Food Safety Authority of Ireland

Microbiology

MONITORING & SURVEILLANCE SERIES

Survey of the Microbiological Safety of Ready-To-Eat Fresh Produce (16ns5)

November 2019

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Abbreviations

BIOHAZ	Biological Hazards
CDC	(USA) Centers for Disease Control and Prevention
CFU	Colony Forming Unit(s)
cm	Centimetres
DAFM	(Irish) Department of Agriculture, Food and the Marine
EC	European Commission
EFSA	European Food Safety Authority
EHO(s)	Environmental Health Officer(s)
EN/ISO	European Standard (German: Europäische Norm) / International Organisation for Standardisation
et al.	and others (Latin: *et alii*)
EU	European Union
FBO	Food Business Operator
FoAO	Foods of Animal Origin
FoNAO	Foods of Non-Animal Origin
FSAI	Food Safety Authority of Ireland
g	Gram(s)
HSE	(Irish) Health Service Executive
ICMSF	International Commission on Microbiological Specifications for Foods
IUNA	Irish Universities Nutrition Alliance
MPN	Most Probable Number
n	Number of sample units
RASFF	Rapid Alert System for Food and Feed
RTE	Ready-To-Eat
USA	United States of America
USDA	United States (of America) Department of Agriculture
VTEC	Verocytotoxigenic *Escherichia coli*

Summary

In recent years an increasing number of fresh fruits and vegetables have been linked to foodborne illness cases and outbreaks worldwide. The aim of this survey was to investigate the prevalence of *L. monocytogenes*, *Salmonella* spp. and *Escherichia coli* (an indicator of faecal contamination) in commonly consumed fruits and vegetables in the Republic of Ireland. A total of 838 samples of ready-to-eat (RTE) whole (uncut) unprocessed fruits and vegetables, consisting of tomatoes (n=248), peppers (n=138), cucumbers (n=103), blueberries (n=142), raspberries (n=87), strawberries (n=77) and blackberries (n=43) were sampled. These samples were collected during a four-month period in 2016 by the Health Service Executive (HSE) and the Department of Agriculture, Food and the Marine (DAFM). They were sampled from primary producers, packers, distributors, wholesalers and retail sectors in the Republic of Ireland. The microbiological analyses were performed by seven laboratories of the Health Service Executive accredited to ISO 17025. The results revealed that none of the samples tested had 'unsatisfactory' levels of any of the three microorganisms. This finding indicates that the surveyed categories of fresh produce produced and on sale in Ireland is of good microbiological quality and suggests that good hygiene practices are carried out along the Irish food supply chain. Despite the satisfactory results of this study, the report discusses recent evidence of the ability of these bacterial species not only to survive but also to grow in and on whole fresh produce products even at refrigeration storage temperatures. The report includes examples of international outbreaks associated with the consumption of RTE vegetables and berries, and provides data from prevalence studies of bacterial contamination in fresh produce carried out in other countries.

Acknowledgements

The FSAI thanks those that participated in this survey: the Environmental Health Officers (EHOs), the laboratory staff of the seven official food microbiology laboratories of the HSE[1] and the staff of the Horticulture and Plant Health Division of DAFM.

[1] (i) Public Health Laboratory, Limerick; (ii) Public Health Laboratory, Sligo General Hospital, Sligo; (iii) Public Health Laboratory, Waterford Regional Hospital, Waterford; (iv) Public Analyst's Laboratory, Sir Patrick Duns Hospital, Grand Canal Street, Dublin; (v) Public Health Microbiology Laboratory, St Finbarr's Hospital, Cork; (vi) Public Health Microbiology Laboratory, Cherry Orchard Hospital, Dublin; and (vii) Public Health Microbiology Laboratory, Galway University Hospitals, Galway.

Introduction

The consumption of fresh produce such as fruit, vegetables and leafy greens, has increased globally over the last 20 years (Olaimat and Holley, 2012). They are an essential part of a healthy lifestyle with current nutritional advice advocating a diet containing at least five to seven 80g portions of fruit and vegetables a day. While the majority of foodborne outbreaks are still attributed to foods of animal origin (FoAO), the incidence of foodborne outbreaks related to consumption of fresh produce has been increasing in recent years demonstrating the importance of food safety along the production chain for primary produce (Berger *et al*., 2010, Callejón *et al*., 2015). The European Food Safety Authority (EFSA) Scientific Opinion on the risk posed by pathogens in food of non-animal origin (FoNAO) found that from 2008 to 2011, there was an increase in the numbers of reported outbreaks (from 22 to 62), cases (from 794 to 5521), hospitalisations (from 92 to 2557) and deaths (from zero in 2008 and 2009, to 2 in 2010, and 54 in 2011) associated with FoNAO (EFSA BIOHAZ, 2013). The BIOHAZ panel noted that trends on data on FoNAO are strongly influenced by including data from the 2011 verocytotoxigenic *E. coli* (VTEC) O104 outbreak in Germany associated with contaminated sprouted seed consumption. The trends in FoNAO data from 2008 to 2011 coincided with a decrease in the numbers of reported outbreaks (from 658 to 208), cases (from 8753 to 3439) and hospitalisations (from 1396 to 564) associated with FoAO. However, the number of reported deaths where FoAO was implicated increased from 8 in 2008 to 24 in 2011 (EFSA BIOHAZ, 2013). In general, trends in the data showed that outbreaks associated with FoNAO tend to involve more cases and to be less severe (i.e. lower proportion of hospitalisations and deaths) than those associated with FoAO (EFSA BIOHAZ, 2013). The data compiled by the BIOHAZ panel was used to develop a multi criteria analysis model to identify and rank specific food/pathogen combinations most often linked to human cases originating from FoNAO in the EU (EFSA BIOHAZ, 2013). Seven criteria were used in the EFSA model which were: (1) the strength of association between food and pathogen, (2) the incidence of illness, (3) the burden of disease, (4) the dose-response relationship, (5) the consumption, (6) the prevalence of contamination and (7) the pathogen growth potential during shelf-life. The top ranking food/pathogen combination was *Salmonella* spp. and leafy greens eaten raw followed by (in equal rank) *Salmonella* spp. and tomatoes, *Salmonella* spp. and bulb and stem vegetables, *Salmonella* spp. and melons, and pathogenic *Escherichia coli* and fresh pods, legumes and grains.

Food consumption data in Ireland

A large proportion of fresh produce is consumed raw, with or without skins, and as such, the presence of pathogens represents a serious risk to public health. Examination of food consumption data between October 2008 and April 2010 from the National Adult Nutrition Survey (IUNA, 2011) revealed that tomatoes, peppers (green, red and yellow bell peppers and chilli peppers) and cucumbers were the most popular food items eaten raw by consumers in Ireland (Table 1). Raw strawberries and blueberries were the most frequently consumed berries followed by raspberries while blackberries were the least commonly consumed.

Table 1: Frequency of consumption of raw tomatoes, peppers, cucumbers and berries in Ireland according to the National Adult Nutrition Survey* (IUNA, 2011)

Food item	Total eating occasions over a 4-day period	Number of days food eaten by survey participants:			
		Eaten on 1 of 4 days surveyed	Eaten on 2 of 4 days surveyed	Eaten on 3 of 4 days surveyed	Eaten on 4 of 4 days surveyed
All tomatoes, raw	1498	460	230	93	44
Green, Red, Yellow, Chilli Peppers, raw	535	324	76	12	1
Cucumbers, raw	280	119	42	18	3
Strawberries, raw	184	83	54	30	1
Blueberries, raw	111	19	26	6	6
Raspberries, raw	42	13	14	4	0
Blackberries, raw	8	5	0	3	0

*Between October 2008 and April 2010, a sample of 1,500 adults (men 740, women 760) aged between 18-90 years from across the Republic of Ireland took part in the National Adult Nutrition Survey. Food intake was determined using a four day semi-weighed food record.

Outbreaks of foodborne illness and contamination incidents

A number of foodborne outbreaks and contamination incidents have been attributed to RTE salad vegetables and fresh berries (Appendix 1). A good illustration of potential risks is given by an outbreak of pathogenic *E. coli* O157:H7 registered in Oregon (USA) in 2011 and associated with the consumption of contaminated fresh strawberries caused by deer defecating in open production fields (Laidler *et al*., 2013). In June 2018, an outbreak in the United States of pathogenic *E. coli* O157:H7 in Romaine lettuce from the Yuma growing region involving 210 cases consisting of 96 hospitalisations, 27 of which involved haemolytic urea syndrome and 5 deaths which were linked to contaminated canal water used for irrigation (CDC, 2018). In October 2018, a further thirteen people were hospitalised with one person developing haemolytic urea syndrome and kidney failure from pathogenic *E. coli* O157:H7 strain linked to Romaine lettuce and leafy greens. This led to the US CDC issuing a food safety alert in November 2018 advising U.S. consumers not to eat any romaine lettuce, and advising retailers and restaurants not to serve or sell these products until the source of the outbreak could be traced (CDC, 2018b). A search of the European Rapid Alert System for Food and Feed (RASFF) for RTE salad vegetables and fresh berries found that from January 2013 to August 2017, there has been 1 foodborne outbreak suspected to be caused by shigatoxin-producing *E. coli* (*stx*+, *eae*+, 3100 CFU/g) in rucola from Denmark, via Sweden (2016) and 1 foodborne outbreak notification suspected to be caused by cherry tomatoes from Morocco (pathogen not reported) (2014). There has been 1 notification on high counts of *E. coli* (7.2×10^5 CFU/g) in raspberries from Spain, via the Netherlands (2015), 1 notification on shigatoxin-producing *E. coli* (presence/25 g) in cherry tomatoes from Morocco, via France (2014) and 2 notifications issued for *Salmonella* in frozen tomatoes both originating from Spain (2013 and 2015)[2].

Potential sources of contamination

Environmental factors, in particular climatic conditions (e.g. heavy rainfall) that increase the transfer of microorganisms from sewage effluents or from agricultural slurry and manure to irrigation water sources or fields; the use of contaminated agricultural water either for irrigation or for the application of agricultural

[2] Source: RASFF portal at https://webgate.ec.europa.eu/rasff-window/portal/?event=SearchForm&cleanSearch=1

chemicals and contamination and cross-contamination by harvesters, food handlers and equipment at harvest or post-harvest represent the greatest risk of contamination of fresh produce (EFSA BIOHAZ, 2014a, b). Water quality is also important for activities such as humidification of greenhouses, hand washing by workers and cleaning/washing/rinsing operations. In the case of hydroponic production, water is used for both irrigation and as the growth medium, and may, therefore present a high risk of microbiological surface and internal contamination (EFSA BIOHAZ, 2013). For all fresh produce that is usually eaten raw/uncooked, water which is to be applied by irrigation or used to make-up pesticides and biocides within two weeks of harvest should be of potable water quality (Monaghan and Hutchinson, 2009; FSAI, 2008; 2016). In 2016, the FSAI published a guidance note covering the good hygiene practices and good agricultural practices involved in the production of fresh produce, i.e. fruit, vegetables, herbs etc., at the primary production level in Ireland with a specific chapter on water and how water can be risk assessed.

Fresh berries such as strawberries, raspberries, blackberries and blueberries are a perishable food which can be consumed fresh or minimally-processed as well as, as a frozen ingredient added to many foods. Fresh berries are usually manually harvested by picking directly and placing in their final packaging for sale to caterers or consumers, and are not normally subject to physical interventions that eliminate or substantially reduce the occurrence of foodborne pathogens (EFSA BIOHAZ, 2014a). As wild and domestic animals – including mammals, birds, reptiles and insects– are also vehicles of pathogenic bacteria such as VTEC and *Salmonella*, the main mitigation options for reducing the risk of contamination of fresh produce are to prevent direct contact with faeces as well as indirect contact through slurries, sewage, sewage sludge, and contaminated soil, water, harvesting equipment or food contact surfaces (EFSA BIOHAZ Panel, 2014a). Farm workers and primary producers of horticultural products have an important impact on the microbiological safety of the produce they handle as the lack of good hygienic practice can lead to cross-contamination (De Roever, 1998; Bassett and McClure, 2008).

Appendix 2 includes a compilation of international studies investigating the prevalence of *Salmonella*, *E. coli* and *L. monocytogenes* in whole fresh tomatoes, cucumbers, peppers and berries.

EU Legislation and Recommendations made by the EFSA BIOHAZ Panel

The current legal European framework includes food safety criteria for *L. monocytogenes* in RTE food, including fruit and vegetables (Commission Regulation (EC) 2073/2005), as amended. According to footnote 4 for food category 1.3 in the aforementioned regulation, regular testing for *L. monocytogenes* in normal circumstances is not required for fresh, uncut and unprocessed vegetables and fruits. The Regulation does not include microbiological criteria for *Salmonella* or *E. coli* for fresh, uncut (whole) and unprocessed fruits and vegetables however in Ireland; we have guideline limits for these microorganisms in RTE food, including fruit and vegetables (FSAI, 2014a).

In 2014, an EFSA Scientific Opinion on the risk posed by *Salmonella* in berries concluded that there is currently insufficient evidence to justify the establishment of a food safety criterion for *Salmonella* for fresh and minimally processed berries (EFSA BIOHAZ, 2014a). A separate EFSA Scientific Opinion on the risk posed by *Salmonella* in tomatoes concluded that a food safety criterion for *Salmonella* in whole tomatoes could be considered as a tool to communicate to producers and processors that *Salmonella* should not be present in the product (EFSA BIOHAZ, 2014b). With regard to *E. coli* and due to the current lack of data, the

EFSA BIOHAZ Panel was unable to assess the suitability of an EU process hygiene criterion for *E. coli* at primary production of berries and tomatoes (EFSA BIOHAZ, 2014a, b). The BIOHAZ Panel recommended studies to collect further data to evaluate the suitability of *E. coli* criteria at both primary production and during minimal processing of tomatoes. *E. coli* is typically found in the intestinal tracts and in the faeces of warm-blooded animals, including humans, and, as such, is often used as an indicator microorganism of faecal contamination. Therefore, *E. coli* could be used to monitor hygiene in whole fresh produce which is frequently eaten raw as unsatisfactory levels could indicate that other pathogenic microorganisms may be present whether they are bacterial (e.g. *Salmonella*, *Shigella*, *Campylobacter*) or viral (e.g. hepatitis A, norovirus, rotavirus).

Aim of the Survey

The aim of this survey was to investigate the microbiological quality and safety of selected types of RTE whole (uncut) unprocessed fresh fruit and vegetables in Ireland. The survey included any variety of tomatoes, cucumbers, peppers, blueberries, raspberries, strawberries and blackberries[3].

Methods

Sample collection

Table 2: Specific details of the collected samples

	EHOs (HSE)	Horticulture and Plant Health Division (DAFM)
Collection period	Between 2nd August and 30th November 2016 (inclusive)	
Sample type	Fresh whole unprocessed **tomatoes, cucumbers, peppers, blueberries, raspberries, strawberries and blackberries** (any variety)	
Pre-packaged	Yes	No
Type of establishments	▪ Supermarkets and corner shops ▪ Green-grocers and health food shops ▪ Market stalls and roadside stalls ▪ Discount retailers ▪ Retail shops in hospitals ▪ Packers, distributors and wholesalers	Primary Producers
Origin	Imported and Irish grown	Irish grown
Sample size	▪ Single samples (n=1) from retailers ▪ Batch samples (n=5) from packers, distributors and wholesalers	Batch samples (n=5)

[3] Technically, tomatoes, cucumbers and peppers are fruits, not vegetables. Fruits are the seed-bearing parts of flowering plants that come from the flower's ovary after flowering. However, it is quite common to refer to cucumbers, tomatoes and peppers as vegetables.

All samples were considered RTE and fell into EU food category 08 'Fruit and Vegetables' as specified in FSAI Guidance Note 2 (FSAI, 2001).

Sample analysis and Interpretation of results

Table 3 summarises the sample analysis and the interpretation of the results for the survey.

Table 3: Sample analysis methods and the interpretation of results

	At producer level	At retail, packer and distributor level
Listeria monocytogenes	▪ Legal criteria set in Regulation (EC) No 2073/2005, as amended. Results were assessed based on **Food Category 1.2*** due to FSAI Guidance Note 27 (FSAI, 2014b) ▪ Method: EN/ISO 11290-1 ▪ Test: **Presence/Absence in 25 g (n=5)** ▪ Interpretation of results and sample designation**: - *Satisfactory*: Absence in 25 g in any sample unit (n=5/5) - *Unsatisfactory*: Presence in 25 g in at least one sample unit (n=1/5)	▪ Legal criteria set in Regulation (EC) No 2073/2005, as amended. Results were assessed based on **Food Category 1.3*** due to footnote 4 of category 1.3 in Regulation (EC) 2073 of 2005 ▪ Method: EN/ISO 11290-2 ▪ Test: **Enumeration (n=1 or 5)** ▪ Interpretation of results and sample designation**: - *Satisfactory*: ≤100 CFU/g in all sample units (n=5/5) - Not designated: ≤100 CFU/g in the sample unit when n=1*** - *Unsatisfactory*: >100 CFU/g in at least one sample (n=1/5 or n=1/1)
	At all levels	
*Salmonella spp.**** *	▪ No legal criteria set in Regulation (EC) No 2073/2005, as amended. Results assessed against Guidance Note 3, Revision 2, Table 1 (FSAI, 2014a) ▪ Method: EN/ISO 6579 ▪ Test: **Presence/Absence in 25 g (n=1)** ▪ Interpretation of results and sample designation: - Satisfactory: Absence in 25 g - Unsatisfactory: Presence in 25 g	
Escherichia coli	▪ No legal criteria set in Regulation (EC) No 2073/2005, as amended. Results assessed against Guidance Note 3, Revision 2, Table 4 (FSAI, 2014a) ▪ Method: ISO 16649-1 or 2 ▪ Test: **Enumeration (n=1)** ▪ Interpretation of results and sample designation: - Satisfactory: <20 CFU/g - Borderline: 20–≤10^2 CFU/g - Unsatisfactory: >10^2 CFU/g	

* **Food Category 1.2** – DAFM assessed samples against food category 1.2 (able to support growth) because FSAI Guidance Note 27 (FSAI, 2014b) advises that where the food business operator (FBO) cannot demonstrate that their food is unable to support the growth of *L. monocytogenes* then it is presumed to fall into food category 1.2.

Food Category 1.3 – HSE assessed samples against food category 1.3 (unable to support growth of *Listeria monocytoegenes*) because footnote 4 of food category 1.3 states that regular testing for *L. monocytogenes* is not required in normal circumstances for fresh, uncut and unprocessed vegetables and fruits. Note at retail level the limit for category 1.2 and 1.3 foods is the same (100 CFU/g). The only difference is that in the case of food category 1.2, the FBO has to be able to show that if counts are found below 100 CFU/g that the legal limit of 100 CFU/g will not be exceeded throughout the remaining shelf life of the product.

** Sample designation under Commission Regulation (EC) No 2073/2005, as amended.

*** This result complies with the limit as specified in Regulation (EC) No 2073/2005, as amended. However, where a single sample unit was taken for Monitoring and Surveillance purposes, the result cannot be designated as "*satisfactory*" under the Regulation (where the criterion includes a sampling plan of n=5).

**** If isolated, serotyping, phage typing and/or pulse field gel electrophoresis were performed by the relevant reference laboratory.

Survey questionnaire

EHOs and staff from the DAFM Horticulture and Plant Health Division completed a survey questionnaire for each sample collected (see Appendix 3).

Results and Discussion

Samples collected

A total of 838 samples were collected in this survey. Figure 1 shows the percentages of each sample type (tomatoes, cucumbers, peppers, strawberries, blueberries, raspberries and blackberries) with respect to the total number of samples. The highest number of samples taken corresponded to tomatoes (n=248/838; 29.6%) while the lowest number corresponded to blackberries (n=43/838; 5.1%).

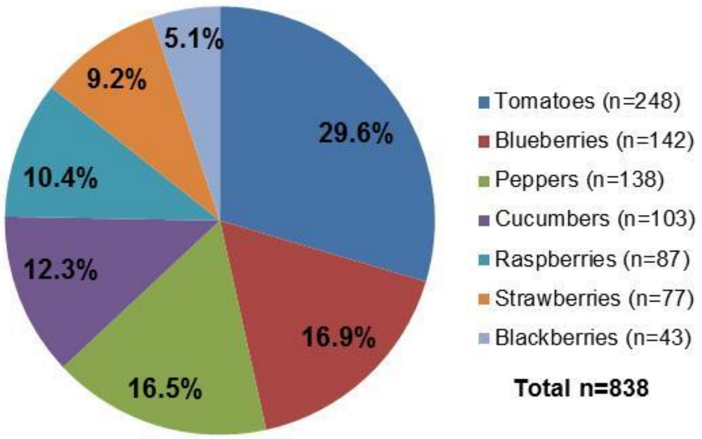

Figure 1: Breakdown of the total number of samples by sample type.

Figure 2 shows the distribution of the samples based on the type of establishments from which they were collected. The majority of the samples were from the retail sector (n=751/838; 89.6%).

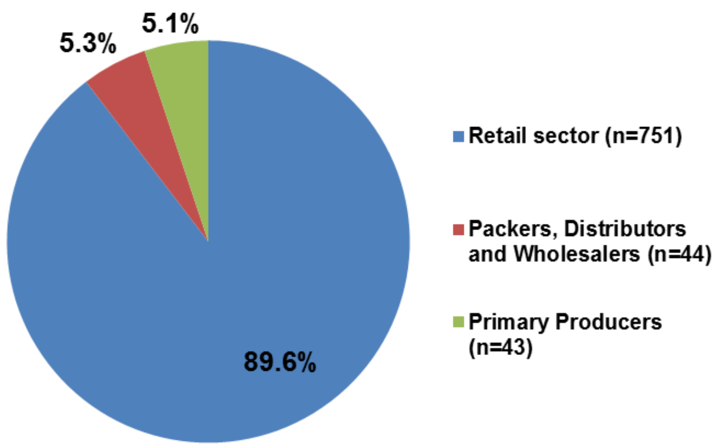

Figure 2: Breakdown of the total number of samples based on the type of establishments where they were collected.

Microbiological results

In total, 1162 tests were performed for *L. monocytogenes* and 846 tests for *E. coli* and *Salmonella* spp. (Table 4). All the samples tested (n=838) were satisfactory for *L. monocytogenes*, *E. coli* and *Salmonella* spp. meaning that (i) *L. monocytogenes* was neither present (presence/absence test) nor at levels above the legal limit of 100 CFU/g (enumeration test) in any of the samples analysed. In fact, all the samples were below the limit of enumeration of the test (10 CFU/g), (ii) *Salmonella* spp. was not detected (presence/absence test) in any of the samples tested and (iii) the levels of *E. coli* in all the samples investigated were below 20 CFU/g (the limit of the enumeration test).

Table 4: Breakdown of the total number of samples by sample type, microorganism and sample size (single sample *vs*. batch sample). The total number of microbiological test performed and unsatisfactory samples is also included.

Listeria monocytogenes

Sample size		Sample type							Totals
		Tomato	Cucumber	Pepper	Strawberry	Blueberry	Raspberry	Blackberry	
HSE	Single	227	91	131	56	135	81	36	757
	Batch[a]	16	9	3	3	5	1	2	39
DAFM	Batch[a]	5	3	4	18	2	5	5	42
Total no. samples		248	103	138	77	142	87	43	**838**
Total Tests		332	151	166	161	170	111	71	**1162**
Unsatisfactory		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**

Salmonella spp. and E. coli

Sample size		Sample type							Totals
		Tomato	Cucumber	Pepper	Strawberry	Blueberry	Raspberry	Blackberry	
HSE	Single	242	100	133	59	140	82	38	794
	Batch[a]	1	-	1	-	-	-	-	2
DAFM	Single	5	3	4	18	2	5	5	42
Total no. samples		248	103	138	77	142	87	43	**838**
Total Tests		252	103	142	77	142	87	43	**846**
Unsatisfactory		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**

[a] A batch sample consists of 5 sample units and consequently entails 5 tests and results.

Based on the literature, contamination of fresh produce with bacterial pathogens appears to be a rare event although it represents a real food safety risk given the increasing number of outbreaks in recent years. However, the previously low association between fresh produce and bacterial pathogens may have been due to foods of animal origin typically being suspected or blamed in the past, leading to an underestimation for the association between foodborne illness and these types of produce. Along with storage temperature and humidity, pH is a principal determinant of growth of bacterial microorganisms on fresh produce. Based on the growth limits for microbial pathogens detailed by the International Commission on Microbiological Specifications for Foods (ICMSF, 1996), Basset and McClure (2008) classified fruits into two categories: high acid fruit (pH≤4) where pathogen's growth is unlikely to occur and low acid fruit (pH>4) where microbial growth is more likely to occur. According to the pH values reported by Bridges and Mattice (1939) and Basset and McClure (2008), blueberries, raspberries and strawberries would fall under the first category (high acid fruit) while blackberries, cucumbers, peppers and tomatoes fall under the second one (low acid fruit). This classification should be taken with caution, as other factors such as the soil characteristics where the produce has grown, the climatic conditions, cultivar variety (nutrient compositional differences) and the ripening stage are strongly related to the ability of pathogens to grow and survive. For instance, climacteric fruits (those that can ripen after removal from the plant) such as tomato, bananas and apples, are more susceptible to microbial infection and spoilage as time from harvest increases (Brackett, 1997). This is attributed to a drop in acid content and a subsequent rise in pH over time. Examples of non-climacteric fruits are cucumbers, strawberries, grapes and citrus fruits. However, there is a lack of information on any (or no) relationship between climacteric fruits and pathogens levels (Basset and McClure, 2008).

A recent study on the prevalence of bacterial contamination in fresh fruits and vegetables sold at retail in Canada performed by the Canadian Food Inspection Agency reported that no pathogens (*L. monocytogenes*, *Salmonella* spp., *E. coli* O157:H7 and *Shigella* spp.) or *E. coli* (at levels >100 CFU or MPN/g)[4] were detected in the 4,837 samples of tomatoes or the 1,776 samples of berries (blueberries, strawberries, blackberries and raspberries) sampled during the survey period (from June 2009 to March 2013) (Denis *et al*., 2016). The Canadian results are consistent with the results obtained in the present survey. Similarly, the USDA's Microbiological Data Program, which ran from 2001 to 2012 and collected information on the prevalence of bacterial pathogens in fresh produce sold in the USA, rarely detected and isolated bacterial pathogens (USDA, 2014; Reddy *et al*., 2016). For instance, *E. coli* O157 was not detected in cantaloupes, (n=9,169), leafy vegetables (n=17,027), tomatoes (n=9,842) and green onions (n=7,192) tested for over a period of several years. *Salmonella*, however, was detected and isolated in several commodities, but its occurrence was rare (<0.1%) in leafy vegetables, cantaloupes, tomatoes and green onions (70,000 samples analysed in total). Considering that tomatoes have been involved in multiple major outbreaks of *Salmonella* infection in North America (Appendix 1; Hanning *et al*., 2009), it is interesting that none of the 4,837 tomato samples collected over three years of USDA's surveillance presented any contamination with *Salmonella*. Similar results were obtained in Japan during a survey period of 10 years (1998-2008), where a prevalence was reported for *Salmonella* spp. and *E. coli* of 0.1% and 2.1% in tomato (1140 total samples) and of 0.2% and 5.9% in cucumber (1315 total samples), respectively (Hara-Kudo *et al*., 2013).

Due to the more common usage of animal manure as a fertilizer in organic production, the microbial safety of organic produce in comparison with conventionally-grown produce has been questioned over the years (Denis *et al*., 2016). Several studies on this matter (Mukherjee *et al*., 2004; Oliveira *et al*., 2010; Tango *et al*., 2014) found that the hygienic quality of produce was impacted by organic production and that organic produce was more susceptible to faecal contamination (Denis *et al*., 2016). In this survey, a total of 17 single samples of cucumber (16.5% of the total cucumber samples) and 4 single samples of tomatoes (1.61% of the total tomato samples) were specified as organic (2.51% of the total samples). However, no conclusions can be drawn as all the samples analysed were satisfactory.

Listeria monocytogenes

Commission Regulation (EC) No 2073/2005, as amended, sets a maximum legal limit of 100 CFU/g for *L. monocytogenes* for ready-to-eat foods during their shelf-life, whether they are able to support the growth of *L. monocytogenes* (food category 1.2) or not (food category 1.3).[5] In addition for food category 1.2, the Regulation sets a limit of absence in 25 g before the food has left the immediate control of the FBO who has produced it when the FBO is not able to demonstrate, to the satisfaction of the competent authority, that the

[4] Under European Regulation EC 2073/2015, 100 CFU/g is the lower limit for *E. coli* in RTE pre-cut fruit and vegetables.
[5] Annex I, Chapter 1, food safety criteria, Food categories 1.2 and 1.3.

product will not exceed the limit of 100 CFU/g throughout the shelf-life. One of the challenges for FBOs complying with, and for competent authorities assessing compliance with, the Regulation with respect to the *L. monocytogenes* criteria is deciding whether a food falls into category 1.2 or 1.3. The Regulation seems to suggest that RTE unprocessed fresh fruit and vegetables fall under food category 1.3, as there is a specific footnote (footnote 4) linked to food category 1.3 which states that regular testing for *L. monocytogenes* is not required in normal circumstances for fresh, uncut and unprocessed vegetables and fruits. The literature, however, indicates that some unprocessed fruit and vegetables do support growth (Table 5). FSAI Guidance Note 27 (FSAI, 2014b) advises that where the FBO cannot demonstrate that their food is unable to support the growth of *L. monocytogenes* then it is presumed to fall into food category 1.2. The issue is less complex once the sample is taken after the food has left the control of the FBO that has produced it, as the same limit applies to both categories; the only difference is that in the case of food category 1.2, the FBO has to be able to show that if counts are found below 100 CFU/g that the legal limit of 100 CFU/g will not be exceeded throughout the remaining shelf life of the product. In the present survey, *L. monocytogenes* was tested in products collected at producer level, retail, packer and distributor levels as indicated in Table 3. DAFM took samples at producer level and assessed them against the absence in 25 g limit of food category 1.2. HSE took samples at retail, packer and distributor level and assessed them against the 100 CFU/g limit of food category 1.3 (Table 3).

L. monocytogenes is a psychrotrophic bacterium i.e. it is able to grow in food products at refrigerated temperatures although its optimum temperature for growth is 20–30°C. Therefore, it is not surprising that *L. monocytogenes* is able to survive and, in some scenarios, grow under refrigeration conditions when mesophilic pathogens cannot. Besides, under abusive storage temperatures bacterial pathogen survival and growth can occur. Regardless of the results obtained in this survey (*L. monocytogenes* was not detected at 'unsatisfactory' levels, i.e. >100 CFU/g, in any of the 838 samples tested) some studies have shown the ability of *L. monocytogenes* to survive and grow on whole fresh produce even at refrigeration temperatures (Table 5). This emphasizes the importance of proper storage conditions in preventing or reducing (any) pathogen survival and growth.

Table 5: Selected studies on the survival and growth of *L. monocytogenes* on whole fresh produce

Product	Observations	Reference
Blueberries	Counts decreased by ~0.5 log_{10} at 4°C (initial=5.8 log_{10}) and by ~1 log_{10} (initial=6.2 log_{10}) at 12°C when stored during 10 days	Concha-Meyer *et al*. (2014)
Raspberries	Slow growth (by ~0.75 log_{10}) after 5 days at 6°C and by ~1 to 1.5 log_{10} after 2 days at 15 or 22°C	Molinos *et al*. (2008)
Strawberries	Survival after 9 days at 4°C (initial and final counts ~10^5 CFU/berry)	Rodgers *et al*. (2004)
	No growth but reduction over 48-h storage at 24°C regardless of the inoculation level (~10^6 or ~10^8 CFU/berry)	Flessa *et al*. (2005)
	Survival beyond the expected market shelf life at 4±1°C. Decline by ~10^3 CFU/berry after extended storage (15 days)	Udompijitkul *et al*. (2007)
Cherry type Tomatoes	Growth occurred at 21°C (from 3.5 to 5 log_{10} in 2 days). No growth at 10°C although remained viable for >20 days	Beuchat and Brackett (1991)
Peppers	Slow growth (from 7.3 to 7.9 log_{10}) on bell-shaped organic green peppers after 2-weeks storage at 7°C	Han *et al*. (2001)
	After 14 days at 12°C populations on jalapeño peppers increased by 1.1 log_{10}. No growth at 7°C although remained viable	Huff *et al*. (2012)

Salmonella spp. and *E. coli*

There are no legal microbiological criteria set for *Salmonella* spp. or *E. coli* in RTE whole fresh produce in Commission Regulation (EC) No 2073/2005, as amended. *Salmonella* was included in the current survey considering the significant number of foodborne outbreaks caused by *Salmonella* spp. and associated with the consumption of RTE salad vegetables and fresh berries (Appendix 1). *E. coli* was included as it is a useful indicator of faecal contamination of food products, and as such, indicates the potential risk of contamination with faecal pathogens. In Ireland we have guideline limits for these microorganisms in RTE food, including fruit and vegetables (FSAI, 2014a). As indicated above, 100% of all samples examined in this survey were 'satisfactory' for *Salmonella* and *E. coli* (Table 4).

Despite these results, fresh produce, with tomatoes specifically implicated, have become increasingly recognized as vehicles of human illness with *Salmonella* and pathogenic *E*. *coli*. According to Zhuang *et al*. (1995), populations of *Salmonella* Montevideo were stable on the surface of mature green tomatoes stored at 10°C for 18 days. Even if pathogens are located on the surface only, these pathogens can also be transferred to the flesh during further handling or cutting. Beuchat and Mann (2008) reported that *Salmonella* can grow in stem scar and pulp tissues of round, Roma, and grape tomatoes stored at 12 and 21°C but not at 4°C. *Salmonella*, however, did not grow on the skin of grape tomatoes regardless of the storage temperature (Beuchat and Mann, 2008) which has been recently attributed to the surface metabolite landscape, including the fatty acids present in tomato fruit surface exudates (Dev Kumar and Micallef, 2017). Although most of the outbreaks involving *Salmonella* spp. or *E. coli* O157:H7 on fresh produce have been associated with contamination post-harvesting; several studies have demonstrated the ability of *Salmonella* spp. and *E. coli* O157:H7 to diffuse into the interior of produce (through both natural apertures and damaged tissues), survive inside and, in some cases, proliferate within (Erickson, 2012). As an example, a study performed by Ibarra-Sánchez *et al*. (2004) indicated that *Salmonella* Typhimurium and *E. coli* O157:H7 were able to diffuse into the interior of ripe field tomatoes and survive inside for at least 72 hours at room temperature (25 to 28°C).

Limited studies have been performed on *Salmonella* and *E. coli* growth in/on whole peppers, cucumbers and berries; though they seem to agree that microbial growth in this type of products is limited. Castro-Rosas *et al*. (2011) reported that several strains of *Salmonella* and *E. coli* did not grow on whole serrano and jalapeño peppers stored at 4 ± 1°C or 25 ± 2°C. In fact, at 25 ± 2°C the populations decreased by 4 and 2.5 $\log_{10}$ on serrano and jalapeño peppers, respectively, and by >3 $\log_{10}$ at 4 ± 1°C after 6 days of storage. With regards to berries, a study carried out by Knudsen et al. (2001) in whole strawberries indicated that *Salmonella* and *E. coli* O157:H7 populations slightly declined over 48 hour storage at 24°C. When stored at 4 to 5°C the populations of both species decreased by 1 to 2-$\log_{10}$ over the 7-day storage period. Another study performed by Nguyen *et al*. (2014) found that *E. coli* O157:H7 and *Salmonella enterica* subsp. *enterica* did not grow, in fact, declined on strawberries at shipping (2 ± 2°C) and retail display (15.5 ± 2°C) temperatures over a period of 7 days of storage, even when they were harvested at a maturity prone to bruising. However, *Salmonella* growth, but not *E. coli*, did occur on bruised (with an intact skin) full ripe blueberries under retail display temperatures (15.5 ± 2°C) after 3 days storage.

There is a wealth of evidence that fresh produce can harbour enteric bacteria and serve as vehicles for pathogenic bacteria causing foodborne illnesses. Aside from the type and variety of commodity and the type of microorganism (species and serotypes) other factors such as the cultivation practices that may affect the ability of microorganisms not only to survive but also to colonise and multiply within.

Information collected on questionnaire

EHOs and staff from the DAFM Horticulture and Plant Health Division completed a survey questionnaire for each sample collected (Appendix 3); in order to provide information about the sample that would not be captured on the National Sample Submission Form (NSSF). A total of 829 questionnaires were received for a total of 838 samples collected and analysed, which corresponds to a response rate of 98.9%. Table 6, showing the number of questionnaires received per sample type, indicates that all the products were represented in a relatively similar proportion. Ten questionnaires received contained no information on the fresh produce sample type. From the 819 filled-out questionnaires, some interesting information can be extracted.

Table 6: Number of questionnaires received per sample type

	Sample type								Totals
	Tomato	**Cucumber**	**Pepper**	**Strawberry**	**Blueberry**	**Raspberry**	**Blackberry**	**NS**	
Total no. samples	248	103	138	77	142	87	43	-	**838**
Total no. questionnaires	241	96	138	75	140	87	42	10	**829**
Response rate (%)	97.2	93.2	100	97.4	98.6	100	97.7	-	**98.9**

Country of origin

As the Irish climate does not permit year-round growth of fresh fruit and vegetables, at certain times of the year imported produce is more likely to be on the market. This survey was conducted between August and November 2016 (Table 2). Figure 3 shows the country of origin labelled on the packaging for each of the samples that had an associated questionnaire. As it can be seen, the country origin varied largely depending on the type of product, with Ireland ranking 1st for strawberries (n=49/75; 65.3%), blackberries (n=19/42; 45.2%) and joint first with the Netherlands for cucumbers (n=32/96; 33.3%), 3rd for raspberries (n=19/87; 21.8%), blueberries (n=25/140; 17.9%), tomatoes (n=47/241; 19.5%) and peppers (n=20/138; 14.5%). Overall, Ireland was the country of origin for 25.8% (n=211/819) of the samples with a questionnaire after the Netherlands (n=297/819; 36.3%) and followed by Spain (n=146/819; 17.8%). As indicated above (Table 4), none of the samples (Irish-grown and imported) analysed in this survey were unsatisfactory for *L. monocytogenes*, *Salmonella* spp. and *E. coli.* In the Canadian (Denis *et al*., 2016) and American (Reddy *et al*., 2016) microbiological surveillance programmes results indicated that the seasonal indices for domestic and imported vegetables were very similar, indicating that the trend in bacterial contamination did not differ based on the product origin.

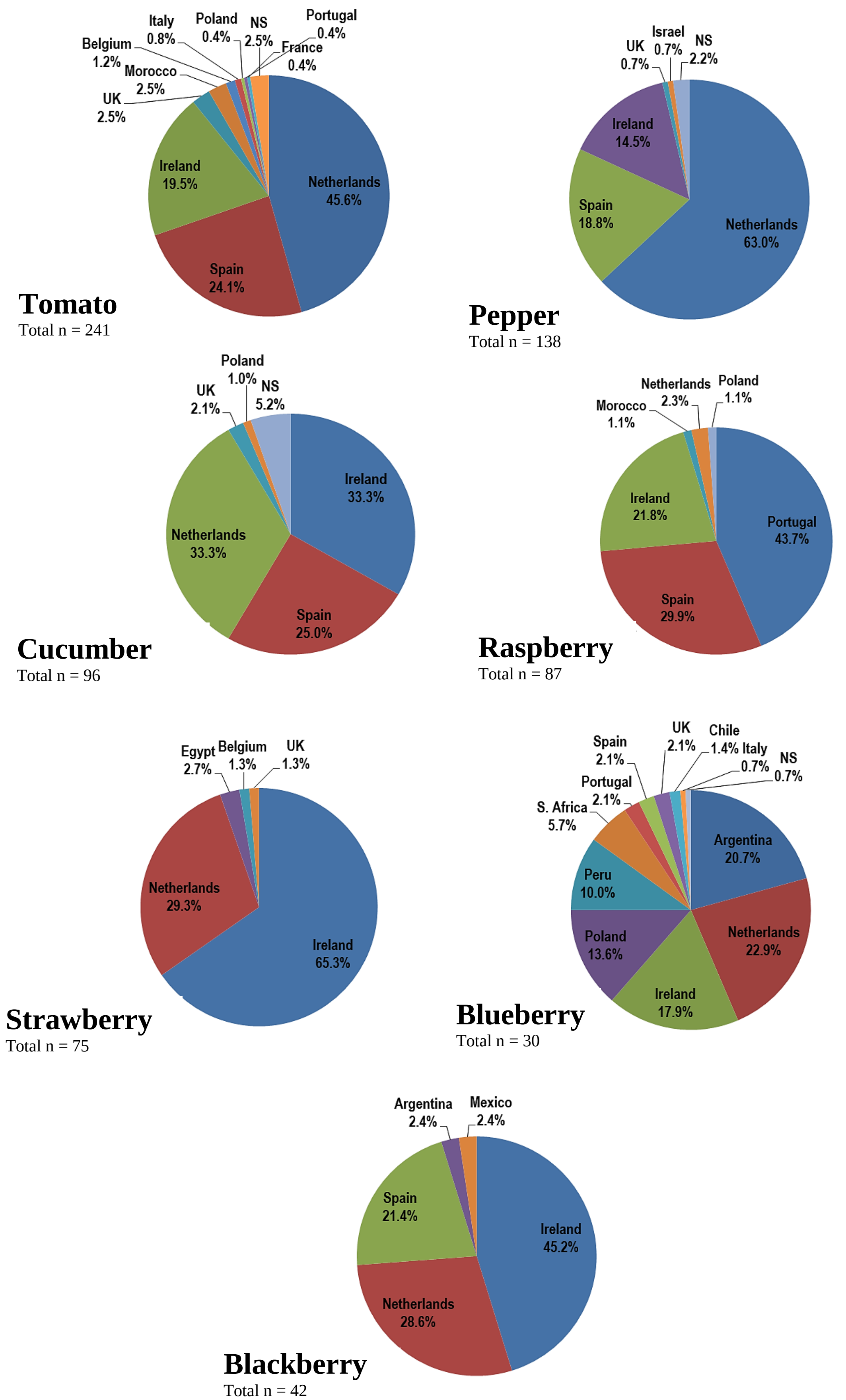

Figure 3: Country of origin labelled on the packaging of the samples with an associated questionnaire.

Types of tomatoes and peppers

Question 6 of the questionnaire (Appendix 3) was intended to collect information regarding the types of tomatoes and peppers surveyed. The information provided in the questionnaires showed that from the 241 tomatoes samples; 117 were cherry tomatoes, 49 were vine tomatoes, 32 were plum tomatoes, 27 were salad tomatoes, 8 were piccolo tomatoes and the remaining 5 were sun-stream (1), sugar-drop (1) and non-specified (6). Regarding the peppers (n=138), 108 were a mix of red, green and yellow peppers, 9 were sweet red Ramiro peppers, 8 were baby peppers, 7 were individual green, red and yellow bell peppers and the 6 remaining were non-specified.

Intact *vs*. damaged samples

In general, the surface of fresh produce is considered a harsh environment for foodborne pathogen survival and growth. Intact surfaces of healthy produce tissue might not provide the environment needed for foodborne pathogens to thrive. However, injured surfaces of fruits and vegetables make nutrients readily available for microorganisms; hence injured produce is generally considered more of a food safety risk than intact produce (Basset and McClure, 2008). Nonetheless, a bruised, punctured or cut surface can leak fluids that contain not only nutrients, but also organic acids that could serve as antimicrobials (Huff *et al*., 2012). Therefore, the survival or growth of bacteria on a plant's surface, either it is intact or injured, is not readily predictable. Question number 7 in the questionnaire (Appendix 3) *'Was the sample bruised or damaged in some way?*' dealt with this circumstance. Out of the 819 filled-out questionnaires, only 7 (0.85%) reported bruised/damaged samples corresponding to 2 tomatoes, 2 raspberries, 1 blueberry, 1 strawberry, 1 cucumber and 1 pepper samples.

Conclusions

Contamination of fresh produce with bacterial pathogens appears to be a relatively rare occurrence. Although based on the increasing number of international outbreaks in recent years, it does represent a food safety risk. Food producers, distributors and retailers are responsible for ensuring that their products are produced hygienically and meet the relevant food safety requirements. The results of this survey indicate that the contamination of the surveyed fresh fruits and vegetables with pathogenic bacteria (*L. monocytogenes*, *Salmonella* spp.) and *E. coli*, as an indicator of faecal contamination, at levels representing a risk to public health is rare in the Irish marketplace. This finding indicates that the fresh produce on sale in Ireland is of good microbiological quality and suggests that the practices carried out along the food supply chain (from agricultural practices by the primary producers to handling practices by the food distributors and retailers) are generally good for the products surveyed (RTE whole unprocessed fresh tomatoes, cucumbers, peppers, blueberries, raspberries, strawberries and blackberries). Nevertheless, studies reporting prevalence, survival and growth of pathogens in/on fresh produce emphasise the need to control pre- and postharvest contamination in order to mitigate risks associated with foodborne illness from fresh produce.

References

Angelo, K.M., Chu, A., Anand, M., Nguyen, T.A., Bottichio, L., Wise, M., Williams, I., Seelman, S., Bell, R., Fatica, M., Lance, S., Baldwin, D., Shannon, K., Lee, H., Trees, E., Strain, E. and Gieraltowski, L. 2015. Outbreak of *Salmonella* Newport infections linked to cucumbers--United States, 2014. *Morbidity and Mortality Weekly Report*, 64(6), 144-147.

Arthur, L., Jones, S., Fabri, M. and Odumeru, J. 2007. Microbial survey of selected Ontario-grown fresh fruits and vegetables. *Journal of Food Protection*, 70(12), 2864-2867.

Bassett, J. and McClure, P. 2008. A risk assessment approach for fresh fruits. *Journal of Applied Microbiology*, 104(4):925-943.

Berger, C.N., Sodha, S.V., Shaw, R.K., Griffin, P.M., Pink, D., Hand, P. and Frankel, G. 2010. Fresh fruit and vegetables as vehicles for the transmission of human pathogens. *Environmental Microbiology*, 12(9), 2385-2397.

Beuchat, L.R. and Brackett, R.E. 1991. Behavior of *Listeria monocytogenes* inoculated into raw tomatoes and processed tomato products. *Applied and Environmental Microbiology*, 57(5), 1367-1371.

Beuchat, L.R. and Mann, D.A. 2008. Survival and growth of acid-adapted and unadapted *Salmonella* in and on raw tomatoes as affected by variety, stage of ripeness, and storage temperature. *Journal of Food Protection*, 71(8), 1572-1579.

Bohaychuk, V.M., Bradbury, R.W., Dimock, R., Fehr, M., Gensler, G.E., King, R.K., Rieve, R. and Romero Barrios, P. 2009. A microbiological survey of selected Alberta-grown fresh produce from farmers' markets in Alberta, Canada. *Journal of Food Protection*, 72(2), 415-420.

Brackett, R.E. 1997. Fruits, vegetables, and grains. In: *Food Microbiology: Fundamentals and Frontiers* pp. 117–126. Washington, DC: ASM Press.

Bridges, M.A., and Mattice, M.R. 1939. Over two thousand estimations of the pH of representative foods, *American Journal of Digestive Diseases*, 9, 440-449.

Callejón, R.M., Rodríguez-Naranjo, I., Ubeda, C., Hornedo-Ortega, R., García-Parrilla, M.C. and Troncoso, A.M. 2015. Reported foodborne outbreaks due to fresh produce in the United States and European Union: Trends and Causes. *Foodborne Pathogens and Disease*, 12(1), 32-38.

Cárdenas, C., Molina, K., Heredia, N. and Garcia, S. 2013. Evaluation of microbial contamination of tomatoes and peppers at retail markets in Monterrey, Mexico. *Journal of Food Protection*, 76(8), 1475-1479.

Castro-Rosas, J., Gómez-Aldapa, C.A., Acevedo-Sandoval, O.A., González-Ramírez, C.A., Villagomez-Ibarra, J.R., Chavarría-Hernández, N., Villarruel-López, A. and Torres-Vitela, Mdel R. 2011. Frequency and behavior of *Salmonella* and *Escherichia coli* on whole and sliced jalapeño and serrano peppers. *Journal of Food Protection*, 74(6), 874-881.

Centers for Disease Control and Prevention (CDC), 2006. Multistate Outbreak of Salmonella Typhimurium Infections Linked to Tomatoes (Final Update). Available at: https://www.cdc.gov/salmonella/2006/tomatoes-11-2006.html (last accessed Nov 2017).

Centers for Disease Control and Prevention (CDC), 2008. Outbreak of *Salmonella* Serotype Saintpaul Infections Associated with Multiple Raw Produce Items - United States, 2008. *Morbidity and Mortality Weekly Report*, 57(34), 929-934.

Centers for Disease Control and Prevention (CDC), 2013. Multistate Outbreak of *Salmonella* Saintpaul Infections Linked to Imported Cucumbers (Final Update). Available at: http://www.cdc.gov/salmonella/saintpaul-04-13/index.html (last accessed Nov 2017).

Centers for Disease Control and Prevention (CDC), 2016. Foodborne Outbreak Online Database [CDC FOOD]. Available at: http://wwwn.cdc.gov/foodborneoutbreaks/Default.aspx (last accessed Nov 2017).

Centers for Disease Control and Prevention (CDC), 2018. Multistate Outbreak of E. coli O157:H7 Infections Linked to Romaine Lettuce (Final Update). Available at: https://www.cdc.gov/ecoli/2018/o157h7-04-18/index.html (last accessed November 2018).

Centers for Disease Control and Prevention (CDC), 2018b. Outbreak of E. coli Infections Linked to Romaine Lettuce. Food Safety Alert. Available at: https://www.cdc.gov/ecoli/2018/o157h7-11-18/index.html (last accessed November 2018).

Concha-Meyer, A., Eifert, J., Williams, R., Marcy, J. and Welbaum, G. 2014. Survival of *Listeria monocytogenes* on fresh blueberries (*Vaccinium corymbosum*) stored under controlled atmosphere and ozone. *Journal of Food Protection*, 77(5), 832-836.

De Roever, C. 1998. Microbiological safety evaluations and recommendations on fresh produce. *Food Control*, 9, 321–347.

Denis N., Zhang, H., Leroux, A., Trudel, R. and Bietlot, H. 2016. Prevalence and trends of bacterial contamination in fresh fruits and vegetables sold at retail in Canada. *Food Control*, 67, 225-234.

Dev Kumar, G. and Micallef, S.A. 2017. Susceptibility of *Salmonella enterica* isolates from tomato farm environments to fatty acids naturally found on tomato fruit. *Foodborne Pathogens and Disease*, 14(5):293-301.

EFSA Panel on Biological Hazards (BIOHAZ), 2013. Scientific Opinion on the risk posed by pathogens in foods of non-animal origin. Part I (outbreak data analysis and risk ranking of food/pathogen combinations). *EFSA Journal*, 11(1), 3025, 138 pp.

EFSA Panel on Biological Hazards (BIOHAZ), 2014a. Scientific Opinion on the risk posed by pathogens in foods of non-animal origin. Part 2 (*Salmonella* and Norovirus in berries). *EFSA Journal*, 12(6), 3706, 95 pp.

EFSA Panel on Biological Hazards (BIOHAZ), 2014b. Scientific Opinion on the risk posed by pathogens in foods of non-animal origin. Part 2 (*Salmonella* and Norovirus in tomatoes). *EFSA Journal*, 12(10), 3832, 75 pp.

Erickson, M.C. 2012. Internalization of fresh produce by foodborne pathogens. *Annual Review of Food Science and Technology*, 3(1), 283-310.

Flessa, S., Lusk, D.M. and Harris, L.J. 2005. Survival of *Listeria monocytogenes* on fresh and frozen strawberries. *International Journal of Food Microbiology*, 101(3), 255-262.

Fletcher, J., Leach, J.E., Eversole, K. and Tauxe, R. 2013. Human pathogens on plants: designing a multidisciplinary strategy for research. *Phytopathology*, 103(4), 306-315.

FSAI, 2001. Guidance Note 2: Guidance Note on the EU classification of food: Available at: www.fsai.ie/WorkArea/DownloadAsset.aspx?id=726 (last accessed Nov 2017).

FSAI, 2008. Food safety implications of land-spreading agricultural, municipal and industrial organic materials on agricultural land used for food production in Ireland. 184 pp.

FSAI, 2014a. Guidance Note 3: Guidelines for the interpretation of results of microbiological testing of ready-to-eat foods placed on the market (Revision 2). Available at: www.fsai.ie/publications_GN3_microbiological_limits/ (last accessed Nov 2017).

FSAI, 2014b. Guidance Note 27: Guidance Note on the enforcement of Commission Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs. Available at: https://www.fsai.ie/publications_GN27_2073/2005/ (last accessed Oct 2018).

FSAI, 2016. Guidance Note 31: Fresh produce safety in primary production in Ireland. Available at: https://www.fsai.ie/publications_GN31_fresh_produce/ (last accessed Oct 2018).

Han, Y., Linton, R.H., Nielsen, S.S. and Nelson, P.E. 2001. Reduction of *Listeria monocytogenes* on green peppers (*Capsicum annuum* L.) by gaseous and aqueous chlorine dioxide and water washing and its growth at 7 degrees C. *Journal of Food Protection*, 64(11):1730-1738.

Hanning, I.B., Nutt, J.D. and Ricke, S.C. (2009). *Salmonellosis* outbreaks in the United States due to fresh produce: sources and potential intervention measures. *Foodborne Pathogens and Disease*, 6(6), 635-648.

Hara-Kudo, Y., Konuma, H., Kamata, Y., Miyahara, M., Takatori, K., Onoue, Y., Sugita-Konishi, Y. and Ohnishi, T. 2013. Prevalence of the main food-borne pathogens in retail food under the national food surveillance system in Japan. *Food additives & contaminants. Part A, Chemistry, analysis, control, exposure & risk assessment*, 30(8), 1450-1458.

Hassan, R., Rounds, J., Sorenson, A., Leos, G., Concepción-Acevedo, J., Griswold, T., Tesfai, A., Blessington, T., Hardy, C. and Basler, C. 2017. Multistate Outbreak of *Salmonella* Anatum Infections Linked to Imported Hot Peppers — United States, May–July 2016. *Morbidity and Mortality Weekly Report*, 66:663–667.

Huff, K., Boyer, R., Denbow, C., O'keefe, S. and Williams, R. 2012. Effect of storage temperature on survival and growth of foodborne pathogens on whole, damaged, and internally inoculated jalapeños (*Capsicum annuum* var. *annuum*). *Journal of Food Protection* 75(2):382-388.

Ibarra-Sánchez, L.S., Alvarado-Casillas, S., Rodríguez-García, M.O., Martínez-Gonzáles, N.E. and Castillo, A. 2004. Internalization of bacterial pathogens in tomatoes and their control by selected chemicals. *Journal of Food Protection* 67(7):1353-1358.

International Commission on Microbiological Specifications for Foods (ICMSF). 1996. Microorganisms in Foods, Volume 5: Characteristics of Microbial Pathogens.

Irish Universities Nutrition Alliance (IUNA), 2011. National Adult Nutrition Survey. *Summary Report.* Available at: http://www.iuna.net/wp-content/uploads/2010/12/National-Adult-Nutrition-Survey-Summary-Report-March-2011.pdf (last accessed Nov 2017).

Johannessen, G.S., Loncarevic, S. and Kruse, H. 2002. Bacteriological analysis of fresh produce in Norway. *International Journal of Food Microbiology*, 77(3), 199-204.

Knudsen, D.M., Yamamoto, S.A. and Harris, L.J. 2001. Survival of *Salmonella* spp. and *Escherichia coli* O157:H7 on fresh and frozen strawberries. *Journal of Food Protection*, 64(10):1483-1488.

Laidler, M. R., Tourdjman, M., Buser, G. L., Hostetler, T., Repp, K. K., Leman, R., Samadpour, M. and Keene, W. E. 2013. *Escherichia coli* O157:H7 infections associated with consumption of locally grown strawberries contaminated by deer. Clinical Infectious Disease 57(8), 1129-1134.

Miller, B.D., Rigdon, C.E., Robinson, T.J., Hedberg, C. and Smith, K.E. 2013. Use of Global Trade Item Numbers in the Investigation of a *Salmonella* Newport Outbreak Associated with Blueberries in Minnesota, 2010. *Journal of Food Protection*, 76(5), 762-769.

Molinos, A.C., Abriouel, H., Ben Omar, N., Lucas, R., Valdivia, E. and Gálvez, A. 2008. Inactivation of *Listeria monocytogenes* in raw fruits by enterocin AS-48. *Journal of Food Protection*, 71(12):2460-2467.

Monaghan, J. and Hutchinson, M. (2009). Salads, slurry and irrigation: quantifying and minimising the risk. *Harper Adams University College*.

Mukherjee, A., Speh, D., Dyck, E. and Diez-Gonzalez, F. 2004. Preharvest evaluation of coliforms, *Escherichia coli*, *Salmonella*, and *Escherichia coli* O157:H7 in organic and conventional produce grown by Minnesota farmers. *Journal of Food Protection*, 67(5), 894-900.

Mukherjee, A., Speh, D., Jones, A.T., Buesing, K.M. and Diez-Gonzalez, F. 2006. Longitudinal microbiological survey of fresh produce grown by farmers in the upper midwest. *Journal of Food Protection*, 69(8), 1928-1936.

Müller, L., Kjelso, C., Frank, C., Jensen, T., Torpdahl, M., Soborg, B., Dorleans, F., Rabsch, W., Prager, R., Gossner, C.M. and Ethelberg, S. 2016. Outbreak of *Salmonella* Strathcona caused by datterino tomatoes, Denmark, 2011. *Epidemiology and Infection*. Feb 5:1-10.

Nguyen, T.P., Friedrich, L.M. and Danyluk, M.D. 2014. Fate of *Escherichia coli* O157:H7 and *Salmonella* on whole strawberries and blueberries of two maturities under different storage conditions. *Journal of Food Protection*, 77(7), 1093-1101.

Olaimat, A.N. and Holley, R.A. 2012. Factors influencing the microbial safety of fresh produce: a review. *Food Microbiology*, 32(1), 1-19.

Oliveira, M. Usall, J., Vinas, I., Anguera, M., Gatius, F. and Abadias, M. 2010. Microbiological quality of fresh lettuce from organic and conventional production. *Food Microbiology*, 27(5), 679-684.

Orozco, L., Rico-Romero, L. and Escartin, E.F. 2008b. Microbiological profile of greenhouses in a farm producing hydroponic tomatoes. *Journal of Food Protection*, 71(1), 60-65.

Orozco, R.L., Iturriaga, M.H., Tamplin, M.L., Fratamico, P.M., Call, J.E., Luchansky, J.B. and Escartin, E.F. 2008a. Animal and environmental impact on the presence and distribution of *Salmonella* and *Escherichia coli* in hydroponic tomato greenhouses. *Journal of Food Protection*, 71(4), 676-683.

Palumbo, M., Harris, L.J. and Danyluk, M.D. 2013. Outbreaks of foodborne illness associated with common berries, 1983 through May 2013. *IFAS Extension University of Florida*, FSHN13-08.

Reddy, S.P., Wang, H., Adams, J.K. and Feng, P.C.H. 2016. Prevalence and characteristics of *Salmonella* serotypes isolated from fresh produce marketed in the United States. *Journal of Food Protection*, 79(1), 6-16.

Rodgers, S.L., Cash, J.N., Siddiq, M. and Ryser, E.T. 2004. A comparison of different chemical sanitizers for inactivating *Escherichia coli* O157:H7 and *Listeria monocytogenes* in solution and on apples, lettuce, strawberries, and cantaloupe. *Journal of Food Protection*, 67(4), 721–731.

Sagoo, S.K., Little, C.L. and Mitchell, R.T. 2001. The microbiological examination of ready-to-eat organic vegetables from retail establishments in the United Kingdom. *Letters in Applied Microbiology*, 33(6), 434-439.

Tango, C.N., Choi, N.J., Chung, M.S. and Oh D.H. 2014. Bacteriological quality of vegetables from organic and conventional production in different areas of Korea. *Journal of Food Protection*, 77(8), 1411-1417.

U.S. FDA (United States Food and Drug Administration), 2001. FDA survey of imported produce FY 1999 Field Assignment. January 2001. Available at: http://wayback.archive-it.org/7993/20171114172351/https://www.fda.gov/Food/GuidanceRegulation/GuidanceDocuments RegulatoryInformation/ProducePlantProducts/ucm118891.htm (last accessed Oct 2018).

U.S. FDA (United States Food and Drug Administration), 2003. FDA Survey of Domestic Fresh Produce FY 2000/2001 Field Assignment. January 2003. Available at: http://wayback.archive-it.org/7993/20171114172340/https://www.fda.gov/Food/GuidanceRegulation/GuidanceDocuments RegulatoryInformation/ProducePlantProducts/ucm118306.htm (last accessed Oct 2018).

Udompijitkul, P., Daeschel, M.A. and Zhao, Y. 2007. Antimicrobial effect of electrolyzed oxidizing water against *Escherichia coli* O157:H7 and *Listeria monocytogenes* on fresh strawberries (*Fragaria×ananassa*). *Journal of Food Science*, 72(9), M397-406.

United States Department of Agriculture (USDA), 2014. USDA Microbiological data Program. Available at: http://www.ams.usda.gov/AMSv1.0/mdp (last accessed Nov 2017).

van Dyk, B.N., de Bruin, W., du Plessis, E.M. and Korsten, L. 2016. Microbiological food safety status of commercially produced tomatoes from production to marketing. *Journal of Food Protection*, 79(3), 392-406.

Yoon, Y., Kim, K., Nam, M., Shim, W.B., Ryu, J.G., Kim, D.H., You, O.J. and Chung, D.H. 2010. Microbiological assessment in strawberry production and recommendations to establish a good agricultural practice system. *Foodborne Pathogens and Disease*, 7(12), 1511-9.

Zhuang, R.-Y., Beuchat, L.R. and Angulo, F.J. 1995. Fate of *Salmonella* Montevideo on and in raw tomatoes as affected by temperature and treatment with chlorine. *Applied and Environmental Microbiology*, 61, 2127–2131.

Appendix 1: Examples of foodborne outbreaks associated with the consumption of RTE vegetables and fresh berries.

Year	Food type (and source if known)	Country	Microorganism	No. of outbreak cases	No. of hospitalisations	No. of deaths	Reference
2016	Hot Peppers (Anaheim variety)	USA	*Salmonella* Anatum	32	8	0	Hassan *et al*. 2017
2015	Tomatoes	USA	*Salmonella* Hartford	19	4	0	CDC, 2016
2015	Cucumbers (Grown in Mexico)	USA	*Salmonella* Poona	907	204	6*	CDC, 2016
2015	Tomatoes	USA	*Salmonella* Newport	119	17	2	CDC, 2016
2014	Cucumbers (Domestic produce)	USA	*Salmonella* Newport	275	48	1	Angelo *et al*. 2015
2013	Cucumbers (Grown in Mexico)	USA	*Salmonella* Saintpaul	84	17	0	CDC, 2013
2011	Strawberries (Domestic produce)	USA	*Escherichia coli* O157:H7	15	6	2	Laidler *et al*. 2013
2011	Datterino cherry tomatoes (Grown in Italy)	Multi-country	*Salmonella* Strathcona	43 Denmark 5 Italy 1 Austria 21 Germany 1 Belgium	20	1	Müller *et al*. 2016
2010	Blueberries (Domestic produce)	USA	*Salmonella* Newport	6	1	0	Miller *et al*. 2013
2009	Blueberries	USA	*Salmonella* Muenchen	14	Not reported	0	CDC, 2016; Palumbo *et al*. 2013
2008	Jalapeño peppers, Serrano peppers. Tomatoes were identified as a possible source early in the outbreak (Grown in Mexico)	USA	*Salmonella* Saintpaul	1,442	286	2	CDC, 2008
2006	Tomatoes	USA	*Salmonella* Typhimurium	183	22	0	CDC, 2006

* *Salmonella* infection was not considered to be a contributing factor in 2 of these death

Appendix 2: Studies on the prevalence of *Salmonella, E. coli* and *Listeria monocytogenes* in tomatoes, cucumbers, peppers and fresh berries.

Year	Country	Food type	Microorganism (type of analytical test)	No. of positives/No. of samples tested (% Prevalence) (detection limit of the test)	Reference
2012-2014	South Africa (Domestic produce)	Tomatoes	*Salmonella* (Detection)	0/905 (0%)	van Dyk *et al*. 2016
			E. coli (Enumeration)	0/905 (0%) (10 CFU/g)	
2009-2013	Canada (Domestic and imported produce)	Tomatoes	*Salmonella* (Detection)	0/4416 (0%)	Denis *et al*. 2016
			E. coli (Enumeration)	0/4837 (0%) (100 CFU or MPN/g)	
			E. coli O157:H7 (Detection)	0/2047 (0%)	
		Berries (blueberries, strawberries, blackberries, raspberries, other)	*Salmonella* (Detection)	0/1370 (0%)	
			E. coli (Enumeration)	0/1776 (0%) (100 CFU or MPN/g)	
			E. coli O157:H7 (Detection)	0/1373 (0%)	
2002-2012	USA (Domestic and imported produce)	Tomatoes	*Salmonella* (Detection, PCR-based method)	6/32,228 (0.02%)	Reddy *et al*. 2016
		Hot peppers		21/8,123 (0.26%)	
2010-2011	Mexico (Domestic produce)	Tomatoes	*Salmonella* (Detection)	1/80 (1.25%)	Cárdenas *et al*. 2013
		Peppers (serrano and jalapeño)		1/80 (1.25%)[1]	
2008	South Korea (Domestic produce)	Strawberries	*Salmonella* (Enumeration)	0/36 (0%) (1 log CFU/100 cm^2)	Yoon *et al*. 2010
			E. coli (Enumeration)	0/36 (0%) (1 log CFU/100 cm^2)	
2007	Canada (Domestic produce)	Tomatoes	*Salmonella* (Detection)	0/120 (0%)	Bohaychuk *et al*. 2009
			E. coli (Detection)	0/120 (0%)	
		Strawberries	*Salmonella* (Detection)	0/31 (0%)	
			E. coli (Detection)	0/31 (0%)	
2004	USA (Domestic produce)	Tomatoes	*Salmonella* (Detection)	1/141 (0.7%)	Arthur *et al*. 2007
			E. coli (Detection)	0/141 (0%)	
2003-2004	Mexico (Domestic produce)	Tomatoes	*Salmonella* (Detection)	66/906 (7.3%)	Orozco *et al*. 2008a[2]
			E. coli (Detection)	166/484 (34.3%)	
2003-2004	USA (Domestic produce)	Berries (included strawberries, raspberries and blueberries)	*Salmonella* (Detection)	0/194 (0%)	Mukherjee *et al*. 2006
			E. coli (Enumeration)	2/194 (1%) (10 CFU/g)	
		Peppers (included Bell and other varieties)	*Salmonella* (Detection)	0/282 (0%)	
			E. coli (Enumeration)	2/282 (0.7%) (10 CFU/g)	
		Tomatoes	*Salmonella* (Detection)	0/238 (0%)	
			E. coli (Enumeration)	0/238 (0%) (10 CFU/g)	
		Cucumbers	*Salmonella* (Detection)	0/179 (0%)	

			E. coli (Enumeration)	6/179 (3.4%) (10 CFU/g)	
2002	USA (Domestic produce)	Strawberries	*Salmonella* (Detection)	0/11 (0%)	Mukherjee *et al*. 2004
			E. coli (Detection)	0/11 (0%)	
		Green peppers	*Salmonella* (Detection)	1/55 (1.8%)	
			E. coli (Detection)	4/55 (7.3%)	
		Tomatoes	*Salmonella* (Detection)	0/108 (0%)	
			E. coli (Detection)	5/108 (4.6%)	
		Cucumbers	*Salmonella* (Detection)	0/40 (0%)	
			E. coli (Detection)	2/40 (5%)	
2000	USA (Domestic produce)	Strawberries	*Salmonella* (Detection)	0/136 (0%)	U.S. FDA, 2003
		Tomatoes		0/198 (0%)	
2000	United Kingdom (Mix of domestic (30%) and imported (70%) organic produce)	Cucumber	*Salmonella* (Detection)	0/221 (0%)	Sagoo *et al*. 2001[3]
			L. monocytogenes (Enumeration)	0/221 (0%) (20 CFU/g)	
		Pepper	*Salmonella* (Detection)	0/184 (0%)	
			L. monocytogenes (Enumeration)	0/184 (0%) (20 CFU/g)	
		Tomato	*Salmonella* (Detection)	0/428 (0%)	
			L. monocytogenes (Enumeration)	0/428 (0%) (20 CFU/g)	
1999-2003	Mexico (Domestic produce)	Tomatoes	*Salmonella* (Detection)	19/681 (2.8%)	Orozco *et al*. 2008b
			E. coli (Enumeration)	5/681 (0.7%) (2.84 MPN/g)	
1999-2001	Norway (Domestic n=94; Imported n=77, Unknown n=2)	Strawberries	*Salmonella* (Detection)	0/173 (0%)	Johannessen *et al*. 2002
			L. monocytogenes (Detection)	1/173 (0.6%)[4]	
1999	USA (Imports from Argentina, Belgium, Canada, Mexico & New Zealand)	Strawberries	*Salmonella* (Detection)	1/143 (0.7%)	U.S. FDA, 2001
		Tomatoes		0/20 (0%)	
1998-2008	Japan (Domestic & imported produce)	Tomatoes	*Salmonella* (Detection)	1/1140 (0.1%)	Hara-Kudo *et al*. 2013
			E. coli (Detection)	24/1140 (2.1%)	
		Cucumber	*Salmonella*	2/1315 (0.2%)	
			E. coli (Detection)	78/1315 (5.9 %)	

CFU/g: Colony forming unit per gram
MPN: Most Probable Number
[1] Note: pepper contamination was found in the stem, which is usually removed prior to consumption.
[2] During the course of this study, two independent natural events affected the farm: water runoff flooded some of the greenhouses and wild animals (opossums, mice and sparrows) gained entry to several of the greenhouses.
[3] *E. coli* was detected in 48 of 3200 of RTE organic vegetables tested in this study; however, the authors do not specify which sample types the microorganism was detected in. *Listeria* spp. (n=6) was detected in a sample of watercress, a sample of radish and four other unspecified samples of RTE organic vegetables.
[4] Domestically produced strawberry.

Appendix 3: Survey questionnaire

EHOs and staff from the DAFM Horticulture and Plant Health Division were asked to complete this survey questionnaire for each sample collected.

1) Name ___________________________________

2) Sample Ref Number ________________________

3) Select Laboratory to which sample was sent for analysis

Cherry Orchard □ Cork □ Galway □ Limerick □ Sligo□ Sir Patrick Dun □ Waterford □

4) Laboratory Reference Number (upon receipt of lab report) __________________

5) Country of origin: Ireland □ Other □ Specify: _____________________________

6) Select the type of fresh whole unprocessed pre-packaged fruit or vegetable sampled

Cucumber □ Pepper □ Tomato □ Blueberry □ Strawberry □
Blackberry □ Raspberry □ Other□ Specify_________________________________

Specify the type of peppers sampled (tick all that apply):

Red bell □ Yellow bell □ Green bell □ Mixed colours □ Baby □
Other □ Specify____

Specify the type of tomatoes sampled (tick all that apply):

Tomatoes:
Salad □
Vine □
Plum □
Beef □
Yellow □
Cherry □
Baby plum □
Yellow/Orange cherry □
Piccolo cherry □
Other □ Specify_________________________

7) Was the sample bruised or damaged in some way? Yes □ No □

To submit questionnaire to FSAI click on the "**Submit Form**" button.

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