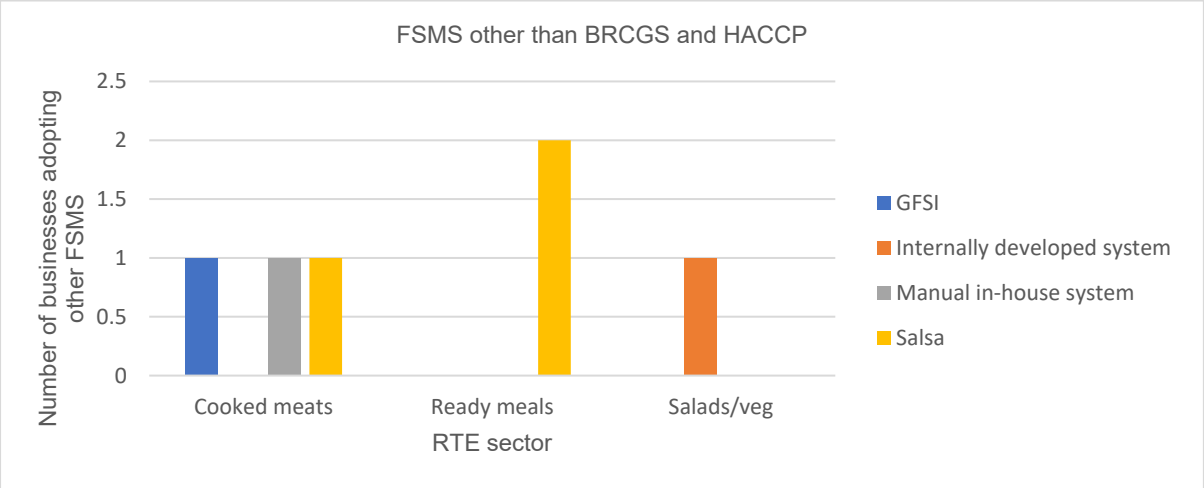
With 1 exception, all survey respondents answered yes to this question. The most common FSMS operated within the survey are summarised in figure 5.4.

Figure 5.4 Most common FSMS implemented by RTE sector



BRCGS was the most common FSMS across all RTE sectors with highest implementation rates in the sandwiches sector followed by dairy, cooked meats ready meals and salads/veg sectors. With the exception of the sandwiches sector, HACCP was the next most common FSMS used for all sectors. All sectors also indicated the use of other FSMS some of which are detailed in figure 5.5. Of these, Salsa, the Global Food Safety Initiative (GFSI) standard and internally developed or in-house systems were the main FSMS in operation.

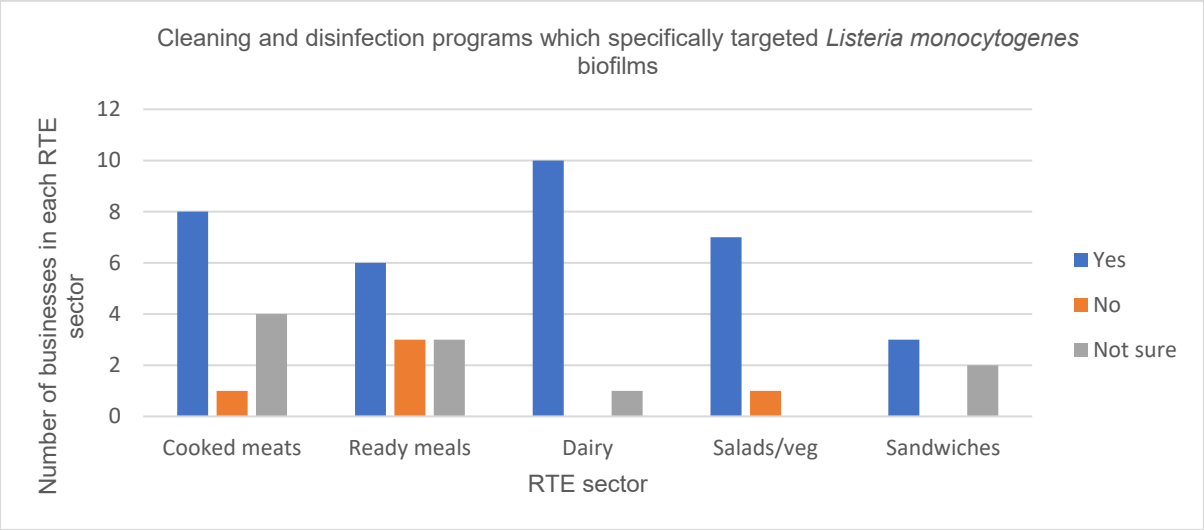
Figure 5.5 FSMS other than BRCGS or HACCP adopted by the Northern Irish RTE food industry



5.1.5 Cleaning and disinfection programmes specifically targeting *Listeria monocytogenes* biofilms

Figure 5.6 summarises the business numbers adopting *Listeria monocytogenes* biofilm specific sanitisation in each sector.

Figure 5.6 Specific *Listeria monocytogenes* biofilm cleaning and disinfection adopted across RTE sectors

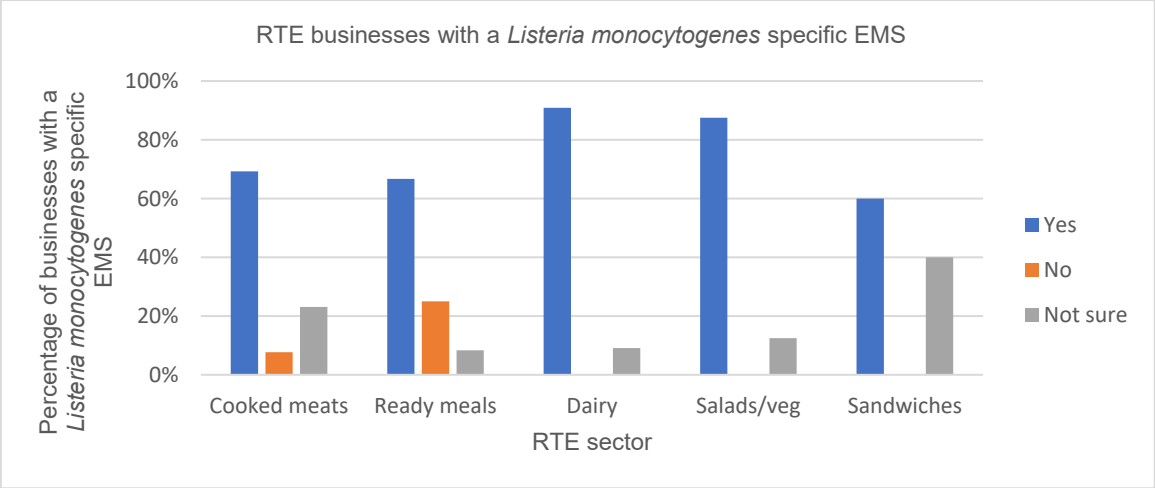


Adoption rates varied across the sectors with the highest rate 90.90% being that of the dairy sector followed by salads/veg (87.5%), cooked meats (61.53%), sandwiches (60%) and ready meals (50%) respectively. 25% of ready meal respondents were either not sure or alternatively did not have a *Listeria monocytogenes* biofilm specific cleaning and disinfection program as did 12.5% of salads/veg respondents and 7.69% of cooked meats respondents. Unsurety levels were greatest in the sandwiches (40%) and cooked meats sectors (30.77%).

5.1.6 *Listeria monocytogenes* environmental monitoring Article 5(2) and Article 9

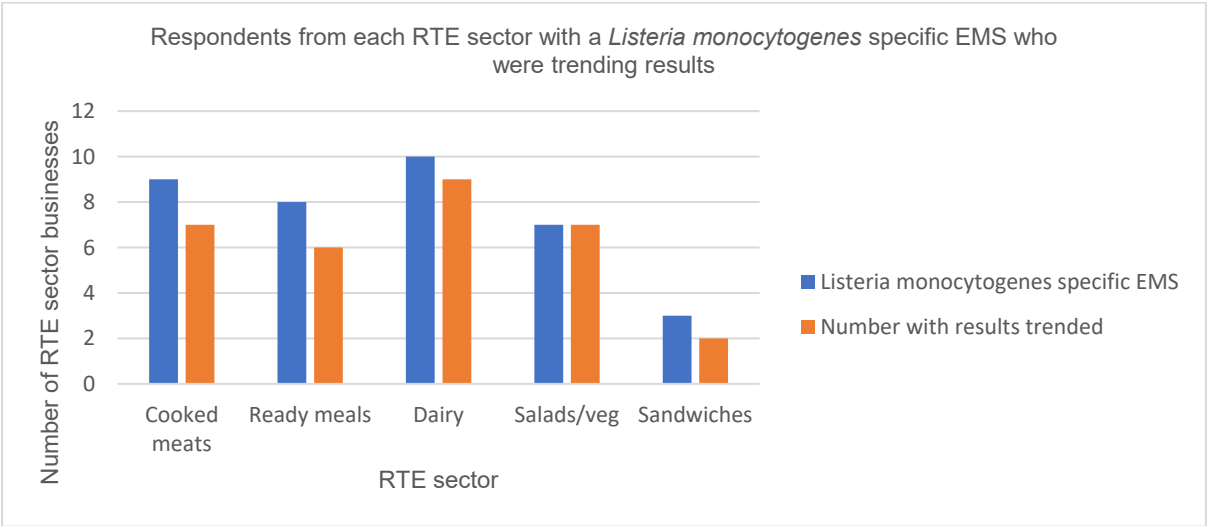
Figure 5.7 summarises survey question 9 responses. Across all RTE sectors, the majority of respondents were conducting environmental monitoring specifically for *Listeria monocytogenes*.

Figure 5.7 RTE sector responses indicating the use of a *Listeria monocytogenes* specific EMS



37 out of 49 respondents indicated that they monitored their environment specifically for *Listeria monocytogenes* with an uptake rate of at least 60% or more across all sectors. The dairy sector was most active in this area with almost 91% actively monitoring. The ready meals sector recorded the greatest proportion of no responses at 25% whilst the highest unsurety levels existed among the sandwiches sector at 40%. Yes respondents to this question were asked if they trended the results from their EMS. Summarised in figure 5.8, the responses indicated that the majority of respondents who monitored their processing environment for *Listeria monocytogenes* were also trending their EMS results.

Figure 5.8 Total numbers of RTE sector businesses with a *Listeria monocytogenes* specific EMS and numbers of respondents also trending its results

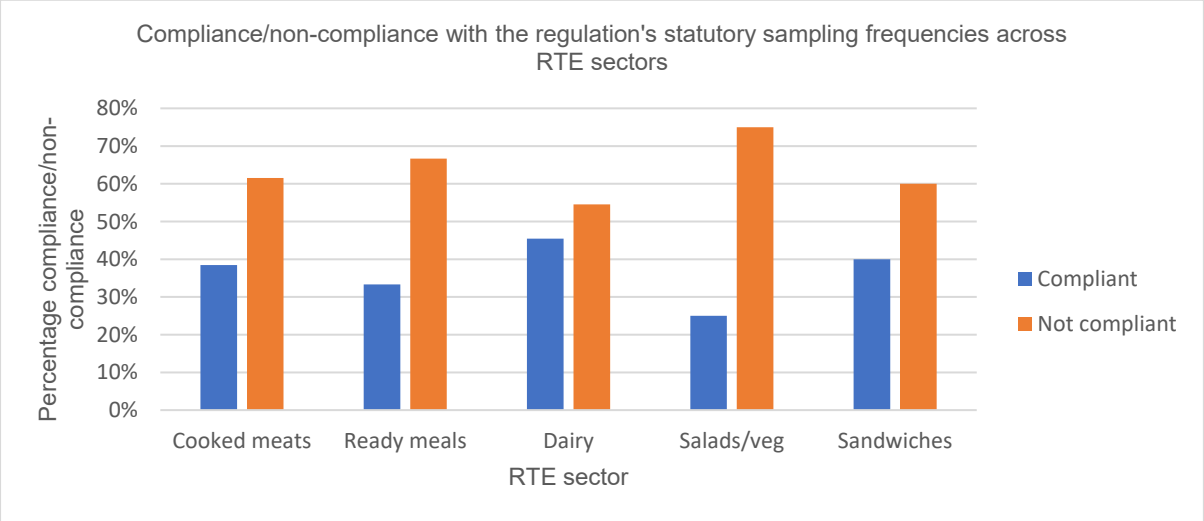


100% of salads/veg sector businesses trended their EMS results with all other sectors noting positive trending responses at >75%. Only the sandwiches sector reported a trend response less than 70%.

5.1.7 Compliance/non-compliance with Chapter 1 Annex 1 FSC

Questions 15 and 16 were taken directly from Chapter 1 Annex 1's *Listeria monocytogenes* FSC and considered the regulation's statutory sampling frequencies required for compliance/non-compliance. The responses to these survey questions are presented in figure 5.9.

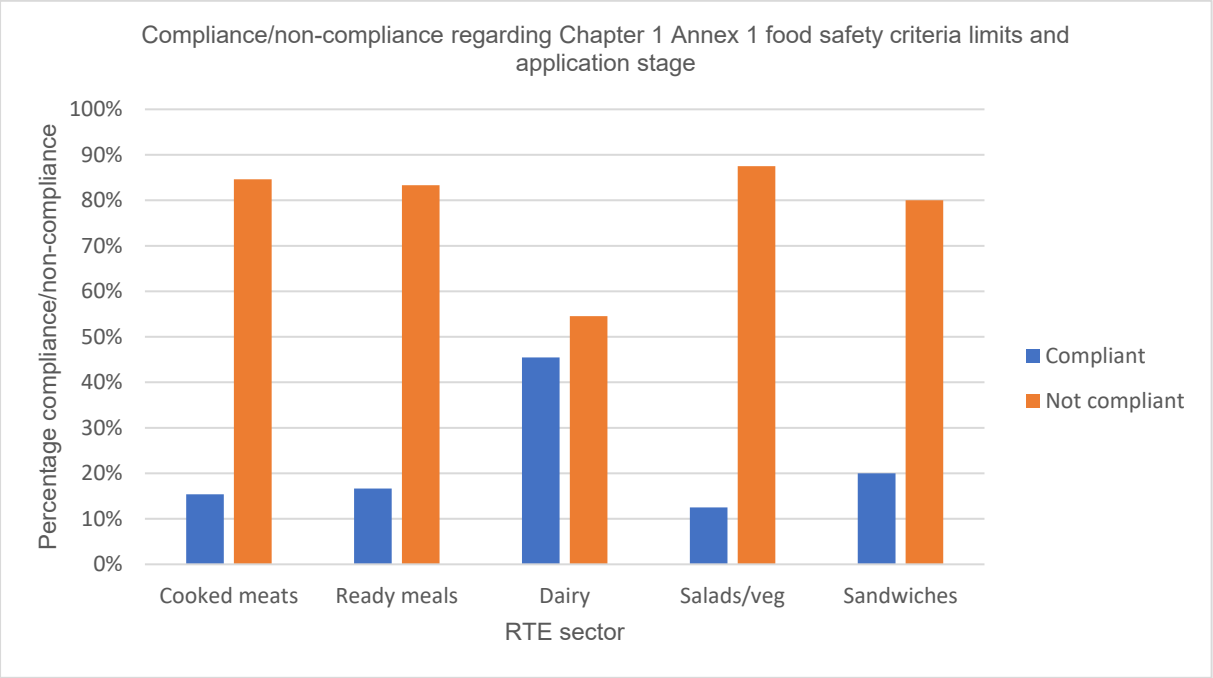
Figure 5.9 Survey responses indicating compliance/non-compliance with the regulation's statutory sampling frequencies



Overall, there were higher levels of non-compliance than compliance with this statutory requirement. None of the sectors demonstrated more than 46% sampling frequency compliance with some high-risk product sectors such as the sandwiches and cooked meats sectors only demonstrating 40% and 38.46% compliance respectively. All sectors demonstrated that more than half of respondents within each sector were submitting incorrect numbers of samples for laboratory analysis with high-risk sectors such as sandwiches, ready meals and cooked meats reporting levels of non-compliance at 60% or greater.

Questions 14, 17 and 18 together considered Chapter 1 Annex 1's statutory requirements for sampling limits and their application stage. A number of possible correct answer combinations depended on whether respondents identified their foods as category 1.2 or 1.3 products. Figure 5.10 summarises survey responses in terms of compliance/non-compliance with this Chapter 1 Annex 1 statutory requirement.

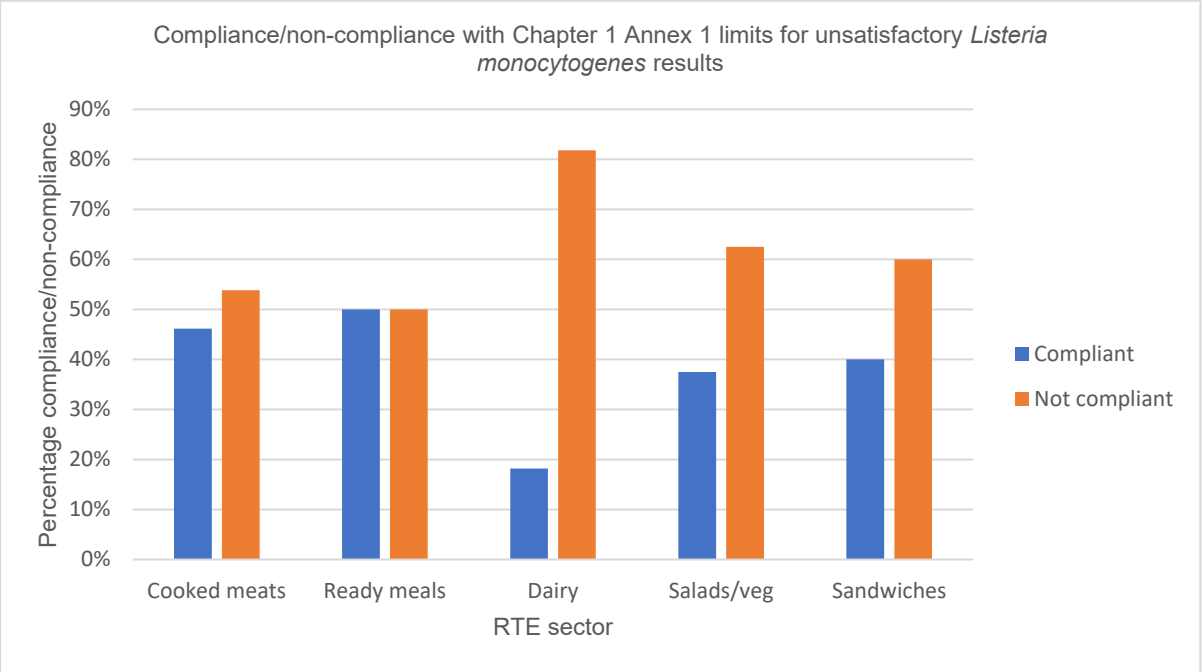
Figure 5.10 Survey responses indicating compliance/non-compliance with Chapter 1 Annex 1 statutory limits and application stage



The survey responses indicated greater non-compliance than compliance with this statutory requirement. Less than 50% of respondents in all sectors demonstrated that they were using the correct limits for their products and applying them at the correct stage in the product life cycle particularly the high-risk product sectors of sandwiches, ready meals and cooked meats who demonstrated 20% or less compliance. All sectors demonstrated more than 50% non-compliance from the dairy sector at 54.55% to the cooked meats sector at 84.62%.

Questions 17 and 19 together considered Chapter 1 Annex 1's statutory requirements regarding correct identification of unsatisfactory results. Figure 5.11 summarises the survey responses in terms of compliance/non-compliance.

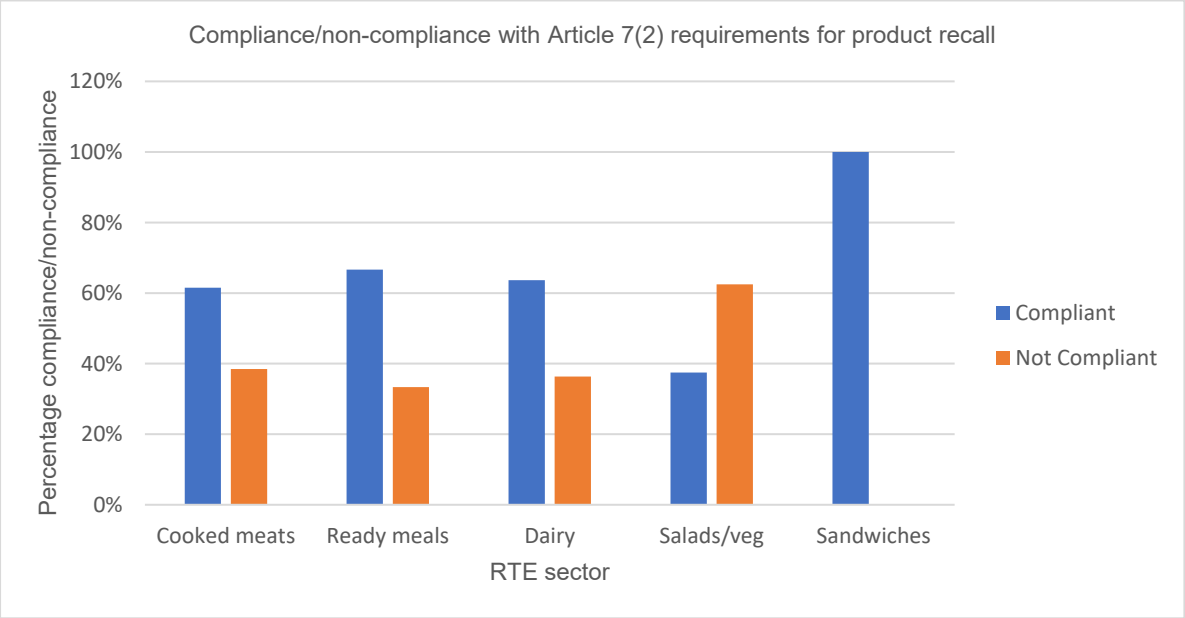
Figure 5.11 Survey responses for compliance/non-compliance with Chapter 1 Annex 1 identification of unsatisfactory results



With the exception of the ready meals sector, there were higher overall levels of non-compliance than compliance with this statutory requirement. The ready meal sector was most compliant with unsatisfactory results identification even though they demonstrated only 50% compliance. Non-compliance predominated in the dairy sector at 81.82% with all other sectors demonstrating non-compliance levels greater than 50%.

Question 20 considered product recall for unsatisfactory results outside Chapter 1 Annex 1's statutory limits. Figure 5.12 summarises respondent's survey replies.

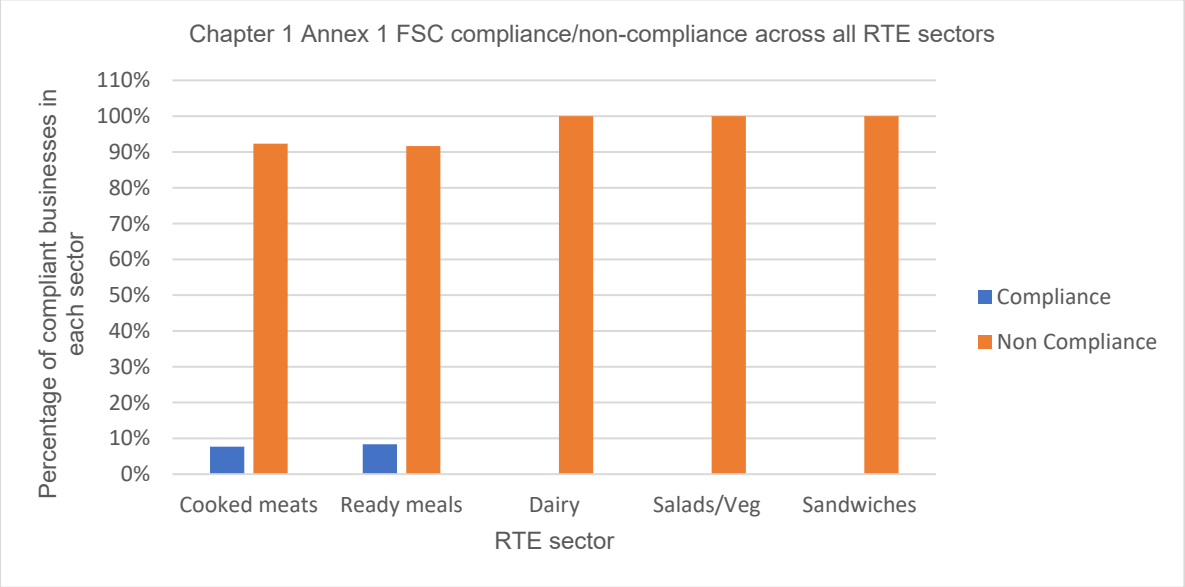
Figure 5.12 Survey responses indicating compliance/non-compliance with Article 7(2) statutory requirements for product recall



With 1 exception, all sectors demonstrated greater compliance than non-compliance with product recall. The sandwiches sector demonstrated 100% compliance whilst the salads/veg sector was the only one where non-compliance was greater than compliance.

The 3 series of survey questions that evaluated compliance/non-compliance with Chapter 1 Annex 1's FSC were assessed together for statistical significance ($p<0.05$). Figure 5.13 indicates those RTE sector respondents who answered all 3 questions correctly and compliance/non-compliance levels demonstrated across all survey sectors for these questions. Each question's individual survey response details have been explained previously.

Figure 5.13 Compliance/non-compliance with Chapter 1 Annex 1 FSC across all RTE sectors



Across the survey, only 1 cooked meat and 1 ready meals respondent answered all 3 FSC compliance questions correctly and statistically significant ($p<0.05$) non-compliance was evidenced across the entire survey. All sectors individually demonstrated statistically significant ($p<0.05$) non-compliance and table 5.3 summarises those 2 respondents who answered all 3 sets of compliance questions together with the 8 respondents who answered all 4 awareness questions correctly.

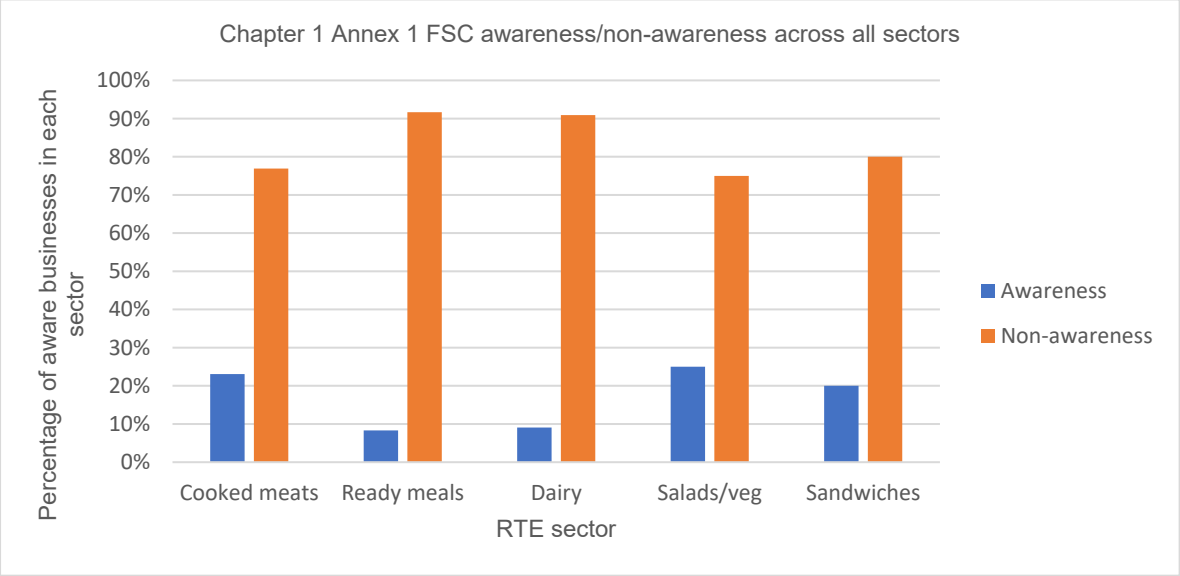
Table 5.3 Survey respondents demonstrating correct answers to composite compliance/non-compliance and composite awareness/non-awareness questions

Respondent number	Correct answers to compliance questions 15/16	Correct answers to compliance questions 14/17/18	Correct answers to compliance questions 17/19	Correct answers to awareness questions 22-25	Personal awareness rating	Sector	Result
1				√	Average	Cooked meats	Aware
12				√	Average	Cooked meats	Aware
13				√	Average	Salads/veg	Aware
17				√	Good	Salads/veg	Aware
18				√	Good	Ready meals	Aware
21				√	Good	Sandwiches	Aware
35	√	√	√		Average	Cooked meats	Compliant
36				√	Average	Cooked meats	Aware
39				√	Good	Dairy	Aware
49	√	√	√		Excellent	Ready meals	Compliant

5.1.8 Awareness/non-awareness of Chapter 1 Annex 1 FSC

The combined responses for questions 22 to 25 tested respondent's awareness/non-awareness of Chapter 1 Annex 1's FSC and were assessed together for statistical significance ($p<0.05$). Figure 5.14 summarises the percentage of respondents from each sector who gave correct answers to these 4 questions.

Figure 5.14 Awareness/non-awareness of Chapter 1 Annex 1 FSC across all RTE sectors

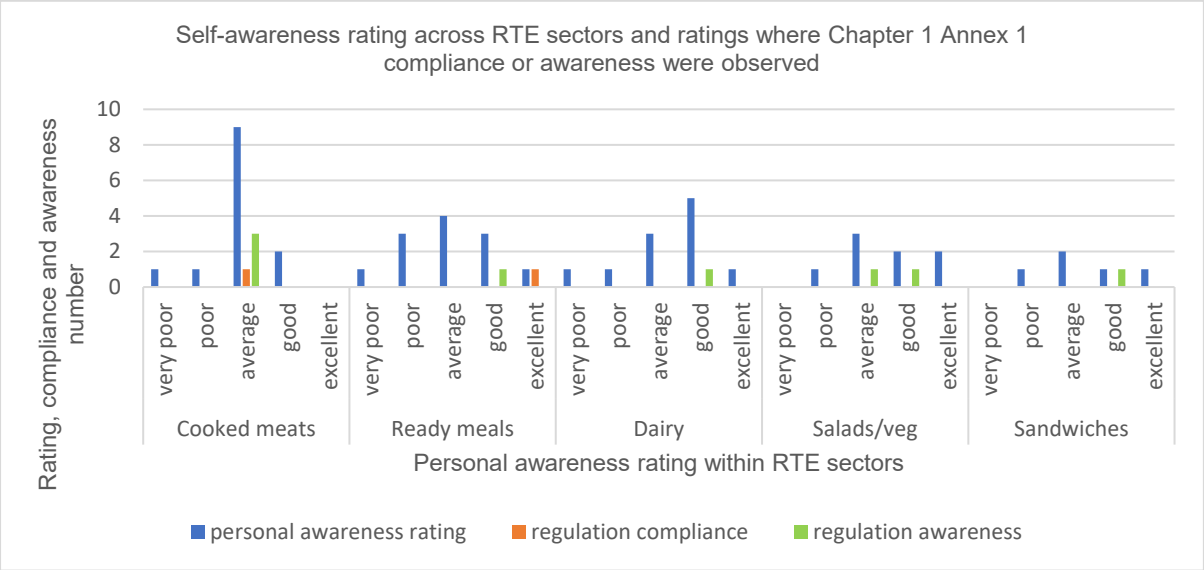


Across the survey, non-awareness of Chapter 1 Annex 1's *Listeria monocytogenes* FSC was observed at much higher levels than was awareness. Less than 25% in each RTE sector demonstrated that they were aware of Chapter 1 Annex 1's FSC requirements. Statistically significant ($p<0.05$) non-awareness was observed across the entire survey. Individually in the sectors, non-awareness also demonstrated statistical significance ($p<0.05$).

5.1.9 Respondent's self-rating of their awareness of the regulation

Question 21 asked respondents to self-rate their level of awareness of the regulation's *Listeria monocytogenes* control requirements. Their responses are summarised in figure 5.15 along with which self-rating gave rise to correct responses regarding Chapter 1 Annex 1 compliance and awareness.

Figure 5.15 Chapter 1 annex 1 self-awareness ratings and ratings where compliance or awareness were observed



In each RTE sector with the exception of the dairy sector, most respondents rated themselves as average. None of the cooked meat sector rated themselves as excellently knowledgeable although 2 salads/veg respondents and 1 respondent from each of the other sectors rated themselves as such. Few respondents rated their awareness as very poor or poor and good was also a strong self-rating particularly in the dairy sector.

Of the 2 respondents who answered the 3 combined compliance questions correctly, only the one from the ready meals sector had self-rated their knowledge as excellent with the other correct respondent coming from the cooked meat sector with an average self-rating.

Regarding the correct responses to the summed awareness questions 22 to 25, 3 cooked meat respondents and 1 from salads/veg who had scored themselves average responded correctly and 1 respondent each from the ready meals, dairy, salads/veg and sandwiches sectors who had scored themselves good responded correctly as well.

5.2 Dependence of Chapter 1 Annex 1 compliance on Chapter 1 Annex 1 awareness

Respondents demonstrating correct compliance and awareness responses are summarised in table 5.3. In total, 2 respondents (1 from cooked meats and 1 from ready meals) answered all Chapter 1 Annex 1 compliance questions correctly whereas

8 answered all awareness questions correctly; 3 of these from cooked meats, 2 from salads/veg and 1 from each of the other sectors. Cooked meats and ready meals were the only sectors that had respondents who gave correct answers to both the compliance and the awareness questions. However, it was not the same respondent from these sectors that got the compliance and awareness questions correct in each case and across the survey, there was no statistical significance ($p=0.958$) that could be attached to the dependence of regulatory compliance on regulatory awareness.

Chapter 6

6.1 Discussion of results

Chapters 2 and 3 of this study established that *Listeria monocytogenes*' public health risk was influenced by effective control measures including those established within the regulation. Chapter 4's main purpose was to establish awareness or non-awareness of and compliance or non-compliance with the regulation within the Northern Irish RTE food industry and this discussion of results will focus on the survey's findings regarding regulation compliance and awareness or lack thereof.

Some interesting findings were observed regarding the Northern Irish RTE food industry and the regulation. Section 2.4 established previous listeriosis outbreaks originated from RTE foods including cooked/processed meats and fish, salads, fresh fruit and vegetables, dairy products including cheeses and sandwiches. This survey's responses (table 5.1) indicated that all of those food vehicles were manufactured in NI. Therefore, products made here could function as potential listeriosis vehicles if effective control measures were absent. Other reports have established a 'reasonably foreseeable contamination' risk attached to these RTE sectors and this study indicates that a similar potential exists in NI (EFSA, p.67, 2022). In fact, a previous study of 24 Northern Irish RTE food manufacturers demonstrated the pathogen's presence in both environmental samples and food samples alike including cooked meat samples at >100 cfu/g (Madden et al., 2018).

6.2 Chapter 1 Annex 1 FSC unawareness

Perhaps a key finding was that the NI RTE food industry demonstrated statistically significant ($p<0.05$) (figures 5.13 and 5.14, tables A7.1 and A7.5) unawareness of and non-compliance with the regulation's microbiological FSC for *Listeria monocytogenes*. Pérez-Lavalle, Carrasco and Valero (2020) previously highlighted that microbiological criterion-based food safety control measures were fundamental to food safety management. Regarding regulation unawareness (tables A7.6 to A7.10), this survey's findings indicated the potential for a lack of such control regarding *Listeria monocytogenes* contamination within RTE food production environments. Survey respondent's regulatory unawareness demonstrated here, indicated the potential for contamination events to happen, the significance of which and their role in listeriosis has already been discussed in sections 2.4 and 3.1. Interestingly, this survey's findings contradict those of a previous survey where 82% of respondents indicated awareness

of the regulation (Everis, 2021). However, that particular survey was based in England not NI and respondents were only asked if they were aware the legislation existed, not the details of its contents, making direct comparisons difficult.

Mirroring survey responses regarding FSC control requirements lack of awareness and despite the majority of survey responses coming from technical and quality managers (table 5.2), most respondents across all sectors self-assessed their knowledge as average (figure 5.15). So why could this have been the case? An anonymous survey, respondents were free to answer this question any way they liked without peer or senior staff pressure to choose higher awareness levels. Previous research suggests that respondents may have lacked the desire to cognitively form an opinion. Alternatively, they may have chosen the first available acceptable answer rather than try to choose a more correct one (Demars and Dary, 2005). However, such bias is unlikely to be the case here since the responses to the 4 awareness level questions indicated unawareness levels reflective of respondent's self-awareness ratings. Furthermore, other research suggests that whilst food business technical staff often have well-defined food safety procedures, *Listeria monocytogenes* on its own is not considered as a singular food safety hazard and they might therefore lack pathogen specific knowledge (Evans et al., 2021).

6.3 Chapter 1 Annex 1 FSC non-compliance

Although statistical significance ($p=0.958$) (tables A7.11 and A7.12) could not be attached to dependence between survey awareness and compliance, there was statistically significant ($p<0.05$) non-compliance with the mandated FSC (figure 5.13, tables A7.2 to A7.4).

It would reason that survey respondent's non-compliance with this part of the regulation could potentially result in undetected batches of contaminated food constituting a public health risk. Again, the food sectors identified within this survey and their potential to cause listeriosis from contaminated foods has previously been discussed in sections 2.4 and 3.1

Respondents indicated statistically significant ($p<0.05$) non-compliance regarding the FSC's mandated sample numbers for *Listeria monocytogenes* laboratory testing (figure 5.9, table A7.1). These mandated sample numbers provide the minimum representative sampling portion levels for *Listeria monocytogenes* detection ensuring

a food's acceptability (Food Standards Agency (FSA), 2022). However, whilst it might be the case that the sampling plan's main purpose is to allow maximum detection probability of contaminated foods, critics argue that adherence to the mandated test numbers does not always guarantee a total absence of contamination since even these numbers cannot be truly representative of a large batch of food with *Listeria monocytogenes* contamination randomly distributed throughout the lot (Ricci et al., 2018; Pérez-Lavalle, Carrasco and Valero Diaz, 2020).

The FSA (2022) indicates that sampling plan sample numbers may be reduced based on risk mitigation evidence and in agreement with local authorities. Some survey respondents indicated reduced sample numbers agreed with their local authority but the majority did not have this agreement. Since EFSA (2023) suggests sampling procedure efficacy depends on use of the correct sample size, this survey again indicated the potential for compromised food safety, further substantiated when considered that single sample sizes do not always guarantee satisfactory food safety within a particular batch of food (EC, 2005).

Non-compliance was also demonstrated regarding the FSC's sampling limits and application stage (figure 5.10). Respondents excelled when it came to identifying which category their particular RTE food belonged to. However, non-compliance arose because many respondents were applying incorrect criterion limits for products unable to support the growth of *Listeria monocytogenes*. Where products could support pathogen growth, many respondents identified the correct limit of not detected in 25g or in some cases used this limit jointly with 100 cfu/g. However, the main non-compliance here was because many manufacturers of foods able to support *Listeria monocytogenes'* growth were applying this criterion throughout the product's shelf life rather than before it left their control. Additionally, many respondents also indicated the use of not detected in 25g as the acceptable limit for products even where 100 cfu/g maximum could apply. Other countries such as the USA adopt this product recall for any positive test results approach. Critics argue that this does not automatically guarantee improved food safety and so there is the case here for potentially compromised food safety from respondent's lack of compliance with this particular criterion (Farber et al., 2021; CDC, 2024).

This survey reinforced previous suggestions that the criterion's specified sampling points have crucial public health significance because they capture food safety hazards at critical control points in the manufacturing chain (Benguerel, 2024). These survey results indicated the potential for compromised food safety since foods containing *Listeria monocytogenes* levels constituting a significant health risk could potentially be placed on the market rather than being identified as hazardous before they left a food business operator's control. Section 2.2.2 considered *Listeria monocytogenes* levels constituting an infective dose. Whereas >1000 cfu/g was established for infection in healthy individuals, it was also identified that some community-based outbreaks had involved numbers <100 cfu/g (Little et al., 2012). Furthermore table 2.7 also identified that hospital listeriosis outbreaks involving immunocompromised individuals had occurred from pathogen levels <100 cfu/g. Little et al. (2012) demonstrated that sandwiches with *Listeria monocytogenes* contamination levels >100 cfu/g had previously been identified from samples taken at retail level and this survey identifies that there is the possibility that that could be the case in NI. This was also evidenced in the final area of non-compliance where respondents demonstrated some confusion regarding what the limits actually were for satisfactory/unsatisfactory results. Previous epidemiological evidence suggested that limits of 100cfu/g ensured elevated levels of consumer safety (Goodburn, 2020). Most respondents indicated correctly that for absence/presence testing, all lab submitted samples should have *Listeria monocytogenes* not detected in all samples submitted. However, many respondents did not realise that the maximum permissible enumeration count of the pathogen was 100 cfu/g and indicated that they would consider counts of <100 cfu/g as unsatisfactory. Whilst these responses erred on the side of caution in terms of food safety, they upheld the general consensus of a lack of understanding amongst survey participants regarding the requirements of the regulation's FSC.

6.4 Compliance

The existing potential for *Listeria monocytogenes* contamination of RTE foods in NI might make the situation appear quite bleak. However, table 2.4 of this study observed that NI listeriosis represented the lowest numbers of gastrointestinal cases with the suggestion that this was because of the way gastrointestinal data was collected in NI. However, there is within this survey, some alternative evidence from a food industry

perspective to suggest other reasons why these infection levels could be so low despite the survey findings suggesting the potential for contamination of RTE foods.

Pérez-Lavalle, Carrasco and Valero (2020) previously questioned sampling's usefulness as a means of ensuring food safety proposing that a preventative approach minimising the risk of food contamination was more effective. This survey's results would support this suggestion indicating compliance within the Northern Irish RTE food industry of the regulation's contamination risk preventative measures. This is somewhat reassuring considering section 3.1 established that cross-contamination of the RTE food types within food sectors in NI are among the most common causes of major food safety issues and product recalls and more so since evidence suggests that *Listeria monocytogenes* can be continually reintroduced into high-risk RTE processing environments (Tompkin 2002; Lee et al., 2021).

6.4.1 HACCP

Regulation Article 3(1) requires RTE food manufacturers to adopt HACCP based procedures. The fact that 42 out of 49 survey respondents and all participating technical and quality managers were HACCP team members (table 5.2), would reinforce that businesses here were committed to this mandated contamination risk reduction strategy. Section 2.4 of this study identified those foods previously responsible for listeriosis outbreaks in NI included sandwiches and cooked meats. This survey (figure 5.3) identified 100% HACCP team membership in the sandwiches sector and similarly strong representation in the cooked meats and other high-risk RTE sectors such as ready meals and dairy products. The likelihood of this elevated level of HACCP team membership positively influencing reduced *Listeria monocytogenes* contamination of RTE products is strong and is supported by other research identifying that HACCP's preventative approach to contamination reduction ensures food safety (Awuchi, 2023). Interestingly non-HACCP team membership was observed to a greater degree in the larger business sizes. This was possibly because in smaller businesses, technical and food safety staff often have to multi-skill and fill a number of distinct roles whereas larger businesses have greater personnel resources and staff here may have had a more dedicated role with other technical staff filling the HACCP team positions.

6.4.2 Cleaning and disinfection and EMS

Section 3.4 identified an Article 5(2) HACCP requirement namely the use of EMS verified cleaning and disinfection as a *Listeria monocytogenes* contamination preventative measure particularly for niche harbourage areas. RTE food processing environments where *Listeria monocytogenes* can pose a public health risk should monitor their environments for the pathogen (FSAI, 2011b). Tompkin (2002) suggested that effective cleaning and disinfection prevented RTE product contamination/recontamination and the results of this survey would indicate strong compliance with both of these contamination prevention strategies. Section 3.3 highlighted *Listeria monocytogenes* biofilm's role in RTE foods recontamination post-listericidal treatment. Reassuringly, most RTE sector respondents identified the use of specific *Listeria monocytogenes* biofilm cleaning and disinfection procedures (figure 5.6). However, several respondents particularly from the cooked meats, ready meals and sandwiches sectors did not. Carpentier and Cerf (2011) had previously identified the ability of persistent *Listeria monocytogenes* to recolonise RTE food processing equipment. Goodburn (2020) had also previously demonstrated effective sanitisation's importance regarding *Listeria monocytogenes* environmental control. Bearing these previous research findings in mind, this survey indicates the opportunity for some improvement in these specific sectors particularly since their foods have previously been responsible for some of the outbreaks outlined in chapter 3.

However, the majority of these respondents were also complying with Article 9's requirements for trending results (figure 5.8). This area of survey compliance was reassuring since mitigation of RTE foods re/cross-contamination potential as previously discussed in chapter 3 is essential. These findings regarding RTE business' cleaning and disinfection adoption in NI as a *Listeria monocytogenes* contamination prevention method is supported by other evidence. This also suggests that effective sanitisation targets those production areas and equipment constituting the root cause of potential listeriosis outbreaks as discussed previously in section 2.4 (Crandall et al., 2024). The findings also reflect that of other surveys where 88% of respondents also relied on compliance with this statutory requirement as an effective means of contamination prevention (Everis, 2021).

Some respondents were unsure if their cleaning and disinfection programmes specifically targeted *Listeria monocytogenes* biofilms (figure 5.6). Mazaheri et al.

(2022) suggest that whilst all food manufacturing environments are sanitised using different *Listeria monocytogenes* preventative measures, no specific standard protocol exists for biofilm removal and it is possible that respondents were effectively cleaning and disinfecting their food processing environments not realising this procedure was also removing biofilms. Furthermore, respondents may have simply been unaware such hazards existed since food industry hygiene training is often generic rather than specific regarding hazards such as biofilms (Chartered Institute of Environmental Health, 2024). Most respondents who were not environmentally monitoring their cleaning and disinfection programme's efficacy or were unsure, came from the cooked meats, ready meals and sandwiches sectors (figure 5.7). These product's potential, if contaminated, to cause listeriosis indicates scope for improvement in this area in these sectors. There is no evidence available to suggest why these 3 sectors lack specific biofilm targeting sanitisation procedures backed up by an EMS. Many of these sector's products receive a heat treatment similar to that outlined in table 3.1 of this study and speculatively, producers might consider that these products do not therefore require the same level of contamination prevention control as some other products. However, the survey results indicate the potential for some work in this area since research suggests that an effective EMS can indicate the pathogen's presence which in turn decreases the risk of final product contamination (FSAI, 2024). And furthermore, previous research demonstrating successful Listeria *monocytogenes* contamination reduction by an EMS in RTE salads/veg products offers further evidence suggesting its beneficial inclusion (Strydom et al., 2016).

6.4.3 Product recall

One of the survey's strongest compliance areas was that of Article 7(2) product recall (figure 5.12). When unsatisfactory, contaminated RTE batches should be withdrawn from the market or recalled in accordance with Regulation (EC) 178/2002 (Gov.UK, 2002). The intention to recall contaminated batches predominated in all but one sector namely the salads/veg sector. Table 2.7 of this study linked sandwiches with NI listeriosis outbreaks and 100% of survey sandwich manufacturers indicated that they would recall *Listeria monocytogenes* contaminated batches. This was reassuring in terms of food safety. However, again whilst the other sectors were mostly compliant, the cooked meats, ready meals and dairy sectors all indicated that there was a degree of product recall non-compliance. Evidence indicates that of the UK's 10 food recalls

in 2023, some involved contamination with *Listeria monocytogenes* (Bone and Anderson, 2023). Product recalls have many disadvantages including food safety implications, financial impacts, brand reputation, consumer trust and enforcement action. Waiting until product recall stage to demonstrate regulatory compliance or more worryingly non-compliance, would seem largely irresponsible for some producers especially since this survey's findings suggest that the compliance areas demonstrated by respondents are capable of achieving elevated levels of consumer protection (Goodburn, 2023). Also, it has been suggested elsewhere that understanding regulatory requirements can help ensure a more proactive food safety approach and diminish the need for excessive product recall (Trustwell, 2023). Since this study has highlighted the existence of unawareness of and non-compliance with these requirements in places, it would seem logical that increasing understanding and compliance particularly in those sectors where it needs strengthened, would facilitate improved *Listeria monocytogenes* food safety throughout NI and reduce the need for product recalls.

6.4.4 FSMS

Perhaps the strongest compliance in the survey was the number of respondents reporting an implemented FSMS (figures 5.4 and 5.5). It is possible that this is the Northern Irish RTE industry's biggest "saving grace" regarding the control of *Listeria monocytogenes* risks. This compliance area is very reassuring in terms of *Listeria monocytogenes* food safety, particularly so since other researchers have demonstrated that a FSMS in a food business was one of the strongest compliance predictive tools (Liggans et al., 2019). Interestingly, the high degree of implemented FSMS in NI reflects that of other countries like Serbia with a >90% uptake of an implemented FSMS (Tomašević et al., 2013).

So why should this be the case? Why such strong FSMS uptake? Regardless of business size, many NI RTE manufacturers produce products for major retailers who require producers to have an implemented FSMS (Tesco, 2014). Section 3.4 of this study identified that an implemented FSMS supported food safety and public health by having pathogen controls built in. Khalid (2024) has previously highlighted the ability of a flexible FSMS to adapt to dynamic food safety risks and within this survey, respondents indicated that their choice of FSMS included those from BRCGS, HACCP, Salsa, GFSI and others. Dependant on choice, some of these FSMS have

specific *Listeria monocytogenes* controls embedded within them (BRC, 2023). But regardless of choice, they all have requirements for manufacturers grounded in food safety legislation including the regulation's requirements for HACCP, effective cleaning and disinfection, environmental monitoring and trending of results. Therefore, it is possible that whilst some survey respondents were unaware of and did not know that they were complying with the regulation's specific *Listeria monocytogenes* controls, they actually were through satisfying the requirements of their implemented FSMS. Furthermore, this may be reinforced by the most recent industry information available at the time of writing suggesting that whilst 2024 UK recalls of *Listeria monocytogenes* contaminated foods continued, none of these involved products made by Northern Irish manufacturers (BRC, 2024). The ongoing monitoring of high-risk areas within RTE food manufacturing environments for preventative *Listeria monocytogenes* control and validation required by FSMS with appropriate corrective measures, would minimise opportunities for pathogen presence/growth and subsequent prevention of re/cross-contamination of high-risk foods that have received a listericidal treatment. Whilst Khalid (2024) also argues that FSMS implementation is not without challenges including those of aligning with existing legislation, it would appear from the results of this survey that those businesses with an implemented FSMS have done so and achieved effective support of key legislative requirements for *Listeria monocytogenes* contamination prevention rather than a juxtaposed position of legislative non-compliance.

6.5 Limitations

Although 49 survey responses were received, it is possible that more responses may have allowed even more accurate interpretation of results particularly for those high-risk production sectors in NI including cooked meats, sandwiches and ready meals. Furthermore, the recent Covid-19 pandemic would appear to have potentially skewed epidemiological data collection. It might take some time yet for accuracy in this area to be re-established.

6.6 Recommendations

This study highlighted that *Listeria monocytogenes* continues to be a microorganism of public health concern. The survey identified statistically significant non-compliance with and unawareness of the regulation's Chapter 1 Annex 1 food safety criteria even though respondents demonstrated compliance with other areas of the regulation

critical for preventative control of the microorganism within RTE food processing environments. Considering the lack of *Listeria monocytogenes* specific training available to production staff, it would be the recommendation of this study that there is scope for a form of *Listeria monocytogenes* specific training/education or instructional information to be created that would allow better familiarisation of current food industry staff with the regulation and that could also be adapted in consideration with any future regulation updates or amendments.

Chapter 7

7.1 Conclusions

Through fulfilment of the objectives of critically evaluating *Listeria monocytogenes*' current literature, its control through cleaning and disinfection and assessment of *Listeria monocytogenes* regulatory control within Northern Ireland, this study has achieved its aim of critically evaluating *Listeria monocytogenes* as a microorganism of public health concern. Since its first epidemiological links with foodborne disease in 1981, *Listeria monocytogenes* continues to be recognised as a significant threat to public health. Rare in comparison to some other foodborne illnesses, listeriosis' wide ranging symptoms can, in invasive form, result in high hospitalisation and mortality rates from complex symptoms including miscarriage, septicaemia, brain abscesses and meningitis particularly in vulnerable groups within the population. The UK and NI benefit from lower listeriosis levels than elsewhere globally and particularly in Europe. Whilst this continues to be the case, the transmission of *Listeria monocytogenes* via contaminated RTE foods presents a problem for both manufacturers and public health professionals alike. Although successfully eradicated via traditional food-industry bactericidal processes, the organism's ability to gain entry to and linger in environments which are often hostile to other bacteria, can lead to post-process contamination of high-risk RTE foods. Effective cleaning and disinfection of these RTE food premises continues to be a crucial preventative measure in the spread of listeriosis and avoidance of product recall scenarios. Enshrined in legislation, the RTE food industry in Northern Ireland exhibits compliance with and awareness of some areas of this legislation. The industry's legislative compliance is strengthened via the use of food safety management systems which increase public health by harmonising quality systems management in these RTE environments. However, although the legislation's food safety criteria allow manufacturers to assess a produced food's acceptability, relying on final product testing which could potentially constitute product recall scenarios would appear somewhat foolhardy and there is potential established within this study for information to be made available that could help manufacturers avoid these finalities.

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Appendices

Appendix 1 Pilot study questionnaire

Listeria monocytogenes questionnaire

This questionnaire is a pilot study for a real research questionnaire which will be issued to the RTE food sector in Northern Ireland regarding their understanding of and compliance with EC Regulation 2073/2005 - The Microbiological Criterion Regulation. The study will meet one of 3 objectives in a Masters Dissertation - *Listeria monocytogenes* as a microorganism of public health significance.

Section 1

1.Please indicate your job role/title
Quality Manager

Technical Manager

2.Are you a member of the HACCP team?
Yes

No

3.What size is your business?
≤10 employees

11 - ≤30 employees

31 - ≤50 employees

>51 employees

4.Have you an implemented Food Safety Management System (FSMS)?
Yes

No

Not sure

5.If you answered yes to question 4 please indicate which FSMS you operate. Choose all that apply. Otherwise please choose 'other' and enter not applicable (N/A).
BRCGS

SALSA

HACCP

6.Do your cleaning and disinfection programmes specifically target *Listeria monocytogenes* biofilms?
Yes

No

Not sure

7.Do you have an environmental monitoring system (EMS) specifically for *Listeria monocytogenes*?
Yes

No

Not sure

8.If you answered yes to question 7 do you trend results from your EMS? If you answered question 7 with any answer other than yes please select 'other' and enter N/A.
Yes

No

9.Please indicate which ready to eat (RTE) foods are manufactured in your facility. Choose all that apply.
Ready meals (ambient, chilled or frozen)

RTE bread based products for example sandwiches, wraps, paninis etc

Cooked pies, sausage rolls, quiches or other RTE savoury foods

Soups

Any cooked, smoked or cured Deli meats, fish, ham, chicken, pork or beef either sold as whole units or sliced

Cured continental style sausages, salamis, chorizo

Pâté

Cooked shellfish

Washed and cut fruit or vegetables or salads or crudités

Coleslaws or potato salads or dips or hummus

Dairy products such as pasteurised milk or yoghurt or cream or cottage or other soft cheese

Chilled desserts such as cheesecakes or custard based desserts or trifle or cakes with fresh or dairy cream

Fresh cheese

Sushi

10.Foods are unable to support the growth of *Listeria monocytogenes* if they fall into 1 or more of the following categories.

1. They have received heat treatment or other processing effective to eliminate *Listeria monocytogenes,* when recontamination is not possible after this treatment (for example, products heat treated in their final package).

2. They are products with pH ≤ 4,4 **or** water availability (aw) ≤ 0,92, **OR**
have pH ≤ 5,0 **and** aw ≤ 0,94, **OR**
have a shelf-life of less than five days.

3. You have other scientific justification to prove *Listeria monocytogenes* won't grow in your product.

Do any of your products fall into categories 1, 2 or 3?
Yes

No

Not sure

11.Choose one of your RTE foods and please

1. Give a brief name and description of the food (for example chicken and coleslaw sandwich)
2. Indicate the temperature of storage for the product shelf life
3. Indicate the product shelf life (for example Date of production (DOP) + 6 days)
4. Indicate the product pH if known (state not known if unknown)
5. Indicate the product's water availability (state not known if unknown)
6. Indicate if the product has received heat treatment or other processing effective to eliminate *Listeria monocytogenes,* when recontamination is not possible after this treatment (state yes if it has, no if it hasn't)

In the answer box please enter 1-6 followed by your answer.

Enter your answer

12.Regarding the RTE food chosen in question 11 please indicate if this is:
A food able to support the growth of *Listeria monocytogenes*

A food unable to support the growth of *Listeria monocytogenes*

Not sure

13.Regarding the RTE food chosen in question 11 please indicate how many samples from a batch you would submit for laboratory analysis for *Listeria monocytogenes*

1

5

10

14.Has this sample number been agreed with your local authority/ environmental health officer?

Yes

No

Not sure

15.Regarding the RTE food chosen in question 11 would your laboratory analysis request be for?

Listeria monocytogenes absence/presence in 25 grams of sample **OR**

Listeria monocytogenes enumeration in colony forming units per gram (cfu/g) **OR**

BOTH *Listeria monocytogenes* absence/presence in 25 grams of sample **AND** *Listeria monocytogenes* enumeration in colony forming units per gram (cfu/g)

Not sure

16.Regarding the RTE food chosen in question 11 and considering the results you receive from the laboratory for *Listeria monocytogenes* analysis, would you apply these results

To the product placed on the market during its shelf-life **OR**

To the product before it has left your immediate control

Not sure

17.Regarding the RTE food chosen in question 11 what would you consider to be an **UNSATISFACTORY** result for *Listeria monocytogenes*?

ONLY if *Listeria monocytogenes* was absent/not detected in 25 grams of product for all samples sent to the lab
ONLY if *Listeria monocytogenes* was present/detected in 25 grams of product for all samples sent to the lab

ONLY if *Listeria monocytogenes* was present/detected in 25 grams of product for all samples sent to the lab but at levels of 20 - ≤100 cfu/g

ONLY if *Listeria monocytogene*s was present/detected in 25 grams of product for all samples sent to the lab but at levels of >100 cfu/g

Not sure

18.Regarding the RTE food chosen in question 11 and for *Listeria monocytogenes* results you consider unsatisfactory; would you recall the product?
Yes

No

Not sure

19.Using the scale below how would you rate your awareness level of the specific requirements of EC Regulation (the Microbiological Criterion Regulations) regarding control of *Listeria monocytogenes*.

	Very poor	Poor	Average	Good	Excellent
Statement 1					

20.Are you aware that EC Regulation 2073/2005 subdivides RTE foods other than those for infants and special medical purposes into 2 different categories?

Yes

No

Not sure

21.Are you aware that EC Regulation 2073/2005 states that for RTE foods **unable to support the growth** of *Listeria monocytogenes* (other than those for infants and special medical purposes) manufacturers should analyse 5 samples from a production batch and none of the 5 should exceed a limit of 100 cfu/g *Listeria monocytogenes* for products placed on the market during their shelf life?
Yes

No

Not sure

22.Are you aware that for RTE foods **able to support the growth** of *Listeria monocytogenes* (other than those for infants and special medical purposes) EC Regulation 2073/2005 offers the following 2 options to manufacturers?

OPTION 1 if a manufacturer can analyse 5 samples from a production batch and demonstrate that none of the 5 samples exceed a limit of 100 cfu/g *Listeria monocytogenes* then he can apply this limit to the product placed on the market during shelf life

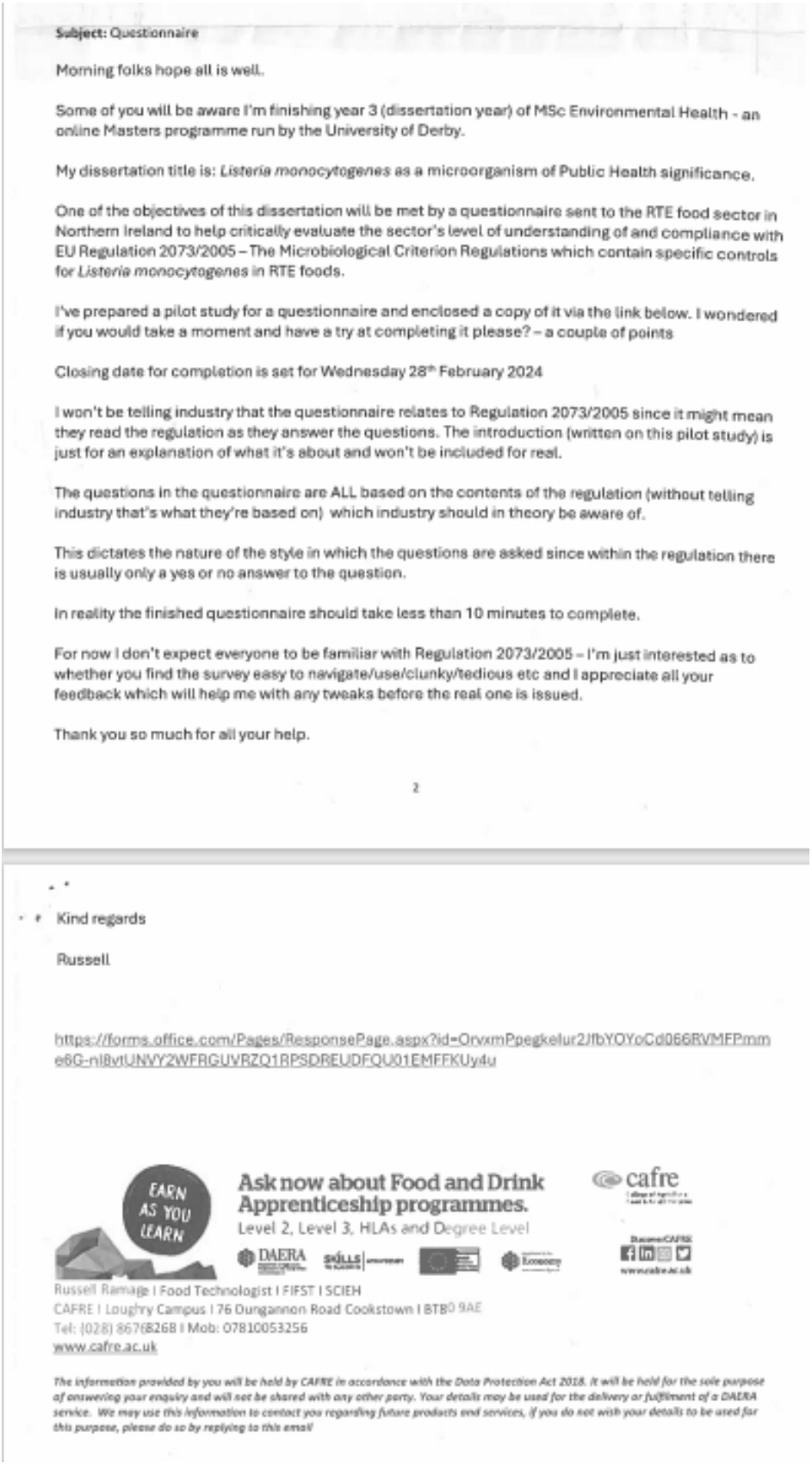
OPTION 2 if the manufacturer lacks the scientific evidence for option 1 then he must demonstrate that before the product has left his immediate control he has analysed 5 x 25 gram samples of the product from the production batch and *Listeria monocytogenes* is absent/not detected in **ALL** 5 x 25 gram samples

Yes

No

Not sure

Appendix 2 Pilot study release



Subject: Questionnaire

Morning folks hope all is well.

Some of you will be aware I'm finishing year 3 (dissertation year) of MSc Environmental Health - an online Masters programme run by the University of Derby.

My dissertation title is: *Listeria monocytogenes* as a microorganism of Public Health significance.

One of the objectives of this dissertation will be met by a questionnaire sent to the RTE food sector in Northern Ireland to help critically evaluate the sector's level of understanding of and compliance with EU Regulation 2073/2005 – The Microbiological Criterion Regulations which contain specific controls for *Listeria monocytogenes* in RTE foods.

I've prepared a pilot study for a questionnaire and enclosed a copy of it via the link below. I wondered if you would take a moment and have a try at completing it please? – a couple of points

Closing date for completion is set for Wednesday 28th February 2024

I won't be telling industry that the questionnaire relates to Regulation 2073/2005 since it might mean they read the regulation as they answer the questions. The introduction (written on this pilot study) is just for an explanation of what it's about and won't be included for real.

The questions in the questionnaire are ALL based on the contents of the regulation (without telling industry that's what they're based on) which industry should in theory be aware of.

This dictates the nature of the style in which the questions are asked since within the regulation there is usually only a yes or no answer to the question.

In reality the finished questionnaire should take less than 10 minutes to complete.

For now I don't expect everyone to be familiar with Regulation 2073/2005 – I'm just interested as to whether you find the survey easy to navigate/use/clunky/tedious etc and I appreciate all your feedback which will help me with any tweaks before the real one is issued.

Thank you so much for all your help.

2

Kind regards

Russell

https://forms.office.com/Pages/ResponsePage.aspx?id=OrvxmPpegkelur2JfbYOYoCd066RVMFPmme6G-nI8vtUNVY2WFRGUVRZQ1RPSDREUDFQU01EMFFKUy4u

Russell Ramage I Food Technologist I FIFST I SCIEH

CAFRE I Loughry Campus I 76 Dungannon Road Cookstown I BT80 9AE

Tel: (028) 86768268 I Mob: 07810053256

www.cafre.ac.uk

Appendix 3 Pilot study feedback

From: Henry, Kevin (DAERA)
Sent: Tuesday, March 5, 2024 4:18 PM
To: Ramage, Russell
Subject: RE: Questionnaire

Hello Russell- looks very good and sequenced well with a story to it.
Just a few suggestions – feel free to accept in part or ignore.
Poss 1a – any pt in ask sector e.g. meat/ dairy/ poultry/ veg/ bakery etc
Q5 – just add NA as a box
Q8 – add an NA box – will save the bypassing- may have to adjust wording if do so
Q9 Danger- Tick All will be problem – all responses will fall into ONE column of Excel responses file- & & you will have sig. prob separating them out – consider make each option a Yes/ No – nice and clean
Q11 – a lot to remember in 1-6- any poss of making it (a) to (f) and having a text box at end of each line?
Q23 Consider finish on an open Question e.g. Have you any other comment to make re food businesses & Listeria ?- may give depth and a few gems of quotes
Best wishes
Kevin

From: O'Neill, Hayley
Sent: Monday, February 26, 2024 2:56 PM
To: Ramage, Russell
Subject: RE: questionnaire draft

- I answered as many in other sections to see if you can see them ok let me know if you got the other answers reported?
- You need a branching Q for Q5 that only goes there if yes from Q4
- Q5 all FSMS will be haccp based (do answers need to be just BRC or SALSA or retailer approved?)
- You need a branching Q for Q8 that only goes there if yes from Q7
- Q8 – don't think you need 'other' as answer option here. Is is just Yes or No?
- Q10– don't think you need 'other' as answer option here. Is is just Yes or No or not sure
- Q12– don't think you need 'other' as answer option here. Is is just Yes or No or not sure
- Q14– don't think you need 'other' as answer option here. Is is just Yes or No or not sure
- Q18– don't think you need 'other' as answer option here. Is is just Yes or No or not sure
- Q19 – theres a way of changing statement 1 to awareness level
- Q20– don't think you need 'other' as answer option here. Is is just Yes or No or not sure
- Q21– don't think you need 'other' as answer option here. Is is just Yes or No or not sure. Also still telling them the answer to above Q's. Maybe we could brainstorm this out again!
- Q22 – change 'he' to 'they'
- Q22– don't think you need 'other' as answer option here. Is is just Yes or No or not sure

Appendix 4 CAFRE Head of Branch permission to access and use food client database

From: Simpson, Peter (DAERA Food & Farming)
Sent: Tuesday, March 12, 2024 8:31 AM
To: Ramage, Russell
Subject: Use of Food Client Database

Good Morning Russell

I am content that you use our client database for the purposes of circulating your dissertation survey.

You have made it clear to clients that their responses will be anonymous.

Your topic is highly relevant, and I look forward to your findings

Peter
Peter Simpson FIFST | **Head of Food Technology Branch** | CAFRE, College of Agriculture, Food and Rural Enterprise| Department of Agriculture, Environment and Rural Affairs | Loughry Campus| email: | **Tel:** | **Mobile:** |

Appendix 5 Redesigned final questionnaire

Listeria monocytogenes questionnaire

Thank you so much for participating in this questionnaire regarding understanding of and compliance with the control of *Listeria monocytogenes* in the Northern Irish Ready to Eat food sector. The study will meet one of 3 objectives in a Masters Dissertation - *Listeria monocytogenes* as a microorganism of Public Health significance. The questionnaire should take no longer than 10 minutes to complete.

Section 1

1.Please indicate your consent to participate in this questionnaire by selecting the consent option below.

I consent to participate in this study and understand that all my responses are anonymous and will be treated with the strictest confidence

2.Please indicate your job role/title.
Quality Manager

Technical Manager

3.Could you please indicate which Ready to Eat (RTE) food sector your business is positioned in? For example meat, poultry, dairy, dairy etc.
Enter your answer

4.Are you a member of the HACCP team?
Yes

No

5.What size is your business?
≤10 employees

11 - ≤30 employees

31 - ≤50 employees

>51 employees

6.Have you an implemented Food Safety Management System (FSMS)?
Yes

No

Not applicable

Not sure

7.If you answered yes to question 6 please indicate which FSMS you operate.

Enter your answer

8.Do your cleaning and disinfection programmes specifically target *Listeria monocytogenes* biofilms?
Yes

No

Not sure

9.Do you have an environmental monitoring system (EMS) specifically for *Listeria monocytogenes*?
Yes

No

Not sure

Not applicable

10.If you answered yes to question 9 please indicate if you trend the results from your *Listeria monocytogenes* EMS.
Yes

No

Not sure

Not applicable

11.From the list below please indicate which option **BEST** categorises the RTE products manufactured in your facility.
Ready meals (ambient, chilled or frozen)

RTE bread-based products for example sandwiches, wraps, paninis etc

Cooked pies, sausage rolls, quiches or other RTE savoury foods

Soups

Any cooked, smoked or cured Deli meats, fish, ham, chicken, pork beef either sold as whole units or sliced

Cured continental style sausages, salamis, chorizo

Pâté

Cooked shellfish

Washed and cut fruit or vegetables or salads or crudités

Coleslaws or potato salads or dips or hummus

Dairy products such as pasteurised milk or yoghurt or cream or cottage or other soft cheese

Chilled desserts such as cheesecakes or custard-based desserts or trifle or cakes with fresh or dairy cream

Fresh cheese

Sushi

12.The following RTE foods are **unable** to support the growth of *Listeria monocytogenes.* Please indicate which (if any) category is applicable to your products.
RTE foods that have received heat treatment or other processing effective to eliminate *Listeria monocytogenes* when recontamination is not possible after this treatment (for example, products heat treated in their final package).

RTE foods with pH ≤ 4,4

RTE foods with water availability (aw) ≤ 0,92

RTE foods with pH ≤ 5,0 AND aw ≤ 0,94

RTE foods with a shelf-life of less than five days.

RTE foods where you have other scientific justification to prove *Listeria monocytogenes* won't grow in your product.

Not applicable

13.Choose one of your RTE foods and please
1. Give a brief name and description of the food (for example chicken and coleslaw sandwich)
2. Indicate the temperature of storage for the product shelf life
3. Indicate the product shelf life
4. Indicate the product pH if known (state not known if unknown)
5. Indicate the product's water availability if known (state not known if unknown)
6. Indicate if the product has received heat treatment or other processing effective to eliminate *Listeria monocytogenes when* recontamination is not possible after this treatment (state yes if it has, no if it hasn't) Required to answer. Multi Line Text.
Enter your answer

14.Regarding the RTE food chosen in question 13 please indicate if this is:
A food able to support the growth of *Listeria monocytogenes*

A food unable to support the growth of *Listeria monocytogenes*

Not sure

15.Regarding the RTE food chosen in question 13 please indicate how many samples from a batch/lot you would submit for laboratory analysis for *Listeria monocytogenes*

1

5

10

16.Has this sample number been agreed with your local authority/ environmental health officer?
Yes

No

Not sure

17.Regarding the RTE food chosen in question 13 would your laboratory analysis request be for?
Listeria monocytogenes absence/presence in 25 grams of sample **OR**

Listeria monocytogenes enumeration in colony forming units per gram (cfu/g) **OR**

BOTH *Listeria monocytogenes* absence/presence in 25 grams of sample **AND** *Listeria monocytogenes* enumeration in cfu/g

Not sure

18.Regarding the RTE food chosen in question 13 and considering the results you receive from the laboratory for *Listeria monocytogenes* analysis, would you apply these results:
To the product placed on the market during its shelf-life **OR**

To the product before it has left your immediate control

Not sure

19.Regarding the RTE food chosen in question 13 what would you consider to be an **UNSATISFACTORY** result for *Listeria monocytogenes*?
ONLY if *Listeria monocytogenes* was absent/not detected in 25 grams of product for all samples sent to the lab

ONLY if *Listeria monocytogenes* was present/detected in 25 grams of product for all samples sent to the lab

ONLY if *Listeria monocytogenes* was present/detected in 25 grams of product for all samples sent to the lab but at levels of 20 - ≤100 cfu/g

ONLY if *Listeria monocytogene*s was present/detected in 25 grams of product for all samples sent to the lab but at levels of >100 cfu/g

Not sure

20.Regarding the RTE food chosen in question 13 and for *Listeria monocytogenes* results you consider unsatisfactory, would you recall the product?
Yes

No

Not sure

21.Using the scale below how would you rate your awareness level of the specific requirements of EC Regulation 2073/2005 (the Microbiological Criterion Regulations) regarding control of *Listeria monocytogenes* in RTE foods?

	Very poor	Poor	Average	Good	Excellent
Statement 1					

22.Are you aware that EC Regulation 2073/2005 subdivides RTE foods other than those for infants and special medical purposes into 2 different categories?
Yes

No

Not sure

23.Are you aware that EC Regulation 2073/2005 states that for RTE foods **unable to support the growth** of *Listeria monocytogenes* (other than those for infants and special medical purposes) manufacturers should analyse 5 samples from a production batch/lot and none of the 5 should exceed a limit of 100 cfu/g *Listeria monocytogenes* for products placed on the market during their shelf life?
Yes

No

Not sure

24.Please read the following statement for RTE foods **able to support** the growth of *Listeria monocytogenes* (other than those for infants and special medical purposes) and indicate if it is true or false.

Statement

If a manufacturer can analyse 5 samples from a production batch/lot and demonstrate that none of the 5 exceed a limit of 100 cfu/g *Listeria monocytogenes* then he can apply this limit of maximum 100cfu/g *Listeria monocytogenes* to the product placed on the market during its shelf life.
True

False

Not sure

25.Please read the following statement for RTE foods **able to support** the growth of *Listeria monocytogenes* (other than those for infants and special medical purposes) and indicate if it is true or false.

Statement

If the manufacturer lacks the scientific evidence presented in the statement in question 24, then he must demonstrate that before the product leaves his immediate control he has analysed 5 x 25g samples from the production batch/lot and *Listeria monocytogenes* is absent/not detected in **ALL** 5 x 25g samples.

True

False

Not sure

26.Are there any other comments you would like to add regarding control of *Listeria monocytogenes* within your RTE food business?

Enter your answer

ChoiceTextRatingDate

Appendix 6 Final questionnaire release to industry

Ramage, Russell Russell.Ramage@daera-ni.gov.uk

Hi everyone. Thank you so much for taking time to read this short communication.

I'm Russell Ramage, a Food Technologist within CAFRE Loughry's Food Science and Innovation group. I'm also a final year student on the University of Derby's MSc Environmental Health programme. For my final year dissertation '*Listeria monocytogenes* as a microorganism of Public Health significance' I'm conducting research regarding the level of knowledge/understanding around this microorganism within the Ready-To-Eat food industry in Northern Ireland. Some producers may have a little knowledge, others may have more extensive knowledge. Both are absolutely fine and I am interested in responses from ALL ranges of understanding and experience.

To facilitate the research, I have prepared a short questionnaire and would be very grateful if you would take a few moments to complete one please. You will find the link to the questionnaire below. It should take around 10-20 minutes to complete and ALL responses are completely anonymous. The questionnaire and its use have been approved by the University's ethics board and as such, once the dissertation is complete, all collected information will be destroyed in line with this ethics policy.

Closing date for responses is Friday June 7th.

Thank you so much in advance for your help.

Kind regards

Russell Ramage

https://forms.office.com/Pages/ResponsePage.aspx?id=OrvxmPpegkelur2JfbYOYoCd066RVMFPmme6G-nI8vtUQUhaWjQ5Vk1RUlp

Appendix 7 Statistics

Table A7.1 Chi square test for overall survey compliance/non-compliance

Number	Compliance	Observed frequency (O)	Expected frequency (E)	(O-E)	$(O-E)^2$	$(O-E)^2/E$
2	Compliance	2	24.5	-22.5	506.25	20.66327
1	Non-compliance	47	24.5	22.5	506.25	20.66327
	Total (N)	49	49		X	41.32653
	No levels (k)	2				

p value	1.28809E-10
Chi square	41.32653034

Table A7.2 Chi square test for cooked meat sector compliance/non-compliance

Number	Compliance	Observed frequency (O)	Expected frequency (E)	(O-E)	$(O-E)^2$	$(O-E)^2/E$
2	Compliance	1	24.5	-23.5	552.25	22.54082
1	Non-compliance	48	24.5	23.5	552.25	22.54082
	Total (N)	49	49		X	45.08163
	No levels (k)	2				

p value	1.8899E-11
Chi square	45.08163003

Table A7.3 Chi square test for ready meal sector compliance/non-compliance

Number	Compliance	Observed frequency (O)	Expected frequency (E)	(O-E)	$(O-E)^2$	$(O-E)^2/E$
2	Compliance	1	24.5	-23.5	552.25	22.54082
1	Non-compliance	48	24.5	23.5	552.25	22.54082
	Total (N)	49	49		X	45.08163
	No levels (k)	2				

p value	1.8899E-11
Chi square	45.08163003

Table A7.4 Chi square test for dairy, salads/veg and sandwich sector compliance/non-compliance

Number	Compliance	Observed frequency (O)	Expected frequency (E)	(O-E)	$(O-E)^2$	$(O-E)^2/E$
2	Compliance	0	24.5	-24.5	600.25	24.5
1	Non-compliance	49	24.5	24.5	600.25	24.5
	Total (N)	49	49		X	49
	No levels (k)	2				

p value	2.55963E-12
Chi square	49.00000452

Table A7.5 Chi square test for overall survey awareness/non-awareness

Number	Awareness	Observed frequency (O)	Expected frequency (E)	(O-E)	$(O-E)^2$	$(O-E)^2/E$
2	Aware	8	24.5	-16.5	272.25	11.11224
1	Not aware	41	24.5	16.5	272.25	11.11224
	Total (N)	49	49		X	22.22449
	No levels (k)	2				

p value	2.4256E-06
Chi square	22.2244898

Table A7.6 Chi square test for cooked meat sector awareness/non-awareness

No	Awareness	Observed frequency (O)	Expected frequency (E)	(O-E)	$(O-E)^2$	$(O-E)^2/E$
2	Aware	3	24.5	-21.5	462.25	18.86735
1	Not aware	46	24.5	21.5	462.25	18.86735
	Total (N)	49	49		X	37.73469
	No levels (k)	2				

p value	8.10502E-10
Chi square	37.73469404

Table A7.7 Chi square test for ready meals sector awareness/non-awareness

No	Awareness	Observed frequency (O)	Expected frequency (E)	(O-E)	$(O-E)^2$	$(O-E)^2/E$
2	Aware	1	24.5	-23.5	552.25	22.54082
1	Not aware	48	24.5	23.5	552.25	22.54082
	Total (N)	49	49		X	45.08163
	No levels (k)	2				

p value	1.8899E-11
Chi square	45.08163003

Table A7.8 Chi square test for dairy sector awareness/non-awareness

No	Awareness	Observed frequency (O)	Expected frequency (E)	(O-E)	$(O-E)^2$	$(O-E)^2/E$
2	Aware	1	24.5	-23.5	552.25	22.54082
1	Not aware	48	24.5	23.5	552.25	22.54082
	Total (N)	49	49		X	45.08163
	No levels (k)	2				

p value	1.8899E-11
Chi square	45.08163003

Table A7.9 Chi square test for salads/veg sector awareness/non-awareness

Number	Compliance	Observed frequency (O)	Expected frequency (E)	(O-E)	$(O-E)^2$	$(O-E)^2/E$
2	Aware	2	24.5	-22.5	506.25	20.66327
1	Not aware	47	24.5	22.5	506.25	20.66327
	Total (N)	49	49		X	41.32653
	No levels (k)	2				

p value	1.28809E-10
Chi square	41.32653034

Table A7.10 Chi square test for sandwich sector awareness/non-awareness

No	Awareness	Observed frequency (O)	Expected frequency (E)	(O-E)	$(O-E)^2$	$(O-E)^2/E$
2	Aware	1	24.5	-23.5	552.25	22.54082
1	Not aware	48	24.5	23.5	552.25	22.54082
	Total (N)	49	49		X	45.08163
	No levels (k)	2				

p value	1.8899E-11
Chi square	45.08163003

Chi square test for awareness/compliance dependence/independence

Comparing Awareness and Compliance

Data: AwareComp [1:2, 1:5]

x-square = 0.64283

df = 4

p value = 0.9582

Now that we have made the claims that there is significant unawareness and non-compliance in the industry of listeria legislation, we want to strengthen these claims. We do this by comparing awareness and compliance. If our second claim is true, we should see that awareness and compliance are independent, if they are not, we would need to rethink our conclusions about compliance.

Table A7.11 Chi square test with Yate's Correction

	0	1	2	3	4	total
Aware	2	2	2	2	0	**8**
Unaware	9	9	13	8	2	**41**
total	**11**	**11**	**15**	**10**	**2**	**49**

What we want to do is perform a test for independence. As we have counts less than 5 and it is not a 2 × 2 table we will use Chi-square test with Yate's correction the same way we have done before. When we did this, we got a p−value of 0.958 which means two classifications are independent. Due to how small our awareness category is there is a possibility that if we adjust our data to make it a simple 2 × 2 table with a compliant and non-compliant category we may see a different result. We combine 0 − 2 to be a category called non-compliant and 3 − 4 to be a category called compliant. We then perform the Fisher's exact test to test for dependence/independence as this is a 2 × 2 table and we have counts under 5. Performing this test does not produce any interesting results and it seems that there is no connection between awareness and compliance. The only test which could potentially point to what this influence is would be a test to see if perceived awareness and compliance are independent. This test could indicate what to look for after collecting more data but due to the concerns discussed previously there is always a possibility that there are no conclusions we can draw from this data especially if they are independent.

Table A7.12 The proportion of each perceived awareness group which was aware and unaware

Level of compliance across perceived awareness (%)

	0	1	2	3	4	total (%)	total
Very poor	33.33	33.33	0.00	33.33	0.00	100	3
Poor	28.57	14.29	42.86	14.29	0.00	100	7
Average	23.81	23.81	23.81	23.81	4.76	100	21
Good	15.38	23.08	38.46	23.08	0.00	100	13
Excellent	20.00	20.00	40.00	0.00	20.00	100	5
total	**22.45**	**22.45**	**30.61**	**20.41**	**4.08**	100	49