New Zealand Food Safety
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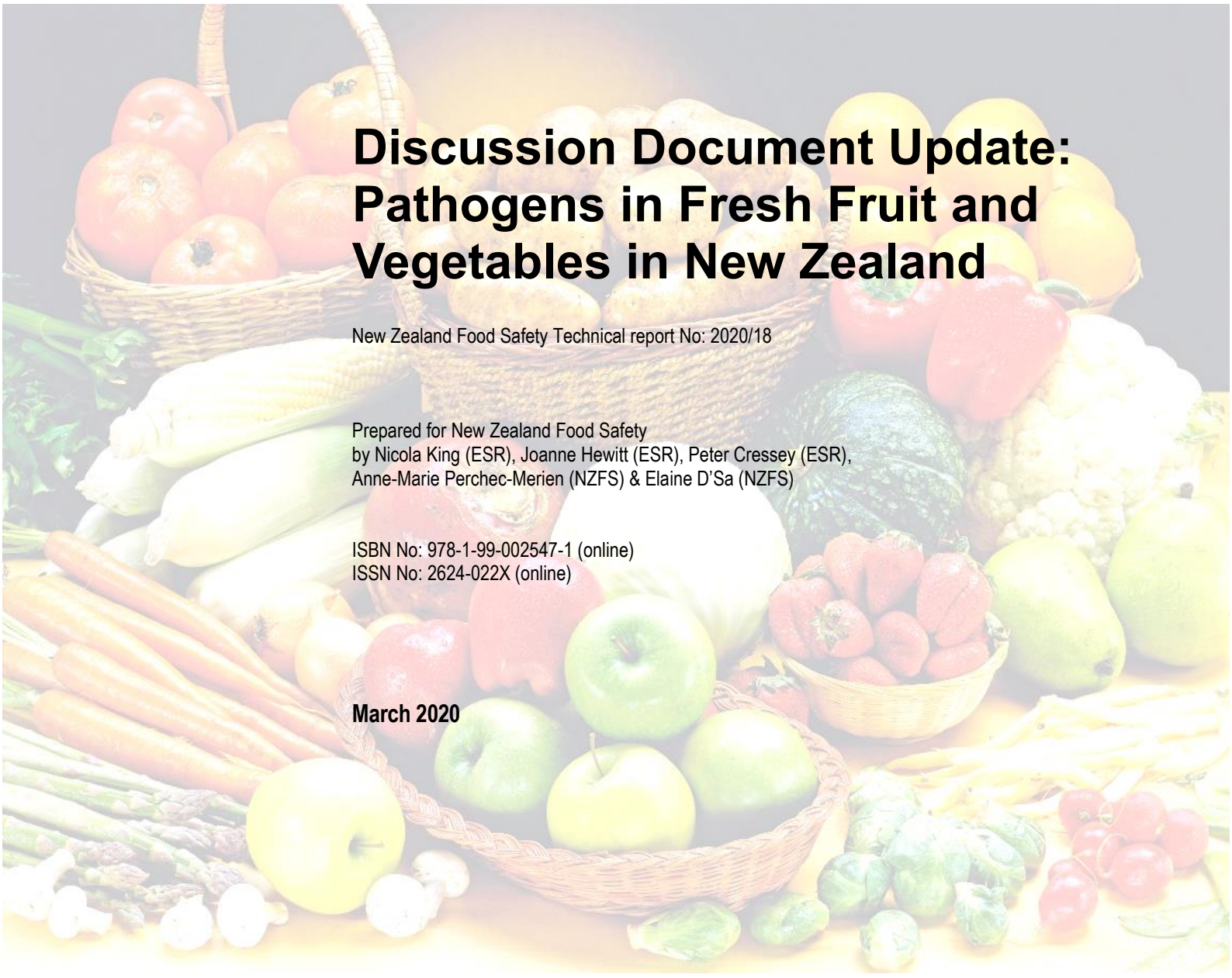

Discussion Document Update: Pathogens in Fresh Fruit and Vegetables in New Zealand

New Zealand Food Safety Technical report No: 2020/18

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Scientific Interpretative Summary

This SIS is prepared by New Zealand Food Safety (NZFS) risk assessors to provide context to the following report for NZFS risk managers and external readers.

Discussion document: Foodborne pathogens in fruit and vegetables, update. July 2015

This document is an update of the 2008 discussion document on pathogens in produce. It reports a review of information published since 2008 and reassesses the human health risks in New Zealand associated with foodborne pathogens in fresh, ready-to-eat (RTE) whole and fresh-cut vegetables and fruits (including fresh herbs), raw frozen berries, fresh juices and sprouted seeds (sprouts).

As in 2008, it is likely that outbreaks associated with produce occur more frequently than the available data suggest because identifying the vehicle is challenging (traceback, analytical methods, short shelf life of foods). Case- control studies do not indicate an elevated risk from fresh fruit and vegetables.

The most important remaining data gaps are:

- Dynamics of internalised pathogens in vegetables and their viability from harvest to consumption,
- Prevalence and concentration of enteropathogenic *Yersinia*, *Aeromonas* spp., Norovirus, Hepatitis A and E viruses, Rotavirus, and protozoan parasites in fresh produce and water,
- Kinetics of sanitisers against the most common foodborne pathogens in produce.

Five microbiological surveys completed in New Zealand since 2008 indicate that only a limited number of pathogenic microorganisms were detected on fresh fruits and vegetables and only at low concentrations: *Salmonella* spp. on leafy greens and sprouts, norovirus GII on RTE leafy salad, *Listeria monocytogenes* on fresh-cut fruit salads, sprouts and RTE coleslaw.

Experiments and outbreaks show that the main risk factors of microbial contamination are contact with animal and/or human faeces, either directly or indirectly (e.g. via soil, water, inadequately treated compost), fresh produce workers (harvesters, food handlers), equipment and surfaces. Data showing that hydroponically grown produce internalise more pathogens and the occurrence of the 2016 Havelock North *Campylobacter* outbreak suggest that water could be an important source of contamination. Measures implemented by growers and food handlers to prevent faecal contamination and the use of contaminated water will reduce the risk of contamination by pathogenic microorganisms.

Pathogenic microorganisms could survive on produce when exposed to normal field conditions, but desiccation, UV radiation, or microbial competition will contribute to their death and cooler temperatures will prevent or slow down their growth.

Some pathogenic microorganisms can become internalised and consequently will be better protected from sanitisers. Similarly, bacterial pathogens can survive in the viable but non-culturable (VBNC) state on fresh produce. However there is little evidence that VBNC organisms can cause human illness.

Since 2008, three outbreaks have been associated with fresh produce based on epidemiological information but with no microbiological evidence (salmonellosis/watermelon, norovirus/fresh fruit salad, yersiniosis/carrots and/or lettuces).

This report clarifies previous issues and identifies the most critical combinations for RTE fresh produce available in New Zealand and pathogens of concern, which are leafy green

vegetables (lettuce, spinach, cabbage, etc.), sprouts, fresh-cut fruit salads, berries, watermelons, carrots, tomatoes and fresh herbs contaminated with *Salmonella* spp., Norovirus, STEC, *Yersinia* spp., Hepatitis A virus and *L. monocytogenes*. *Aeromonas* spp., Rotavirus, and protozoan parasites (*Cryptosporidium* spp., *Toxoplasma gondii*) can be potentially important pathogens too.

Information regarding the microbiological safety of fresh juices produced in New Zealand is scarce, and survival or slow growth of pathogenic microorganisms in fresh juices, especially at room temperature, should be investigated.

Sprout contamination occurrence is a combination of seed contamination, bacterial growth favoured by sprout germination conditions and survival on sprouts stored at cooler temperatures.

Although they are often grown in soils amended with organic wastes, there is no evidence that organically produced fruits and vegetables are any more contaminated by pathogenic microorganisms than those conventionally produced. A review of organic assurance programmes found that these did not provide much support for growers to manage food safety risks.

Under the Food Act 2014, horticultural producers and manufacturers of fresh RTE salads are subject to one of three risk-based control measures, depending on the activities they undertake. There are mandatory microbiological standards for *Salmonella* spp. on sprouts, and *L. monocytogenes* on RTE foods such as fresh-cut and packaged fruit and vegetables. Acidic dressings make a less favourable environment for bacterial growth in salads. However, specific ingredients such as raw nuts can introduce microorganisms, particularly *Salmonella* spp, and the presence of animal proteins can promote pathogen survival and growth.

UPDATE: DISCUSSION DOCUMENT ON PATHOGENS IN FRESH FRUITS AND VEGETABLES IN NEW ZEALAND

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We also thank FSANZ for provision of consumer recall data.

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DISCLAIMER

ABBREVIATIONS

ANS	The 2009 Adult Nutrition Survey
aOR	Adjusted odds ratio (multivariate analysis)
CFU	Colony forming unit
CI	Confidence interval
CNS	The 2002 National Childrens' Nutrition Survey
DNA	Deoxyribose nucleic acid
EFSA	European Food Safety Authority
EU	European Union
FSANZ	Food Standards Australia New Zealand
GAP	Good Agricultural Practices
GHP	Good Hygienic Practices
GMP	Good Manufacturing Practices
HACCP	Hazard analysis and critical control points
HAV	Hepatitis A virus
HUS	Haemolytic uraemic syndrome
MAF	Ministry of Agriculture and Forestry (New Zealand)*
MAP	Modified atmosphere packaging
MPI	Ministry for Primary Industries (New Zealand)*
MPN	Most probable number
NNS	The 1997 National Nutrition Survey
NZFSA	New Zealand Food Safety Authority
OR	Odds ratio (univariate analysis)
PCR	Polymerase chain reaction
pH	Measure of acidity (min. = 0 = most acidic; max. = 14)
RTE	Ready-to-eat
STEC	Shiga toxin-producing (or shigatoxigenic) *E. coli* (synonym = VTEC)
USFDA	United States Food and Drug Administration
VBNC	Viable but non-culturable

* On 1 July 2010, NZFSA and MAF were amalgamated. On 30 April 2012, MAF was renamed as MPI. This document uses the names NZFSA and MAF for documents produced during the existence of these organisations.

CONTENTS

LIST OF TABLES

SUMMARY

This document is an update of an earlier discussion document considering human pathogens in fruit and vegetables, published in 2008.[1] This update critically reviews information published since 2008 to reassess the human health risks in New Zealand associated with foodborne microbiological pathogens in fresh, ready-to-eat (RTE) whole and fresh-cut vegetables and fruits (including fresh herbs), raw frozen berries, fresh juices and sprouted seeds (sprouts). This update also considers new information on organic and hydroponically grown vegetables, and the role of salad 'additives' (dressings, nuts, animal proteins) in changing risk.

The foodborne pathogens considered in this update are *Aeromonas* spp., *Bacillus cereus*, *Campylobacter* spp. (focussing on *C. jejuni* and *C. coli*), *Clostridium botulinum*, *Clostridium perfringens*, Shiga toxin-producing *Escherichia coli* (STEC, including *Escherichia coli* O157:H7), *Listeria monocytogenes*, non-typhoidal serotypes of *Salmonella enterica* ssp. *enterica*, *Shigella* spp., *Staphylococcus aureus*, *Yersinia* spp. (focussing on *Y. enterocolitica* and *Y. pseudotuberculosis*), *Cryptosporidium parvum*, *Giardia duodenalis*, *Cyclospora cayetanensis*, *Toxoplasma gondii*, hepatitis A virus (HAV), human norovirus and rotavirus.

Contact with animal and/or human faeces, either directly or indirectly (e.g. via soil, water, inadequately treated compost) is the most important risk factor for fresh fruit and vegetables becoming contaminated with pathogenic microorganisms. Soils amended with organic wastes, including manures, sludges and composts, can be a source of microbial pathogens, but their survival in these soils is highly variable. The transfer of pathogenic bacteria from soil to plants has been demonstrated under experimental conditions but these usually represent "worst-case" scenarios and transfer under standard practices is less well substantiated. There is no evidence that organically produced fruits and vegetables are more significantly contaminated by pathogenic microorganisms than those conventionally produced.

Experiments have shown that contaminated water can introduce pathogenic microorganisms onto fresh produce, particularly when the water is sprayed onto the plants when irrigating or applying agrichemicals. There is also limited evidence from outbreak investigations that supports water as a source of contamination. Evidence from experiments and outbreaks also show that fresh produce workers (harvesters, food handlers), equipment and surfaces are important sources of microbiological contamination.

A small survey of New Zealand fruit and vegetable growers identified some practices that increased the risk of fresh produce being contaminated with pathogenic microorganisms, such as applying untreated animal-based fertilisers close to or at crop planting, or using water of uncertain quality for irrigation or post-harvest washing.

Viruses and parasites pathogenic to humans cannot replicate outside a host so will not multiply on any foods, but can survive. Bacteria may multiply (grow) if conditions are suitable. The few field studies that have been done indicate that pathogenic microorganisms on growing plants or fruits could survive for a month or more when exposed to normal field conditions. Dessication, UV radiation from sunlight, or competition with commensal microbiota can all contribute to the death of pathogenic bacteria under field conditions.

Pathogenic microorganisms can move from the outside to the inside of fruit and vegetable plants and fruits, in a process called internalisation. There is good evidence to support internalisation, at least for some pathogenic bacteria, which is of concern because internalised

[1] McIntyre L, Cressey P and Lake R (2008) Discussion document on pathogens in fruits and vegetables in New Zealand. Institute of Environmental Science and Research, Christchurch. Available from: http://www.foodsafety.govt.nz/elibrary/industry/discussion-document-pathogens-research-projects/index.htm (accessed: 17 March 2015).

pathogens are better protected from sanitisers. But there is still uncertainty around the extent of internalisation under normal horticulture production and the length of time pathogenic microorganisms can remain viable inside a food plant. Similarly, there is currently insufficient evidence to support the existence of bacterial pathogens in the viable but non-culturable (VBNC) state on fresh produce under normal horticulture production, or to support them as an undetected cause of human illness. Prolonged survival in fresh water or in biofilms can induce the VBNC state so there is potential for them to be present in irrigation or wash water.

After harvest, fresh, RTE fruits and vegetables can provide a suitable substrate for bacterial growth, particularly if they are cut into pieces. The ability of a pathogenic microorganism to survive or multiply on raw produce (whole or cut) depends on the characteristics of the pathogen and the food, other microflora present on the food and the environment the food is in. Data from recent studies and recent reviews of older studies show that the behaviour of pathogenic bacteria on harvested produce is difficult to predict, but cooler temperatures do prevent growth or slow it down. Most studies have focused on *Salmonella* spp., *L. monocytogenes* and STEC. Data for other pathogenic bacteria, and pathogenic protozoa and viruses, are limited. Where data are available, these show survival (or growth, for some bacteria), except for *Campylobacter* spp.

Fresh juices are juices of fruit and/or vegetables that have not been treated by pasteurisation or any other antimicrobial treatment (e.g. high pressure processing). Such juices are usually sold through juice bars and other food service outlets. There is a higher risk of fresh juices becoming contaminated with pathogenic microorganisms if fallen fruit are used. Fresh juices provide an excellent supply of water and nutrients that favour bacterial growth. The behaviour of pathogenic microorganisms in fresh juices depends on the microbiological species, the ingredients of the juice and the resulting characteristics (e.g. pH, presence of natural antimicrobials and/or other microflora), plus the storage time/temperature before consumption. Recent studies (mostly focussing on *L. monocytogenes* or viral pathogens) demonstrate survival or growth of pathogenic microorganisms in fresh juices. However, most experiments were conducted at low temperatures (≤10ºC) and any bacterial growth measured at these temperatures was slow. The potential for growth at room temperature over a 24-hour period (a more likely scenario for fresh juices) has not been well studied.

The most common cause of sprout contamination is outgrowth of microorganisms from contaminated seeds. The germination conditions for sprouts are highly favourable for bacterial growth. Evidence from experiments and outbreaks prove that pathogenic microorganisms (chiefly bacteria and protozoa) can contaminate and grow on sprouts. Recent studies showed that survival and growth of bacterial pathogens were enhanced by warmer temperatures, and when the seeds were the source of contamination rather than contamination occurring after sprouting. Microbial pathogens can survive well on sprouts stored at cooler temperatures.

The presence of a salad dressing, which is typically acidic, makes the salad a less favourable environment for bacterial growth. A few recent studies reported death of pathogenic bacteria in dressed salads. However, the presence of animal proteins (e.g. poultry, seafood, egg) will promote survival or even growth of *L. monocytogenes* in a dressed salad. Raw nuts, if contaminated, can introduce microorganisms to salads, particularly *Salmonella* spp.

Five microbiological surveys of fresh produce have been completed in New Zealand since 2008. The results indicate that pathogenic microorganisms can be found on these products, but the prevalence and concentrations are low. *Salmonella* spp. were detected on 2% of 891 samples of fresh fruits and vegetables, *E. coli* O157 and *Campylobacter* spp. were not detected. *L. monocytogenes*, *Salmonella* spp. and *Campylobacter* spp. were not detected in 307 samples of pre-packaged RTE leafy salads, but norovirus GII was detected in three samples. *L. monocytogenes* was detected in 5% of 75 samples of fresh-cut fruit salads (concentration <100 CFU/g). *Salmonella* spp. and *L. monocytogenes* were each detected in

2% of 50 sprout samples at low concentrations (≤0.04 MPN/g, <100 CFU/g, respectively). *L. monocytogenes* was detected in 13% of RTE coleslaw samples with dressing.

Recalls for fresh produce are rare in New Zealand; only three recalls have been issued since 2008, for spinach and parsley potentially contaminated with *L. monocytogenes*, and for pipfruit potentially contaminated with HAV.

Fresh produce has been a vehicle of infection in outbreaks of enteric disease in New Zealand but the available public health surveillance data do not provide enough information to determine the frequency of illness caused by fruits, vegetables, sprouts or fresh juices. Since 2008 there have been three outbreaks where fresh produce was the most likely vehicle for infection based on epidemiological information (salmonellosis/watermelon, norovirus/fresh fruit salad, yersiniosis/carrots and/or lettuces). There has only been one outbreak confirmed by microbiological evidence (i.e. same outbreak strain found in food and cases) since this is difficult to achieve for fresh produce outbreaks. This was a 2002 outbreak of Hepatitis A caused by domestically-grown blueberries.

From the information available from New Zealand and other countries, the pathogens can be categorised with respect to RTE fresh produce available in New Zealand:

- The pathogens considered to be of most concern are *Salmonella* spp., norovirus, STEC, *Yersinia* spp., HAV and *L. monocytogenes*.
- The pathogens considered to be of less concern are *B. cereus*, *Campylobacter* spp., *C. botulinum*, *C. perfringens*, *S. aureus* and *Shigella* spp.
- The pathogens that are potentially important, but for which there is currently scarce evidence, are *Aeromonas* spp., the protozoan parasites *C. parvum*, *G. duodenalis*, *C. cayetanensis* and *T. gondii*, and rotavirus.

The RTE fresh produce commodities available in New Zealand that are of most concern for their potential for contamination with pathogenic microorganisms, are leafy green vegetables (lettuce, spinach, cabbage, etc.), sprouts, fresh-cut fruit salads, berries, watermelons, carrots, tomatoes and fresh herbs. There is still very little information to evaluate the microbiological safety of fresh juices produced in New Zealand.

The absence of a pathogen elimination step for fresh fruit and vegetables means that measures for reducing the risk of contamination from pathogenic microorganisms must be implemented by the grower in the first instance, then by subsequent food handlers. There are no on-farm practices that can guarantee fresh produce will be free from pathogenic microorganisms but there are practices that will reduce opportunities for contamination. Strategies that prevent faecal contamination are priorities, and this includes preventing use of contaminated water supplies.

The *Food Act 2014* will be fully in force by 1 March 2016 and will replace the *Food Act 1981*, the Food Hygiene Regulations 1974 and the Food (Safety) Regulations 2002. Under the new Act, horticultural producers and manufacturers of fresh RTE salads will be subject to one of three risk-based control measures, depending on the activities they undertake. This is the first New Zealand regulation that applies during the growing of fresh produce. The Food Handler Guidance and National Programmes are still under development. Registered Food Safety Programmes will be recognised as Food Control Plans under the *Food Act 2014* provided they meet certain conditions. There are already mandatory microbiological standards for *Salmonella* spp. on sprouts, and *L. monocytogenes* on RTE foods such as fresh-cut and packaged fruit and vegetables. The Ministry for Primary Industries will propose additional microbiological standards as part of administrating the *Food Act 2014* if these are considered necessary.

1. INTRODUCTION

This document updates a discussion document considering human pathogens in fruit and vegetables, completed in 2008 (hereafter referred to as the '2008 Document') (McIntyre *et al.*, 2008). Like the 2008 Document, this update adopts the general structure of a Risk Profile, but reflects ESR's current format for Risk Profiles, so some differences in layout will be noticed.

This update will critically review information published since 2008 to reassess the risks in New Zealand associated with foodborne pathogens in fresh, ready-to-eat (RTE) whole and fresh-cut vegetables and fruits (including fresh herbs), raw frozen berries, fresh (unpasteurised) juices and sprouted seeds (sprouts). This update will also consider new information on organic and hydroponically grown vegetables, and the role of salad 'additives' (dressings, nuts, animal proteins) in changing risk.

This is not a stand-alone document and readers are referred to the 2008 discussion document which can be accessed from: http://www.foodsafety.govt.nz/elibrary/industry/discussion-document-pathogens-research-projects/index.htm (accessed 30 June 2015).

1.1 SCOPE: FOODBORNE PATHOGENS

The foodborne pathogens considered in this update are almost the same as those listed in the 2008 Document. These are:

- Bacteria: *Aeromonas* spp., *Bacillus cereus*, *Campylobacter* spp. (focussing on *C. jejuni* and *C. coli*), *Clostridium botulinum*, *Clostridium perfringens*, Shiga toxin-producing *Escherichia coli* (STEC, including *Escherichia coli* O157:H7), *Listeria monocytogenes*, non-typhoidal serotypes of *Salmonella enterica* ssp. *enterica*, *Shigella* spp., *Staphylococcus aureus* and *Yersinia* spp. (focussing on *Y. enterocolitica* and *Y. pseudotuberculosis*).
- Protozoan parasites: *Cryptosporidium parvum*, *Giardia duodenalis*, *Cyclospora cayetanensis* and *Toxoplasma gondii*.
- Enteric viruses: Hepatitis A virus (HAV), human norovirus and rotavirus.

The 2008 Document only considered *E. coli* O157:H7 and not the wider STEC group of pathogenic *E. coli*. STEC (synonymous with VTEC) encompasses all *E. coli* carrying one or more of the *stx* toxin genes, including *stx*-positive non-O157 serotypes.

Note that in this document "norovirus" refers only to human norovirus (unless otherwise specified), and *Giardia lamblia* is referred to by the more accepted synonym *Giardia duodenalis* (also synonymous with *Giardia intestinalis*). The typhoidal serotypes of *Salmonella* spp. are not considered in this update, but some information on these has been included in Appendix C.3.

1.2 SCOPE: FOODS

RTE fruit includes fresh (not blanched, cooked, dried) whole or cut fruits that can be eaten without any further preparation, although the consumer may choose to wash whole fruit in water. Examples include berries, pipfruit, stonefruit, fruit salad and tomatoes. Melons, mangoes and pineapples are also included as these are often sold in a RTE form (e.g. halved/sliced in supermarkets, diced in fruit salads). Melons grow in contact with soil and have caused gastroenteritis outbreaks in many countries. Raw, frozen berries are also included. The scope does not include fruits that require peeling, such as citrus, kiwifruit and bananas, since the act of peeling will largely remove external contaminants. However, these

fruits may also be served fresh-cut (e.g. as part of fruit salads) and the edible flesh can become contaminated during peeling and cutting in the same way as tropical fruit, so while the focus is on RTE fruit, some information from these other fruits has been included where it was located as part of data collation.

Similarly, RTE vegetables include fresh (not blanched, cooked, dried) whole vegetables ready for consumption (e.g. leafy green vegetables, celery, broccoli, mushrooms, carrots),[2] plus those that have been minimally prepared, e.g. shredded cabbage, salad mixes such as packaged salads, sliced or baby raw carrots. Vegetables that are cooked before eating are not considered, e.g. potatoes, kumara and aubergine. While there is a risk of cross-contamination in the kitchen from root vegetables such as potatoes, the cooking step kills any pathogenic microorganisms that might be present on the vegetables.[3]

Fresh juices are juices of fruit and/or vegetables that have not been treated by pasteurisation or any other antimicrobial treatment (e.g. high pressure processing). Refrigerated juices sold in supermarkets are usually pasteurised or pressure-treated to extend shelf-life, so this document only considers fresh juices sold through juice bars and other food service outlets.

Sprouts are the emergent plantlets germinated from a variety of seeds, including alfalfa, mung bean, chickpea, radish, broccoli, soybean and sunflower.

[2] Broccoli and mushrooms are included as examples of vegetables that are often cooked, but also consumed raw. Carrots may or may not be peeled by consumers. Unpeeled, well-washed carrots are increasingly common at retail.

[3] Handling raw leeks and potatoes in the kitchen and cross-contamination from these vegetables was the likely cause of an eight month outbreak of *E. coli* O157 infection in the UK during 2010/11 (Launders *et al.* 2015). There were 252 cases including 80 hospitalisations, two HUS cases and one death.

2. HAZARD AND FOOD

2.1 THE PATHOGENS

Appendix A.1 contains additional information on the pathogens.

Key findings

Fruits and vegetables can be contaminated by any of the pathogens considered in this document since these pathogens are present in soil, contaminated water and/or faeces (human or animal) that may come into contact with the produce. Some pathogens can also be excreted by asymptomatic people and transmission to fresh produce can occur when these people handle fresh produce. Most pathogens are inactivated in adequately prepared compost, but the variable nature of compost production means survival is possible.

The 2008 Document provides a brief summary of information about each of the pathogens of most concern in fresh produce, in a New Zealand horticultural context. To better understand the potential for each of these pathogens to contaminate fresh produce, data on their detection and survival in horticultural inputs were assembled (see Appendix A.1), focussing on New Zealand data where available. TABLE 1 summarises the findings.

It is clear from this table that most of the pathogens considered in this document may be introduced to fresh produce from the soil, surface water or direct or indirect contact with human or animal faeces. Some pathogens (*Aeromonas* spp., *Campylobacter* spp.) have also been detected in potable water in New Zealand but, other than *Aeromonas* spp. which is a natural inhabitant of freshwater and can often be isolated from drinking water, the presence of pathogenic microorganisms in New Zealand drinking water is usually a result of failed water quality controls. While symptomatic workers are an obvious risk factor for contamination, many of the pathogens can also be excreted by asymptomatic people (sometimes without a recent episode of illness), so fresh produce handlers who do not adhere to good hand hygiene may transfer pathogens onto the foods.

In New Zealand, the standard for adequate thermal treatment for compost is 55-76°C for at least three consecutive days (or equivalent) (Standards New Zealand, 2003; 2005). The initial hot composting may be followed by a period of maturation, where temperatures remain steady below 45°C. While most of the pathogens should not survive adequate composting, composts vary greatly in their inputs and production and survival is possible, e.g. *Aeromonas* spp. have been isolated from various composts (Lim *et al.*, 2014; Priyadarshini *et al.*, 2012). In a recent summary of studies on bacterial pathogen survival during the composting of animal manure, it was evident that the period of pathogen survival in compost is highly variable and subject to a number of factors. These included internal temperature, type of animal manure, carbon:nitrogen (C:N) ratio, pH, moisture, size of heaps, ambient temperature, frequency of turning and initial numbers of pathogens (Jiang and Shepherd, 2009; Lake *et al.*, 2011b). Bacterial pathogens were particularly able to survive in the surface of compost heaps where temperatures were cooler.

TABLE 1: The potential for pathogens to contaminate horticultural products as indicated by their presence in horticultural inputs

Key to table:
- ✓ present in this source
- × not present in this source
- NZ present in this source and isolated in New Zealand from this source
- NI no information located

PATHOGEN	SOIL[1]	SURFACE WATER[1]	ANIMAL FAECES	COMPOST[2]	ASYMPTOMATIC HUMANS
Aeromonas spp.	✓	✓	✓	✓	✓ (<1%)
B. cereus	✓	✓	✓	✓	✓ (uncommon)
Campylobacter spp.	✓	NZ	NZ	×	✓ (uncommon)
C. botulinum	✓	✓	✓	✓	✓ (uncommon)
C. perfringens	✓	NZ	✓	✓	✓ (uncommon)
L. monocytogenes	✓	✓	✓	×	✓ (0.6-3.4%)
Salmonella spp.	✓	NZ	NZ	×	✓ (0.5%)
Shigella spp.	✓[3]	✓	×	×[4]	×[5]
S. aureus	✓	✓	✓	×	✓ (20-30%)
STEC	✓	✓	NZ	×	✓ (uncommon)
Yersinia spp.	✓	✓	NZ	×	✓ (1%)
C. parvum	✓	NZ[6]	NZ[6]	×	✓ (0.4%)
G. duodenalis	✓	NZ[6]	NZ[6]	×	✓ (1.6%)
C. cayetanensis	✓	✓	×	NI	✓ (uncommon)
T. gondii	✓	✓	(cats only)	NI	×
HAV	NI	✓	×	×	✓
Norovirus[7]	NI	NZ[3]	×	×	✓
Rotavirus	NI	NZ[3]	✓	NI	✓

1. For most of these pathogens, their presence in soil or surface water is as a result of being introduced from faecal matter directly or indirectly (e.g. run-off, wastewater outfall).
2 That has undergone adequate thermal treatment.
3 Detection by PCR.
4 Human biowaste.
5 Shigellosis is not widespread in New Zealand so it would not be expected to be excreted by asymptomatic humans in this country.
6 Only identified to species level, i.e. *Cryptosporidium* spp. or *Giardia* spp.
7 Noroviruses are classified into at least six genogroups (GI – GVI) and some genogroups are further divided into genotypes (for example GII.4). See Appendix A.1.17 for additional details.

2.2 THE FOODS: RTE FRUIT AND VEGETABLES, FRESH JUICES, SPROUTS

Key findings

RTE fruit and vegetables can be eaten without further preparation, as can sprouts. Fresh juices are prepared without any microbial kill steps such as pasteurisation.

RTE fruit and vegetables can provide a suitable substrate for bacterial growth, particularly if they are cut. Fresh juices provide an excellent supply of water and nutrients that favour bacterial growth. The germination conditions for sprouts are highly favourable for bacterial growth.

Viruses and parasites pathogenic to humans cannot replicate outside a host so will not multiply on any foods.

A wide range of fruit and vegetables are grown in New Zealand, but data on tonnage are not readily available, so the proportion of each product sold on the domestic market is not known. The largest weight of fruits imported into New Zealand in 2014 were grapes, pears, melons and tropical fruits. Capsicums, tomatoes and asparagus were the largest fresh vegetable imports (by weight). A large proportion of imported fruit and vegetables came from Australia and the USA.

Organic horticultural production is increasing in New Zealand.

The fresh-cut and fresh juice markets appear to be growing in New Zealand.

2.2.1 Relevant characteristics of the foods

The relevant characteristics of the foods have been discussed in the 2008 Document, but key points to note are:

- Pathogenic bacteria have been associated more with fresh vegetables (by prevalence studies and outbreaks) than fruits because of the differences in production (i.e. most fruits have little direct contact with soil during growth) and the generally lower pH of fruits (which inhibits pathogen survival);
- Bacteria may be able to multiply on fresh fruit and vegetables given the right conditions, but protozoa and viruses pathogenic to humans cannot (they require a host to replicate i.e. humans in the case of viruses pathogenic to humans, or animals or humans in the case of protozoa);
- Damaged or processed plant products (e.g. when peeled, chopped, juiced) provide greater opportunity for bacteria to multiply (if pH and storage conditions are suitable) because nutrients and water are released.

The perishable nature of RTE fruit and vegetables means that, unless frozen, the products have a limited shelf life and this limits opportunity for most pathogenic bacteria to grow, particularly if the food is stored at ≤4ºC throughout its shelf life. The “best before” dates on products viewed in New Zealand supermarkets indicate that the expected shelf life of packaged salads, lettuces, cabbage-halves, baby carrots and fresh herbs is approximately seven days. Bagged salads can be packed in air or nitrogen.[4] Fruit salads may carry best before or use by dates of up to six days (D’Sa and Hudson, 2014).[5]

[4] http://www.snapfreshfoods.com/FoodSafetyQualityAssurance.aspx (accessed 3 June 2015).
[5] “use by” means the food should not be eaten after the date; “best before” means the food can be eaten after the date but the quality may have declined.

Fresh juices provide an excellent supply of water and nutrients that favour bacterial growth. One potential limitation to growth is the pH, which varies widely depending on the juice ingredients. The 2008 Document cited a pH range of 3.0-6.7 for fresh juices containing fruits and vegetables. It is expected that fresh juices sold at juice bars or other food service outlets are consumed within the same day of purchase.

The seeds used to produce sprouts are soaked in water, and then put in warm, humid conditions to sprout. These conditions are near optimal for bacteria to grow, including pathogenic bacteria (Yang *et al.*, 2013). For example, the concentration of native aerobic bacteria increased by approximately 3 $\log_{10}$ CFU/g during sprouting under commercial conditions that did not undertake a seed decontamination step (Kim *et al.*, 2013). Sprouts can be sold still attached to the germination mat and the discarded seed coats are often still within the product. Sprouts are often consumed raw in New Zealand, and the shelf-life, as indicated by "use by" or "best before" dates, is typically 5-7 days (S. Paulin, ESR, pers. comm.).

2.2.2 Vegetable and fruit production in New Zealand

TABLE 2 presents data on New Zealand production of fruits and vegetables relevant to this document. Consolidated statistics are not available for the weight of vegetables and fruits produced in New Zealand, so the data presented in TABLE 2 have been assembled from the websites of Product Groups affiliated with Horticulture New Zealand.[6]

Around 200 hectares are also used to produce 3,500 tonnes of silverbeet and spinach.[7] A small number of kiwiberry growers also operate in New Zealand with around 22 hectares in production.[8]

Most of the outdoor (field grown) tomatoes in New Zealand are processed into pastes and canned products. The majority of fresh tomatoes available at retail are grown hydroponically in greenhouses.[9] Around 40% of cherries, 70% of apricots and almost all of the nectarines, peaches and plums grown in New Zealand are sold on the domestic market.[10]

[6] http://www.hortnz.co.nz/about-us/product-groups/ (accessed 9 April 2015).
[7] Data for the 2013 year, http://www.freshvegetables.co.nz/crops/leafy-crops/ (accessed 9 April 2015).
[8] http://www.nzkiwiberry.com/growing.html (accessed 9 April 2015).
[9] http://www.tomatoesnz.co.nz/about/industry-overview/ (accessed 9 April 2015).
[10] Data for the 2012/13 year, http://www.summerfruitnz.co.nz/Overview/NZ-market/The-New-Zealand-Market.htm (accessed 9 April 2015).

TABLE 2: Production of selected fruits and vegetables in New Zealand

FRUITS	AREA HARVESTED (HECTARES, 2012)[1]	WEIGHT PRODUCED (x1000 TONNES)[2]	VEGETABLES	AREA HARVESTED (HECTARES, 2012)[1]	WEIGHT PRODUCED (x1000 TONNES)[2]
Apples	8,845	488	Asparagus	820	NR
Apricots	434	3,344	Broccoli	1,977	19
Blackcurrants[3]	1,408	6-9	Cabbage	793	35
Blueberries	579	NR	Capsicum (indoors)[4]	57	15
Boysenberries	259	NR	Carrots	2,047	78
Cherries	619	2,476	Cauliflower	852	38
Grapes (table)	43	NR	Cooking herbs	314	NR
Melon (water/rock)	273	NR	Cooking herbs (indoors)	9	NR
Nashi pears	76	NR	Green beans	1,186	NR
Nectarines	409	3,587	Lettuce	1,250	NR
Peaches	452	2,822	Lettuce/salad greens (indoors)	24	NR
Pears	617	NR	Mushrooms (indoors)	15	NR
Plums	362	2,332	Peas (fresh/processed)	6,672	NR
Raspberries	132	NR	Tomatoes	669	50
Strawberries	220	NR	Tomatoes (indoors)	118	NR

[1] Data are from the 2012 Agricultural Census and are provided by Statistics New Zealand (http://www.stats.govt.nz/browse_for_stats/industry_sectors/agriculture-horticulture-forestry/2012-agricultural-census-tables/horticulture.aspx, accessed 9 April 2015).

[2] NR, not reported. No data were located from the websites of product groups affiliated with Horticulture New Zealand, for years within the range 2012-2014.

[3] It appears that a large proportion of the blackcurrants produced in New Zealand are processed into concentrate (http://blackcurrant.co.nz/blackcurrants/the-nz-industry/, accessed 11 June 2015).

[4] Indoors refers to production under cover, e.g. in greenhouses.

Organic horticultural production in New Zealand

An overview of the New Zealand organic industry published in 2012 shows that organic horticultural production is increasing (Cooper *et al.*, 2012). It was estimated that 9% of horticultural land in New Zealand was under organic certification in 2012, and from 2009 to 2012, the land area used for certified organic horticulture production increased from 8,175 ha to 11,188 ha. In 2012 there were 720 horticulture organic operators and 413 mixed/other organic operators, some of whom would undertake some horticultural activities. In addition, the value of organic fresh fruit and vegetable exports rose from $85.9 million in 2009 to $96.9 million in 2012, and these products (mostly kiwifruit and apples) made up 45% of the total organic exports, by value, in 2012.

The fresh-cut RTE market in New Zealand

Data on the fresh-cut RTE market in New Zealand is still sparse but the range of products available in supermarkets (e.g. salad mixes, baby leaves, baby carrots, cut herbs), and the size of the retail space they take up, both indicate that this is a stable (if not growing) market. A comparison of 2007 and 2012 agricultural censuses shows that the area in indoor lettuce

and salad greens harvested increased by approximately 13,000 m^2.[11] Two brands appear to dominate the bagged green salad market: Leaderbrand and Snap Fresh Foods (previously New Zealand Fresh-cuts Ltd.). A 2012 survey of leafy green salads initially identified that 147 different RTE products containing leafy salads were available in New Zealand, and went on to test 92 different products from nine different processors and/or distributors (Hewitt and Rivas, 2015)(J. Hewitt, ESR, pers. comm.). A 2013/14 survey of fresh-cut fruit salads included 14 different brands (D'Sa and Hudson, 2014). Fresh-cut products such as half-cabbages, half-pineapples and half-celery bunches tend to be produced on-site by supermarkets or fresh produce stores and their availability varies (N. King, ESR, personal observations).

Production of fresh juices in New Zealand

The 2008 Document mentioned juice bars as being one source of fresh juices in New Zealand, and these might be fixed shops or mobile outlets. Fresh juices might also be offered by other food service outlets such as restaurants, bars or fast food premises, or as part of services from other parts of the hospitality industry such as hotels. There are no data on fresh juice production in New Zealand but the market is clearly growing: One major fresh juice company has opened 56 stores since 2001.[12]

Production of sprouts in New Zealand

The 2008 Document did not include information on production of sprouts. A 2013/14 survey of sprouts available in New Zealand identified four major producers and seven minor producers supplying farmers' markets, independent grocery stores and supermarkets (D'Sa and Paulin, 2015; Hudson *et al.*, 2014). Sprouts are readily available in most supermarkets, suggesting that this is a fairly stable market, but data on the amount produced were not located.

2.2.3 International trade

Import and export data for fruits and vegetables relevant to this document are included in Appendix A.2.[13] All percentages and values below are by weight.

The major fresh fruits imported into New Zealand in 2014, by weight, were grapes, pears, melons and tropical fruits (pineapples, guavas, mangoes, mangosteens and papayas). The amount of grapes was largest at 13,927 tonnes, with the next largest amount being for pineapples (7,018 tonnes). Most of the grapes were imported from the USA (48% by weight) and Australia (34%), with 10% coming from Chile. These three countries were also the main sources of imported grapes to New Zealand in 2008. The majority of imported pineapples come from the Philippines (95% in 2014), but the origin countries for other tropical fruits vary (Equador, Peru, Mexico, USA, Australia). Australia is the major source of watermelons (96% in 2014) and other melons (100% in 2014), and has been since 2008. Around half of the pears imported also come from Australia, with the remainder mainly from the USA (33%) and China (10%). Strawberries were the main fresh berry imported during 2014, and these were imported from Australia (83%), the USA (12%) and Peru (5%).

The major fresh vegetables imported into New Zealand in 2014, by weight, were capsicums, tomatoes and asparagus. There was a notable decrease in imported tomatoes from 3,079 tonnes in 2008 (99% by weight from Australia) to 371 tonnes in 2014 (100% from Australia). The decrease was first reported in 2012 and was as a result of changes to Australian

[11] Data from http://www.stats.govt.nz/browse_for_stats/industry_sectors/agriculture-horticulture-forestry (accessed 18 February 2015).
[12] http://www.tankjuice.co.nz/ (accessed 9 June 2015).
[13] Data discussed in this section and presented in the appendix are sourced from Statistics New Zealand Infoshare (http://www.stats.govt.nz/infoshare/Default.aspx), accessed 23 April 2014.

agrichemical requirements in 2011, which meant the majority of Australian tomatoes no longer met New Zealand's biosecurity requirements.

Australia was the main country of origin for many other vegetables imported in 2014: 100% of cauliflowers and cucumbers, 96% of lettuce and 63% of capsicums (35% from The Netherlands). The USA was the country of origin for 98% of the asparagus and 35% of the carrots. Most (65%) of the carrots were from China. Spinach imported into New Zealand in 2014 came from the USA (92%) and China (8%), compared with in 2008, when spinach came from Fiji (81%) and Australia (19%).

Data on frozen fruits are available but those imported as cooked product are not separated from those uncooked. During 2014 there were just over 6,000 tonnes of frozen fruit (cooked or uncooked, without any added sweeteners) imported into New Zealand. Almost 60% of this, by weight, came from Chile (29%) and China (28%).

A comparison between data on exported fruit and vegetables and TABLE 2 suggests that a large proportion of fruit is exported from New Zealand, but without domestic production weights it is difficult to know the proportion of vegetables exported. It is probable that leafy vegetables such as lettuces and spinach that are prone to damage and have a shorter shelf-life are largely sold on the domestic market.

2.3 CONTAMINATION OF FRUIT AND VEGETABLES, FRESH JUICES AND SPROUTS BY PATHOGENIC MICROORGANISMS

Key findings

Contact with animal and/or human faeces, either directly or indirectly (e.g. via soil, water, inadequately treated compost) is the most important risk factor for fresh fruit and vegetables becoming contaminated with pathogenic microorganisms.

Soils amended with organic wastes, including manures, sludges and composts, can be a source of microbial pathogens, but their survival in these soils is highly variable. The transfer of pathogenic bacteria from soil to plants has been demonstrated under experimental conditions but these usually represent "worst-case" scenarios and transfer under standard practices is less well substantiated.

There is evidence from experiments that shows contaminated water can introduce pathogenic microorganisms onto fresh produce, particularly when the water is sprayed onto the plants when irrigating or applying agrichemicals. There is also some limited evidence from outbreak investigations that supports water as a source of contamination.

There is evidence from outbreaks and experimental studies to indicate that workers (harvesters, food handlers), equipment and surfaces are important sources of contamination.

Climate change will alter the likelihood of fresh produce becoming contaminated with pathogens, but whether the likelihood increases or decreases will depend on the balance of a number of factors including changes in temperature, rainfall, wind, insect populations and grower practices.

The 2008 Document did not find evidence that organically produced fruits and vegetables were more significantly contaminated by pathogenic microorganisms than those conventionally produced. Studies published since, continue to support this position.

There is a higher risk of fresh juices becoming contaminated with pathogenic microorganisms if fallen fruit are used.

The most common cause of sprout contamination is outgrowth of microorganisms from contaminated seeds.

2.3.1 Contamination of fruit and vegetables

The 2008 Document presents information from a number of studies that show how fruit and vegetables can become contaminated by pathogens through contact with contaminated soils, natural fertilisers (manure, compost, chicken litter, vermiculture products, etc.), irrigation water or liquid pesticides during the growing phase, and through contact with contaminated wash water or infected food handlers after harvesting.

In summary, along the food chain from growing to retail sale, pathogens can be transferred to fruit and vegetables before they reach the consumer from:

- The environment in which the plants are grown (soil, water, air, animals);
- Seeds contaminated prior to growth or sprouting, e.g. it was demonstrated that *Salmonella* spp. and *E. coli* O157:H7 can survive on lettuce seeds for two years and be transferred to the subsequent seedlings (Van der Linden *et al.*, 2013);
- Water, fertilisers or pesticides applied during growth;

- The environment in which the produce is harvested and processed ready for distribution (e.g. equipment, surfaces, wash water tanks, packaging, pests), including any slicing or shredding stages; and/or
- Containers, equipment, and surfaces involved in transportation and retail display.
- Workers involved in harvesting, processing, transport and retail display.

After evaluating the risk posed by pathogens in several fruits and vegetables (see Appendix B.3.2), the European Food Safety Authority (EFSA) determined that the risk factors for contamination at primary production were:

- Environmental factors, in particular proximity to animal rearing operations and climatic conditions (e.g. heavy rainfall events) that increase the transfer of pathogens from animal reservoirs to the food;
- Contact with animals (domestic or wild) gaining access to growing fields;
- Use of untreated or insufficiently treated manure or compost;
- Use of contaminated agricultural water either for irrigation or for application of agricultural chemicals such as fungicides; and
- Contamination and cross-contamination by harvesters, food handlers and equipment at harvest or post-harvest.

A systematic review of the risk factors for contamination of fruits and vegetables with *L. monocytogenes*, *Salmonella* spp., and *E. coli* O157:H7 at the preharvest level identified manure (from animals or via soil amendments) and irrigation water as the two most important modes of pathogen transmission (Park *et al.*, 2012). A FSANZ review of foodborne illness associated with selected RTE fresh produce identified the use of poor quality water for pre- and post-harvest activities as the most common cause of product contamination (FSANZ, 2011). Univariate analyses of management practices that influence the likelihood of *Salmonella* spp. or *Listeria* spp. being detected in the soil of a produce growing field identified a number of risk and protective factors, but when subjected to multivariate analysis it was reported that (Strawn *et al.*, 2013):

- *Salmonella* spp. were more likely to be detected in the soil if manure was applied within a year of sample collection, but less likely to be detected if the field was surrounded by a buffer zone where no produce was grown.
- *Listeria* spp. were more likely to be detected in the soil if wildlife were observed in the field within three days of sample collection, if the field was irrigated within three days of sample collection, or if the field was cultivated within seven days of sample collection.

Overall, contamination from faeces, either directly or indirectly (e.g. via soil, water, inadequately treated compost) is the most important risk factor for fresh fruit and vegetables and there have been several outbreaks that demonstrate this point. In 2011 an outbreak of STEC infection in the USA (15 cases, including four of haemolytic uraemic syndrome (HUS), two deaths) was caused by strawberries contaminated with faeces from wild deer (Laidler *et al.*, 2013). After four multistate outbreaks caused by tomatoes contaminated with a specific strain of *Salmonella* Newport, gull faeces were identified as the most likely source (Gruszynski *et al.*, 2014). In 2005 an outbreak of STEC infection in Sweden (135 cases, 11 HUS) was caused by lettuces that had been irrigated with water from a small stream contaminated with cattle manure from an upstream farm (Soderstrom *et al.*, 2008). A large outbreak of norovirus in Germany (10,950 cases) was caused by frozen strawberries that were found to contain three different norovirus genotypes, which makes sewage contamination a more likely scenario rather than contamination by an infected worker (Mäde *et al.*, 2013).

Soil

Soils amended with organic wastes, including manures and composts, can be a source of pathogens. Survival of pathogens in organically-amended soils varies and depends on the interaction of biotic (microbial) and abiotic factors (e.g. temperature, moisture, ammonia content, pH, nutrient availability, soil type, weather, and timing and nature of application) (Avery *et al.*, 2012). For example, *Salmonella* spp. and *E. coli* O157:H7 survived better in soil amended with poultry manure than with cattle manure slurry (although the inoculum was still detectable by enrichment throughout the 180-day experiments, regardless of manure type)(Nyberg *et al.*, 2010). A recent study found that *L. monocytogenes*, *Salmonella* Dublin and a non-toxigenic *E. coli* O157 strain all declined over time in a range of soils, but it was the native microflora of the soils that had a significant influence over the death rates, rather than the chemical and physical characteristics of the soils (Moynihan *et al.*, 2015).[14]

The contamination of plants from pathogens present in the soil has been demonstrated experimentally for some pathogens. *E. coli* O157:H7 and *Listeria innocua* were detected on lettuce leaves from plants grown in soils mixed with organic compost that had been inoculated with these bacteria (Oliveira *et al.*, 2011; Oliveira *et al.*, 2012b), and *B. cereus* was detected on lettuces after these plants were grown in soil artificially contaminated by this pathogen (Kim *et al.*, 2012). However, large inoculums or use of untreated animal wastes are features of such studies, thus representing worst-case scenarios. From a model estimating the probability of lettuce contamination with *E. coli* O157:H7 from manure-amended soil under constant environmental conditions, the probability of contamination was most highly correlated with the initial concentration and prevalence of the pathogen in manure, and the storage time of the manure before application to soil (Franz *et al.*, 2008). In a USA study of large, chemically-fertilised tomato plantations and small tomato plantations fertilised with animal products (e.g. poultry manure), *Salmonella* spp. were only detected in the soil of the large plantations (where irrigation water was a potential source of contamination), thus indicating that application of the organic fertilisers was well-managed on the small plantations (Micallef *et al.*, 2012).

Water

There is evidence to support water as a source of fresh produce contamination with pathogens, particularly when the water is sprayed onto the plants when irrigating or applying agrichemicals. Pathogens are better protected from environmental conditions when contaminated water lands on the abaxial (underside) of leaves (e.g. through splashing or natural leaf movement while being irrigated), and this position also facilitates better access to plant stomata (pores, found in the epidermis of leaves, stems, and other organs that are used to control gas exchange) (Park *et al.*, 2012). Norovirus genogroup II (GII) was detected in 2/56 samples of irrigation water used on commercial strawberry plants in a survey of European berry production (Maunula *et al.*, 2013). Results from laboratory survival experiments with murine noroviruses (used as a surrogate for human norovirus) showed that the presence of pesticides in water may not necessarily inactivate norovirus (Verhaelen *et al.*, 2013), and *Escherichia coli* O157:H7 and *Salmonella* spp. can also survive, and even grow, in the presence of pesticides (Dobhal *et al.*, 2014; Lopez-Velasco *et al.*, 2013). Section 2.4.3 contains further examples of field studies using contaminated irrigation water.

Salmonella spp. were isolated from pond water used for irrigating tomatoes on USA plantations, however the growers used drip irrigation lines under plastic sheets to prevent contact between the soil and the leaves and fruits, so the risk of contamination was very low in these cases (Micallef *et al.*, 2012). An important finding of this study was that the presence and serotypes of *Salmonella* spp. detected in the irrigation ponds was not consistent over time, thus indicating that this pathogen was transient in irrigation waters and perhaps

[14] Although it might be argued that the chemical and physical aspects of the soil influence the native microflora composition.

introduced by wildlife visiting the ponds. The possibility of increased contamination of New Zealand waterways with *G. duodenalis* as a result of increased conversion of land into dairy production has been raised (Winkworth, 2010). Another study observed that the prevalence of *L. monocytogenes* was highest in the soils of spinach fields 24 hours after irrigation with creek water, but the pathogen was not detected five days later (Weller *et al.*, 2015). While experimental evidence indicates the potential for irrigation waters to contaminate fresh produce, a recent review noted that "direct evidence of irrigation water causing foodborne disease is relatively rare" (Pachepsky *et al.*, 2011). Identifying the source of contamination in an outbreak is usually very difficult (see Section 3.1).

Climate change

The impact of climate change on fresh produce contamination is also being increasingly studied. Several recent reviews all agree that the likelihood of fresh produce becoming contaminated with pathogens will change with increased temperatures and changes in rainfall patterns, but whether the likelihood increases or decreases will depend on the balance of a number of factors (Hellberg and Chu, 2015; Liu *et al.*, 2013; Tirado *et al.*, 2010). The likelihood of contamination will increase in areas experiencing more frequent and intensive rainfall events (contamination from flooding or rain splash, prolonged pathogen survival in soils due to higher soil moisture), but will decrease where higher temperatures or greater exposure to UV reduce pathogen survival in soil and water (EFSA, 2014c; Liu *et al.*, 2013). Transfer of *Salmonella* Typhimurium from soil or organic mulch to tomato plants during a heavy rainfall event (110 mm/h rain for 10 min) was demonstrated using a rain simulator (Cevallos-Cevallos *et al.*, 2012). Dry, windy weather can promote airborne spread of pathogens, e.g. *E. coli* O157:H7 was detected in leafy green vegetables sampled as far as 180 metres downwind of a cattle feedlot (Berry *et al.*, 2015). Warmer temperatures may increase insect vectors, and contamination of food plants with bacterial pathogens by insects (flies, worms) has been reported (Fremaux *et al.*, 2008). Furthermore, climate change may also bring about changes in grower practices which further alter likelihood of contamination, e.g. switching from overhead to trickle irrigation in areas with a depleting water supply (Liu *et al.*, 2013).

Food handlers, equipment and surfaces

In addition to outbreaks attributed to infected food handlers (Section 3), there are a number of recent studies that provide evidence that workers (harvesters, food handlers), equipment and surfaces are also important sources of contamination:

- Using indicator viruses, two studies identified toilets and workers' hands as specific carriers of human enteric viruses that can lead to contamination of fresh berries and leafy vegetables at the farm, and HAV and norovirus genogroups I and II (GI and GII) were also detected at these sampling locations on leafy vegetable farms (Kokkinos *et al.*, 2012; Maunula *et al.*, 2013). Samples from berry processing plants were negative for norovirus and HAV, but a swab from a food handler's hand was positive for a viral indicator (Maunula *et al.*, 2013). Samples of rinsing water from leafy vegetable processing plants were positive for a viral indicator (Kokkinos *et al.*, 2012).

- Norovirus on gloved fingertips was transferred to blueberries, grapes and raspberries, and transferred between fingertips and a stainless steel surface, with wetter conditions increasing the number of viruses transferred (Sharps *et al.*, 2012). Norovirus genotypes GI.4 and GII.4 were transferred from naked fingertips to tomato skins and cut cucumbers, and this transfer was demonstrated for GI.4 even when the fingerpad was allowed to dry and sequentially touch the food seven times (Tuladhar *et al.*, 2013). Norovirus GII.4 was transferred from pieces cut from food grade gloves or a stainless steel disc to lettuce pieces and vice-versa (Stals *et al.*, 2013).

- Experiments have indicated that there is a risk of transferring *E. coli* O157:H7 to the inside of lettuces when they are cored in the field, but the risk of contamination is low when the

stems are cut and no field coring occurs (McEvoy *et al.*, 2009; Taormina *et al.*, 2009; Yang *et al.*, 2012). Field coring is not common practice in New Zealand.

- *Salmonella* spp. transference between surfaces (ceramic, stainless steel, glass, plastic) and fresh-cut produce (celery, carrot, watermelon) occurred even when the inoculated surfaces or foods were allowed to dry for one hour before the experiment. The same report also gave evidence of transference of *E. coli* O157:H7 between these surfaces and lettuce (Jensen *et al.*, 2013). Variable transfer rates were reported between the different produce items although in general, more bacteria were transferred in the direction of surfaces to foods than the reverse direction.

- A series of experiments has demonstrated that *E. coli* O157:H7 inoculated onto leafy green vegetables can be transferred throughout a simulated shredding and washing system, and further transferred onto uncontaminated vegetables processed afterwards (Buchholz *et al.*, 2012a, 2012b). No sanitisers were added to the washwater.

Contamination of fruits and vegetables, such as soft fruits, by enteric viruses is more likely to occur from food handlers who do not adhere to good hand hygiene, or from contaminated irrigation waters, rather than directly from the soil.

2.3.2 Organic vs. conventional production

Natural fertilisers (e.g. compost, manure, chicken litter) used in organic horticultural production can introduce pathogenic microorganisms to the growing areas. The 2008 Document did not find evidence that organically produced fruits and vegetables were more significantly contaminated by pathogenic microorganisms than those conventionally produced. Studies published since, continue to support this position:

- Sampling of leafy green vegetables produced organically and conventionally in Spain or Brazil found very little difference in the levels of indicator bacteria (pathogenic bacteria were not isolated from any samples) (Maffei *et al.*, 2013; Oliveira *et al.*, 2010b).

- A two-year field experiment investigating the effect of different fertiliser treatments (fresh manure, composted manure, stinging nettle extract, calcium ammonium nitrate) on the microbial quality of head lettuce did not find a higher risk of bacterial contamination if organic fertilisers were used (Wießner *et al.*, 2009).

- Two studies in the USA did not find the growing method (organic or conventional) to be a significant factor in tomato or leafy green microbiological contamination (Marine *et al.*, 2015; Pagadala *et al.*, 2015). *Salmonella* spp. were detected on leafy green vegetables from organic and conventional farms.

- A model estimating the probability of lettuce contamination with *E. coli* O157:H7 from manure-amended soil under constant environmental conditions predicted contamination of 0.10 lettuce heads/hectare for an organic production scenario, and 0.89 lettuce heads/hectare for a conventional production scenario (Franz *et al.*, 2008). In the model, several organic practices reduced the final concentration of *E. coli* O157:H7 in the manure-amended soil before planting, e.g. feeding cattle a high-fibre diet, greater use of manure rather than slurry for fertilising, storage of manure for longer periods before applying to soil, and longer time between applying manure to soil and planting crops.

Furthermore, tomato plants grown in soil used for organic production demonstrated better resistance to *Salmonella* Typhimurium colonisation when this pathogen was inoculated onto leaves than plants grown in soil used for conventional production (Gu *et al.*, 2013). The endophytic bacterial diversities of tomato plants grown in the organic soils were significantly higher than those in the conventional soils, and this increased diversity may be associated with the plants' increased colonisation resistance. The natural microflora of fresh fruit and vegetables can inhibit pathogen colonisation (Critzer and Doyle, 2010).

It has been noted that where surveys of fresh produce detected pathogenic bacteria on organic products, there is usually no evidence to prove that the contamination arose from organic practices, since fresh produce can become contaminated at multiple points along the supply chain (Lima and Vianello, 2011).

2.3.3 Contamination of fresh juices

Aside from the sources of contamination discussed above for fruit and vegetables, fresh juices can also become contaminated if fallen fruit is used (a greater likelihood of contamination from soil or faecal material) or where contaminated water is used for washing or processing the fruit (Vojdani *et al.*, 2008). Other sources of contamination are workers hands/gloves, work or storage surfaces and utensils, and the juicing equipment.

2.3.4 Contamination of sprouts

The most common cause of sprout contamination is growth from contaminated seeds (Yang *et al.*, 2013). Seeds are produced for several end-uses (e.g. edible seeds, animal feeds, oil production and horticulture) and not specifically for sprout production, so their production may not include controls for human pathogens (EFSA, 2011a). The seeds can become contaminated during their production from the same sources as already discussed for fruit and vegetables, i.e. irrigation water, organic soil amendments, faeces, infected workers, etc. Additionally, if contaminated seeds are scarified (rubbed between two hard surfaces to create microscopic cracks that improve water uptake and germination of the seed), pathogenic bacteria can be forced into the cracks where they are better protected from disinfection (Dechet *et al.*, 2014). Pathogenic microorganisms that are able to survive dry conditions are well suited to surviving on sprout seeds. This includes *Salmonella* spp. (can remain viable on seeds for years) and *B. cereus* (has been detected on samples of alfalfa seeds and rapeseed intended for sprout germination at concentrations of 0.1-2.7 $\log_{10}$ CFU/g) (Dechet *et al.*, 2014; Kim *et al.*, 2013). It is also possible that pathogens can colonise the inside of sprouts once the seeds have germinated, and internalisation of *Salmonella* Typhimurium (introduced with irrigation water) has been demonstrated with mung bean sprouts (Ge *et al.*, 2014).

2.4 THE BEHAVIOUR OF PATHOGENIC MICROORGANISMS ON FRUIT AND VEGETABLES, FRESH JUICES AND SPROUTS

Key findings

Since the 2008 Document there has been a lot of work investigating the ability of pathogenic microorganisms to move from the outside to the inside of fruit and vegetable plants and fruits (internalisation). There is good evidence to support internalisation, at least for some pathogenic bacteria, which is of concern because internalised pathogens are better protected from sanitisers. But there is still uncertainty around the extent of internalisation under normal horticulture production and the length of time pathogenic microorganisms can remain viable inside a food plant.

Entry into the viable but non-culturable (VBNC) state has been demonstrated for all of the bacterial pathogens considered in this document except for the clostridia, which are spore-formers, but there is currently insufficient evidence to support the existence of VBNC bacterial pathogens on fresh produce or to support them as an undetected cause of human illness. Prolonged survival in fresh water or in biofilms can induce the VBNC state so there is potential for them to be present in irrigation or wash water.

The few field studies that have been done indicate that pathogenic microorganisms on plants or fruits could survive for a month or more when exposed to normal field conditions. Dessication, UV radiation by sunlight, or competition with commensal microbiota can all contribute to the death of pathogenic bacteria under field conditions.

The ability of a pathogenic microorganism to survive or multiply on raw produce (whole or cut) depends on the characteristics of the pathogen and the food, other microflora present on the food and the environment the food is in. Viruses and parasites pathogenic to humans cannot replicate outside a host so will not multiply on any foods, but can survive. Data from recent studies and recent reviews of older studies show that the behaviour of pathogenic bacteria on harvested produce is difficult to predict, but cooler temperatures do prevent growth or slow it down. Most studies have focused on *Salmonella* spp., *L. monocytogenes* and STEC. Data for other pathogenic microorganisms, including protozoa and viruses, is limited, but most have demonstrated survival or growth, except for *Campylobacter* spp.

The behaviour of pathogenic microorganisms in fresh juices depends on the microbiological species, the ingredients of the juice and the resulting characteristics (e.g. pH, presence of natural antimicrobials and/or other microflora), and the storage time/temperature before consumption. Recent studies (mostly focussing on *L. monocytogenes* or viral pathogens) demonstrate survival or growth of pathogenic microorganisms in fresh juices. However, most experiments were conducted at low temperatures (≤10ºC) and any bacterial growth measured at these temperatures was slow (days). The potential for growth at room temperature over a 24-hour period (a more likely scenario for fresh juices) has not been well studied.

Evidence from experiments and outbreaks prove that pathogenic microorganisms (chiefly bacteria and protozoa) can contaminate and grow on sprouts. Recent studies showed that survival and growth of bacterial pathogens were enhanced by warmer temperatures, and when the seeds were the source of contamination rather than contamination occurring after sprouting. Microbial pathogens can survive well on sprouts stored at cooler temperatures.

The presence of a salad dressing, which is typically acidic, generally makes the salad a less favourable environment for bacterial growth. There are a few recent studies that report death of pathogenic bacteria in dressed salads. However, the presence of animal proteins (e.g. poultry, seafood, egg) will promote survival or even growth of *L. monocytogenes* in a dressed salad. Raw nuts, if contaminated, can introduce pathogenic microorganisms to salads, particularly *Salmonella* spp.

2.4.1 Pathogen internalisation

The 2008 Document mentioned the possibility of pathogenic bacteria being drawn into the inside of some fruits and vegetables (tomatoes, celery, unwaxed apples) via stomata, stem scars or damaged tissues. Bacteria can also enter plants via other natural openings such as pollen tubes, lenticels or root cracks, and be pulled into the internal tissues along with water, e.g. via the roots following irrigation or via the calyx or other openings during post-harvest washing (Deering *et al.*, 2012; Lynch *et al.*, 2009; Melotto *et al.*, 2014). Once inside, bacteria can move to different parts of the plant (Melotto *et al.*, 2014). There has been a lot of work done in this area since the 2008 Document, and there is good evidence to support internalisation, at least for some pathogens, which is of concern because internalised pathogens are better protected from sanitisers.

One criticism of much of the work is that experiments are usually conducted with inoculums at high concentrations, so there is still uncertainty around the extent of internalisation under normal horticulture production where pathogen contamination levels are likely to be lower and affected by natural soil and plant microbiota, and climate. It has also been shown that some bacterial pathogens (*Salmonella* spp., *E. coli* O157:H7) are able to multiply inside plants, exploiting plants as alternative hosts (Deering *et al.*, 2012). Long-term colonisation, e.g. a pathogen remaining viable inside a food plant from seedling to harvest is a data gap, and understanding plant responses to human pathogens is an emerging research area (Holden *et al.*, 2009; Melotto *et al.*, 2014).

Most of the research on internalisation focusses on *Salmonella* spp. and STEC. Two 2012 reviews of the scientific literature present evidence of pre-harvest internalisation of *Salmonella* spp. and *E. coli* O157:H7 when these pathogens are introduced to plants on the seed or through the soil and/or irrigation water (Deering *et al.*, 2012; Hirneisen *et al.*, 2012). There are inconsistencies between results, and differences in experimental design are likely to contribute to this. However, there is sufficient evidence to support internalisation by these pathogens, particularly as microscopy and immunolocalisation techniques have shown the presence of these pathogens within a number of parts of the plant (Deering *et al.*, 2012).

When focussing only on studies evaluating root uptake of *Salmonella* spp. and STEC, there was more evidence to support internalisation via hydroponic growing systems than via soil, but the overall risk of pathogen internalisation via the roots of plants appears to be low (Hirneisen *et al.*, 2012). Studies published since these reviews continue to show variable results, with some studies unable to demonstrate internalisation, and others showing that uptake from leaves is more likely than roots (Erickson *et al.*, 2014; Ge *et al.*, 2013; Gorbatsevich *et al.*, 2013; Lopez-Velasco *et al.*, 2012; Macarisin *et al.*, 2014; Nicholson *et al.*, 2015; Zheng *et al.*, 2013).

Salmonella spp. and *E coli* O157:H7 have also been detected inside fruits (tomatoes, mangoes, apples, oranges) and leafy green vegetables (lettuce, spinach, parsley) following post-harvest contamination through wash water (Deering *et al.*, 2012). The 2008 Document mentions that a wash water temperature of at least 10ºC higher than that of incoming produce will minimise internalisation of pathogens. Using wash water at a temperature cooler than that of incoming produce (a negative temperature differential) causes air inside the fruit or vegetable to contract and the internal pressure of the product to lower, so water outside the product, and any microbes in that water, are drawn into the tissues by the resulting vacuum (Lake *et al.*, 2011a). However, recent studies with tomatoes and baby spinach leaves using post-harvest dump tank conditions typically used by growers found that the temperature differential was not a significant factor in *Salmonella* spp. internalisation (Gomez-Lopez *et al.*, 2013; Xia *et al.*, 2012; Zhou *et al.*, 2014). The temperature differential only became important when the immersion time was extended past the typical two minutes (Zhou *et al.*, 2014).

There have been a few studies that demonstrate the potential for enteric viruses to internalise after plants are exposed to contaminated water or soil, although the virus levels used in these

experiments may be higher than found in natural growing conditions. Hepatitis A virus (HAV) was internalised by spinach and green onion plants grown in spiked hydroponic solutions, and by one spinach plant grown in a peat-based growing medium (Hirneisen and Kniel, 2013). For example, the average concentration of HAV inside two spinach plants floating in a hydroponic solution (containing 7 $\log_{10}$ RT-qPCR HAV units/ml)[15] was 4.5 $\log_{10}$ RT-qPCR units/plant after one day. Spinach plants growing on oasis cubes in HAV-spiked circulating hydroponic solution (average HAV titre 3.6 $\log_{10}$ RT-qPCR units/ml) contained approximately 2-3 $\log_{10}$ RT-qPCR HAV units when measured weekly over three weeks.

In other studies, norovirus was internalised by romaine lettuces growing in hydroponic solution (Dicaprio *et al.*, 2012), but was not internalised by lettuces held in spiked water or turf briquettes (Urbanucci *et al.*, 2009). The human norovirus surrogate, murine norovirus, was internalised via the roots in lettuces and strawberries (DiCaprio *et al.*, 2015; Wei *et al.*, 2011).

Studies on other pathogens are rare. *L. monocytogenes* was detected in the surface-sterilised leaves of lettuce and rocket seedlings, and was still detectable 80 days after the lettuces were contaminated using soil inoculation (Chitarra *et al.*, 2014). *L. monocytogenes* and *S. aureus* were internalised by lettuce seedlings grown in vermiculite and hydroponically grown mature lettuce plants (Standing *et al.*, 2013). *Bacillus* spp. have been detected inside lettuce leaves at a high prevalence but *B. cereus* was not detected (Hou *et al.*, 2013). An analysis of 16S rDNA gene sequences from the interior (endosphere) of grapevine leaves and stems detected sequences that suggested the presence of *S. aureus*, *C. botulinum* and *C. perfringens* (Yousaf *et al.*, 2014).

2.4.2 Potential for bacteria to enter a viable but non-culturable (VBNC) state

The VBNC state is understood to be a transient physiological phase in which bacterial cells switch off activities typical for growing organisms but maintain a low level of metabolic activity yet non-culturable on routine microbiological media (Dinu and Bach, 2011). It is thought that this state is used for bacteria to survive unfavourable conditions and cells can be resuscitated under appropriate conditions, however, the specific mechanisms that induce the VBNC state or resuscitate the cells back to a culturable state are still being investigated (Li *et al.*, 2014; Ramamurthy *et al.*, 2014). Pathogens in the VBNC state may be responsible for causing a proportion of human foodborne disease since they cannot be detected by standard methods and have greater resistance to physical and chemical sanitisers (Li *et al.*, 2014). Some pathogens continue to express virulence genes while in the VBNC state (e.g. VBNC *Shigella dysenteriae* type 1 and *E. coli* O157:H7 continue to produce enterotoxin), but for others, their virulence characteristics only return upon resuscitation (e.g. *A. hydrophila*, *L. monocytogenes*) (Ramamurthy *et al.*, 2014).

Entry into the VBNC state has been demonstrated for all of the bacterial pathogens considered in this document except for the clostridia (Appendix A.1). There is evidence that bacterial pathogens can exist in the VBNC state in the soil and rhizosphere of plants but there is very little information on the VBNC state on fresh produce (Dinu *et al.*, 2009). Three strains of *L. monocytogenes* were observed to enter the VBNC state on the leaves of parsley plants held under low relative humidity but the VBNC cells were not resuscitated after a return to 100% relative humidity (Dreux *et al.*, 2007). There are, as yet, no reports of successful resuscitation of pathogenic bacteria from the VBNC state on plant surfaces, nor evidence to show a link between VBNC cells on fresh produce and human illness (Dinu *et al.*, 2009). Prolonged survival in fresh water or in biofilms (both nutrient-deprived environments) can induce the VBNC state in many of these pathogens, so there is potential for them to be present in irrigation or wash water.

[15] RT-qPCR, Reverse-transcriptase real-time quantitative PCR.

2.4.3 Behaviour of pathogens on fruit and vegetables

The ability of a pathogen to survive or multiply on raw produce depends on the characteristics of the pathogen and the food, other microflora present on the food and the environment (e.g. temperature, humidity) the food is in.

Pre-harvest

UV radiation from sunlight, drying, or competition with commensal microbiota can all contribute to the death of pathogenic bacteria under field conditions (EFSA, 2014d).

There have been experiments to better understand pathogen survival on fruits and vegetables before harvesting, although experiments under normal growing conditions are few because of the obvious difficulties in working with pathogenic microorganisms outside a controlled environment. The focus of these studies has been to measure survival of bacterial pathogens after being applied to fresh produce with irrigation water, i.e. simulating a scenario when contaminated irrigation water has been used:

- Field-grown **lettuces**: A non-pathogenic strain of *E. coli* was detected on lettuces up to seven days after being applied via sprinkler irrigation (note a high application rate of 10^8-10^9 cells/ml) (Fonseca *et al.*, 2011). When applied using furrow-irrigation, *E. coli* was detected in soil for five days during summer (17-27ºC) and 17 days during winter (8-16ºC).

- Field-grown **lettuces**: The concentration of a non-pathogenic strain of *E. coli* O157:H7 sprayed on young plants decreased quickly, as did the number of *E. coli*-positive plants, but the *E. coli* could still be detected by enrichment on some plants after 35 days in the field (Moyne *et al.*, 2011). Neither drip nor overhead irrigation significantly impacted bacterial survival. The *E. coli* test organism was not detected on lettuces when these were planted in soil inoculated with the *E. coli*.

- Field-grown **lettuce** and **spinach** seedlings: When watered with inoculated bore water containing *Salmonella* Enteritidis, *C. jejuni* or non-verotoxin-producing *E. coli* O157, the concentration of these bacteria decreased from an initial 3-4 $\log_{10}$ CFU/g to <10 CFU/g after one week, and after two weeks, only *Salmonella* Enteritidis was detected by enrichment (Hutchison *et al.*, 2008). A normal spray irrigation regime with non-inoculated water was maintained throughout the experiment.

- Field-grown **tomato** plants: An attenuated strain of *Salmonella* Typhimurium spray irrigated at a low concentration (100 CFU/ml) onto fruiting plants was still detectable on the tomatoes 15 days after initial inoculation (Lopez-Velasco *et al.*, 2013). Survival was enhanced in the presence of some pesticides (those with the active ingredients pyraclostrobin, spinetoram and spinosyn).

- Field-grown **coriander**: When an attenuated strain of *E. coli* O157:H7 and *Salmonella* Enteritidis were sprayed onto plants, some of the plants were still positive for these bacteria after 12 days, particularly when the bacteria were applied at a higher inoculum (approximately 4 $\log_{10}$ CFU/g measured 0.5 days after application) (Suslow, 2012). Positive plants could still be detected after harvesting, washing in sanitiser and after additional storage for 14 days at 2.5 ºC.

- Greenhouse-grown **basil** and **parsley**: *Salmonella* Typhimurium spray irrigated onto basil or parsley was still detected on the leaves, stalks and roots, and in the soil, 28 days after irrigation (Kisluk *et al.*, 2013; Kisluk and Yaron, 2012). It was still detected on the basil leaves 100 days after irrigation. However, the initial inoculation was high in these studies (approximately 8 $\log_{10}$ CFU/g leaves), and further studies with parsley did not recover *Salmonella* Typhimurium when it was applied at concentrations approximately 5-fold lower (Kisluk and Yaron, 2012).

These field studies indicate that pathogenic microorganisms on plants or fruits could survive for a month or more when exposed to normal field conditions. One common observation was that the majority of the inoculated bacteria died quickly and only a minority survived throughout the experiments. Mathematical modelling of several data sets derived from field-based experiments observing *E. coli* O157:H7 on lettuces also identified this overall pattern of biphasic decay, i.e. an initial rapid decrease in concentration followed by a more gradual decrease (McKellar *et al.*, 2014). However, laboratory-grown bacterial cultures were used in these experiments. Populations within the environment are naturally more variable and adapted to the conditions, so the "tail" of survivors observed in these experiments is probably more informative than the initial decrease.

Pathogenic bacteria may form biofilms on the leaves and roots of fresh produce where they are better protected from environmental stresses (Yaron and Römling, 2014). Biofilms are an accumulation of live and dead bacteria, and a variety of secreted compounds (e.g. polysaccharides, proteins). Biofilm formation on fresh produce has been recently reviewed (Jahid and Ha, 2012; Yaron and Römling, 2014). Biofilm formation on fresh produce has been reported for *E. coli* O157:H7, *Salmonella* spp., *Campylobacter* spp., *Listeria* spp. and *Shigella* spp. While biofilm formation is an important survival strategy for pathogenic bacteria in the environment, biofilms also provide protection from sanitisers used on harvested fresh produce. Thus, most biofilm research is focussed on post-harvest treatments that are effective against pathogenic bacteria in biofilms. This research includes investigating natural plant-related compounds (e.g. those released as part of a plant's immune response) or microorganisms (e.g. *B. subtilis*, bacteriophages) that inhibit bacterial biofilm formation on growing produce.

Post-harvest

Bacterial pathogens

The behaviour of bacterial pathogens on fresh produce can change after harvest, when the plant is no longer alive. Cocktails of different *Salmonella* serotypes, *E. coli* O157:H7 and *L. monocytogenes* all died when inoculated onto the leaves of growing spinach plants and stored for 24 hours at 23ºC and 50% relative humidity (to try to replicate field growing conditions), but grew by 2 $\log_{10}$ CFU/leaf when inoculated onto freshly cut leaves and stored under the same conditions (Koseki *et al.*, 2011).

Data from recent studies and recent reviews of older studies show that bacterial behaviour on harvested produce is difficult to predict, but cooler temperatures do prevent growth or slow it down.

Cutting fruit or vegetables releases nutrients to microorganisms, so after produce is sliced, diced or shredded, contamination can lead to high pathogen counts if factors such as pH, temperature or the presence of plant-produced antimicrobials or natural plant microflora are not inhibitory (Lynch *et al.*, 2009). A review of seven studies comparing growth of *L. monocytogenes* on intact and cut produce found that *L. monocytogenes* grew on intact but not cut tomatoes, grew on cut but not intact carrots, cabbage, peppers and cantaloupe, grew on cut and intact cabbage, and did not grow on strawberries whether these were cut or not (Hoelzer *et al.*, 2012).

The behaviour of *Salmonella* spp., *L. monocytogenes* and STEC on fresh produce has been well studied, and TABLE 3 provides some examples of studies carried out at cooler temperatures, which better indicate potential for growth during post-harvest storage. Higher temperatures can enhance survival or promote growth on fresh-cut produce, e.g. *E. coli* O157:H7 did not grow on grated carrots or melon at 5ºC, but rapidly grew at 25ºC (an increase of up to 4.0 $\log_{10}$ after only one day) (Abadias *et al.*, 2012). Higher temperatures (with high humidity) may also encourage microbial growth on whole fruits or vegetables (Park *et al.*, 2012). For example, a cocktail of *Salmonella* serotypes did not grow during seven days storage at 4ºC on whole tomatoes, jalapeño peppers or coriander, but grew by 2-4 $\log_{10}$ CFU/g

at 21ºC (Ma *et al.*, 2010). Reviews assembling data from older studies have been recently published and these show how variable pathogen behaviour can be on different produce types under different conditions (Chauret, 2011; Erickson, 2010; Hoelzer *et al.*, 2012; Park *et al.*, 2012).

L. monocytogenes is of particular concern for post-harvest fresh produce since this pathogen can grow at refrigeration temperatures, however Hoelzer *et al.* (2012) compared the maximum daily growth rates at 10ºC for *L. monocytogenes* from 119 studies and found that growth in most experiments was ≤1 $\log_{10}$ CFU/g/day. Commodity-specific differences were evident and mathematical modelling predicted a growth rate of 0.4 $\log_{10}$ CFU/g/day (95% CI 0.3-0.5) for *L. monocytogenes* on lettuce and endive, based on 33 observations and averaged across experimental temperatures (Hoelzer *et al.*, 2012). The highest and lowest commodity-specific growth rates were predicted for asparagus (1.4 $\log_{10}$ CFU/g/day, 95% CI 0.9-2.1) and cantaloupe melon (0.0 $\log_{10}$ CFU/g/day, 95% CI 0.0-0.2), but there were fewer observations to base these predictions on (four and two, respectively). A recently published model predicted that the maximum growth of *L. monocytogenes* (and *E. coli* O157:H7) in bagged lettuce along a distribution chain (including transport, storage and display) where temperatures were <10ºC would, in most cases, be <2 $\log_{10}$ CFU/g (Zeng *et al.*, 2014). Another model predicted that *L. monocytogenes* on fresh-cut melons would take six days to increase by 1 $\log_{10}$ CFU/g at 4ºC (i.e. growth rate of 0.17 $\log_{10}$ CFU/g/day), and around one day at 10ºC (Danyluk *et al.*, 2014). Despite these predictions, some of the more recent studies that are presented in TABLE 3 show that growth of *L. monocytogenes* can be significant. Hudson *et al.* (2014) have recently compiled data from a number of studies that demonstrate the ability of *L. monocytogenes* to grow in fruit salads, particularly where melon is an ingredient (Hudson *et al.*, 2014).

TABLE 3: Examples of recent studies on the behaviour of *Salmonella* spp., *L. monocytogenes* and STEC on fresh produce at cooler temperatures

PATHOGENIC BACTERIA	CHANGE IN CONCENTRATION OF PATHOGEN IN EXAMPLES OF STUDIES REPORTING:		
	DEATH	SURVIVAL	GROWTH
Salmonella spp.	• 4ºC (Typhimurium): Survived 1 day on **blueberries**, then decreased by 1 $\log_{10}$ CFU/g over the next 2 days (Li and Wu, 2013). • 4ºC (serotype cocktail): Decreased on the cut and uncut surfaces of **celery** stored in bags or containers over 7 days (Vandamm *et al.*, 2013). • 4ºC (serotype cocktail): Decreased on whole **strawberries** by 2.5-3.9 $\log_{10}$ CFU/g over 7 days and on **basil** by 0.3-1.8 $\log_{10}$ CFU/g (Delbeke *et al.*, 2015).	• 4ºC (Newport): Remained stable for 15 days on the stems or leaves of **spring onions** (Xu *et al.*, 2015). • 4ºC (serotype cocktail): Concentration remained fairly stable over 7 days on sliced **mangoes** and **papayas** inoculated at 3 $\log_{10}$ CFU/g or less (Strawn and Danyluk, 2010). • 4ºC (serotype cocktail): Remained stable for 7 days on intact **tomato**, **jalapeño**, and **coriander** (Ma *et al.*, 2010) • 10ºC (serotype cocktail): Remained stable for 1 week on unpeeled **oranges** (died off thereafter) (Danyluk *et al.*, 2013).	• 4ºC (Seftenberg): Grew just under 1 $\log_{10}$ CFU/g in 3 days on **basil** leaves (Kisluk *et al.*, 2013). • 7ºC (serotype cocktail): Grew just over 0.5 $\log_{10}$ CFU/g in 3 days on **spinach** (Calix-Lara *et al.*, 2014). • 7ºC (serotype cocktail): Grew approx. 1 $\log_{10}$ CFU/g in 6 days under MAP (5% O_2, 15% CO_2, 80% N_2) on a RTE **lettuce** or **brassica** mix (Sant'Ana *et al.*, 2013).

Continues next page

TABLE 3 continued

PATHOGENIC BACTERIA	CHANGE IN CONCENTRATION OF PATHOGEN IN EXAMPLES OF STUDIES REPORTING:		
	DEATH	SURVIVAL	GROWTH
L. monocytogenes	• 4ºC: Decreased on the cut and uncut surfaces of **celery** stored in bags or containers over 7 days (Vandamm *et al.*, 2013). • 6ºC: Decreased on sliced **blackberries** and sliced **strawberries** (survived on whole **raspberries** and all berries when frozen at -20ºC) (Cobo Molinos *et al.*, 2008a) • 7ºC: Decreased by 3 $\log_{10}$ CFU/g over 6 days on grated **carrot** stored under modified atmosphere packaging (Sant'Ana *et al.*, 2012).	• 6ºC: Remained stable for 7 days on sliced **pear** and sliced **kiwifruit** (Cobo Molinos *et al.*, 2008a). • 10ºC: Minimal decline in concentration (approx. 1 $\log_{10}$ CFU/g) over 7 days on **parsley** (Likotrafiti *et al.*, 2013).	• 4ºC: Grew by about 4.5 $\log_{10}$ CFU/g over 12 days on **broccoli** florets (Takala *et al.*, 2013). • 5ºC: Grew by about 1 $\log_{10}$ over 10 days on shredded **lettuce** stored under different packaging films, including one with perforations to replicate packaging under normal atmospheric conditions (Oliveira *et al.*, 2010a). • 6ºC: Grew 2-3 $\log_{10}$ CFU/g over 7 days on sliced **melon** and sliced **watermelon** (Cobo Molinos *et al.*, 2008a). • 7ºC: Grew by 1-1.8 $\log_{10}$ CFU/g in most samples of a RTE **brassica** mix stored for 6 days under MAP or perforated film (Sant'Ana *et al.*, 2013). • 10ºC: Grew by >2 $\log_{10}$ CFU/g over 8 days on shredded **lettuce** under modified atmosphere (Oliveira *et al.*, 2012a). • 10ºC: Grew 1.8 $\log_{10}$ CFU/cm^2 on the surface of **cucumber** stored 4 days at 90% relative humidity (slow decrease in concentration thereafter) (Likotrafiti *et al.*, 2013).

TABLE 3 continued

PATHOGENIC BACTERIA	CHANGE IN CONCENTRATION OF PATHOGEN IN EXAMPLES OF STUDIES REPORTING:		
	DEATH	SURVIVAL	GROWTH
STEC (all used *E. coli* O157:H7)	• 4ºC: Decreased on the cut and uncut surfaces of **celery** stored in bags or containers over 7 days (Vandamm *et al.*, 2013). • 4ºC: Decreased on whole **strawberries** by 2.5-3.9 $\log_{10}$ CFU/g over 7 days, and on **basil** by 0.4-1.6 $\log_{10}$ CFU/g (Delbeke *et al.*, 2015). • 5ºC: Slow decrease on cut escarole (curly **endive**) and grated **carrot** (up to 1.3 $\log_{10}$ CF/g over 10 days) (Abadias *et al.*, 2012).	• 3ºC: cocktail of three STEC strains remained stable on whole **carrots** for 15 days (Gomez-Aldapa *et al.*, 2013c). • 4ºC: Remained fairly stable over 7 days on sliced **mangoes** and **papayas** (Strawn and Danyluk, 2010). • 4ºC: Survived 12 days on **broccoli** florets (concentration appeared to decrease then regrow to initial levels) (Takala *et al.*, 2013). • 5ºC: Remained fairly stable over 6 days on sliced **pineapple** and **melon** stored under air (Abadias *et al.*, 2012). • 5ºC: Remained fairly stable over 10 days on cut **apples** and **peaches** (Alegre *et al.*, 2013). • 10ºC: Remained relatively stable on most samples of shredded **lettuce** for 8 days under modified atmosphere (Oliveira *et al.*, 2012a).	• 4ºC: Grew approx. 2 $\log_{10}$ CFU/g in 15 days on **kale** (Mansur *et al.*, 2014). • 7ºC: Grew just over 1 $\log_{10}$ CFU/g in 3 days on **spinach** (Calix-Lara *et al.*, 2014).

Limited information is available on the behaviour of the other bacterial pathogens:

- ***A. hydrophila*** grew by approximately 2 $\log_{10}$ CFU/g in a RTE vegetable salad stored at 25ºC for 24 hours (Kumar *et al.*, 2012). Biofilm formation on fresh produce has been suggested by one study. Strains of *A. hydrophila* isolated from drinking water biofilms were able to attach to lettuce and cabbage leaves within an hour of contact and the concentration of attached cells and strength of attachment increased over 24 hours at 10ºC (Elhariry, 2011). No other recent studies on *Aeromonas* spp. behaviour were located, but older reports show that these bacteria can survive and potentially grow on fresh produce, and have some resistance to chlorination (see (Uyttendaele *et al.*, 2004) and (Stratev *et al.*, 2012) and references therein).

- The concentration of naturally present ***B. cereus*** (presumably as spores) remained stable on RTE baby carrots stored under Modified Atmosphere Packaging (MAP) for 15 days at 4ºC (Leceta *et al.*, 2015).

- As mentioned in the 2008 Document, ***Campylobacter*** **spp.** do not grow on fresh produce and the concentration slowly decreases so the final concentration at consumption will depend on the initial load. This has been supported by a recent study that reported death of *C. jejuni* on spinach leaves stored at 4ºC and 12ºC (Guévremont *et al.*, 2015).

- No new information was located for **clostridia** but these bacteria will be able to survive in spore form for long periods of time. Clostridia are most likely to grow in fruits and vegetables kept in MAP, and older studies demonstrate that *C. botulinum* can grow and produce toxin on a variety of fresh-cut fruit and vegetables stored under modified atmospheres (Institute of Food Technologists, 2001). Data on the potential for growth of *C. botulinum* and *C. perfringens* and their ability to produce toxins on a wide variety of produce stored in MAP at mildly abusive temperatures such as 7-12°C are needed to better understand the risk for products sold in the current market.

- ***S. aureus*** grew by approximately 2 $\log_{10}$ CFU/g in a RTE vegetable salad stored at 25ºC for 24 hours (Kumar *et al.*, 2012). The concentration of *S. aureus* decreased by almost 2 $\log_{10}$ CFU/g when inoculated on to fruit salad and stored at 24ºC for 60 hours (Estrada *et al.*, 2013). *S. aureus* could attach to the surface of whole peaches and plums but could not survive subsequent storage at 0.5ºC (Collignon and Korsten, 2010).

- The concentration of ***S. sonnei*** remained stable when inoculated onto coriander, parsley or spinach and stored for 7 days at 4ºC (Orue *et al.*, 2013).

- The concentration of a two-strain cocktail of ***Y. enterocolitica*** remained stable when inoculated on tomatoes or lettuce leaves and stored for seven days at 4ºC, but was able to grow by up to 1 $\log_{10}$ CFU/g on lettuce leaves and 3 $\log_{10}$ CFU/g on tomatoes when these foods were chemically sanitised, thus removing competitive microflora (Velázquez *et al.*, 2009). Similarly, *Y. enterocolitica* grew in the absence of competitive microflora on pre-sterilised bell pepper disks, increasing by 5 $\log_{10}$ CFU/disk after two days at 20ºC (Liao, 2009).

Protozoan pathogens

Protozoa only progress through their reproductive life cycle when they have infected their definitive host. *Cryptosporidium* spp., *Giardia* spp., *C. cayetanensis* and *T. gondii* have all been detected on fresh produce though surveys (Appendix A.3.1), but there are very few studies of survival and maintenance of infectivity on fresh produce over time. *C. parvum* oocysts inoculated onto apples and stored at 6ºC remained viable for 4 weeks at 6ºC, as measured by microscopy and mouse infectivity assays, and were firmly attached to the peel (Macarisin *et al.*, 2010b). *C. parvum* oocysts were also observed to adhere to the root hairs and leaves of spinach plants (Macarisin *et al.*, 2010a). No recent studies were located for the

other protozoans. Survival of *C. cayetanensis* on basil (23ºC, 6 days) was reported in an older review (Erickson and Ortega, 2006).

Viral pathogens

Like protozoa, viruses only multiply when they have infected their animal host. Evidence from outbreaks indicates that norovirus can remain infectious on fresh salad vegetables for several days, possibly more, and HAV and norovirus can survive for several months on frozen fruit (EFSA, 2011d, 2014f). Older studies have found that viruses remained viable for periods exceeding the shelf lives of fresh produce (see summary in Butot *et al*., 2008), and more recent laboratory studies provide further evidence for survival on fresh produce:

- HAV was detected from spinach leaves six weeks after they were inoculated and stored at 5.4ºC (albeit at 2% of the initial HAV concentration), implying that HAV can persist under normal domestic storage conditions for extended periods of time (Shieh *et al.*, 2009).
- The concentration of norovirus GI and GII (as measured by PCR) did not change when inoculated on to strawberries or raspberries and stored for seven days at 4ºC, and neither did the cultural concentration of the surrogate murine norovirus (Verhaelen *et al.*, 2012). Slow viral decay was reported at 10ºC (e.g. 7 days for cultured murine norovirus to reduce by 1 log on strawberries), and viral decay was hastened at 21ºC (e.g. PCR copies of norovirus GI reducing by 1 log in 1-4 days on strawberries).
- Norovirus, HAV and rotavirus survived storage for 90 days at -20ºC on blueberries, raspberries, strawberries, basil and parsley (Butot *et al.*, 2008).
- When stored for nine days at 7ºC on mixed baby leaf salad, the concentration of infectious feline calicivirus, a norovirus surrogate, initially reduced during the first day, then remained relatively stable (Azizkhani *et al.*, 2013).
- Porcine rotavirus, a human rotavirus surrogate, was able to attach to leaves from 21 leafy green cultivars and fruits from three tomato cultivars (Lu *et al.*, 2015). The extent of viral adsorption was related to the physicochemical properties of the plant.

In addition, virus-like-particles for the norovirus GII.4 strain were shown to preferentially attach to cut edges, stomata and along minor veins of romaine lettuce leaves (Esseili *et al.*, 2012).

2.4.4 Behaviour of pathogens in fresh juices

The 2008 Document cites many examples of outbreaks of enteric disease caused by contaminated fruit juices, and this provides evidence that pathogens can either contaminate juices at high enough concentrations to cause disease, or grow to a sufficient concentration. The behaviour of pathogenic microorganisms in fresh juices will vary with the species, the ingredients of the juice and the resulting characteristics (e.g. pH, presence of natural antimicrobials and/or other microflora) and the storage time/temperature before consumption.

It is apparent from recent literature that survival of pathogenic microorganisms is possible in juices, however very few experiments used fresh juice that had not been modified in any way before pathogen inoculation. In addition, most experiments have focussed on *L. monocytogenes* or viral pathogens, and most were conducted at low temperatures (≤10ºC) so any growth of *L. monocytogenes* measured at these temperatures was slow (days):

- The concentration of *L. monocytogenes* increased in filtered and sterilised **melon juice** (pH 5.77), decreased in **apple juice** (pH 3.70) and did not change in **pear juice** (pH 4.61) when stored at 10ºC for eight days (Oliveira *et al.*, 2014). At 5ºC, the concentration of *L. monocytogenes* remained fairly stable (<0.5 $\log_{10}$ CFU/ml change) in filtered and sterilised melon, apple and pear juices over five days (Raybaudi-Massilia *et al.*, 2009).

- *L. monocytogenes* grew very slowly in **coconut water** (pH 4.9) stored at 4ºC (lag time of about 15 days, growth rate of about 0.1 $\log_{10}$ CFU/ml/day) but at 10ºC the lag time was reduced to about three days and the growth rate was approximately 0.5 $\log_{10}$ CFU/ml/day (Walter *et al.*, 2009).

- The concentration of *L. monocytogenes* decreased by just over 1 $\log_{10}$ CFU/ml in **watermelon juice** stored for three days at 6ºC, but the concentration did not change with further storage for another four days (Cobo Molinos *et al.*, 2008a).

- The concentration of a cocktail of four *L. monocytogenes* strains inoculated into filtered and radiation-sterilised **carrot juice** decreased by 1.5 $\log_{10}$ CFU/ml when stored for 14 days at 4ºC, and by 0.6 $\log_{10}$ CFU/ml when stored at 8ºC (Patterson *et al.*, 2012). At 12ºC, the inoculum grew by almost 1 $\log_{10}$ CFU/ml. When inoculated into irradiation-sterilised and heat-treated carrot juice, the concentration of *L. monocytogenes* increased at all temperatures, possibly because the heat treatment inactivated the natural antimicrobial activity of the carrot juice.

- The concentration of a human norovirus surrogate, murine norovirus, slowly decreased when stored in **pomegranate juice** (pH 3.3) at 4ºC for 21 days, but remained stable in **orange juice** (pH 3.8) (Horm and D'Souza, 2011). The concentration of two other surrogates, feline calicivirus and MS2 bacteriophage, decreased in both juices or remained stable in both juices, respectively. All three viruses are acceptable human norovirus surrogates so these results indicate that human norovirus can potentially survive in these juices at 4ºC.

- The concentration of three norovirus surrogates, murine norovirus, feline calicivirus and bacteriophage MS2 all decreased in a **blueberry juice drink** stored for 21 days at 4ºC, but the rate of reduction varied. Approximately half of the initial inoculum (approximately 4 $\log_{10}$ plaque-forming units per ml) of murine norovirus was still detected at the end of this period (Horm *et al.*, 2012).

- The concentration of *Salmonella* Saintpaul inoculated into fresh **orange juice** (pH 3.5) or **mango juice** (pH 4.0) decreased by 3-4 $\log_{10}$ CFU/ml during storage at 5ºC for 21 days (Raybaudi-Massilia *et al.*, 2012). The concentration of *E. coli* O157:H7 also decreased by almost 4 $\log_{10}$ CFU/ml in fresh mango juice under the same conditions, but only decreased by approximately 1.3 $\log_{10}$ CFU/ml in fresh orange juice, despite significant growth of background microflora.

- The concentration of a cocktail of three STEC strains (two non-O157) remained stable in fresh **carrot juice** stored at 3ºC for 10 days, but grew by 2 $\log_{10}$ CFU/ml when the juice was stored for 24 hours at 12ºC (Gomez-Aldapa *et al.*, 2013c).

The potential for growth at room temperature over a 24 hour period has not been extensively studied. This is relevant for scenarios when fresh juices are prepared and given directly to the consumer. Acid-adapted *L. monocytogenes* were able to grow 4 $\log_{10}$ CFU/ml over 6 hours in diluted **orange juice** with a pH of 2.6, an acidity level that should not permit growth, but the storage temperature was 37ºC (unrealistic) (Caggia *et al.*, 2009). It is not known whether acid-adapted *L. monocytogenes* can grow in low-pH juices stored in refrigeration or ambient conditions.

2.4.5 Behaviour of pathogens on sprouts

Evidence from surveys and outbreaks cited in the 2008 Document, and this update, prove that pathogenic microorganisms (bacteria and protozoa) can contaminate sprouts. Pathogenic viruses might also contaminate sprouts (e.g. through contaminated seed, irrigation water or infected workers) but no outbreaks or survey data were located.

Some recent studies have investigated the behaviour of (mostly bacterial) pathogens on this food. These show that the seeds were the source of contamination rather than contamination after sprouting, and that survival and growth were enhanced by warmer temperatures but pathogens can survive well on sprouts stored at cooler temperatures:

- The concentration of *B. cereus* applied as vegetative cells decreased when inoculated on asparagus, soybean sprouts or alfalfa sprouts and stored at 6ºC, but grew at 15ºC (Cobo Molinos *et al.*, 2008c). Naturally present *B. cereus* (presumably spores) were detected on commercially-produced alfalfa and rapeseed sprouts throughout germination, growth and storage (the producers did not use a seed decontamination step prior to sprouting) (Kim *et al.*, 2013). The final concentration on sprouts ranged 4.4-2.7 $\log_{10}$ CFU/g.
- A cocktail of three non-O157 STEC inoculated onto sanitised mung bean seeds survived storage on the seeds for 90 days at 22ºC and 60% relative humidity, and grew on the sprouts when the seeds were subsequently germinated, growing by approximately 4 $\log_{10}$ CFU/g at 20ºC and almost 6 $\log_{10}$ CFU/g at 30ºC over 10 days (Gomez-Aldapa *et al.*, 2013a). These STEC also survived for 10 days on sprouts grown from contaminated seeds and stored at 3ºC. When uncontaminated seeds were germinated and inoculated with non-O157 STEC within two days of germination, the STEC did not grow during continued sprouting at 20ºC, and only grew by approximately 1 $\log_{10}$ CFU/g at 30ºC, before slowing dying off at both temperatures. The authors suggested that the absence of growth compared with that observed on sprouts produced from contamined seeds was possibly due to competitive microflora or less available nutrients. Similar results were observed on alfalfa sprouts (Gomez-Aldapa *et al.*, 2013b).
- *S. enterica* (serotype not specified), *E. coli* O157:H7, *S. sonnei*, *S. flexneri* and *A. hydrophila* all survived when inoculated on soybean sprouts and stored for two days at 6ºC, but *Y. enterocolitica* grew by approximately 3 $\log_{10}$ CFU/g (Cobo Molinos *et al.*, 2008b).
- *E. coli* O157:H7 and *Salmonella* Typhimurium both grew on pre-sterilised buckwheat sprouts stored at 4ºC for eight days (growth was ≤1 $\log_{10}$ CFU/g) (Chun and Song, 2013).
- HAV and two human norovirus surrogates, murine norovirus and tulane virus, survived on alfalfa seeds for 50 days at 22ºC with some decrease in infectivity, and remained infectious on subsequent sprouted seeds (Wang *et al.*, 2013) HAV demonstrated greater persistence than the norovirus surrogates.

2.4.6 The influence of salad 'additives' on the behaviour of pathogens

Three 'additives' to fresh vegetable salads will be considered: Salad dressings, nuts and animal proteins (meat, fish).

Salad dressings (e.g. mayonnaise, vinaigrette) typically contain an acid component which reduces the pH of a salad, which generally makes the salad a less favourable medium for pathogens to survive or grow. However, the behaviour of pathogens in a dressed salad will still be affected by the overall characteristics of the salad and the pathogen being considered, e.g. the presence of animal proteins (e.g. poultry, seafood, egg) will promote survival or even growth of *L. monocytogenes* in a dressed salad (Erickson, 2010; Skalina and Nikolajeva, 2010; Uyttendaele *et al.*, 2009). The few recent experiments with dressed vegetable salads have focussed on *L. monocytogenes* (the pathogen most likely to contaminate and grow on RTE dressed salads), and *Salmonella* spp. (can tolerate acidic conditions and contaminate eggs so can be transferred to mayonnaise (Zhu *et al.*, 2012)):

- The concentration of a cocktail of *L. monocytogenes* serotypes decreased slowly when inoculated into coleslaw with mayonnaise (pH 3.9) and stored at 4°C, but the pathogens were still detected after 27 days despite the initial concentration being only 2 $\log_{10}$ CFU/g

(Alali *et al.*, 2012). A cocktail of *Salmonella* serotypes inoculated into coleslaw at approximately 2 $\log_{10}$ CFU/25g was not detectable after 13 days storage at 4ºC (i.e. concentration was <1 CFU/25 g). Inactivation was faster at 10ºC for both pathogen cocktails.

- When spinach leaves pre-inoculated with a cocktail of *Salmonella* spp. or *L. monocytogenes* were treated with a mixture of white vinegar and canola oil (1:2), after 40 minutes at room temperature, the concentrations of *Salmonella* spp. and *L. monocytogenes* decreased by approximately 2 $\log_{10}$ CFU/g and 0.5 $\log_{10}$ CFU/g, respectively (Faith *et al.*, 2012).

STEC can also tolerate acidic conditions but no recent studies of STEC behaviour in dressed vegetable salads were located. In studies with pickling brines, strains of *E. coli* O157:H7 were significantly more acid resistant than strains of *Salmonella* spp. and *L. monocytogenes* (Breidt *et al.*, 2013).

The addition of heat-treated nuts (e.g. roasted) and cooked proteins will not increase the prevalence of any pathogenic microorganisms in a fresh vegetable salad unless these foods have been contaminated after cooking. Raw nuts can introduce pathogenic microorganisms to salads, particularly *Salmonella* spp.

2.5 EXPOSURE ASSESSMENT

Key findings

The results from recent surveys of New Zealand fresh produce (whole and cut) indicate that pathogenic microorganisms can be found on these products, but the prevalence and concentrations are low:

- *Salmonella* spp. were detected on 2% of 891 samples of fresh fruits and vegetables, *E. coli* O157 and *Campylobacter* spp. were not detected. Leafy green vegetables were most likely to be contaminated.
- *L. monocytogenes*, *Salmonella* spp. and *Campylobacter* spp. were not detected in 307 samples of pre-packaged RTE leafy salads. Norovirus GII was detected in three samples and the presence of other *Listeria* species in 19 samples indicate that *L. monocytogenes* could contaminate these products.
- *L. monocytogenes* was detected in 5% of 75 samples of fresh-cut fruit salads (concentration <100 CFU/g). Melon-based salads were most likely to be contaminated.
- *Salmonella* spp. and *L. monocytogenes* were each detected in 2% of 50 sprout samples at low concentrations (≤0.04 MPN/g, <100 CFU/g, respectively).
- *L. monocytogenes* was detected in 13% of RTE coleslaw samples with dressing.

A. hydrophila has been detected in RTE food in a New Zealand survey from 1991, but there are no recent prevalence studies. No prevalence studies were located for the other pathogens considered in this document and there is a particular absence of data for pathogenic protozoa.

Recalls for fresh produce are rare in New Zealand; only three recalls have been issued since 2008, for spinach and parsley potentially contaminated with *L. monocytogenes*, and for pipfruit potentially contaminated with HAV.

Comparison of data from national nutrition surveys in 1997 and 2009 suggest that, in general, consumption of fresh fruit and vegetables has decreased among adult New Zealanders. The proportion of adults consuming berries and other small fruits (e.g. grapes), or consuming fresh juices, has increased. Other than root and tuber vegetables (and salads made from these), fresh juices and pome fruit, children are generally less likely to eat raw fruit or vegetables in a 24 hour period, and consume smaller quantities.

2.5.1 New Zealand prevalence studies

The 2008 Document reported the results from three microbiological surveys. One of these, a survey of *Listeria* spp. in RTE salads with dressings (2006/07) had not been fully completed at the time. Upon completion, 4/31 (13%) samples of RTE coleslaw with dressing purchased from supermarkets and delicatessens were positive for *L. monocytogenes* (Wong, 2008). The concentrations of *L. monocytogenes* in these samples were 100 CFU/g (1 sample), 30 CFU/g (1 sample, also contaminated with *L. innocua* at <10 CFU/g) and <10 CFU/g (2 samples). The 2008 Document did not report results from an older survey: *A. hydrophila* was isolated from one sample of coleslaw (no mayonnaise) in a survey of 396 New Zealand retail food samples for aeromonads (Hudson and Delacy, 1991). Aeromonads were also isolated from other RTE salads, but these contained seafood. This survey is worth noting as it is the only New Zealand survey for *Aeromonas* spp. on RTE foods.

Four other microbiological surveys of fruit and vegetables sold in New Zealand have been completed since the 2008 Document.

Survey of produce (2008/09)

Over a 15 month period, 891 samples of fresh fruits and vegetables were purchased from retail outlets in Auckland and Christchurch and tested for faecal coliforms, generic *E. coli*, *E. coli* O157, *Salmonella* spp. and *Campylobacter* spp. (McIntyre and Cornelius, 2009). The samples included imported produce (25%) and produce grown in New Zealand using organic (35%) and conventional (39%) methods. Melons, tomatoes, strawberries, apples, table grapes, leafy green vegetables (lettuce, spinach, kale), capsicums, carrots and sprouted seeds were sampled.

Salmonella Typhimurium phage type RDNC-May 06 was detected on two samples of domestically-produced organic lettuce from the same grower. Bird faeces were noted on hail netting above the lettuce growing site, and while no testing was carried out, this is a potential source of sporadic contamination. *Campylobacter* spp. and *E. coli* O157 were not detected. These results were similar to results from produce surveys in other countries (as summarised in McIntyre and Cornelius (2009)) where *Salmonella* spp. are often detected at low prevalence.

Elevated levels of faecal coliforms (≥100 CFU/g) were detected in 22/108 (20.4%) samples of leafy green vegetables, 2/129 (1.6%) capsicum samples and 5/150 (3.3%) strawberry samples. It was noted that 18/22 of the leafy green vegetables samples with elevated faecal coliforms were organically grown, but of these, 15 were samples of organic kale purchased from the same premises at the same time (the rest were spinach). Elevated levels of generic *E. coli* (≥100 CFU/g) were detected in 20/108 (18.5%) samples of leafy green vegetables, 1/129 (0.8%) capsicum sample and 5/150 (3.3%) strawberry samples. Faecal coliforms and *E. coli* were both present on all three foods at concentrations up to 1.1×10^4 MPN/g. All of the samples with elevated levels of these microbiological hygiene markers were domestically grown.

The results of this survey show that, of the fruit and vegetables tested, leafy green vegetables were more likely to be contaminated.

Survey of packaged leafy vegetable salads (2012)

A total of 307 samples of pre-packaged RTE leafy vegetable salads were purchased from 29 retail stores in Auckland, Wellington and Christchurch and tested for *Listeria* spp. (and *L. monocytogenes*), *Salmonella* spp., generic *E. coli* (followed by STEC testing if *E. coli* was present), *Campylobacter* spp. and norovirus genogroups I and II (GI and GII) within +/- two days of the best before date (Hewitt and Rivas, 2015).[16] The total sample set included 92 different products (single and mixed salads including different lettuce varieties, endive, baby spinach, salad rocket, watercress, herbs and mesclun mixes) from nine processors/distributors. All samples were grown in New Zealand (two organically, eight hydroponically). Any non-leafy ingredients (e.g. cucumber, capsicum, cabbage) were removed before testing, but potential cross-contamination within the unopened bag could not be avoided.

Generic *E. coli* were not detected (<3 MPN/g) in 227 (96.2%) of the 236 samples tested for these bacteria. Nine samples yielded 3-43 MPN/g generic *E. coli*. STEC were not detected in any of the leafy salads confirmed to contain *E. coli*.

Listeria spp. were detected in 19/307 samples (6.2%, all at concentrations of <100 CFU/g) but *L. monocytogenes* was not detected. No *Salmonella* spp. or *Campylobacter* spp. were detected in any samples.

[16] The testing also included human adenoviruses to investigate whether these could be used as an alternative hygiene indicator to generic *E. coli*.

Viral testing was only possible for 305 samples, and norovirus GI was not detected. Norovirus GII was detected in 3/305 (1.0%) of samples at a concentration of <50 genome copies per 25g. Generic *E. coli* were below the limit of detection in these three samples (<3 MPN/g).

The results of this survey indicate that microbiological contamination of packaged salads is minimal. The pathogens that may be of concern are norovirus (detected, but the viability was not established) and *L. monocytogenes* (the presence of other *Listeria* species indicates that *L. monocytogenes* could contaminate these products). Also, the presence and concentration of generic *E. coli* appears to be unsuitable for indicating the potential for viral contamination.

Survey of fresh-cut fruit salads (2013/14)

Packets of fresh-cut, RTE, non-retorted fruit salads were purchased from supermarkets or online retail stores and tested at the end of shelf-life for the presence of *Salmonella* spp. and *Listeria* spp., and the concentration of *E. coli*, coagulase-producing *Staphylococcus* spp. (CPS) and mesophilic aerobic microflora (D'Sa and Hudson, 2014). Seventy-five composite samples were analysed, comprised of up to five samples in the same batch.

L. monocytogenes was detected in 4/75 (5%) composite samples (three melon, one mixed fruit including melon) at concentrations <100 CFU/g. The same three melon samples also tested positive for *L. innocua*, and an additional seven samples also tested positive only for *L. innocua* (prevalence of 13% for *Listeria* spp.). The counts of *L. innocua* were <100 CFU/g for seven samples, and were 350-900, 200-1250 and 200 (for one sub-sample) CFU/g in the remaining three samples. *Salmonella* spp., *E. coli* and CPS were not detected. The aerobic plate counts were highly variable, ranging from 3.6 $\log_{10}$ CFU/g (citrus mix) to 8.9 $\log_{10}$ CFU/g (melon and grape).

The pH of the samples ranged from 3.2 (pineapple) to 6.7 (rockmelon), and melon samples were less acidic (lowest pH recorded was 5.4). *Listeria* spp. were not detected in samples where pH≤4.0.

The results of this survey indicate that melon-based fresh-cut fruit salads are most likely to be contaminated, and *L. monocytogenes* is the pathogen of most concern.

Survey of sprouts and shoots (2014)

Fifty lots of pre-packed sprouted seeds and shoots were purchased from retail outlets and tested for generic *E. coli*, mesophilic aerobic microflora, *Listeria* spp., *Salmonella* spp. and STEC (serogroups O26, O45, O103, O111, O121, O145 and O157) within +/- two days of the expiry or best before date (D'Sa and Paulin, 2015).

Of the 50 composite samples, one (2%) tested positive for *Salmonella* Adelaide (mixed alfalfa sprouts and snow pea shoots), one (2%) tested positive for *L. monocytogenes* (serotype O1/2, sunflower seed sprouts) and six (12%) tested positive for other *Listeria* spp. (sprouts of soybean, chickpeas, alfalfa and broccoli). STEC were not detected in any of the samples.

Enumeration of five subsamples of the *Salmonella*-positive sample found a low concentration of the pathogen: Not detected in three subsamples, and 0.04 MPN/g in the other two subsamples. All *Listeria* spp. counts were <100 CFU/g, including *L. monocytogenes*.

Generic *E. coli* were detected in 2/50 (4%) samples (detection limit 3 MPN/g) at concentrations of ≤23 MPN/g. Both samples were also positive for *Listeria* spp. (one *L. monocytogenes*, the other *L. innocua*). Mesophilic aerobic microflora concentrations ranged from 2.1×10^6 to 7.7×10^8 CFU/g.

The results of this survey indicate that *L. monocytogenes* and *Salmonella* spp. can be present in sprouts, but the prevalence and concentrations are low.

2.5.2 Product recalls

Between 2008 and June 2015 there were three New Zealand food recalls relevant to this document:

- January 2011: Recall for a range of RTE packaged salads containing spinach that may have been contaminated with *L. monocytogenes* (MPI, 2011).[17] The contamination appeared to have been caused by unusually severe weather in the 48 hours before harvest causing leaf damage and high soil loadings on the produce from rain splash, plus inadequate antimicrobial treatment during post-harvest washing (Lowry, 2011).
- March 2014: Apples and pears potentially contaminated with HAV. A packhouse worker was diagnosed with Hepatitis A.
- June 2015: Fresh Italian parsley potentially contaminated with *L. monocytogenes* (the cause of the contamination was not reported).[18]

2.5.3 Import food testing

There are currently no Imported Food Requirements (IFRs) for the foods considered in this document (MPI, 2015a).

2.5.4 Food consumption: Fruit and vegetables

The 2008 Document presented data from the 1997 National Nutrition Survey (1997NNS; adults 15+ years, *n* = 4,636) (Russell *et al.*, 1999) and some comparison data with the 2002 National Children's Nutrition Survey (2002CNS; children 5-14 years, *n* = 3725) (Ministry of Health, 2003). Since the 2008 Document, results from the 2009 Adult Nutrition Survey (2009ANS, adults 15+ years, *n* = 4721) have become available (University of Otago and Ministry of Health, 2011).

Information was taken from 24-hour dietary recall (24HDR) records collected as part of the three nutrition surveys, where people report what they recalled eating over a 24-hour period. The parameters reported in TABLE 4 are:

- Percent consuming: The proportion of respondents who reported consumption of the food or foods during the 24-hour recall period.
- Respondent mean consumption: The total amount of the food or foods consumed during the 24-hour recall period divided by the total number of survey respondents. This is an estimate of the ongoing mean daily consumption of the food across the whole population.

Fruits and vegetables have been grouped according to the Codex Classification of Foods and Animal Feeds (FAO/WHO, 1993). Additionally, salads have been included in the group corresponding to their predominant ingredient. For example, lettuce salads, green salads, Greek salads and Caesar salads have been included with leafy vegetables, as their predominant ingredient is lettuce. Several foods of specific interest (fruit salads, sprouts, unpasteurised juices) have been identified separately.

The food consumption information summarised in this section has been selected to align as closely as possible to the definitions used in the current discussion document. Consequently the following conventions have been adopted:

[17] New Zealand food recalls are advertised at http://www.foodsmart.govt.nz/food-safety/recalls/latest-recalls/ (accessed 25 March 2015).
[18] http://www.foodsmart.govt.nz/elibrary/consumer/tasman-bay-herbs-brand-italian-parsley-50g-bag.htm (accessed 8 July 2015).

- Descriptors for fruits and vegetables were included if they contained the words ‘raw’ or ‘fresh’, without mention of any cooking method.
- Salads based on leafy vegetables, brassica vegetables (cabbage), fruiting vegetables (tomato) and carrots were assumed to meet the definition used in this discussion document.
- When salad was identified as a component of a composite food (eg. filled roll) and was not further specified, it was assumed that this would be a leafy vegetable salad.
- Canned fruits and vegetables were not included, even when the foods were described as ‘raw’, as canned foods are subjected to a retorting process.
- Frozen berries were included, as these products are frozen from fresh.
- Olives were not included as these foods undergo a brining process.
- Fermented vegetable products, such as sauerkraut and kimchi, were not included.

Data were also collected on fruit and vegetable juices described as ‘from fresh fruit/vege’.

The 2008 Document reported that salads (including pasta salads) were consumed by 17.6% of respondents in the 1997NNS in a 24-hour period, and fresh fruit salads by 0.98%, then suggested that consumption of these foods had increased since the 1997 survey. Comparison of data from the 1997NNS and 2009ANS in TABLE 4 shows that the proportions of adults consuming these food groups on any given day have not increased. Significantly fewer adults consumed leafy vegetables and leafy vegetable salads in a 24 hour period in the 2009 survey (19.9%, 95% confidence interval (CI) 18.8-21.1) compared with the 1997 survey (22.8%, 95% CI 21.6-24.0), and the proportion of adults consuming fruit salads appears to have decreased, but the change is not statistically significant. Those who do consume leafy vegetables and leafy vegetable salads appear to be consuming larger amounts, and those consuming fruit salads appear to be consuming smaller amounts.

It is also notable that the proportion of adults consuming berries and other small fruits (such as grapes) has increased from 3.9% (95% CI 5.1-6.4) in 1997 to 5.7% (95%CI 3.4-4.6) in 2009. The proportion of adults consuming fruiting vegetables and fruiting vegetable salads (e.g. tomatoes) has decreased from 25.4% (95% CI 24.2-26.7) in 1997 to 19.3% (95% CI 18.1-20.4) in 2009.

Consumption of fresh juices by adults has significantly increased (TABLE 4).

The 2008 Document also reported that sprouts were consumed by 2.9% of adults in any given 24 hour period with an average daily consumption of 0.6 g/day. The data reported for the 1997NNS in TABLE 4 are different due to a different method of analysis (focussing on raw consumption), but comparing data between the two surveys of adults does not show any significant change in sprout consumption.

Other than root and tuber vegetables and salads, fresh juices and pome fruit, children are generally less likely to eat raw fruit or vegetables in a 24 hour period, and consume smaller quantities.

TABLE 4: Consumption of fruit and vegetables, and fresh juices, by New Zealand adults (aged 15+ years; 2009ANS and 1997NNS) and New Zealand children (5-14 years; 2002CNS)

FOOD/FOOD GROUP	PERCENT CONSUMING (%)			MEAN DAILY CONSUMPTION, ALL RESPONDENTS (g/day)		
	2009ANS	1997NNS	2002CNS	2009ANS	1997NNS	2002CNS
Berries and other small fruit[1]	5.7	3.9	2.3	4.9	4.3	1.7
Brassica vegetables and brassica vegetable salads	4.1	4.9	2.3	4.1	4.0	1.4
Bulb vegetables	5.3	6.1	1.7	1.5	1.3	0.3
Cucurbits[2]	6.8	6.3	3.2	8.1	5.2	3.9
Fruiting vegetables and fruiting vegetable salads[3]	19.3	25.4	8.8	17.7	23.3	6.2
Fungi	0.4	0.5	0.1	0.1	0.1	<0.1
Herbs	1.0	1.1	0.3	0.1	0.1	<0.1
Leafy vegetables and leafy vegetable salads	19.9	22.8	11.8	12.8	10.8	4.8
Legume vegetables	0.4	0.4	0.2	0.2	0.2	0.1
Pome fruit	23.0	24.6	34.6	41.7	55.4	73.9
Root and tuber vegetables and salads	4.9	6.3	5.6	2.8	2.7	2.5
Stalk and stem vegetables[4]	1.0	2.2	0.5	0.4	0.6	0.1
Stone fruit	5.4	4.1	1.7	8.7	8.4	2.6
Tropical fruit – edible peel[5]	0.5	0.5	0.2	0.6	0.6	0.4
Fruit salad	0.9	1.3	0.2	1.9	2.4	0.6
Juice (fresh)	2.4	0.8	1.7	5.3	2.1	4.1
Sprouts	0.4	0.8	0.4	0.2	0.2	0.1

[1] Includes table grapes.
[2] This is a diverse group that includes fruits (e.g. melons) and vegetables (e.g. cucumbers, courgettes).
[3] Includes peppers and tomatoes.
[4] Includes asparagus and celery.
[5] Mainly tamarillos.

2.6 DATA ON PATHOGENS IN FRUIT AND VEGETABLES FROM OTHER COUNTRIES

Appendix A.3 contains detailed data summarised in this section.

Key findings

Based on recent review documents and some key studies that largely focus on prevalence in developed countries, most of the pathogens considered in this document have been detected on fresh produce. Exceptions are *C. perfringens* and *Shigella* spp., which were not detected in the few surveys including these bacteria, and HAV (no recent data were located for this virus). Only one recent study was located for *T. gondii*, and the presence of this protozoan was only indicated by PCR.

Concentration data are rarely available for any pathogens which prevents assessing the potential for infection if these foods were consumed.

Most surveys of fresh fruits and vegetables focus on *L. monocytogenes*, *Salmonella* spp., norovirus and STEC or *E. coli* O157. Results from these show that *L. monocytogenes* and *Salmonella* spp. can both be detected on fresh fruits and vegetables, but *Salmonella* spp. are less likely to be found on fruits compared with vegetables (except for melons due to their ground contact), and are generally less prevalent when compared with *L. monocytogenes*. The data also indicate that norovirus is widespread on fruits and vegetables, but the viruses detected on the positive samples may not be infectious, since there is currently no robust method for determining whether noroviruses present on foods are infectious. STEC or *E. coli* O157 have been rarely detected.

Surveys do not often test for the other pathogens so data are scarce.

Pathogenic microorganisms were most often detected on sprouts, fresh herbs, berries and salads/leafy vegetables, but many of the surveys specifically focussed on these foods and did not test other fresh produce types.

Prevalence or concentration data for fresh juices are very scarce for developed countries. *Salmonella* spp. and *S. aureus* were detected in one survey in Spain.

Of eight recalls in Australia since 2008, only three were for potential contamination with a pathogenic microorganism: Sprouts/*Salmonella* spp., frozen berries/HAV (two related recalls).

From recalls issued for the EU since 2001 it was apparent that the foods most of concern due to potential contamination with pathogenic microorganisms were leafy green vegetables, tropical fruit and melons, berries (including frozen), sprouts and tomatoes. There were also multiple recalls for mushrooms but there was not enough information to determine whether these were fresh or preserved.

Recalls for fresh juices are rare, presumably because they are consumed quickly and a recall would yield no products. There was one notification in the EU for acai berry juice but there was no information to determine whether this was pasteurised.

3. EVALUATION OF ADVERSE HEALTH EFFECTS

3.1 FRUIT AND VEGETABLE CONSUMPTION AS A RISK FACTOR FOR INFECTION

Key findings

Fresh produce has been a vehicle of infection in outbreaks of enteric disease in New Zealand but the available public health surveillance data do not provide enough information to determine the frequency of illness caused by fruits, vegetables, sprouts or fresh juices. Confirming the source for enteric infections as caused by the pathogens relevant to this document is rare, and this is especially difficult for illnesses and outbreaks where fresh produce are suspected.

Since 2008 there have been three outbreaks where fresh produce was the most likely vehicle for infection based on epidemiological information (salmonellosis/watermelon, norovirus/fresh fruit salad, yersiniosis/carrots and/or lettuces). There has only been one outbreak confirmed by microbiological evidence (i.e. same outbreak strain found in food and cases), since this is difficult to achieve for fresh produce outbreaks. This was a 2002 outbreak of Hepatitis A caused by raw, domestically-grown blueberries.

Between 2009 and 2014 fruit and vegetables were implicated (not the proven vehicle) in 10% of the foodborne outbreaks reported during this period that involved 19% of the foodborne outbreak cases. This is indicative only since the produce items may have been cooked, more than one food can be implicated in an outbreak and other non-food modes of transmission may have been reported.

All of the pathogens considered in this document were the reported causative organism in at least one outbreak reported overseas since 2008 where fresh fruits, vegetables, fresh juices or sprouts were the vehicle of infection. Exceptions were *Aeromonas* spp., *B. cereus* and *Giardia* spp., for which there was insufficient information to confirm these as causative organisms in such outbreaks. *Salmonella* spp., norovirus, STEC and *L. monocytogenes* were the most common causative organisms. The fresh produce items most often implicated by strong evidence were leafy green vegetables, sprouts, berries, tomatoes and melons, but a range of other vegetables were implicated by strong evidence.

From investigations of outbreaks in other countries, the most commonly cited causes of contamination were the use of contaminated water for irrigating or washing produce, and handling of the food by sick or asymptomatic people. Faecal contamination from animals was also commonly reported. Using contaminated seeds for sprout production was a common failure for this food.

Two New Zealand case control studies that were not associated with outbreak investigations, found that eating raw vegetables or raw fruits and vegetables was protective for STEC infection and yersiniosis, respectively. Most of recent case controls studies in other countries, which investigated campylobacteriosis, listeriosis, STEC infection or yersiniosis, also reported consumption of fruit and/or vegetables to be protective or not significant. One study identified consumption of lettuce as a risk factor for campylobacteriosis, and another identified fruits/berries imported into Finland as a risk factor for infection from *Y. enterocolitica* serotype 1A (but not other serotypes).

Identifying and confirming fresh and fresh-cut produce, sprouts or fresh juices as vehicles of infection is difficult because these foods have a short shelf life and are either consumed or discarded in homes and food service outlets within a short period of time, thus the implicated foods are rarely available for microbial testing. The incubation period for some foodborne diseases exceeds the period that fresh produce would reasonably be kept. In addition, fresh produce such as sprouts or fresh herbs are often added in small amounts to foods, including foods that are already cooked such as pasta salads, so they are not as readily recognised as potential vehicles of infection.

3.1.1 Sporadic cases

The 2008 Document did not include an analysis of enteric sporadic cases for evidence of illness being caused by fruit, vegetable, juice or sprouts. An analysis of 263 sporadic listeriosis cases for the period 2003-2013 found no cases for which packaged RTE salads (fruit or vegetable) were confirmed as the source of infection, either by laboratory analysis or epidemiological investigation (Hudson *et al.*, 2014).

There were 85,407 sporadic cases of notifiable enteric disease reported to EpiSurv for the period 2008-2014 and 11,893 in 2014 alone.[19] Neither data set was analysed for cases attributable to RTE fruit and vegetables because confirming the food causing infection is rarely accomplished in investigations of sporadic cases.

3.1.2 Outbreaks

The 2008 Document describes one outbreak for the period 1997-2008 where fresh produce was the confirmed cause (2002, Hepatitis A, raw blueberries) and a further three where fresh produce was a possible, but unconfirmed cause (giardiasis, salmonellosis, STEC infection). A case control study strongly suggested that carrots washed in stream water were the cause of a 2005 outbreak of *Salmonella* Saintpaul infection (King *et al.*, 2011a; Neuwelt *et al.*, 2006).

For the period 2009-2014 there were three outbreaks where fresh produce was the most likely cause of infection based on epidemiological evidence (laboratory evidence was inconclusive in all of these outbreaks):

- January 2009, salmonellosis (*Salmonella* Typhimurium phage type 1), 19 cases in Gisborne (5 hospitalised): Epidemiologically linked to consumption of locally grown watermelon (McCallum *et al.*, 2010). Further investigation identified one watermelon supplier who sold the fruit via a roadside stall. *Salmonella* spp. were not isolated from leftover watermelon but multiple hygiene failures were noted at the premises. Note that this watermelon was not sold RTE (i.e. not cut).

- April 2011, norovirus infection, 31 cases, Auckland: Epidemiologically linked to fresh fruit salad served in a restaurant that was most likely contaminated by an infected food handler (Lim *et al.*, 2012).[20]

- September/October 2014, yersiniosis (*Y. pseudotuberculosis*), 220 cases (70 hospitalised), nationwide: A case control study (96 cases, 112 controls) identified that lettuces and/or carrots were potential vehicles of infection but the source of infection was not established (Health Intelligence Team, 2014c; MPI, 2014a).

Examination of the annual foodborne disease reports from 2008-2014 identified five additional outbreaks where fresh produce was a suspected vehicle but the evidence was limited to the identification of critical control point failures linked to the implicated source and/or cases had a history of exposure to the implicated source:

[19] EpiSurv is the New Zealand public health surveillance database.
[20] Additional information retrieved from EpiSurv report.

- *C. perfringens* intoxication (2 outbreaks), 2008, 6 confirmed and 18 probable cases in total, suspected foods were rice, **beans** and salad (Williman *et al.*, 2009);
- Norovirus infection, 2009, 2 confirmed and 15 probable cases, suspected foods were **sushi**, sauces, sausage rolls and salmon sandwiches (Lim *et al.*, 2010).
- Norovirus infection, 2013, 6 confirmed and 2 probable cases, suspected food handler contaminating **avocado** sandwiches and chocolate cake (Horn *et al.*, 2014).
- *C. perfringens* intoxication, 2014, 4 confirmed and 13 probable cases, suspected food was chicken **salad** (Horn *et al.*, 2015).
- Norovirus infection, 2014, 10 confrmed and 44 probable cases, suspected food was berry trifle (frozen **raspberries**, custard, sponge) (Horn *et al.*, 2015).

Further data were collated from the annual outbreak summary ESR reports for New Zealand, for the years 2009 to 2014 (Health Intelligence Team, 2011, 2012, 2013, 2014a, 2015b; Population and Environmental Health Group, 2010). Data prior to 2008 were not included as the food vehicle/source categorisation scheme was different. For the 2009-14 period, the total number of outbreaks where "foodborne" was one of the modes of transmission reported was 686 and there were 5,038 cases associated with these outbreaks. Of these foodborne outbreaks, a food vehicle was identified in 299 (44%), involving 2,574 cases, of which:

- Root vegetable(s) were implicated in 27/299 (9%) outbreaks involving 401/2574 (16%) cases (65% (260/401) of these cases were reported in 2014, when there was a large outbreak probably caused by lettuces or carrots);
- Leafy vegetables were implicated in 17/299 (6%) outbreaks involving 327/2574 (13%) cases (72% (237/327) of these cases were reported in 2014, when there was a large outbreak probably caused by lettuces or carrots);
- Vine/stalk or stalk vegetables were implicated in 11/299 (4%) outbreaks involving 65/2574 (3%) cases; and
- Fruit/nuts were implicated in 13/299 (4%) outbreaks involving 183/2574 (7%) cases.

In total, these four produce groups were implicated in 68/686 (10%) foodborne outbreaks and involved 976/5038 (19%) cases. This is not too dissimilar to the statistics reported in the 2008 Document for the year 2007, when fresh produce was implicated in 16.2% of all foodborne outbreaks, which involved 18.2% of foodborne outbreak cases.

These data are indicative only since the foods are not necessarily uncooked and more than one food can be implicated in an outbreak (all foods are scored equally). Moreover, other modes of transmission may have been reported in these outbreaks (e.g. drinking water, contact with animals). See the outbreak summary reports for a full explanation of how these data were prepared and the applicable caveats.[21]

The causative agent was only identified in 42/68 of the foodborne outbreaks where fruits/nuts or any of the vegetable groups were implicated, and norovirus was the causative agent in 20 of these. Foodborne norovirus outbreaks are often characterised by a mixture of foods contaminated by an infected food handler and person-to-person transmission. The causative agents identified in the other 22 outbreaks were *Clostridium* spp. (10 outbreaks, non-botulinal), *Salmonella* spp. (4 outbreaks), *Campylobacter* spp., *Yersinia* spp. and *Vibrio parahaemolyticus* (2 outbreaks each), and *S. aureus* and enteropathogenic *E. coli* (one outbreak each).

[21] Available from https://surv.esr.cri.nz/surveillance/annual_outbreak.php (accessed 1 July 2015).

3.1.3 Case control and attribution studies

The 2008 Document did not report any New Zealand case control or attribution studies. Two case controls studies have been reported since. These were carried out to identify risk factors for foodborne disease and were not associated with specific outbreak investigations.

A case control study of yersiniosis conducted during the period 1995-1996 found that eating raw fruit and vegetables was protective (OR 0.98, 95% CI 0.97-0.99) (Satterthwaite *et al.*, 1999).

A prospective case control study of STEC infection for the period 2011-12 (113 cases, 506 controls) found that eating raw vegetables and drinking refrigerated fruit juice from a supermarket each had a protective effect (adjusted odds ratio (aOR) for raw vegetables 0.52 (95% confidence interval (CI) 0.27-0.99), for juice aOR 0.25 (95% CI 0.14-0.47)) (Jaros *et al.*, 2013).

An analysis of 204 non-typhoid salmonellosis outbreaks between 2000 and 2009 did not identify any outbreaks involving uncooked fruit or vegetables, unpasteurised fruit juice or sprouts, where the same *Salmonella* serotype was isolated from one or more cases and from the implicated source (i.e. strong evidence for the mode of transmission) (King *et al.*, 2011a). Only two outbreaks involving produce were identified where the mode of transmission was supported by case control or cohort study: The 2009 outbreak linked to watermelon and the 2005 outbreak linked to carrots (both cited above).

A systematic review of the aetiology of salmonellosis in New Zealand found that fresh produce was “likely” to be a “minor cause” of New Zealand foodborne salmonellosis cases (i.e. <10% of foodborne cases) (Wilson and Baker, 2009).

3.1.4 Overseas surveillance data

The 2008 Document reported outbreaks of bacteria, parasites and viruses where fresh vegetables or fruits had been implicated as the vehicle of infection. Most of the reported vegetable-linked outbreaks were due to lettuce and salad, and most of the fruit-linked outbreaks were due to berries and melons. Of the 15 outbreaks caused by contaminated unpasteurised fruit juices, all but three were caused by *E. coli* O157:H7, enterotoxigenic E. coli or *Salmonella* spp.

Outbreaks reported in other countries caused by vegetables, fruit, unpasteurised juice and sprouts are described in Appendix B.1, and the findings are not too dissimilar to that reported in the 2008 Document.

All of the pathogens considered in this document were the reported causative organism in at least one outbreak reported since 2008, except for *Giardia* spp. and *Aeromonas* spp. (no outbreaks of infection caused by fresh produce were located for these pathogens). In addition, the reports citing *B. cereus* as the causative organism did not provide enough detail to determine whether the food vehicles implicated were made from fresh fruits or vegetables, so surveillance data are inconclusive for this pathogen.

The pathogens most often cited were *Salmonella* spp. and norovirus, and to a lesser extent, STEC and *L. monocytogenes*. The fresh produce items most often implicated by strong (epidemiological and/or microbial) evidence were leafy green vegetables (often contaminated with norovirus, *Salmonella* spp. or STEC), sprouts (*Salmonella* spp., STEC, *L. monocytogenes*), berries (norovirus), and to lesser extent tomatoes (*Salmonella* spp.) and melons (*Salmonella* spp., norovirus). However, a range of other vegetables were implicated by strong evidence indicating that contamination events can occur in a range of fresh produce, e.g. baby corn, baby carrots, peas, cucumber.

From the outbreak reports that included information on the failure(s) that lead to contamination, the most commonly cited causes were the use of contaminated water for

irrigating or washing produce (e.g. using surface waters, water contaminated by a septic tank) and handling of the food by sick or asymptomatic food workers. Other causes of contamination were faeces from wild or domestic animals in the field or packhouse, inadequate post-harvest washing, and in the case of a botulism outbreak, inappropriate refrigeration of fresh juice. The commonly cited cause of sprout-associated outbreaks was the use of contaminated seeds (failing to ensure the seed supply was uncontaminated or failing to use a suitable seed-sanitising step before germination).

These findings differ from those reported in the 2008 Document, which found contamination from food handlers to be the most commonly cited cause of outbreaks (at least for leafy vegetables), alongside cross-contamination from other foods or surfaces. The 2008 Document acknowledged that "the occurrence of contamination at the farm level is under-represented, mainly due to the difficulties associated with identifying problems at this level and possible traceability issues". These difficulties still exist, but perhaps there are now more resources invested in traceback and environmental investigations in an effort to identify the "failures" leading to fresh produce contamination, since there is now more information available on key points of contamination at farm-level. The increased use of traceability systems will aid in these investigations.

The 2008 Document did not report any case control studies carried out in other countries. Appendix B.2 summarises the results from seven case control studies and two meta-analyses where fruit and/or vegetable consumption was one of the risk factors investigated for campylobacteriosis, listeriosis, STEC infection or yersiniosis. In all but two studies consumption of fruit and/or vegetables was found to be protective or not a significant risk factor.

Sporadic cases of campylobacteriosis in Ireland were 2.6 times more likely to have consumed lettuce than controls and the authors suggested lettuces may become contaminated on-farm or by cross-contamination in the kitchen (Danis *et al.*, 2009). Given that chicken was also identified as a risk factor, and that available data suggest that *Campylobacter* spp. do not survive well in the soil or on fresh produce, contact with contaminated water or cross-contamination are probably important. However, consumption of prepared salad other than lettuce was found to be protective in this study, and presumably cross-contamination events are equally likely for all leafy vegetables. Perhaps this study unknowingly identified an outbreak of campylobacteriosis caused by lettuces. It is also possible that a Finnish study identified an outbreak of yersiniosis caused by imported fruits/berries, since cases with *Y. enterocolitica* serotype 1A infection were 3.5 times more likely to consume these foods, and all other serotype/produce combinations were either protective or not significantly associated with yersiniosis (Huovinen *et al.*, 2010). The authors did not offer any possible reasons for this finding.

3.2 NEW ZEALAND HUMAN HEALTH SURVEILLANCE

Key findings

There were reported cases of disease caused by all of the pathogens considered in this document during the period 2012-2014, except cyclosporiasis. The highest rates of notifiable disease in New Zealand were for campylobacteriosis, giardiasis and salmonellosis, and this has not changed since 2006.

Pathogens that are often associated with foodborne transmission in outbreak reports are *Campylobacter* spp., *Salmonella* spp., *Shigella* spp., *B. cereus*, *C. perfringens*, *Listeria* spp. and *S. aureus*.

Of the diseases caused by the 18 pathogens considered in this document, nine of them are notifiable diseases in New Zealand: Campylobacteriosis, cryptosporidiosis, giardiasis, hepatitis A, listeriosis, salmonellosis, shigellosis, STEC infection and yersiniosis (Ministry of Health, 2013).

The remaining nine (*Aeromonas* spp. infection, *B. cereus* intoxication, *C. botulinum* intoxication (botulism), *C. perfringens* intoxication, cyclosporiasis, norovirus infection, rotavirus infection, *S. aureus* intoxication and toxoplasmosis) will only be notified under the category of 'acute gastroenteritis' where any of the following has occurred (Ministry of Health, 2013):

- There is a suspected common source (i.e. an outbreak).
- A 'high risk' person has been identified as being ill, e.g. a food handler or an early childhood service worker (signalling potential for an outbreak). This includes *B. cereus* intoxication, intoxication from clostridia, norovirus infection, rotavirus infection.
- There is a single case of bacterial or toxic food poisoning such as botulism.

3.2.1 Sporadic infection in New Zealand

The 2008 Document presented the rates of infection per 100,000 and case numbers for relevant notifiable diseases, for the period 2001-2006. TABLE 5 presents the rates of infection for acute gastroenteritis and the nine notifiable diseases relevant to this document, for the years 2006 (for comparison) and 2012 to 2014, plus estimates for the percentage that are foodborne from a recent expert elicitation exercise. The data are ranked based on the 2014 rate and while the rates of campylobacteriosis and salmonellosis are lower in 2014 compared with 2006, and giardiasis is higher, these remain the top three diseases based on rate.

In 2014 there were 755 cases of acute gastroenteritis notified in New Zealand (Health Intelligence Team, 2015a). A causal agent was reported for 326 of these cases:

- 181 (24%) cases were for rotavirus infection;
- 115 (15%) cases were for norovirus infection;
- 3 (0.4%) cases were for *Aeromonas* spp. infection;
- 3 (0.4%) were for *B. cereus* intoxication; and
- 1 (0.1%) was for staphylococcal intoxication.

Based on the 2014 resident population, the calculated rate of notified rotavirus infection is 4.0 per 100,000, and the rate of norovirus infection is 2.5 per 100,000, however large numbers of rotavirus and norovirus cases will not be reported because symptoms are typically of a

duration and severity that people do not seek healthcare.[22] The expert elicitation process also produced estimates for the percentage of norovirus infection cases that were foodborne (32.7%; 95% credible interval 10.0-66.4%), and the percentage of toxoplasmosis cases that were foodborne (27.6%; 95% CI 3.8-57.1%) (Cressey and Lake, 2013).

TABLE 5: Rate per 100,000 for notifiable (potentially foodborne) diseases in New Zealand (2012-2014) and the estimated percentage of cases that are foodborne

DISEASE	2006[1]	2012[2]	2013[3]	2014[3]	ESTIMATED PERCENT FOODBORNE (95% CI)[4]
Campylobacteriosis	379.3	158.3	153.9	150.3	63.8 (44.1-83.2)
Giardiasis	29.0	38.7	38.9	37.9	NE
Salmonellosis	31.9	24.4	25.7	21.2	62.1 (35.2-86.4)
Acute gastroenteritis	22.4	17.2	12.6	16.7	NE
Yersiniosis	11.6	11.6	10.9	15.1	63.2 (29.0-91.5)
Cryptosporidiosis	17.6	19.8	30.3	12.9	NE
STEC infection	2.1	3.3	4.6	4.1	STEC O157 29.9 (3.5-60.7) Non-O157 STEC 34.0 (3.5-63.5)
Shigellosis	2.4	3.0	3.1	2.8	NE
Hepatitis A	2.9	1.8	2.0	1.6	NE
Listeriosis	0.5	0.6	0.4	0.6	87.8 (57.9-98.5)

[1] (Population and Environmental Health Group, ESR, 2008)
[2] (Health Intelligence Team, 2014b)
[3] (Health Intelligence Team, 2015a)
[4] (Cressey and Lake, 2013). Percentages determined through an expert elicitation process involving a panel of 10 New Zealand experts. Percentages presented are the mean aggregate estimates from a weighting scheme based on each individual's self-assessment of expertise. Values in brackets are the 95th percentile credible interval. NE, not estimated for this disease.

There was also one case of botulism reported in 2014, the first since 1985, and was caused by an improperly refrigerated rice snack (Health Intelligence Team, 2015a).

There were no sporadic cases of *C. perfringens* intoxication reported in 2014 but four cases were reported in 2013 and two in 2012 (this disease is more likely to be reported when it is the cause of an outbreak, see next section). There were no cases of toxoplasmosis reported to EpiSurv in 2014, but hospital discharge data show that cases do occur every year (e.g. seven cases in 2014) and present with different clinical manifestations (Cressey and Lake, 2015). No cases of cyclosporiasis have been reported to EpiSurv since 2006.

3.2.2 Reported outbreaks

The 2008 Document presented data on the number of outbreaks and associated cases for notifiable diseases for the year 2007. TABLE 6 summarises the number of notifiable outbreaks of disease for the pathogens considered in this report for the years 2008 to 2014, and the number likely to be foodborne. An outbreak was classified 'foodborne' if food was recorded as one of the likely modes of transmission applicable to the outbreak, but it is important to

[22] Calculation based on the estimated resident population in New Zealand for the year ending December 2014 (4,513,100 people). Statistics New Zealand Infoshare, http://www.stats.govt.nz/infoshare/Default.aspx (accessed 16 June 2015).

note that a single outbreak may have multiple pathogens and modes of transmission (Horn *et al.*, 2014). The foodborne outbreaks listing any fresh produce items as potential vehicles of transmission have already been listed in Section 3.1.2. There were no reported outbreaks of *Aeromonas* spp. infection, botulism caused by *C. botulinum*, cyclosporiasis, toxoplasmosis or rotavirus infection in any of these years.

In terms of the overall number of outbreaks reported as foodborne each year, norovirus infection, campylobacteriosis, salmonellosis, giardiasis and *C. perfringens* intoxication usually made up the largest proportions, but the importance of foodborne transmission for each of these diseases is better evaluated by observing the percentage of outbreaks that were reported as foodborne for each disease.

Between 34 and 58% of campylobacteriosis outbreaks each year were classed as foodborne, and for salmonellosis and shigellosis the ranges were 27-53% and 0-40% respectively. These ranges were much lower for the protozoan pathogens: 0-10% for cryptosporidiosis and 0-13% for giardiasis, since water is a more probable route of transmission for these pathogens. The most likely route of transmission for norovirus is person-to-person, so the percentage foodborne each year was in the range 2-17%. There were few outbreaks reported for the remaining diseases, but all outbreaks of *B. cereus* intoxication, *C. perfringens* intoxication, listeriosis and *S. aureus* intoxication were classed as foodborne across all years.

TABLE 6: Total number of reported outbreaks (OB) of notifiable diseases in New Zealand and the number that were foodborne (FB), for the years 2008-2014

DISEASE	2008		2009		2010		2011		2012		2013		2014	
	OB	FB	OB	FB	OB	FB	OB	FB	OB	FB	OB	FB	OB	FB
B. cereus intoxication	1	1	0	0	0	0	1	1	0	0	1	1	1	1
Campylobacteriosis	16	8	12	7	29	14	29	11	32	11	40	16	35	18
C. perfringens intoxication	7	7	3	3	4	4	4	4	4	4	9	9	3	3
Listeriosis	0	0	1	1	0	0	0	0	1	1	0	0	0	0
Salmonellosis	15	4	12	6	23	10	15	8	27	11	18	9	23	7
Shigellosis	6	1	3	0	5	1	11	4	12	4	10	4	11	4
S. aureus intoxication	0	0	0	0	2	2	0	0	1	1	1	1	1	1
STEC infection	4	1	4	0	5	1	2	0	1	0	16	2	10	4
Yersiniosis	0	0	2	1	2	2	2	1	5	0	3	1	7	2
Cryptosporidiosis	7	0	20	0	43	2	29	3	47	1	98	3	20	0
Giardiasis	50	2	41	0	97	4	72	6	69	6	78	10	85	6
Hepatitis A	3	1	1	0	1	1	0	0	1	0	5	0	1	0
Norovirus infection	152	26	270	29	152	19	181	20	249	26	169	16	820	18

References: (Horn *et al.*, 2014; Horn *et al.*, 2015; Lim *et al.*, 2012; Lim *et al.*, 2011; Lim *et al.*, 2010; Lopez *et al.*, 2013; Williman *et al.*, 2009).

3.3 RISK ASSESSMENTS AND RELATED ACTIVITIES

Key findings

A small survey of New Zealand fruit and vegetable growers identified some practices that increased the risk of fresh produce being contaminated with pathogenic microorganisms, such as applying untreated animal-based fertilisers close to or at crop planting or using water of uncertain quality for irrigation or post-harvest washing.

An analysis of assurance programmes available to New Zealand growers found that many of these required activities that would minimise the opportunity for pathogens to contaminate fresh produce from natural fertilisers or water, specific details such as withholding periods for fertilisers and microbiological standards were lacking (e.g. for water and natural fertilisers).

A variety of risk assessments and risk-related activities have been completed in other countries since 2008. Together (and based on the available data) these indicate that:

- Fresh produce does not cause as large a public health burden when compared with other food commodities, but the burden is significant enough to warrant attention.
- The most important pathogens with respect to fresh produce are *Salmonella* spp., pathogenic *E. coli* and norovirus.
- The foods considered most at risk of being contaminated (and causing human illness) are leafy green vegetables, sprouts, tomatoes, melons, berries and fresh herbs.

3.3.1 New Zealand risk assessments and related activities

A review of the use of water and natural fertilisers during the growing, harvesting and packing of horticultural produce was completed in 2011 (King *et al.*, 2011b; King *et al.*, 2011c; Lake *et al.*, 2011a, 2011b). This study evaluated whether chemical and microbiological hazards are likely to be present on New Zealand horticultural produce at levels of concern as a result of applying natural fertilisers (e.g. composts, manures) and water (irrigation, post-harvest), through literature review, a grower survey and a comparison of New Zealand assurance programmes.

A small survey of 40 leafy vegetable and berry growers (including 11 organic certified growers) found that (King *et al.*, 2011c; Lake *et al.*, 2011b):

- Half used one or more natural fertilisers on their crops. Most natural fertilisers containing animal manure were applied to the soil prior to planting, although the time period between final application and planting was not always clearly specified. Some growers increased the possibility of introducing pathogens onto the edible parts of the plants by applying untreated animal-based natural fertilisers close to (or at) planting. Sprays made from commercially-produced fish or seaweed extracts were commonly applied by these growers to the edible parts of plants, right up to harvest.
- Physical barriers (e.g. roads, buffer zones) were important for minimising any contamination from livestock farming activities adjacent to growing areas.
- Most growers surveyed used groundwater (bores) as a water source for irrigation and applying plant products, and many had access to town supplies of potable water. Testing of other water supplies was infrequent and treatment was rare. Most growers took steps to keep their produce free from any potential contamination from irrigation water, but some growers increased the possibility of their crops becoming contaminated by using irrigation water of uncertain quality up to the point of harvest.

- Most growers using water on produce during or after harvest reduced the possibility of contamination by using groundwater or water from a roof or town supply, but these water sources were not always treated or tested regularly. Several growers took additional action to reduce the possibility of contamination by monitoring or adding antimicrobial chemicals to water used for immersing produce (tub/sink) and changing this water regularly, by using two wash steps, or by washing the plant roots separately. One grower increased the possibility of contaminating their produce by applying water from a stream to their produce during harvest.

A comparative analysis of New Zealand and international assurance programmes,[23] focussing on requirements for the use of water and natural fertilisers, found that (King *et al.*, 2011b; Lake *et al.*, 2011b):

- Food safety issues concerning natural fertilisers are addressed by non-organic assurance programmes, but mostly in general terms, and there is reliance on the supplier that the fertilisers have been adequately treated.
- Food safety issues concerning natural fertilisers are less extensively addressed by organic programmes, but they do prohibit human sewage and raw manures being used as fertilisers.
- Food safety issues concerning water are generally well covered by non-organic programmes, with *E. coli* and some chemical limits specified, but ice/cooling water was not specifically addressed by some assurance programmes.
- Food safety issues concerning irrigation water are less extensively addressed by organic programmes.
- All assurance programmes require potable water to be used wherever it comes into contact with produce during post-harvest processing.

While many of the assurance programmes required activities that would minimise the opportunity for pathogens to contaminate fresh produce from natural fertilisers or water, specific details such as withholding periods for fertilisers and microbiological standards (e.g. for water and natural fertilisers) were lacking.

3.3.2 Risk assessments and related activities from other countries

Information on risk-related activities from other countries has been included in Appendix B.3. In summary:

- Three reports published prior to 2008 that considered the risk of *L. monocytogenes* in RTE food concluded that nearly all listeriosis cases were the result of eating RTE foods that can support the growth of *L. monocytogenes* and contain high numbers of this pathogen (well above 100 CFU/g), and that raw vegetables and raw/dried fruits were considered to be “low risk” for listeriosis when compared with other RTE foods such as delicatessen meats, smoked seafood and pasteurised liquid milk.
- Three reports published since 2008 by the Food and Agriculture Organisation of the United Nations (FAO) and the World Health Organisation (WHO) identified a large number of risk factors that contribute to microbial contamination of fresh and fresh-cut leafy vegetables and herbs (both pre- and post-harvest), and have also identified norovirus,

[23] Codex Code of Hygienic Practice for Fresh Fruits and Vegetables (CAC/RCP 53-2003), New Zealand GAP, New Zealand GAP (GLOBALG.A.P. Equivalent), GLOBALG.A.P., Safe Quality Food, New Zealand Standard for Organic Production (NZS 8410:2003), NZFSA Official Organic Assurance Programme, BioGro New Zealand Organic Standards, AsureQuality Organic Standard and Demeter Standards.

HAV, *Cryptosporidium* spp., *G. dudoenalis* and *C. cayetanensis* as being important viral and protozoan hazards associated with fresh produce.

- From EU outbreak data, EFSA estimated that foods of non-animal origin were associated with 10% of all reported outbreaks and 26% of the cases. A subsequent risk ranking exercise identified several hazard/fresh produce combinations (see Appendix B.3.2), but overall *Salmonella* spp., pathogenic *E. coli*, norovirus, *Shigella* spp. and *Yersinia* spp. were identified as the most important pathogens associated with fresh produce, and the foods commonly associated with reported illness were leafy green vegetables, sprouted seeds, bulb and stem vegetables, tomatoes, melons, raspberries, fresh herbs, carrots, and fresh pods, legumes or grains. EFSA has since assessed several of these hazard/fresh produce combinations, but when these are compared, similar risk factors were identified in all of them.
- An EFSA risk assessment of pathogenic bacteria in sprouts concluded that *Salmonella* spp. and pathogenic *E. coli* were the most often reported etiological agents in sprout-related outbreaks, and that outbreaks can be caused by seeds contaminated at a very low concentration (e.g. 4 MPN/kg). EFSA also found that there were no reliable methods for decontaminating all types of seeds.
- A quantitative risk assessment model for the European lettuce food chain found that contamination from workers' hands was the most important risk factor for contamination of lettuces with norovirus or HAV.
- After a literature review on the occurrence and control of foodborne viruses, EFSA recommended that food producers and handlers focus on preventing viral contamination rather than trying to remove or inactivate viruses from foods.
- The top three pathogen/fresh produce pairs ascertained from a risk ranking tool produced for the USA were enterohaemorrhagic *E. coli* (EHEC)/leafy green vegetables, followed by *Salmonella* spp./tomatoes and *Salmonella* spp./leafy green vegetables.[24]
- The results of the New Zealand survey of growers (Section 3.3.1) was supported by a survey of USA growers, some of whom applied raw manure close to harvest or used water of uncertain quality.

Appendix B.3 also includes information from attribution studies published since 2008 that considered multiple pathogens and fresh produce as a commodity. Although the studies take different approaches these suggested that:

- *Salmonella* spp. were the main pathogen associated with 'produce' outbreaks, followed by norovirus and pathogenic *E. coli*. Based on percentage attribution, the next most important pathogen were *Cyclospora* spp. (6.5% of produce outbreaks). This study used published data from all countries for the period 1988-2007.
- Contaminated fruit and nuts or contaminated vegetables are not large contributors to salmonellosis or campylobacteriosis outbreaks in Europe (the attribution percentages for these food/hazard groups were very low, <0.5% of outbreaks and <2% of cases). This study used data from Europe for the period 2005-2006.
- Outbreaks of salmonellosis can occur from a wide variety of fresh produce, but *E. coli* O157 infection is more often associated with vegetable row crops. Campylobacteriosis is

[24] The pathotype enterohaemorrhagic *E. coli* (EHEC) is still used occasionally to refer to the subset of STEC that are capable of causing haemorrhagic colitis (HC) and haemolytic uraemic syndrome (HUS).

not often associated with fresh produce. This study used data from the USA for the period 1998-2012.

4. CONTROLS

Key findings

The absence of a pathogen elimination step for fresh fruit and vegetables means that measures for reducing the risk of contamination from pathogenic microorganisms must be implemented by the grower in the first instance, then by subsequent food handlers. There are no on-farm practices that can guarantee fresh produce will be free from pathogenic microorganisms but there are practices that will reduce opportunities for contamination. Strategies that prevent faecal contamination are priorities, and this includes preventing use of contaminated water supplies.

The *Food Act 2014* will be fully in force by 1 March 2016 and will replace the *Food Act 1981*, the Food Hygiene Regulations 1974 and the Food (Safety) Regulations 2002. Under the new Act, horticultural producers and manufacturers of fresh RTE salads will be subject to one of three risk-based control measures, depending on the activities they undertake. This is the first New Zealand regulation that applies during the growing of fresh produce. The Food Handler Guidance and National Programmes are still under development. Registered Food Safety Programmes will be recognised as Food Control Plans under the *Food Act 2014* provided they meet certain conditions.

There are mandatory microbiological standards for *Salmonella* spp. on sprouts, and *L. monocytogenes* on RTE foods such as fresh-cut and packaged fruit and vegetables. MPI will propose additional microbiological standards as part of administrating the *Food Act 2014* if these are considered necessary.

There are currently no mandatory controls over the source or quality of water used for irrigation or post-harvest washing of fresh produce, nor for the quantity or quality of manures or animal wastes applied to land, unless such controls are imposed through a Regional Plan or resource consent conditions. In most regions of New Zealand these activities do not require resource consent.

Assurance programmes and guidance documents (both voluntary) focus on managing food safety risks using good agricultural practices (GAP) and Hazard Analysis and Critical Control Points (HACCP) principles.

Sanitisers that come into contact with plant foods are considered to be processing aids, and only processing aids permitted by the Australia New Zealand Food Safety Code can be used. It is likely that chemical sanitisers, particularly those using chlorine as an active ingredient (e.g. hypochlorite), are still most commonly used for post-harvest washing in New Zealand.

A number of reviews have concluded that there is no one sanitising option that controls all pathogens and spoilage microflora, and still maintains product integrity. The current view is that the primary purpose of chemical sanitisers is not to eliminate microbial contamination from fresh produce, but to reduce the microbial load overall and keep the process water free from microbial contamination, so that opportunity for cross-contamination is reduced.

There are no regulatory requirements for New Zealand sprout producers to decontaminate seeds. Evaluations of different seed decontamination methods found that there was no satisfactory chemical method. The combination of multiple treatments, including physical treatments, appears more effective (e.g. heat and a chemical sanitiser).

Refrigeration of fresh produce is not an effective control for all pathogenic microorganisms. MAP is an effective packaging system for control of spoilage organisms but is not a control for pathogenic bacteria.

Food safety controls relevant to leafy vegetables include:

- New Zealand food legislation and associated regulations and standards (mandatory);
- New Zealand legislation and standards that control the taking of water for irrigation, water quality and application of fertilisers to land (mandatory);
- Non-mandatory food safety standards; and
- Assurance programmes (non-mandatory).

TABLE 7 lists controls described in the 2008 Document (all non-mandatory) and provides an update, and further information is included in the following sections. Much of the information in these sections has been summarised from a document prepared in 2011 that discussed the above four types of controls as they applied to the use of water and natural fertilisers in horticultural production (King *et al.*, 2011b).

TABLE 7: Update on controls included in the 2008 Document

CONTROL INCLUDED IN THE 2008 DOCUMENT	UPDATE ON THIS CONTROL
New Zealand GAP (2006)	Still current. Updated version released in 2009 (version 5.0).
New Zealand Standard: Composts, soil conditioners and mulches (NZS4454:2005)	Still current.
BioGro New Zealand Organic Standards (2001)	Still current. Updated version released in 2009.

4.1 NEW ZEALAND FOOD LEGISLATION

The *Health Act 1956* and *Food Act 1981*, and their associated regulations, are currently the most relevant legislation for controlling microbiological hazards in fresh fruit and vegetables. These Acts apply when the fruit or vegetables are harvested for sale to the public. Briefly, everyone who is involved in the sale of food intended for human consumption must either meet the requirements of the Food Hygiene Regulations 1974 or be exempt from these Regulations by meeting the requirements of the *Food Act 1981*, which requires an approved Food Safety Programme to be in place to identify and control food safety hazards. The Food (Safety) Regulations 2002 associated with this Act require that water added to food is of a potable quality. Under the *Food Act 1981*, sprouts are considered a 'high risk' food.

There are three microbiological standards under the Australia New Zealand Food Standards Code (Standard 1.6.1) that are relevant to this document (FSANZ, 2014b):

- Cultured seeds and grains (bean sprouts, alfalfa etc): *Salmonella* spp. not detected in five samples of 25 g (i.e. n=5, c=0, m=not detected in 25 g).
- RTE food in which growth of *L. monocytogenes* will not occur: In five samples of 25 g, the concentration of *L. monocytogenes* will not exceed 100 CFU/g (i.e. n=5, c=0, $m=10^2$ CFU/g). RTE foods with a refrigerated shelf-life of no more than five days, or frozen RTE foods, are considered to be RTE foods in which *L. monocytogenes* will not grow.
- RTE food in which growth of *L. monocytogenes* can occur: *L. monocytogenes* not detected in five samples of 25 g (n=5, c=0, m=not detected in 25 g).

The standards for *L. monocytogenes* do not apply to whole raw fruits and vegetables but do apply to fresh-cut and packaged fruit and vegetables.

Irradiation of some fresh fruits and vegetables is permitted in New Zealand (FSANZ, 2014c; MPI, 2015b). Examples include apples, capsicums, tomatoes, strawberries, courgettes and a variety of stonefruit.

4.1.1 *Food Act 2014*

The *Food Act 2014* will be fully in force by 1 March 2016 and will replace the *Food Act 1981* and, over time, replace the Food Hygiene Regulations 1974 and the Food (Safety) Regulations 2002. Under the new Act, any person involved in the trade of food must ensure that it is safe and suitable, and must operate under one of three risk-based measures: Food Handler Guidance, National Programmes or Food Control Plans (MPI, 2014b). This is the first New Zealand regulation that applies during the growing of fresh produce (the old legislation only applied upon harvesting, i.e. when the fresh produce was ready for sale).

Horticultural producers that only minimally process and handle the produce they have grown themselves (e.g. wash or rinse), and only sell this produce directly to consumers (e.g. roadside stalls) will be subject to food handler guidance. Food handler guidance will outline the steps that are necessary to achieve safe and suitable food, but does not carry with it any registration or verification requirements.

Growers who sell their own minimally processed fruit and vegetables to another outlet (e.g. retail store, wholesaler) need to operate under a National Programme Level 1. Packhouses, transporters and distributers of fruit and vegetables also need to operate under this National Programme. The National Programme will specify the minimum regulatory requirements that these food businesses will need to comply with to assure food safety. Growers who operate under an audited good agricultural practice programme (i.e. an assurance programme) that need to operate under the Level 1 National Programme can choose to operate under a Food Control Plan instead.[25] Growers not under an assurance programme can opt for a Food Control Plan if they choose to.

Manufacturers of fresh RTE salads (including fruit salads) that are purchased in packaging and do not require further preparation by the consumer before consumption will require a Food Control Plan. Food Control Plans identify, control, manage, and eliminate or minimise hazards or other relevant factors for the purpose of achieving safe and suitable food. If a RTE salad manufacturer already operates a registered Food Safety Programme, this will become their Food Control Plan under the *Food Act 2014*, subject to conditions set out in the Act.

In 2015, MPI undertook a public consultation on regulations under the *Food Act 2014*, with submissions closing 31 March 2015. The consultation included proposed regulations to control microbiological hazards (MPI, 2015c):

- A requirement that the food business operator takes all practicable measures to eliminate or control the level of any microorganisms (particularly harmful pathogens) that may be present on or in the food, and to protect the food from the likelihood of contamination at any stage.
- A requirement that the temperature of the food is controlled effectively wherever it is critical to the food's safety and/or suitability (when processing and handling food that will not undergo further pathogen controls, the time the food remains at temperatures that permit the growth of pathogenic micro-organisms must be minimised).
- A requirement that, in a finished food product that does not have an associated microbiological standard, the operator provides evidence that the food is safe, including by undertaking sampling and testing of the product, if necessary.

[25] This is an option that may be taken up by growers whose requirements under their assurance programme are more aligned with a Food Control Plan.

Food must continue to comply with microbiological limits set by Standard 1.6.1 in the Australia New Zealand Food Standards Code. MPI will conduct separate consultations where there is need for additional microbiological requirements, i.e:

- Further requirements about managing the level of pathogens in specific foods (specifying the types of food, the particular organisms of interest, and the level (if any) at which they might be permitted in the food).

Sector or process-specific requirements setting microbiological levels or requirements at specified points in the food chain (e.g. for high risk products or as a result of pathogen control strategies).

Importers of fruit and vegetables must be registered and it is proposed that imported foods should be categorised according to risk, with high risk foods requiring more assurances, and this may include sampling and testing. Categorisation of imported foods will require the MPI Chief Executive to consider a number of matters, including potential hazards in the food, the severity of any potential effects on human health, and how hazards are managed in the exporting country.

4.2 NEW ZEALAND LEGISLATION AND STANDARDS THAT CONTROL THE TAKING OF WATER FOR IRRIGATION, WATER QUALITY AND APPLICATION OF FERTILISERS TO LAND

The taking of water for irrigation and the application of natural fertilisers to land require resource consent under the *Resource Management Act 1991*, unless a Regional Authority permits these activities under their regional plan. In most New Zealand regions the application of manure or animal effluent and the taking of irrigation water are permitted activities provided the grower meets any conditions set by the Authority (King *et al.*, 2011b). This system is designed to sustainably manage the environment and does not provide food safety controls.

The Drinking Water Standards for New Zealand define what is meant by potable water (Ministry of Health, 2008). The microbiological limits specified in the Standards are based on *E. coli* as an indicator for the potential presence of pathogens. There are no specific standards for any of the pathogenic bacteria or viruses considered in this discussion document but there is a standard for pathogenic protozoa: Less than one infectious (oo)cyst per 100 litres of sample.[26] There are currently no mandatory microbiological standards for water used for irrigating or washing fruit or vegetables.

4.3 NON-MANDATORY FOOD SAFETY STANDARDS

The 1995 Ministry of Health Microbiological Criteria for Foods (Ministry of Health, 1995) and 2001 FSANZ microbiological criteria for RTE foods (FSANZ, 2001) are both relevant to fresh fruits and vegetables. These include the following recommended standards:

- Salads – vegetable or fruit – excluding combination with meat: *Salmonella* spp. not detected in five samples of 25 g (i.e. n=5, c=0, m=0) (Ministry of Health, 1995).
- Concentrations for what is considered a “satisfactory”, “marginal”, “unsatisfactory” or “potentially hazardous” RTE food, for the following microbes (FSANZ, 2001): Coagulase positive staphylococci, *C. perfringens*, *B. cereus* and other pathogenic *Bacillus* spp., *Campylobacter* spp., *Salmonella* spp. and *L. monocytogenes*.

[26] Results are currently required to be reported as verified *Giardia* or *Cryptosporidium* (oo)cysts since methods for routine monitoring of drinking water are currently not suitable for assessing the viability or infectivity of these (oo)cysts (Ministry of Health, 2008).

4.4 ASSURANCE PROGRAMMES AND GUIDELINES

An assurance programme usually consists of a set of standards or requirements that ensure the production of safe and high quality food, or to assure consumers that food is produced according to its labelling (e.g. organic foods). Assurance programmes might be put in place by overseas governments, large retailers such as supermarket chains, or by credible, independent industry bodies. If a horticultural producer is to gain access to specific markets, such as export markets, organic markets or major retail outlets, they usually need to be certified under one or several assurance programmes (Lake *et al.*, 2011b). Good Agricultural Practices (GAP) – activities that support the production of safe food – are often required by assurance programmes. Findings from the 2011 analysis of New Zealand assurance programmes (including New Zealand GAP and various New Zealand organic programmes) have already been summarised in Section 3.3.1.

The "Guidelines for on-farm food safety for fresh produce" is a document initially developed for the Australian horticultural industry (DAFF, 2004). In March 2015 the Fresh Produce Safety Centre launched a review of the guidelines.[27] The updated guidelines, due for publication in September 2015, will be equally applicable to the New Zealand and Australian horticultural industries. The guidelines contain good practices for preventing microbial contamination of fruit and vegetables during growing and packing of fresh produce, and include risk-based decision trees to support best practice for the use of water and soil amendments. The updated guidelines will be expanded to include the food chain steps up to and including retail sale.

MPI has published guidance for the control of *L. monocytogenes* in RTE foods that sets out regulatory requirements, Good Operating Practices and microbiological testing regimes.[28] It has been designed to be applicable to all RTE food producers.

4.5 ADDITIONAL OPTIONS FOR RISK MANAGEMENT

There are no steps during the production of fresh fruits and vegetables, and fresh-cut or juices made from these, that will guarantee the final product is pathogen-free. However, there are practices that will:

- Reduce opportunities for pathogen contamination, i.e. Good Operating Practices, including Good Agricultural Practices (GAP), Good Manufacturing Practices (GMP), and Good Hygenic Practices (GHP);
- Reduce the concentration of microorganisms on produce (sanitising); and
- Control growth on the final whole, cut or juiced product (storage conditions).

There is a large volume of literature and research considering each of the three points above, and this section will only provide a brief summary. Sprouts are discussed separately. The production of fresh juices at juice bars or other food service outlets is subject to requirements under the *Food Act 1981* or Food Hygiene Regulations 1974 with the former requiring a Food Safely Plan based on Hazard Analysis and Critical Control Point (HACCP) principles, which, if operating effectively, should minimise the opportunity for pathogen contamination of juices at the premises.[29] Food premises preparing RTE products and fruit and vegetable retailers

[27] http://freshproducesafety-anz.com/guidelines/ (accessed 9 March 2015). The Fresh Produce Safety Centre was established in 2014 to support fresh produce food safety across Australia and New Zealand.

[28] Available from http://www.foodsafety.govt.nz/science-risk/programmes/hazard-risk-management/listeria.htm (accessed 16 June 2015).

[29] Unless they have already transitioned to the *Food Act* 2014 and have a registered Food Control Plan in place, which operates under the same principles.

are also subject to this legislation and must take steps to prevent contamination of fresh fruit and vegetables during storage, any cutting and packaging steps, and display.

While there is opportunity for consumers to further decrease exposure to any pathogens on fruit or vegetables in the home (e.g. by washing fresh produce) and consumer advice is available,[30] the behaviour of consumers is highly variable (e.g. (Jacxsens *et al.*, 2015)). Controls applied before the products are purchased would be more universally effective.

4.5.1 Reduce opportunities for pathogen contamination

The strict application of a HACCP-based approach on the farm for growing and harvesting fresh fruit and vegetables is difficult because there are no critical control points that ensure pathogenic microorganisms are controlled, but there are a number of steps that can be taken to reduce the opportunities for pathogens to contaminate fresh produce. One report suggests calling these "risk reduction points" (Leifert *et al.*, 2008), however the terms GAP and GHP, included in Good Operating Practices, are currently more widely accepted and understood, and perhaps capture better the wider range of factors that can lead to contaminated produce (e.g. wildlife faeces, flooding, previous land use, sick workers) rather than focussing on specific inputs and interactions with the standing crop.[31]

Codex Alimentarius has produced the "code of hygienic practice for fresh fruits and vegetables", the "code of practice general principles of food hygiene" and the "code of practice for packaging and transport of fresh fruit and vegetables", plus a large number of individual standards for specific fruits or vegetables, and these provide a consolidated set of recommended practices to prevent fruit and vegetable contamination (CAC, 2003, 2004, 2013).[32] Since 2008, the code for fresh fruits and vegetables has received new annexes specific to fresh leafy vegetables, melons and berries. Many of the recommended practices within the Codex documents have been incorporated into assurance programmes available to growers in New Zealand (King *et al.*, 2011b).

Strategies that prevent faecal contamination (animal and human) are priorities, and this includes preventing use of contaminated water supplies coming in contact with fresh produce. A useful review has recently been published that summarises activities to prevent microbial contamination of leafy vegetables, and this draws largely on the Codex documents and other government guidelines (Gil *et al.*, 2015). In an organic vegetable production system, careful management of manure production, storage and application was key to reducing the risk of vegetables being contaminated by enteric pathogens (Leifert *et al.*, 2008). Another paper proposes a "pre-requisite programme" for fresh produce during the primary production stage, which includes elements not often considered by other publications such as allergen controls, purchasing procedures and traceability/recall systems (Manning and Soon, 2013). The United States Center for Produce Safety has published reports that have investigated different growing and harvesting practices for specific crops with the goal of minimising pathogen contamination.[33]

Fresh produce can also become contaminated post-harvest, particularly during preparation of fresh-cut RTE products, and application of HACCP, GMP and GHP in fresh-cut operations is important for minimising the risk of a contamination event.

[30] http://www.foodsmart.govt.nz/elibrary/fresh_produce_food.htm (accessed 8 June 2015).
[31] A useful summary of Good Operating Practices (GAP, GMP, GHP) and how this differs to HACCP is available at http://foodsafety.govt.nz/industry/general/gop/overview.htm (accessed 8 June 2015).
[32] All standards are available from http://www.codexalimentarius.org/standards/list-of-standards/ (accessed 8 June 2015).
[33] http://www.centerforproducesafety.org/grant_opportunities_awards.php (accessed 11 June 2015).

4.5.2 Sanitising

Sanitising may be by chemical, biological or physical methods. The 2008 Document included information on sanitation options. Briefly:

- Hypochlorite is used in New Zealand for washing produce such as lettuce and carrots. Typical concentrations are 50-200 ppm with treatment for 1-2 minutes. The use of hypochlorite in wash water can be improperly controlled and this reduces its effectiveness. Hypochlorite, at the levels routinely used in the produce industry, is of limited effectiveness against protozoan (oo)cysts and viruses, and some bacterial spores.
- Iodine and peroxyacetic acid are other sanitisers reportedly used in New Zealand.
- Alternative chemical sanitisers (e.g. chlorine dioxide, organic acids), biological sanitisers (bacteriophages) and physical sanitisers (e.g. ultrasound, irradiation) were noted, but those that were available as commercial products that could be used by the horticulture industry in New Zealand were not identified.
- Multiple treatments (hurdles) may offer better decontamination.

There is no change to these findings. There is no centralised source of information to indicate which sanitisers are used post-harvest. It is likely that chemical sanitisers, particularly those using chlorine as an active ingredient, are still most commonly used. Sanitisers that come into contact with plant foods are considered to be processing aids, and only processing aids permitted by Standard 1.3.3 of the Australia New Zealand Food Safety Code can be used (FSANZ, 2015). This Standard includes a list of 20 washing agents permitted for fresh fruits and vegetables, of which six contain a chlorine component. Sanitisers need to be registered in New Zealand and a search of the ACVM Register identified five products registered as biocides and/or viricides where the label indicated these could be used for use in post-harvest dump tanks.[34] These products used the following active ingredients:

- 3-bromo-1-chloro-5,5-dimethylhydantoin;[35]
- Hydrogen peroxide;[36] or
- Bitter orange oil.[37]

Snap Fresh Foods, a major supplier of RTE vegetable products, uses chlorine at a concentration of 150 ppm in the first two of the three wash tanks.[38]

Because of the range of foods and pathogens considered in this document, the efficacy of various sanitisers will not be discussed here. Interested readers are referred to recent reviews by Goodburn & Wallace (2013) and Ramos *et al*. (2013) that summarise knowledge on sanitising options for fresh produce with a focus on bacterial pathogens, and EFSA (2011d) for a summary addressing viruses.[39,40] From both of these reviews it can be seen that there

[34] Agricultural Compounds and Veterinary Medicines register, https://eatsafe.nzfsa.govt.nz/web/public/acvm-register (accessed 8 June 2015). The primary purpose for some of these products was for the control of plant fungal pathogens.
[35] Product registration numbers P008011, P008400 and P008679.
[36] Product registration number P007819.
[37] Product registration P007997.
[38] http://www.snapfreshfoods.com/FoodSafetyQualityAssurance.aspx (accessed 8 June 2015).
[39] Two additional reviews are also worth noting that specifically address *L. monocytogenes* (Hoelzer *et al*., 2014) and *Salmonella* spp. (Mukhopadhyay and Ramaswamy, 2012). A recent review considering studies of sanitiser efficacy against parasites was not located.
[40] The United States Center for Produce Safety has also published reports that have investigated optimal post-harvest conditions for specific produce. See http://www.centerforproducesafety.org/grant_opportunities_awards.php (accessed 11 June 2015).

is no one sanitising option that controls all pathogens and spoilage microflora, and still maintains product integrity. While chemically sanitising fresh produce as part of post-harvest processing may be largely effective in reducing microbial loads, this process still carries the risk of cross-contamination to fresh produce previously uncontaminated with pathogenic microorganisms (e.g. (Lopez-Velasco *et al.*, 2013)), plus the process may be ineffective if pathogens are internalised or resistant (e.g. *Cryptosporidium* spp. oocysts are resistant to chlorine at the levels usually used in the fresh produce industry (Duhain *et al.*, 2012)). Lack of an infectivity assay for norovirus makes measuring the efficacy of different sanitisers against this pathogen difficult (EFSA, 2014d).

The current view is that the primary purpose of chemical sanitisers is not to eliminate microbial contamination from fresh produce, but to reduce the microbial load overall and keep the process water free from microbial contamination, so that any opportunity for cross-contamination is reduced (Gil *et al.*, 2015). A study of two Belgian fresh produce processing companies identified failures in wash tank management that resulted in cross-contamination and poor microbial quality of the end product (Holvoet *et al.*, 2012). In Canada, samples of RTE spinach were of poorer microbial quality after washing and packing (Ilic *et al.*, 2008). A model developed to understand cross-contamination of fresh-cut lettuces by *E. coli* O157:H7 during commercial washing identified the mean wash time and free chlorine concentration as critical parameters (Munther and Wu, 2013). Interestingly, a study of three RTE vegetable production systems in Italy found that, based on microbial quality indicators (aerobic mesophilic count and generic *E. coli*), RTE leafy vegetables produced using GAP, GMP and HACCP but without a disinfection step showed better microbiological quality than those processed using chemical (chlorine washes) or physical (tunnel freezer) decontamination (De Giusti *et al.*, 2010). This emphasises the importance of reducing contamination on-farm and not relying on post-harvest sanitation processes for microbial control.

Application of multiple hurdles might be suitable for some products but the uptake of new (or different) technologies by fresh fruit and vegetable producers can often be inhibited by cost, customer perceptions and uncertainties around the efficacy of any new technologies when upscaling to a commercial setting.

4.5.3 Storage

Data included in Section 2.4.3 show how the behaviour of pathogens on fresh produce can vary, even when held under refrigeration. Modified atmosphere packaging (MAP) can be used to extend the shelf-life of fresh produce and is often used for RTE vegetable salads (Zhang *et al.*, 2014). The atmospheric gases around the food may be replaced by a specific gas or mixture of gases (usually oxygen is reduced or removed), and the packaging material is used to control (or prevent) the flow of gases between the inside and outside of the package. Gas scavengers are sometimes included for additional control (e.g. oxygen absorbers). While MAP is successfully used to control spoilage microorganisms and plant respiration, a recent review found that there were no particular MAP conditions that would ensure all pathogenic bacteria could not grow (Caleb *et al.*, 2013). Concerns about specific pathogenic bacteria were raised:

- *L. monocytogenes*: Facultatively anaerobic and psychrotrophic, and while reports of survival and growth on refrigerated fresh-cut produce are inconsistent (likely to be due to differences in MAP conditions, temperature and produce), growth was observed in some studies.
- *C. botulinum*: Anaerobic (favouring high carbon dioxide atmospheres under some MAP) and the non-proteolytic group can grow at 3ºC, but studies indicate that outgrowth and toxin production only occurs at non-refrigeration temperatures (>20ºC).
- *Aeromonas* spp: Also able to grow under high (up to 50%) carbon dioxide or low (1.5%) oxygen, and at refrigeration temperatures, and some studies have reported growth on fresh produce in MAP.

The findings for *L. monocytogenes* were supported by a second analysis of 12 studies under MAP, which reported that in four studies (mushrooms, endive x 3) the growth rate of *L. monocytogenes* was higher under MAP compared with atmospheric packaging and in the remaining eight studies (asparagus, broccoli, cactus pear, carrots, cauliflower, sprouts, lettuce, tomatoes) there was no significant difference in growth rates between MAP and atmospheric packaging (Hoelzer *et al.*, 2012). MAP is not a control for *L. monocytogenes*.

Certain MAP conditions may even enhance survival. The concentration of *Salmonella* Typhimurium on lettuce leaves reduced when stored at 8ºC for 7 days under permeable film that had been perforated (no MAP) or not (passive MAP), but remained stable when the atmosphere within the bag was replaced with 10% oxygen, 10% carbon dioxide and 80% nitrogen (active MAP) (Horev *et al.*, 2012).

4.5.4 Sprouts

The 2008 Document notes two control options: Using high levels (up to 3,500 ppm) of the chemical sanitiser hypochlorite to soak seeds before sprouting, and testing spent irrigation water during sprouting.

In 2012 FSANZ added the Production and Processing Standard for Seed Sprouts (Standard 4.2.6) to the Australia New Zealand Food Standards Code, but this Standard only applied in Australia (FSANZ, 2014a). The Standard requires seeds to be decontaminated but does not specify acceptable decontamination methods.

Guidelines have been published by the Australian New Zealand Sprouters' Association.[41] These guidelines advocate a HACCP-based food safety programme that includes:

- Pre-production seed sampling and testing: Sprouting of samples of seed and testing spent irrigation water for *Salmonella* spp.;
- Sanitising seeds prior to sprouting: The recommended treatment varies depending on the seed type, but the most intensive treatment, for alfalfa seed, requires a minimum of 5,000 ppm available chlorine at pH 6-7 with an approximate water/seed ratio of at least 2:1 for a minimum of 10 minutes.
- Verification of controls by testing spent irrigation water for *Salmonella* spp. and testing the final product for *Salmonella* spp., *L. monocytogenes* and generic *E. coli*.
- Verification of GHP by testing the final product for *Staphylococcus* spp.

EFSA has also concluded that food safety management based on HACCP principles should be the objective of operators producing sprouted seeds, and this should incorporate GAP, GHP and GMP along the whole chain from seed production to the final sprouted product (EFSA, 2011a).

EFSA also evaluated different seed decontamination methods and concluded that there was no chemical method of disinfection that can ensure all seed types will be pathogen free, and there were very few disinfection treatments that consistently achieve a substantial (i.e. >5 $\log_{10}$ CFU/g) reduction in pathogen numbers for all seed types (EFSA, 2011a). This view was echoed in two more recent reviews (Dechet *et al.*, 2014; Yang *et al.*, 2013). The EFSA review included considering the United States Food and Drug Administration (USFDA)'s recommended treatment of soaking seeds in 20,000 ppm of calcium hypochlorite.

Yang et al. (2013) summarised the efficacy of various treatments on reducing *E. coli*, *Salmonella* spp. and *Listeria* spp. on seeds. The physical treatments of dry heat, hot water, high hydrostatic pressure and irradiation were all effective treatments, reducing pathogen

[41] Available online at http://www.sproutnet.com/Guidelines-for-Australian-and-Nz (accessed 8 June 2015).

concentrations by 5-8 $\log_{10}$ CFU/g. The results of chemical treatment experiments were highly variable and difficult to compare because of the range of chemicals, concentrations and experimental conditions employed. Overall, seed chlorination treatments reduced the pathogen burden on sprout seeds but did not eliminate the risk of human infection because the pathogens were not necessarily completely eliminated. Biological treatments (antagonistic microorganisms, antimicrobial metabolites and bacteriophages) were less effective, resulting in reductions of 1-7 $\log_{10}$ CFU/g, but may be a sanitising option after seeds have sprouted.

It has been reported that combination treatments using one or more physical sanitisers, such as heat and high pressure or heat and chemical treatment, were most effective in eliminating pathogens on seeds (Dechet *et al.*, 2014). Such treatments could greatly improve the efficacy of seed decontamination but these treatments need optimising for different seed types (EFSA, 2011a).

Testing spent irrigation water has been recommended as a monitoring method for microbial contamination (guidelines, above). The 2008 Document reported that, given a lack of peer-reviewed studies to validate this monitoring method, any decision-making around implementing this activity in New Zealand was to be postponed until further information was published. EFSA has evaluated testing spent irrigation water as a control option for EU sprout growers (EFSA, 2011a). They determined that a composite sample of water was required, taken from multiple sampling points from each production lot. Testing might target *Salmonella* spp., STEC and *L. monocytogenes*. Concerns about the monitoring method included:

- Non-detection of pathogens if water samples were drawn too far apart (if water is collected at distance from the initial contaminated seeds then there is significant risk that the pathogens will not be detected); and
- Non-detection because the concentration of pathogens in the water is lower than on the contaminated sprouts.

It appears that there is still a need to validate irrigation water testing in a New Zealand context.

5. DISCUSSION AND DATA GAPS

5.1 DISCUSSION

Key findings

There have been five surveys completed since the 2008 Document, covering a range of fresh produce commodities. The results from these surveys indicate that pathogenic microorganisms can be found on these products, but the prevalence and concentrations are low.

The pathogens currently considered to be of most concern with respect to RTE fresh produce in New Zealand are *Salmonella* spp., norovirus, STEC, *Yersinia* spp., HAV and *L. monocytogenes*.

The pathogens currently considered to be of less concern with respect to RTE fresh produce in New Zealand are *B. cereus*, *Campylobacter* spp., *C. botulinum*, *C. perfringens*, *S. aureus* and *Shigella* spp.

The pathogens that are potentially important with respect to RTE fresh produce in New Zealand, but for which there is scarce evidence, are *Aeromonas* spp., the protozoan parasites *C. parvum*, *G. duodenalis*, *C. cayetanensis* and *T. gondii*, and rotavirus.

The RTE fresh produce commodities available in New Zealand that are of most concern for their potential for contamination with pathogenic microorganisms are leafy green vegetables (lettuce, spinach, cabbage, etc.), sprouts, fresh-cut fruit salads, berries, watermelons, carrots, tomatoes and fresh herbs.

There is still very little information to evaluate the microbiological safety of fresh juices produced in New Zealand.

The Food Handler Guidance and National Programme Level 1 risk-based measures apply to growers under the new *Food Act 2014*, and these regulatory requirements (still under development) will ensure that all growers are subject to food safety controls, irrespective of any accreditation to an assurance programme.

The 2008 Document's conclusions, with commentary from this update are:

- Exposures to pathogens in fruits and vegetables in New Zealand are not responsible for as high a proportion of reported outbreaks as overseas.

  The difficulties in identifying the vehicle of infection in outbreak investigations means that it is likely that fresh produce-associated outbreaks occur more frequently than the available data suggest. The public health surveillance and reporting systems also differ between countries, as do food consumption patterns and food chains, so this discussion will not attempt to use a comparison of New Zealand and overseas outbreak data to comment on risk. The 2008 Document did note that New Zealand recalls and outbreak data for fresh produce were limited and under-reporting of outbreaks may be hiding produce-related issues. This finding has not changed, although produce-related outbreaks are more likely to be under-reported because identifying the vehicle of infection is challenging in general, and especially for short shelf life foods.

- Information on the prevalence of pathogens on fruit and vegetables in New Zealand is very limited.

There have been five surveys completed since the 2008 Document, covering a limited range of fresh produce commodities and pathogens. The results from these surveys indicate that pathogenic microorganisms can be found on these products, but the prevalence and concentrations are low.

- The operation of growers and/or processors under certification and food safety programmes provides some reassurance that many in the sector are aware of, and take steps to control, pathogens. There may be issues regarding water quality.

  More information is available to support this finding. A small survey of New Zealand fruit and vegetable growers identified some practices, including using water of uncertain quality, that increased the risk of fresh produce being contaminated with pathogenic microorganisms. Assurance programmes require at least some activities that minimise the opportunity for pathogens to contaminate fresh produce from natural fertilisers or water, but not all assurance programmes have food safety as their primary purpose, e.g. the purpose of organic programmes is to assure the consumer that the product has been produced using organic standards.

- It is likely that New Zealanders are changing the ways in which they consume fruits and vegetables. There is now greater availability of RTE fruit and vegetable products, organic produce and farmers' markets.

  There are no additional data to support or oppose this finding. Data from national nutrition surveys suggests that, other than berries and other small fruits, and fresh juices, consumption of fresh fruit and vegetables has decreased among adult New Zealanders, although those that do consume fresh fruit or vegetables may be consuming more. Children are generally less likely to eat raw fruit or vegetables than adults, and consume smaller quantities.

As already stated, there is no control step during the production of RTE fresh produce, fresh juices or sprouts that will ensure the consumer-ready product is pathogen-free. Steps for reducing the risk of contamination from pathogenic microorganisms must be implemented by the grower in the first instance, since outbreak investigation information shows that contamination events often occur on-farm. Subsequent contamination controls must be implemented by food handlers involved in the transport, storage, further processing (e.g. fresh-cut) and retailing of fresh produce products. The Food Handler Guidance and National Programme Level 1 risk-based measures apply to growers under the new *Food Act 2014*, and these regulatory requirements (still under development) will ensure that all growers are subject to food safety controls, irrespective of any accreditation to an assurance programme.

The remainder of this discussion will first focus on each pathogenic microorganism considered in this document, then the different food commodities.

5.1.1 Which pathogenic microorganisms are of most concern for RTE fresh produce available in New Zealand?

From the information available, the pathogens considered in this document can be categorised with respect to RTE fresh produce available in New Zealand. The categories are: "of most concern", "of less concern", and "potentially important" but the lack of information makes a quantitative assessment difficult.

The pathogens currently considered to be **of most concern** with respect to RTE fresh produce in New Zealand are *Salmonella* spp., norovirus, STEC, *Yersinia* spp., HAV and *L. monocytogenes*.

Salmonella spp.

Salmonella spp. were detected at low prevalence on lettuces and sprouts in New Zealand and were the etiological agent in one outbreak where fresh produce was the confirmed vehicle (watermelon). *Salmonella* spp. have been detected in surface water and animal faeces in New Zealand. Overseas, *Salmonella* spp. have been isolated from a range of fresh produce, and even though the prevalence and concentrations (where known) are usually low, there have been multiple produce-associated salmonellosis outbreaks reported.

Salmonella spp. have been shown to colonise the inside of plants and harvested fruits and vegetables, where growth may occur. *Salmonella* spp. can survive or grow on a wide range of fruits and vegetables at cooler temperatures, and while survival on plants in the field appears to be less successful, survival in greenhouse conditions is more likely and the pathogen may survive post-harvest washing and storage. *Salmonella* spp. have been detected on conventional and organic produce and have caused outbreaks as a result of faecal contamination on-farm. *Salmonella* spp. are a particular issue for sprouts as the bacterium can survive long periods on the seeds and grow during sprouting.

Norovirus

Norovirus has been detected on fresh produce in the only New Zealand study that looked for this virus, although the prevalence was low (1%). Lack of a suitable culture method means that the viability of the virus could not be established in this, or any overseas studies. Overseas, norovirus has been detected in multiple surveys of fresh produce and caused many outbreaks linked to fresh produce, some involving large numbers of people. There was one confirmed norovirus outbreak in New Zealand caused by an infected food handler contaminating fruit salad. Based on outbreak information from other countries, and the large number of norovirus outbreaks reported in New Zealand, there have probably been other norovirus outbreaks caused by fresh produce in New Zealand.

The importance of fresh produce as the vehicle of infection relative to infected food workers is difficult to establish. There are multiple potential viral sources and pathways for contamination, and viral survival and cross-contamination from food handling equipment and work surfaces have also been demonstrated. In addition, evidence suggests that norovirus can survive in a viable state on fresh produce, even when frozen (e.g. berries), and there is some suggestion that norovirus can be internalised in produce.

STEC

STEC have not been detected on fresh produce in New Zealand and have not been the etiological agent in any New Zealand outbreaks where fresh produce was the confirmed vehicle of infection. STEC are considered to be a pathogen of concern based on:

- the severity of health outcomes should a contamination event occur in New Zealand;
- the potential for this pathogen to contaminate fresh produce in the field via soil, natural fertilisers or water;
- the ability of this pathogen to survive and grow on a range of fresh produce (even at cooler temperatures or in the field);
- the ability to survive on sprout seeds and grow in sprouts;
- the ability to be internalised in plants and fruit where it may grow; and
- the ability to express virulence genes, even in the VBNC state.

Further support comes from overseas prevalence and outbreak data. Although STEC have been detected at low prevalence on fresh produce overseas, STEC-contaminated fresh produce have caused large outbreaks (the largest involving 4,300 cases).

Yersinia spp. (*Y. enterocolitica, Y. pseudotuberculosis*)

Prior to 2014, *Yersinia* spp. may not have been considered pathogens of concern with respect to fresh produce. While the rate of reported sporadic yersiniosis in New Zealand is notable, previously there was little information to indicate that produce was a vehicle in this country. There are a few studies that demonstrate the ability for *Y. enterocolitica* to survive and grow on fresh produce, but data are scarce and growth appears to be strongly inhibited by other microflora. There are reports of yersiniosis outbreaks overseas (carrots, fresh-cut produce) but while these are few, both *Y. enterocolitica* and *Y. pseudotuberculosis* have been involved.

In 2014 *Y. pseudotuberculosis* caused an outbreak in New Zealand involving 220 people nationwide, 70 of which were hospitalised. While the outbreak investigation did not confirm the vehicle of infection, a case control study identified fresh carrots and/or lettuces as possible vehicles. There is a need to obtain more data on the prevalence and concentration of *Yersinia* spp. on New Zealand fresh produce and in the environment to better understand the importance of this pathogen in horticulture.

HAV

The reported rate of Hepatitis A in New Zealand is low relative to enteric diseases, and there are only a few outbreaks reported each year, but HAV has caused an outbreak in New Zealand linked to fresh produce (domestically-produced blueberries) and recently caused outbreaks in Australia and other countries. There are no recent prevalence studies but outbreak data strongly suggest that berries are an important vehicle for this virus. The outbreak data (and multiple recalls overseas) also prove that HAV can survive in a viable state on berries, and there are studies that reported the virus's ability to survive on fresh spinach leaves and frozen herbs. There is some evidence to suggest that HAV can be internalised in fresh produce. HAV survives well in the environment, but contamination can only come from human sewage since humans are the only known host. Therefore, as for norovirus, the importance of fresh produce as the vehicle of infection relative to infected food workers is difficult to establish; toilets and workers' hands have been identified as key on-farm contamination points for HAV. There has been one recall in New Zealand for apples and pears potentially contaminated with HAV from an infected food handler.

L. monocytogenes

In New Zealand, *L. monocytogenes* has been detected on fresh fruit salads and sprouts at low prevalence, and was not detected in RTE leafy vegetable salads (although other *Listeria* spp. were). The concentrations measured in these surveys were all <100 CFU/g, which has a very low probability of causing illness even in susceptible people. *L. monocytogenes* was detected in 13% of dressed coleslaw samples from delicatessens, but these salads may have been contaminated at the food premises and were not necessarily contaminated from the raw vegetables. Prevalence and concentration data from overseas surveys of fresh produce are generally similar to that found in New Zealand, although higher prevalence and concentrations have been reported.

Despite these data, and in disagreement to the conclusions of the 2008 Document, *L. monocytogenes* is considered to be a pathogen of concern for fresh produce because the pathogen is able to survive or grow on a range of fresh produce at lower temperatures (growth can be slow but an increase of >1 $\log_{10}$ CFU/g over a few days is possible), there is some evidence *L. monocytogenes* can be internalised in fresh produce, there have been listeriosis outbreaks overseas linked to fresh produce (one involved 147 cases and 33 deaths), and severe health outcomes are likely should a contamination event occur in New Zealand. *Listeria* spp. can be widespread in the environment, particularly near animal farms where environmental contamination might be enhanced through the use of silage or a listeriosis outbreak among livestock. There has been one recall in New Zealand for fresh produce that was contaminated with *L. monocytogenes* on-farm.

The pathogens currently considered to be **of less concern** with respect to RTE fresh produce in New Zealand are *B. cereus*, *Campylobacter* spp., *C. botulinum*, *C. perfringens*, *S. aureus* and *Shigella* spp.

B. cereus

B. cereus is ubiquitous in the environment and survives well as spores, so it has been detected in surveys of fresh produce overseas (there are no New Zealand fresh produce surveys for this pathogen). There have been some reports of fresh produce-associated outbreaks overseas where *B. cereus* has been the etiological agent identified, but there was not enough information to confirm that the fresh produce was contaminated with either a very high concentration of *B. cereus* or pre-formed toxin.

Emetic food poisoning usually involves food products with high starch content, such as pasta, rice, mashed potatoes, bread and pastries, where *B. cereus* spores have germinated, and the cells multiplied and produced toxin (Ceuppens *et al.*, 2013). Fresh produce may introduce *B. cereus* spores to these foods (e.g. fresh produce added to cooked pasta or potato salads), but the spores will almost always already be present in the cooked foods. Diarrhoeal food poisoning is caused by ingesting foods containing high numbers of *B. cereus* (>10^5 CFU/g), and for fresh produce such significant growth is likely to be accompanied by spoilage.

Campylobacter spp.

New Zealand has a comparatively high rate of reported campylobacteriosis and large numbers of reported (small) campylobacteriosis outbreaks each year, but no outbreaks have yet been linked to fresh produce. *Campylobacter* spp. were not detected on fresh produce in New Zealand, and when this species has been detected in surveys overseas, the prevalence was low (<2%). A small number of survival studies have shown that *Campylobacter* spp. do not survive well on fresh produce before or after harvesting.

Together, this information suggests that *Campylobacter* spp. is not of concern with respect to fresh produce, but it should be noted that the high prevalence of *Campylobacter* spp. in the faeces of New Zealand livestock and New Zealand surface waters shows the potential for contamination of fresh produce from these sources. Fresh produce should only pose a risk for human illness if the initial load of *Campylobacter* spp. is high (a gross contamination event) as growth is unlikely to occur. Only one recent overseas outbreak was reported, caused by peas in Alaska contaminated with wild bird faeces.

C. botulinum, *C. perfringens*

C. botulinum and *C. perfringens* are also spore-forming bacteria that are ubiquitous and stable in the environment, although there are very little data on their presence in soils, dust or sediments in New Zealand. There have been some recent surveys overseas that have detected *C. botulinum* on fresh produce, but *C. perfringens* was not detected. While spores might be present on fresh produce in New Zealand, they are unlikely to germinate and grow under the conditions typically applied to these foods. The only notable exception is fresh produce stored under the MAP; data are needed to determine whether these clostridia can germinate and grow under the atmospheric and temperature conditions typically found for New Zealand MAP produce. No outbreak reports were located where clostridia intoxication occurred from people eating MAP fresh produce. One recent overseas outbreak of botulism was caused by fresh juice that had been temperature-abused, and another outbreak of *C. perfringens* intoxication was reported where contaminated fresh herbs were added to a pasta salad (the salad, not the herbs, provided the substrate for growth). *C. perfringens* outbreaks are usually associated with improperly cooled proteinaceous foods.

S. aureus

S. aureus is ubiquitous in the environment and on people's skin and mucous membranes. There are only a few outbreaks of *S. aureus* intoxication in New Zealand each year and none have been linked to fresh produce. There have been some fresh produce outbreaks reported overseas and *S. aureus* has been detected on fresh produce in a small number of surveys in other countries. A few studies have indicated potential for this pathogen to grow on fresh produce and internalisation might be possible, however data are limited and *S. aureus* does not compete well with indigenous microflora in raw foods (Argudín *et al.*, 2010).

Because *S. aureus* is commonly found in the nose and on the skin of humans, food handlers, particularly at the retail end of the food chain, are considered to be an important source of food contamination. An outbreak in the USA was caused by coleslaw containing enterotoxin produced by a community-acquired methicillin resistant *S. aureus* from an asymptomatic food handler (Jones *et al.*, 2002). Growth and/or formation of enterotoxin is usually associated with protein-rich foods (meat, dairy) that have been improperly prepared or stored (Schelin *et al.*, 2011). The 2008 Document also considered *S. aureus* to be an unlikely risk associated with fresh produce.

Shigella spp.

Humans are the only known host for *Shigella* spp. so fresh produce will only be contaminated with this bacterial species if it comes into contact with human faeces directly or via water or soils containing sewage, or via an infected food worker. Contamination of foods with *Shigella* spp. is usually the result of an infected food handler with poor personal hygiene (USFDA, 2012). Because of this, *Shigella* spp. contamination of fresh produce is likely to be sporadic, which is one possible reason why *Shigella* spp. were not detected in a small number of fresh produce surveys overseas. There have been shigellosis outbreaks reported overseas caused by fresh produce, and available information shows that the source of contamination was either a food worker at the retail end of the food chain or a packhouse located in developing countries where shigellosis is more common. There are some data to show that *Shigella* spp. can survive on fresh produce (including sprouts), and this pathogen can still express virulence genes when in the VBNC state.

The pathogens that are **potentially important** with respect to RTE fresh produce in New Zealand, but for which there is scarce evidence, are *Aeromonas* spp., the protozoan parasites (*C. parvum*, *G. duodenalis*, *C. cayetanensis* and *T. gondii*), and rotavirus.

Aeromonas spp.

Aeromonas spp. have been detected in RTE food in New Zealand (including one sample of coleslaw), but there are no recent surveys for this species. *Aeromonas* spp. have been detected in surveys of fresh produce overseas, sometimes at high concentrations (>10^5 CFU/g). A high concentration is thought to be necessary to cause human illness (Sarvikivi *et al.*, 2012). *Aeromonas* spp. infection has recently been reported in New Zealand but there have been no reported outbreaks. No outbreak reports of *Aeromonas* spp. infection associated with fresh produce were located. There is limited information to suggest that *Aeromonas* spp. can survive and grow on fresh produce, and many strains are able to grow and produce toxins at refrigeration temperatures (Tomas, 2012).

Water is the most likely source of fresh produce contamination by *Aeromonas* spp., since this bacterium is well-suited to survival in aquatic systems, including potable water distribution systems where they can form biofilms and resist chlorination. Data on the prevalence and concentration of *Aeromonas* spp. on fresh produce and irrigation/wash water in New Zealand would help to determine the potential for fresh produce to be a cause of *Aeromonas* spp. infection in New Zealand.

C. parvum

There are a notable number of sporadic cases and outbreaks of cryptosporidiosis each year in New Zealand, but infection by *Cryptosporidium* spp. is largely considered to be waterborne in this country. *Cryptosporidium* spp. have been detected in livestock faeces and water in New Zealand, so there is potential for fresh produce contamination, but the prevalence and concentration of this protozoan on fresh produce in New Zealand is not known, and growth on produce is not possible. Two studies from Canada have found *Cryptosporidium* spp. on fresh produce at prevalences of 5.9% and 0.6%. There is limited evidence to show that viable oocysts can adhere to fresh produce and remain stable on this food during storage, but outbreaks of cryptosporidiosis from contaminated fresh produce have been reported overseas. One of these outbreaks involved over 500 cases in UK.

G. duodenalis

Like *Cryptosporidium* spp., *Giardia* spp. have also been the reported etiological agent in sporadic cases and outbreaks of giardiasis in New Zealand each year, most of which are assumed to be waterborne or from contact with farm animals. *Giardia* spp. have been detected in livestock faeces and water in New Zealand, so there is potential for fresh produce contamination. Work in other countries has found that in most cases animals do not share identical *Giardia* spp. genotypes with those isolated from infected humans, providing little support for zoonotic transmission (or transmission via fresh produce contaminated by animal faeces) (Cacciò, 2015). But in New Zealand, genotypes of *G. duodenalis* known to infect humans have been isolated from calves (Abeywardena *et al*., 2012). There are no surveys of fresh produce in New Zealand for *Giardia* spp., and this genus was detected at a low prevalence (1.8%) in a recent survey in Canada. There have been no recent reports of outbreaks caused by fresh produce contaminated with *Giardia* spp.

C. cayetanensis

C. cayetanensis has not been detected in New Zealand so there are no data on the distribution of this pathogen in the environment or on fresh produce, or its importance as a cause of acute gastroenteritis in this country. There is very little information available on the prevalence of *Cyclospora* spp. on fresh produce from other developed countries (1.7% in a Canadian study), and as there are currently no tests to determine if *Cyclospora* spp. detected on foods is infectious, this makes it difficult to interpret prevalence data in terms of human health risk (Ortega and Sanchez, 2010). Data on survival of *C. cayetanensis* on fresh produce are scarce, but several outbreaks of cyclosporiasis from fresh produce have been reported overseas, which provides evidence that survival in a viable state on these foods is possible. *Cyclospora* spp. are resistant to many disinfectants, including chlorination at levels used in water treatment (Chacin-Bonilla, 2010).

T. gondii

Fresh vegetables contaminated from the soil have often been cited as a potential vehicle of infection for toxoplasmosis, but there is currently little evidence to support this. There are no New Zealand data on the prevalence of *T. gondii* on fresh produce, or in horticultural soils and waters. Only one recent survey was located and this used PCR to detect this protozoan, so the presence of oocysts was not confirmed. There has only been one recently reported outbreak of toxoplasmosis linked to fresh produce (Brazil). Evidence for fresh vegetables as a cause of toxoplasmosis is largely from case control or cohort studies (mostly in developing countries) that use seropositivity of cases as the indicator for them having been infected (Alvarado-Esquivel *et al.*, 2013; Alvarado-Esquivel *et al.*, 2011; Alvarado-Esquivel *et al.*, 2010; Hofhuis *et al.*, 2011; Lopes *et al.*, 2012).

Rotavirus

Rotavirus sporadic infection is reported each year in New Zealand but there were no outbreaks reported for the period 2008-2014. Person-to-person spread is currently considered to be the most important route of transmission, but there are no data to indicate whether fresh produce in New Zealand is contaminated with this virus. Rotavirus has been detected at a high prevalence (64%) in samples of river water in New Zealand. It is possible that if fresh produce contamination occurs, this is a sporadic event brought on by use of contaminated water or handling of the food by a worker shedding the virus.

Data from overseas are also scarce: Rotavirus was detected in one survey of packaged leafy green vegetables at a low prevalence of 0.4%, and has been the cause of one recently-reported outbreak where raw sliced cabbage was contaminated by an infected food handler.

Rotavirus vaccines used for humans are effective (reviewed by (Bines and Kirkwood, 2015)) and were introduced as part of the National Immunisation Schedule for New Zealand in June 2014 (Ministry of Health, 2014). As in countries where the vaccine has been introduced, it is expected that not only will the number of hospitalisations due to rotavirus significantly reduce but may also provide indirect protection to other population groups (Dey *et al.*, 2012; Lopman *et al.*, 2011).

5.1.2 Which RTE produce commodities available in New Zealand are of most concern given their potential for contamination with pathogenic microorganisms?

Together, the foods associated with strong evidence outbreaks in New Zealand, the foods on which pathogenic microorganisms have been detected through surveys in this country, and the foods that have been recalled in New Zealand for potential pathogenic microbial contamination, are: Leafy green vegetables (lettuce, spinach), sprouts, fresh-cut fruit salad, coleslaw, watermelon, blueberries, carrots, apples and pears, and parsley.

This list shows similarity with information from other countries:

- The fresh produce items most often implicated by strong evidence in overseas outbreaks were leafy green vegetables, sprouts, berries, tomatoes and melons, but a range of other vegetables were also implicated by strong evidence, e.g. baby corn, baby carrots, cucumbers, peas.
- In surveys of fresh produce pathogenic microorganisms were often detected on sprouts, fresh herbs, berries and salads/leafy vegetables, but it should be noted that many of these surveys specifically focussed on these foods and did not test other fresh produce types.
- From recalls issued for the EU since 2001 it was apparent that the foods most of concern due to potential contamination with pathogenic microorganisms were leafy green vegetables, tropical fruit and melons, berries (including frozen), sprouts and tomatoes. There were also multiple recalls for mushrooms but there was not enough information to determine whether these were fresh or preserved.
- From risk assessments and risk related activities, the foods considered most at risk of being contaminated by pathogenic microorganisms (with resultant human illness) were leafy green vegetables, sprouts, tomatoes, melons, berries and fresh herbs.

The 2008 Document concluded, largely from international literature, that lettuce and spinach were the most important vegetables in terms of risk for foodborne disease, and berries and melons were the most important fruits. In the survey of New Zealand fresh fruit and vegetables around half of the products considered “unsatisfactory” based on the concentration of generic *E. coli* were leafy green vegetables. However, the information above shows that there are other types of fresh produce that might be important in New Zealand in addition to leafy green vegetables, melons and berries.

There is still very little information to evaluate the microbiological safety of fresh juices produced in New Zealand. It is expected that these drinks will be consumed within the day of purchase, if not immediately, so exposure will depend more on the initial concentration of the pathogenic microorganism than subsequent storage temperatures/times, but it should be noted that there are no data to substantiate these assumptions.

The 2008 Document found no evidence to suggest that sprouts were a concern with respect to foodborne illness in New Zealand, but the recent New Zealand survey shows that pathogenic bacteria are detectable on sprouts available in this country. The results of this survey have prompted the suggestion that the practices applied along the primary production and processing chain for seed sprouts should be reviewed, including seed production on-farm, seed processing and sprout production (D'Sa and Paulin, 2015).

The 2008 Document found no evidence to suggest that organic produce carried a greater risk of being contaminated with pathogens when compared with non-organic produce. This finding has not changed, although the review of organic assurance programmes found that these did not provide much support for growers to manage food safety risks.

The 2008 Document found no evidence to suggest that hydroponically grown produce carried a greater risk of being contaminated with pathogens when compared with non-hydroponic

produce. While no surveys were identified that were specifically designed to compare microbial contamination of hydroponic and conventionally grown fresh produce, studies of pathogen internalisation found that bacterial pathogens were more likely to become internalised in plants grown in hydroponic solutions. This signals an increased risk associated with irrigation water but, as already signalled in this document, there is a lack of data on long-term survival on internalised pathogens so it is not known if they might still be present at the point of consumption.

Outbreak reports, outbreak dataset reviews, epidemiological studies and risk assessments help to build a picture of common food/hazard combinations. However, this can sometimes mask a new food/hazard combination, and an often cited example is that of the 2008 *Salmonella* Saintpaul outbreak in the USA. Contaminated raw tomatoes were the suspected vehicle of transmission, since the tomato/*Salmonella* spp. pairing was well recognised and consumption of salsa was a risk factor for infection, but it was not until the outbreak was nearly over that raw jalapeño and serrano peppers were identified as the cause (Anonymous, 2008; Jungk *et al.*, 2008).

Changes in consumer preferences, partly driven by demographic changes, are influencing the range of fresh produce grown and made available in New Zealand. Obvious examples include watercress, green vegetables used in Asian cuisine, and imported table grapes. New trends in the way vegetables are used also change consumer exposure, e.g. consumption of raw beetroot, ginger root or frozen berries. Relying on historical information to assess risk from fresh produce in New Zealand may overlook emerging foods and consumer behaviours.

Despite all of the information above, most of the case control studies of enteric disease conducted in New Zealand and overseas found that consumption of fresh fruit and vegetables did not present elevated risk.

5.2 DATA GAPS

The 2008 Document did not provide a list of specific data gaps. Those that were apparent in the text are presented in TABLE 8.

TABLE 8: Data gaps identified in the 2008 Document

DATA GAP	COMMENTARY
Validation of testing spent irrigation water for pathogens during the sprouting process	EFSA has evaluated testing spent irrigation water as a control option for EU sprout growers, see Section 4.5.4.
The presence of *Y. enterocolitica* in New Zealand waters*	No new data are available.
The level of health risk associated with *C. cayetanensis**	No new data are available for New Zealand. Overseas outbreaks associated with fresh produce have been reported.
Description of the grower, processor and retail sectors in New Zealand	A collation of data up to 2011 has been conducted (Lake *et al.*, 2011a, 2011b) but a complete data set is not available.
Prevalence of pathogens in fruits and vegetables in New Zealand, particularly viruses and protozoa	Microbiological surveys of fresh produce have been completed including testing for norovirus in packaged salads. There are no data for other viruses or protozoa.
Information on actions taken when pathogens are detected by growers	This information has not been collated.

* New Zealand specific data gaps highlighted by the 2008 Document but initially identified by Walker and Faulkner (2001).

Continuing and additional data gaps identified in this report that impact on the report's discussion are:

- The length of time that internalised pathogenic microorganisms remain viable inside the edible parts of plants (i.e. can they survive through the growing period, and can they survive from harvesting until consumption).
- The prevalence and concentration of pathogenic microorganisms in fresh juices available in New Zealand.
- Growth of pathogenic bacteria in fresh juices stored at ambient temperatures.
- Data on the potential for growth of *C. botulinum* and *C. perfringens* and their ability to produce toxins on a wide variety of produce stored in MAP under mild temperature abuse such as 7-12°C.
- The prevalence and concentration of *Yersinia* spp. on New Zealand fresh produce and in the environment (water, soil, compost, manure).
- The prevalence and concentration of *Aeromonas* spp. on fresh produce and irrigation/wash water in New Zealand.
- The prevalence and concentration of *Giardia* spp., and *Cryptosporidium* spp., *C. cayetanensis* and *T. gondii* on fresh produce and irrigation/wash water in New Zealand.
- The prevalence and concentration of norovirus, HAV and rotavirus on fresh produce and irrigation/wash water in New Zealand.
- A review of data on the efficacy of sanitisers against the protozoans considered in this document.

The tables presented in Appendix A.1 also show a lack of New Zealand-specific data that would better inform each microorganism's potential for contaminating fresh produce.

The 2002 document "Risks associated with bacterial pathogens in exported fruit and vegetables" (Hudson and Turner, 2002) contains useful tables of results from fresh produce surveys (prevalence, concentrations) published as far back as the 1970s. There is value in updating these tables as a resource for future work.

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APPENDIX A: HAZARD AND FOOD

A.1 RELEVANT CHARACTERISTICS OF THE PATHOGENS

General information on the growth, survival and inactivation of the pathogens considered in this discussion document is presented in microbiological datasheets available from:

http://www.foodsafety.govt.nz/science-risk/hazard-data-sheets/pathogen-data-sheets.htm

and a more recent document published by FSANZ (FSANZ, 2013), available from:

http://www.foodstandards.govt.nz/publications/pages/agentsoffoodborneill5155.aspx

This section contains additional information on each of the pathogens considered in this document that helps to assess their potential for contaminating fresh produce. The information presented here is only that obtained during preparation of this current document and could be expanded with further resources.

New Zealand data are prefixed "**NZ**". Overseas data are used where New Zealand data were not located.

A.1.1 *Aeromonas* spp.

Foodborne disease	Gastroenteritis
Pathogenic strains	*A. hydrophila*, *A. caviae*, and *A. veronii* biovar *sobria* are the predominant species isolated from humans with gastrointestinal disease, and while some scientists think that there is only a subset of aeromonads that are pathogenic, others have shown that cytotoxicity and adhesivity (capabilities necessary for enteric infection) are widespread amongst *Aeromonas* spp. isolates from food and water (Janda and Abbott, 2010; Martino *et al.*, 2014; Ottaviani *et al.*, 2011; Parker and Shaw, 2011).
Presence and survival in soil	Can be detected in soil and may survive for a month (Brandi *et al.*, 1996).
Presence and survival in water	Colonise aquatic environments and *A. hydrophila* is the predominant species isolated from freshwater (Janda and Abbott, 2010). Detected in an urban river and its tributaries (Pettibone, 1998). Recovered from spent irrigation water used for growing mung beans (McEgan *et al.*, 2008). **NZ**: *Aeromonas* spp. were recovered from 16/125 samples of roof-collected drinking water from rural houses in the Auckland area (Simmons *et al.*, 2001).
Presence and survival in manure	*A. caviae*, *A. sobria* and *A. hydrophila* were isolated from rectal swabs from sheep, cows and a horse (Ceylan *et al.*, 2009). Faecal carriage rates for animals have been reported in the range <1 to 7% (Igbinosa *et al.*, 2012).
Presence and survival in compost	Laboratory experiments suggest *Aeromonas* spp. should not survive adequate composting (Nishikawa *et al.*, 1993), but it has been isolated from compost (Lim *et al.*, 2014; Priyadarshini *et al.*, 2012).
Excretion from asymptomatic humans	Carriage rate in asymptomatic people in industrialised countries is typically <1% (Janda and Abbott, 2010).

VBNC state	Has been demonstrated for *A. hydrophila* in water (Maalej *et al.*, 2004; Mary *et al.*, 2002).

A.1.2 *B. cereus*

Foodborne disease	Two forms: Emetic, from consumption of toxin produced by *B. cereus* that have multiplied in a food, and diarrhoeal, from consumption of high concentration of *B. cereus* cells that produce toxin in the intestine.
Pathogenic strains	All considered potentially pathogenic.*
Presence and survival in soil	Ubiquitous in soil and able to multiply (Altayar and Sutherland, 2006; Vilain *et al.*, 2006).
Presence and survival in water	Spores recovered from eight rivers and two drinking water samples in Norway (Ostensvik *et al.*, 2004).
Presence and survival in manure	Recovered from cow and horse manure (Altayar and Sutherland, 2006; Bagge *et al.*, 2010).
Presence and survival in compost	Likely to survive adequate composting as spores and the compost maturation stage can favour germination and vegetative cell multiplication (Avery *et al.*, 2012).
Excretion from asymptomatic humans	Can become part of the transitory intestinal flora (Bottone, 2010).
VBNC state	Able to enter a VBNC state (Li *et al.*, 2014).

* Some are non-pathogenic but diarrhoeal and emetic toxin genes are highly prevalent among *B. cereus* strains (Ceuppens *et al.*, 2013).

A.1.3 *Campylobacter* spp.

Foodborne disease	Campylobacteriosis
Pathogenic strains	*C. jejuni* is most frequently isolated from campylobacteriosis cases in New Zealand. *C. coli* also causes disease in New Zealand.
Presence and survival in soil	Only if introduced from faeces. *C. jejuni* decreased by around 4 $\log_{10}$ CFU/g soil in the rhizosphere of spinach plants grown in organic soil when stored at 10 or 16°C for 28 days, but the rate of decrease slowed over time (Brandl *et al.*, 2004).
Presence and survival in water	Only if introduced from faeces. **NZ**: *Campylobacter* spp. detected in 430/725 (59%) surface water samples from 25 sites at a mean concentration of 0.9 MPN/100 ml (43 samples >110 MPN/100 ml) (Till *et al.*, 2008). Almost half of the positive samples contained *C. jejuni*. **NZ**: *Campylobacter* spp. detected in 45/53 (85%) of Canterbury surface waters at concentrations up to 110 MPN/ml and in a rural drinking water supply where treatment failed (Bartholomew *et al.*, 2014; Bigwood and Hudson, 2009). **NZ**: *Campylobacter* spp. detected in 16/135 (12%) shallow aquifers in a Canterbury dairying region using border dyke irrigation at concentrations ranging 0.6 to >3.1 MPN/L (Close *et al.*, 2008).
Presence and survival in manure	**NZ**: Detected in the faeces of wild birds, waterfowl, domestic chickens, cats, dogs, horses, cows, goats and sheep in New Zealand (Anderson *et al.*, 2012; Irshad *et al.*, 2015; Mohan, 2015; Mohan *et al.*, 2013; Moriarty *et al.*, 2011a; Moriarty *et al.*, 2011c;

	Moriarty *et al.*, 2008; Rapp and Ross, 2012; Rapp *et al.*, 2012). **NZ**: Detected in sheep manure at up to $2.7x10^7$ CFU/g but declined rapidly (not detectable after 2-7 days, depending on season), detected in cow manure at up to $2.1x10^4$ CFU/g but not detectable after 14 days (T90 2.2 days) (Gilpin *et al.*, 2009; Moriarty *et al.*, 2011b).
Presence and survival in compost	Should not survive adequate composting (Avery *et al.*, 2012).
Excretion from asymptomatic humans	Reported in very young children in developing countries and in occupationally-exposed adults (e.g. poultry workers) (Lee *et al.*, 2013; Pazzaglia *et al.*, 1991; Quilliam *et al.*, 2013). An Australian study found a prevalence of 0.1% (Hellard *et al.*, 2000).
VBNC state	*C. coli*, *C. jejuni* and *C. lari* can enter a VBNC state (Li *et al.*, 2014). **NZ:** Two strains of *C. jejuni* entered a VBNC state in a saline solution but the VBNC cells were killed upon exposure to simulated human saliva, gastric fluid and intestinal fluid, and the VBNC cells could not be resuscitated (Brandt and Podivinsky, 2008).

A.1.4 *C. botulinum*

Foodborne disease	Two forms: Botulism from consumption of preformed toxin (intoxication), or botulism/infant botulism from consumption of spores and their subsequent outgrowth and toxin production in the intestines of babies or adults with compromised intestinal functions (toxico-infection).
Pathogenic strains	Strains produce one of eight known types of botulinal neurotoxins (A to H) and only those producing types A, B, E and F (rarely) cause botulism in humans (Espelund and Klaveness, 2014). Proteolytic and non-proteolytic *C. botulinum* producing these toxins are pathogenic, but the latter can grow and form toxin at 3ºC.
Presence and survival in soil	A common soil organism. Type E producing strains are marine inhabitants. Survived for over two years in soil amended with contaminated compost (Gessler and Bohnel, 2006). **NZ**: Fragments of the botulinal neurotoxin genes (B, E or F) were detected by PCR in DNA isolated from samples taken from farm environments but bacteria were not isolated (Gilbert *et al.*, 2006).
Presence and survival in water	Can be found in surface waters but greater concentrations are found in aquatic sediments and algal mats (Espelund and Klaveness, 2014; Lan Chun *et al.*, 2015). A case of infant botulism in Japan was caused by untreated well water likely contaminated by spores mobilised from the soil and sediments in the well (Kobayashi *et al.*, 2014). **NZ**: The gene for Type A botulinum neurotoxin was detected in 1/501 sediment samples from coastal marine locations (no toxin was detected by mouse bioassays in any sample) (Fletcher *et al.*, 2008). The C and D toxins (not pathogenic to humans) have been detected in lake and marine sediments (Gill and Penney, 1982).
Presence and survival in manure	Has been detected in pig and cow faeces (Dahlenborg *et al.*, 2001, 2003).
Presence and survival in compost	Likely to survive adequate composting as spores and the compost maturation stage can favour germination and vegetative cell multiplication (Avery *et al.*, 2012).

Excretion from asymptomatic humans	Usually only in cases of toxico-infection (up to 158 days from onset of constipation until well after recovery was reported for infant botulism) (Brook, 2007).
VBNC state	No information located.

A.1.5 *C. perfringens*

Foodborne disease	Intoxication
Pathogenic strains	Strains produce one of five types of toxins (A, B, C, D, E) and most *C. perfringens* food poisoning cases reported in developed countries are caused by type A strains.
Presence and survival in soil	A common soil microorganism. Detected in soils used for cultivating root, bulb and other vegetable crops (Voidarou *et al.*, 2011). Detected in soils with or without compost or manure applications at concentrations ranging <10-2.9x10^2 CFU/g, and detectable between applications which were two years apart (Brochier *et al.*, 2012).
Presence and survival in water	**NZ**: Spores detected in 418/725 (58%) surface water samples taken from 25 sites at a mean concentration of 1.5 CFU/100 ml (maximum 120 CFU/100 ml) (Till *et al.*, 2008).
Presence and survival in manure	Recovered from cow manure (Bagge *et al.*, 2010; McAuley *et al.*, 2014).
Presence and survival in compost	Likely to survive adequate composting as spores and the compost maturation stage can favour germination and vegetative cell multiplication (Avery *et al.*, 2012). A number of studies reported concentrations of 10^2-10^3 CFU/g (Brochier *et al.*, 2012).
Excretion from asymptomatic humans	Toxigenic strains have been detected in faeces from healthy individuals (Heikinheimo *et al.*, 2006).
VBNC state	No information located.

A.1.6 *L. monocytogenes*

Foodborne disease	Two forms: Invasive listeriosis (“listeriosis”) or non-invasive listeriosis (“febrile listeriosis” or “listerial gastroenteritis”).
Pathogenic strains	All strains potentially pathogenic. Serotypes O1/2 and O4 are commonly isolated from infected humans in New Zealand (Health Intelligence Team, 2014b).
Presence and survival in soil	Only if introduced from infected animals or humans. Detected in soil from produce growing fields in the USA (Strawn *et al.*, 2013). Death rate of up to 0.4 CFU/g/d in a range of soils (survived 65 days at 10ºC) (Moynihan *et al.*, 2015).
Presence and survival in water	Only if introduced from infected animals or humans. Detected in 43% of 1,405 surface water samples from 30 sites in a leafy green growing area of the USA (Cooley *et al.*, 2014). Detected in 1/31 surface water samples from dairy farms in Australia (McAuley *et al.*, 2014).

Presence and survival in manure	Detected in faeces from dairy animals (Borucki *et al.*, 2005; Ho *et al.*, 2007). Concentration slowly decreases in the presence of manure (Erickson *et al.*, 2015).
Presence and survival in compost	Should not survive adequate composting (Avery *et al.*, 2012). Survived in in freshly prepared compost mixes stored at 20ºC for 12 weeks, but notable die-off in compost mix using chicken manure (Erickson *et al.*, 2015).
Excretion from asymptomatic humans	0.6–3.4% of healthy people with unknown exposure to *Listeria* spp. were found to shed *L. monocytogenes* in their faeces (FSANZ, 2013). People affected by the invasive form of the disease do not always shed *L. monocytogenes* in their faeces.
VBNC state	*L. monocytogenes* can enter a VBNC state (Li *et al.*, 2014). **NZ:** Two strains of *L. monocytogenes* entered a VBNC state in a saline solution, and while the VBNC cells could not be resuscitated, they were able to survive exposure to simulated human saliva and intestinal fluid, but were sensitive to simulated gastric fluid (Brandt and Podivinsky, 2008).

A.1.7 *Salmonella* spp.

Foodborne disease	Salmonellosis
Pathogenic strains	All *Salmonella* spp. considered potentially pathogenic. The serotypes *Salmonella* Typhimurium and *Salmonella* Enteritidis are most often isolated from infected humans in New Zealand (Health Intelligence Team, 2014b).
Presence and survival in soil	Only if introduced from faeces. Detected in 2/14 soil samples from dairy farms in Australia (McAuley *et al.*, 2014). Detected in soil from growing fields in the USA (Strawn *et al.*, 2013). Survival highly variable but >5 months possible in manure amended soil under field conditions (Ongeng *et al.*, 2013). Death rate of up to 0.3 CFU/g/day in a range of soils (survived 110 days at 10ºC) (Moynihan *et al.*, 2015). Survival is less successful at warmer temperatures or in the presence of manure (Garcia *et al.*, 2010; You *et al.*, 2006).
Presence and survival in water	Only if introduced from faeces. Detected in ground, surface and drinking waters (Levantesi *et al.*, 2012). Survival depends on chemistry and natural microflora of irrigation water (Van Der Linden *et al.*, 2014). **NZ**: Detected in 69/725 (10%) surface water samples taken from 25 sites (Till *et al.*, 2008). The concentration in 65/69 of the positive samples was <11 MPN/100 ml.
Presence and survival in manure	**NZ**: Not detected in a survey of 155 cow pats or in 320 droppings from waterfowl (Moriarty *et al.*, 2011a; Moriarty *et al.*, 2008). **NZ**: Detected in faeces from lambs at slaughter, but not sheep on pasture (Moriarty *et al.*, 2011c). **NZ**: Detected in faeces from calves (Al Mawly *et al.*, 2015). Able to survive (e.g. decimal reduction time 25 days), lower temperatures prolong survival (Avery *et al.*, 2012). Survival highly variable but several months possible under field conditions (Ongeng *et al.*, 2013).
Presence and survival in compost	Should not survive adequate composting (Avery *et al.*, 2012). Survived in in freshly prepared compost mixes stored at 20ºC for 12 weeks (Erickson *et al.*, 2015).

Excretion from asymptomatic humans	Shedding can continue for about 4 weeks after illness in adults and 7 weeks in children, and an estimated 0.5% of infected people become long-term carriers (FSANZ, 2013). An Australian study found a prevalence of 0.4% amongst 1,091 asymptomatic people (Hellard *et al.*, 2000).
VBNC state	Can enter a VBNC state (Li *et al.*, 2014). *Salmonella* Typhimurium entered a VBNC state when inoculated into grapefruit juice and was able to be resuscitated (Nicolo *et al.*, 2011). **NZ:** *Salmonella* Typhimurium and *Salmonella* Brandenberg entered a VBNC state in a saline solution and while the VBNC cells could not be resuscitated, they were able to survive exposure to simulated human saliva, gastric fluid and intestinal fluid (Brandt and Podivinsky, 2008).

A.1.8 *Shigella* spp.

Disease	Shigellosis (bacillary dysentery)
Pathogenic strains	The genus *Shigella* consists of four species: *S. dysenteriae* (subgroup A), *S. flexneri* (subgroup B), *S. boydii* (subgroup C), and *S. sonnei* (subgroup D) all of which cause human disease. *S. flexneri* and *S. sonnei* are most associated with foodborne disease (EFSA, 2014d).
Presence and survival in soil	Only if introduced from human faeces. Detected by PCR in soils irrigated by grey water (Benami *et al.*, 2013).
Presence and survival in water	Only if introduced from human faeces (commonly detected by studies of surface or drinking water in developing countries). Has caused a waterborne outbreak from a public drinking water supply in Spain (Godoy *et al.*, 2011).
Presence and survival in manure	Animals are not a known reservoir, but will be present in faeces from infected humans.
Presence and survival in compost	Should not survive adequate processing of human biowaste.
Excretion from asymptomatic humans	Only adults who live in areas where shigellosis is endemic (FSANZ, 2013).
VBNC state	*S. sonnei*, *S. flexneri* and *S. dysenteriae* can enter a VBNC state (Li *et al.*, 2014). *S. flexneri* entered a VBNC state when inoculated into grapefruit juice and was able to be resuscitated (Nicolo *et al.*, 2011).

A.1.9 *S. aureus*

Foodborne disease	Staphylococcal food poisoning caused by ingestion of preformed staphylococcal enterotoxin.
Pathogenic strains	All isolates are considered potentially pathogenic. Strains (clonal lineages) of *S. aureus* can be host specific, but some strains can colonise multiple hosts (Fitzgerald, 2012). Full epidemiological characterisation of most clonal lineages is still lacking, and changes in host range can occur (Smith, 2015).
Presence and survival in soil	Widespread in the environment and can be found in soil (USFDA, 2012). Coagulase-positive staphylococci were not detected in 14

	samples of soil taken from Australian dairy farms (McAuley *et al.*, 2014).
Presence and survival in water	Widespread in the environment and can be found in water (USFDA, 2012). Coagulase-positive staphylococci were not detected in 31 surface water samples taken from Australian dairy farms (McAuley *et al.*, 2014).
Presence and survival in manure	*S. aureus* has been detected in animal faeces (Friese *et al.*, 2013; He *et al.*, 2013). Coagulase-positive staphylococci were not detected in 16 faecal samples taken from Australian dairy farms (McAuley *et al.*, 2014).
Presence and survival in compost	Should not survive adequate composting (Avery *et al.*, 2012).
Excretion from asymptomatic humans	Commonly found in the nose and on the skin of humans, with estimates of 20–30% for persistent and 60% for intermittent colonisation (Argudín *et al.*, 2010). Food handlers carrying enterotoxin-producing *S. aureus* in their noses or on their hands are regarded as the main source of food contamination via direct contact or through respiratory secretions.
VBNC state	Can enter a VBNC state (Li *et al.*, 2014).

A.1.10 STEC, including *E. coli* O157:H7

Foodborne disease	Gastroenteritis, STEC infection
Pathogenic strains	STEC are *E. coli* carrying the genes for producing Shiga-like toxins. Serotype O157:H7 is most commonly isolated from patients in New Zealand, but routine testing of faecal samples for non-O157 STEC is not standard in New Zealand laboratories.
Presence and survival in soil	Only if introduced from faeces. Survival highly variable but several months possible in manure amended soil under field conditions (Ongeng *et al.*, 2013). Survival variable and depends on climate, soil type, land use, etc., but low temperatures (<5 ºC) and high-quality soil structure that enhances the air, water and nutrient circulation through soil spaces may favour STEC survival (Fremaux *et al.*, 2008). Death rate of up to 0.5 CFU/g/d in a range of soils (survived 110 days at 10ºC) (Moynihan *et al.*, 2015).
Presence and survival in water	Only if introduced from faeces. STEC O157 and non-O157 STEC were detected in 8% and 11% (respectively) of 1,386 surface water samples from 30 sites in a leafy green growing area of the USA, with highest prevalence near cattle operations (Cooley *et al.*, 2014). Culturable for extended periods, e.g. 109 days in cattle drinking water, 14 days in farm ponds (Fremaux *et al.*, 2008).
Presence and survival in manure	**NZ**: STEC detected in faeces from 2/155 (1.3%) cows and 2/220 (0.2%) sheep, plus composite samples of calf faeces, all collected from New Zealand farms (Irshad *et al.*, 2015; Moriarty *et al.*, 2011c; Moriarty *et al.*, 2008). Able to survive (e.g. decimal reduction time 22 days) and lower temperatures prolong survival (Avery *et al.*, 2012). Survival of a month possible under field conditions (Ongeng *et al.*, 2013).

Presence and survival in compost	Should not survive adequate composting (Avery *et al.*, 2012). Poor survival in freshly prepared compost mixes stored at 30ºC for 12 weeks (Erickson *et al.*, 2015).
Excretion from asymptomatic humans	STEC was detected in the faeces of asymptomatic farm workers and farm residents, and in workers in meat processing plants (Silvestro *et al.*, 2004; Stephan *et al.*, 2000).
VBNC state	Sublethal stresses induce VBNC state, e.g. low pH, osmotic shock, shifts in temperature and low level enterotoxin production by VBNC cells in water has been reported (Dinu and Bach, 2011). VBNC STEC still detected on the surface of lettuce leaves after 16 days storage at 8ºC (Dinu and Bach, 2011). **NZ:** A non-toxigenic strain of *E. coli* O157:H7 entered a VBNC state in a saline solution and while the VBNC cells could not be resuscitated, they were able to survive exposure to simulated human saliva, gastric fluid and intestinal fluid (Brandt and Podivinsky, 2008).

A.1.11 *Yersinia* spp.

Foodborne disease	Yersiniosis
Pathogenic strains	*Y. enterocolitica* is most commonly isolated from yersiniosis cases in New Zealand but *Y. pseudotuberculosis* is also recovered. The *Y. enterocolitica* biotypes recovered recently were 1A, 2, 3 and 4 (Health Intelligence Team, 2014b).
Presence and survival in soil	Has been detected in soils grazed by sheep in Canada (Sutherland *et al.*, 2009). Not detected in 14 samples of soil from dairy farms in Australia (McAuley *et al.*, 2014).
Presence and survival in water	Only if introduced from faeces. Has been detected in surface water near grazing sheep (Sutherland *et al.*, 2009).
Presence and survival in manure	**NZ**: Detected in manure of infected goats (Lanada *et al.*, 2005). Not detected in 16 samples of faeces from dairy farms in Australia (McAuley *et al.*, 2014). Detected in faeces from sheep, pigs and domestic pets (Farzan *et al.*, 2010; Stamm *et al.*, 2013; Sutherland *et al.*, 2009).
Presence and survival in compost	Should not survive adequate composting (Avery *et al.*, 2012).
Excretion from asymptomatic humans	Biotype 1A isolated from 9/811 (1.1%) of faecal samples from asymptomatic humans in Switzerland (no other *Y. enterocolitica* types were isolated) (Stephan *et al.*, 2013).
VBNC state	VBNC state reported under polar marine environment conditions (Smith *et al.*, 1994).

A.1.12 *C. parvum*

Foodborne disease	Cryptosporidiosis
Pathogenic strains	A number of *Cryptosporidium* species have been isolated from humans but *C. parvum* is the most frequently reported zoonotic species (Erickson and Ortega, 2006; USFDA, 2012). Humans are also the primary host for *C. hominis*.

Presence and survival in soil	Only if introduced from faeces. Has been detected in the soils of beef cattle farms (Boyer and Kuczynska, 2010). Highly variable results reported for survival – weeks to years (Erickson and Ortega, 2006).
Presence and survival in water	Only if introduced from faeces. **NZ**: Oocysts of *Cryptosporidium* spp. detected in 33/725 (5%) surface water samples from 25 sites (Till *et al.*, 2008). Survival of up to 240 days has been reported, but warmer temperatures decrease survival (Erickson and Ortega, 2006).
Presence and survival in manure	**NZ**: *Cryptosporidium* spp. detected in faeces from 8/155 (5%) cows at concentrations of 1-25 oocysts/g, in 7/320 (2%) droppings from waterfowl and in faeces from 8/220 (4%) sheep (Moriarty *et al.*, 2011a; Moriarty *et al.*, 2011c; Moriarty *et al.*, 2008). **NZ**: Detected in faeces from calves and foals (Al Mawly *et al.*, 2015; Grinberg *et al.*, 2009). Survival variable: Up to 400 days in animal faeces, 178 days in human faeces.
Presence and survival in compost	Should not survive adequate composting (Avery *et al.*, 2012).
Excretion from asymptomatic humans	*Cryptosporidium* spp. have been detected in stools from asymptomatic children in the USA (Denno *et al.*, 2012). An Australian study found a prevalence of 0.4% for *Cryptosporidium* spp. (Hellard *et al.*, 2000).

A.1.13 *G. duodenalis*

Foodborne disease	Giardiasis
Pathogenic strains	There are eight major genetic groups (assemblages), but only two (A and B) are capable of causing disease in humans (Winkworth, 2010).
Presence and survival in soil	Only if introduced from faeces. Can remain infectious for over a month at low temperatures, but infectivity is not well sustained at higher temperatures (Feng and Xiao, 2011).
Presence and survival in water	Only if introduced from faeces. **NZ**: Cysts of *Giardia* spp. were detected in 59/725 (8%) surface water samples from 25 sites (Till *et al.*, 2008).
Presence and survival in manure	**NZ**: *Giardia* spp. detected in faeces from 7/155 (4%) cows (concentrations of 1-17 cysts/g), 39/105 (37%) lambs at slaughter and 11/180 (6%) calves (Abeywardena *et al.*, 2012; Moriarty *et al.*, 2011c; Moriarty *et al.*, 2008).
Presence and survival in compost	Should not survive adequate composting (Avery *et al.*, 2012).
Excretion from asymptomatic humans	A large percentage of people infected with *G. duodenalis* remain asymptomatic (Erickson and Ortega, 2006). An Australian study found a prevalence of 1.6% for *Giardia* spp. (Hellard *et al.*, 2000).

A.1.14 *C. cayetanensis*

Foodborne disease	Cyclosporiasis
Pathogenic strains	*C. cayetanensis* is the only species of *Cyclospora* identified in humans (Erickson and Ortega, 2006; USFDA, 2012). All *C. cayetanensis* are currently considered pathogenic.
Presence and survival in soil	Introduced to soil through human faeces but survival not known (Chacin-Bonilla, 2010).
Presence and survival in water	Has been detected in drinking, irrigation and surface waters in developing countries. Oocysts survive in water at 4ºC for 2 months and waterborne disease has be reported (Chacin-Bonilla, 2010).
Presence and survival in manure	Animals are not a known reservoir. Oocytes shed from humans are non-infectious and need time in the environment to mature (sporulate) into the infectious form (little is known about the environmental conditions required for maturity but the process may take 7-15 days) (Chacin-Bonilla, 2010; Ortega and Sanchez, 2010).
Presence and survival in compost	No data located.
Excretion from asymptomatic humans	Can be excreted from asymptomatic carriers, particularly those who have had recurrent infections and are no longer symptomatic (Chacin-Bonilla, 2010).

A.1.15 *T. gondii*

Foodborne disease	Toxoplasmosis. Most human infections are asymptomatic.
Pathogenic strains	Most virulence studies have involved genotypes I, II and III, but there is very little known about the virulence of each of these in humans (FSANZ, 2013).
Presence and survival in soil	Detected in 71/243 soil samples from a village and surrounding rural area with contamination decreasing with increased distance from cat home ranges (Gotteland *et al.*, 2014). Oocysts can survive in moist soil or sand for 12-18 months (Innes, 2010). **NZ**: A study of *T. gondii* seroprevalence in sheep found widespread exposure to the oocysts, indicating that they are present on farms (Dempster *et al.*, 2011).
Presence and survival in water	Can contaminate water and oocysts have remained viable in freshwater for 54 months (Torrey and Yolken, 2013). Waterborne outbreaks of toxoplasmosis have been reported (Ekman *et al.*, 2012).
Presence and survival in manure	Only excreted in the faeces of cats, the definitive host (Innes, 2010). Can survive in cat faeces outdoors for more than a month if uncovered, and more than a year if buried in soil (Jones and Dubey, 2012).
Presence and survival in compost	No information located but adequate composting should inactivate a proportion of oocysts.
Excretion from asymptomatic humans	Not excreted.

A.1.16 HAV

Foodborne disease	Hepatitis A
Pathogenic strains	One serotype and six genotypes in the genus *Hepatovirus*, within the family *Picornaviridae*. Only genotypes I (a and b subtypes), II (a and b subtypes) and III are associated with human infections. There is no information on the HAV genotypes circulating in New Zealand.
Presence and survival in soil	HAV persists for long periods (>3 months) in soil at ambient temperatures (Blanc and Nasser, 1996; Sobsey *et al.*, 1986). No published data are available on the presence and concentration in New Zealand soils.
Presence and survival in water	Extremely stable, especially at low temperatures. No published New Zealand data on presence in water. Wastewater treatment processes are often ineffective for virus removal or inactivation so these viruses can be present and persist in receiving waters (Greening, 2006).
Presence and survival in manure	HAV does not infect non-primates so is unlikely to be present in farmyard manure. In laboratory experiments, a reduction in infectivity of 1-2 logs was observed after 2 months storage in contaminated manure at low ambient temperature, although results were matrix type dependent (Wei *et al.*, 2010). In another study, HAV was rapidly inactivated at 25°C in a mixture of human effluent and animal manure (Deng and Cliver, 1995). No published New Zealand data.
Presence and survival in compost	HAV is the most heat-resistant human enteric virus studied (Gerba *et al.*, 2002) but as it can be inactivated when exposed to 60°C for 10 hours (Murphy *et al.*, 1993) it should not survive adequate composting. Virus inactivation increases with storage, increased pH (e.g. lime treatment) and low moisture conditions. No published New Zealand data.
Excretion from asymptomatic humans	Most HAV infections are asymptomatic or subclinical, and occur in young children. For adults, HAV infection is more likely to be symptomatic. Viruses are usually shed at the highest concentrations (10^{11} viral particles/g faeces) in faeces between 1-3 months after infection, but are also excreted 2-3 weeks prior to clinical symptoms. Prolonged excretion can occur for up to 6 months. Virus concentrations excreted from asymptomatic/subclinical cases are as high as for symptomatic individuals (Costafreda *et al.*, 2006; Hollinger and Emerson, 2007).

A.1.17 Norovirus

Foodborne disease	Norovirus infection
Pathogenic strains	Genetically diverse. Noroviruses that infect humans belong to three genogroups (GI, GII and GIV). There are at least nine and 19 distinct genotypes for GI and GII respectively (Kroneman *et al.*, 2013). The prevalence of GIV in the New Zealand population appears to be low. Norovirus GII, genotype 4 (GII.4) is the predominant genotype associated with gastroenteritis outbreaks in New Zealand and is found elsewhere in the world (Greening *et al.*, 2012; van Beek *et al.*, 2013). Compared to other genotypes, GII.4 are less frequently associated with foodborne outbreaks (Verhoef *et*

	al., 2015). Unlike other enteric viruses that cause gastroenteritis, humans remain susceptible to infection throughout life.
Presence and survival in soil	Limited data on presence in soil. Survival characteristics expected to be similar to other enteric viruses such as HAV, and so expected to persist for several weeks. No published data on the presence and concentration in New Zealand soils.
Presence and survival in water	Commonly found in human sewage overseas and in New Zealand, so their presence in receiving waters is common (J. Hewitt, ESR, pers. comm.). **NZ**: Has been detected in rivers, urban streams and estuarine waters (Hewitt *et al.*, 2013; Williamson *et al.*, 2011). **NZ**: Norovirus gastroenteritis outbreaks have been associated with the consumption of contaminated drinking water, including in New Zealand (Hewitt *et al.*, 2007; Jack *et al.*, 2013).
Presence and survival in manure	Although the presence of human norovirus-like strains have been reported in cattle manure (Mattison *et al.*, 2007) and samples of manure from leafy vegetable farms (Kokkinos *et al.*, 2012), the ability of these viruses to infect farmyard animals has not been demonstrated experimentally. Porcine noroviruses, closely related to human noroviruses, may be present in pig slurry. No data on survival in manure as human noroviruses are uncultivable.
Presence and survival in compost	**NZ:** Three compost samples were negative for norovirus GI and GII by qPCR (Hewitt, 2015).
Excretion from asymptomatic humans	Concentrations in human faeces vary but typically are 10^8 viral particles/g but can be up to 10^{11} particles/g (Atmar *et al.*, 2008). Viruses can persist for up to 4 weeks following resolution of clinical symptoms and so present risk for food-borne transmission. Although prolonged shedding for months and even years has been reported, there is a lack of knowledge of the extent of asymptomatic shedding in the community. Outbreaks associated with asymptomatic food handlers are described, and in 2012, multiple gastroenteritis outbreaks associated with an infected post-symptomatic food handler occurred in New Zealand (Thornley *et al.*, 2013). Asymptomatic carriage of norovirus by workers in a catering facility on Japan has been reported, but no cases of illness were linked with the facility (Okabayashi *et al.*, 2008).

A.1.18 Rotavirus

Foodborne disease	Gastroenteritis, rotavirus infection
Pathogenic strains	Rotaviruses belong to the genus *Rotavirus* in the family *Reoviridae*. Highly antigenically and genetically diverse. There are at least seven groups/species (A-G) of which A, B and C infect humans and cause gastroenteritis (Estes and Kapikian, 2007). Group A, B and C also infect other animals especially young domestic and farm animals including birds. Group A is the most prevalent group (Parashar *et al.*, 1998). In humans, while varying by country and in time, five strains predominant globally (Banyai *et al.*, 2012).
Presence and survival in soil	No published data on the presence and concentration in New Zealand soils. Temperature and viral (including rotaviruses) adsorption to soil particles affected their survival (Hurst *et al.*, 1980).

Presence and survival in water	Wastewater treatment processes are often ineffective for virus removal or inactivation so these viruses can be present and persist in receiving waters (Greening, 2006). Rotaviruses are highly stable in the environment and can remain infectious for weeks at 4°C and 20°C in environmental waters. **NZ:** Rotaviruses were present in 70/109 (64.2%) samples from two rivers (Williamson *et al.*, 2011).
Presence and survival in manure	Little data on the presence and persistence of viruses in animal waste. Animal disease most frequently occurs in piglets less than 8 weeks of age, but can occur in adults too (Yuan *et al.*, 2006). The severity of illness decreases with age. Infected calves can shed up to 10^{10} virus particles/g faeces and subsequently contaminate water, so can be a source of infection. It is likely that zoonotic transmission occurs (Greening, 2006). Enteric viruses present in animal wastes can survive for long periods.
Presence and survival in compost	No specific data for rotavirus survival in compost.
Excretion from asymptomatic humans	Shed (up to 10^{10} virus particles/g faeces) prior to the onset of symptoms and for up to a month after the onset of symptoms (Pickering *et al.*, 1988). Median rotavirus shedding (as determined by RT-PCR) is 10 days (Richardson *et al.*, 1998).

A.2 NEW ZEALAND IMPORT AND EXPORT DATA

All data presented in this section have been sourced from Statistics New Zealand Infoshare (http://www.stats.govt.nz/infoshare/Default.aspx, accessed 23 April 2014). Data presented here are only for fruits and vegetables relevant to this document.

A.2.1 Imported fruits and vegetables

The tonnes of fresh fruits imported into New Zealand for the period 2008-2014 are presented in TABLE 9, and TABLE 10 presents data for fresh vegetables. There were no kiwiberries, musk melons, prince melons, sour cherries, sloes or boysenberries imported in any of these years.

TABLE 9: Fresh fruits imported into New Zealand (2008-2014), values in tonnes

FRUIT	2008	2009	2010	2011	2012	2013	2014
Grapes	10962	9488	11042	10508	11577	12927	13927
Pineapples	7660	5301	5827	5873	6332	7126	7018
Pears	3739	3158	3710	3081	3729	3465	3699
Guavas, mangoes, and mangosteens	1951	1444	1886	2240	2195	2907	2982
Other melons	3111	2658	1892	1667	1807	2115	2616
Watermelons	2684	2730	1850	1684	2040	2283	2502
Papayas	892	542	775	819	853	916	938
Apples, whole	1631	1249	1140	984	1129	872	842
Strawberries	371	230	184	226	208	258	448
Nectarines	2062	1451	1421	1300	778	272	293
Plums	1084	841	712	670	484	285	210
Peaches	567	448	549	438	370	87	115
Cherries	103	82	95	77	103	52	86
Fresh dates	46	67	31	35	25	24	17
Cranberries, Bilberries, other *Vaccinium* spp.	1	1	3	<1	1	10	1
Apples (fresh, pulped, grated, sliced, chopped, shredded)	14	4	11	0	0	1	1
Apricots	18	<1	<1	1	0	0	0
Blueberries	5	<1	2	2	<1	1	0
Currants, and gooseberries	4	<1	0	1	0	0	0
Raspberries	0	0	0	2	0	0	0
Blackberries	3	1	0	2	0	0	0
Mulberries and loganberries	1	2	0	5	0	0	0

TABLE 10: Fresh vegetables imported into New Zealand (2008-2014), values in tonnes

VEGETABLE	2008	2009	2010	2011	2012	2013	2014
Capsicum or pimenta	683	798	750	414	325	349	593
Tomatoes	3079	3086	2196	1181	27	196	371
Asparagus	65	13	71	50	42	21	146
Carrots	52	11	0	8	1	0	76
Cucumbers and gerkins	100	77	66	28	27	76	46
Other brassica	16	7	12	15	19	11	43
Mushrooms	6	6	7	9	51	34	36
Other lettuce	13	65	35	207	24	87	26
Cauliflowers and broccoli	11	47	24	211	5	6	19
Spinach	2	45	3	1	1	31	12
Salad beetroot, salsify, celeric, radishes and similar edible roots	22	15	6	5	4	6	3
Cabbages	0	0	0	0	<1	0	<1
Brussel sprouts	0	0	<1	0	<1	0	0
Head lettuce	8	0	3	0	2	18	0
Witloof chicory	<1	<1	<1	<1	0	0	0
Chicory	<1	0	0	0	0	0	0
Celery	<1	0	0	0	19	0	0

Data on frozen fruits are available but those imported as cooked product are not separated from those uncooked. During 2014 there were just over 6,000 tonnes of frozen fruit (cooked or uncooked, without any added sweeteners) imported into New Zealand. Almost 60% of this, by weight, came from Chile (29%) and China (28%).

Imports of these frozen fruits were dominated by four categories:

- Blueberries (24% of total imports, by weight): 1,508 tonnes; main countries of origin were the USA (32%), Chile (32%) and Canada (30%).
- Fruit other than boysenberries, blueberries or kiwifruit (24%): 1,492 tonnes; 27% from China, 25% from Chile, 14% from the USA and 10% from Thailand;=.
- Strawberries (22%): 1,383 tonnes; 54% from China and 26% from Mexico.
- Raspberries (20%): 1,259 tonnes, 55% from Chile, 28% from China and 10% from Poland.

A.2.2 Exported fruits and vegetables

The tonnes of fresh fruits exported from New Zealand for the period 2008-2014 are presented in TABLE 11, and TABLE 12 presents data for fresh vegetables. There were no prince melons, sloes or boysenberries exported in any of these years. In terms of the total weight of these fruits exported, the major markets in 2014 were the USA, the UK and The Netherlands. For the same year, the major markets for these vegetables were Japan, Fiji and Australia.

TABLE 11: Fresh fruits exported from New Zealand (2008-2014), values in tonnes

FRUIT	2008	2009	2010	2011	2012	2013	2014
Apples, whole	260807	302775	259652	299380	284312	322052	307755
Pears	4732	5476	5008	4290	2922	4665	5316
Cherries	1301	1572	1591	1318	1023	1481	1696
Apricots	941	1329	1350	1094	1106	1082	1318
Blueberries	707	697	925	830	934	1110	1136
Strawberries	660	497	531	552	509	598	707
Other melons	229	261	230	228	179	203	153
Kiwiberries	63	54	58	73	32	60	140
Apples (fresh, pulped, grated, sliced, chopped, shredded)	13	2	1	3	31	17	101
Peaches	18	13	60	52	39	47	72
Watermelons	7	77	50	75	19	39	59
Grapes	66	31	77	64	36	19	54
Plums	36	71	41	52	25	38	39
Nectarines	41	47	12	34	8	12	4
Musk melons	0	<1	0	<1	0	4	2
Pineapples	8	7	6	8	5	4	2
Currants and gooseberries	12	4	1	1	1	1	1
Guavas, mangoes and mangosteens	1	2	1	2	1	<1	1
Raspberries	<1	1	1	1	1	1	1
Blackberries	<1	1	1	<1	<1	1	<1
Papaws/papayas	<1	1	2	3	1	<1	<1
Mulberries and loganberries	<1	1	1	0	<1	<1	<1
Fresh dates	<1	5	<1	<1	<1	<1	<1
Cranberries, bilberries, and other fruits of the genus Vaccinium, other than blueberries	<1	<1	0	3	<1	6	<1
Sour cherries	0	0	0	0	204	0	0

TABLE 12: Fresh vegetables exported from New Zealand (2008-2014), values in tonnes

VEGETABLE	2008	2009	2010	2011	2012	2013	2014
Carrots	10899	10773	9219	13148	11382	10911	9846
Capsicum or pimenta	5317	5671	5927	5679	7969	6870	7078
Tomatoes	1621	2932	3446	4891	3197	2686	2980
Cabbages	530	701	818	736	947	808	805
Celery	212	204	273	277	357	422	461
Cauliflowers and broccoli	149	172	207	174	220	253	176
Asparagus	429	366	337	332	315	250	164
Mushrooms	155	212	217	199	155	165	138
Other brassica	2	12	57	1	24	181	107
Other lettuce	99	125	133	166	222	237	97
Salad beetroot, salsify, celeric, radishes and similar edible roots	356	282	158	172	792	304	90
Head lettuce	47	25	35	71	73	83	82
Witloof chicory	37	64	72	64	61	67	65
Cucumbers and gerkins	38	74	60	96	45	29	28
Brussel sprouts	2	2	22	26	24	18	13
Spinach	3	8	6	11	13	13	8
Chicory	2	1	1	2	2	4	4

A.3 PATHOGENS ON THE FOODS CONSIDERED IN THIS DOCUMENT: DATA FROM OVERSEAS

A.3.1 Detection of pathogens on fruit and vegetables (including sprouts) overseas

Because of the number of pathogens and variety of foods considered in this document, this section reports summary data from recent review papers that have already summarised food surveys, and augments these with data from large surveys from developed countries or surveys for pathogens not included in the aforementioned sources.

Survey data from recent reviews are presented in TABLE 13. Only one of the reviews presented data on pathogen concentration: From 10 surveys of *L. monocytogenes* in RTE leafy salads and fresh-cut vegetables, 171/177 samples contained <100 CFU/g, six samples contained >100 CFU/g and the maximum reported concentration was 750 CFU/g (Hudson *et al.*, 2014). It is likely that additional concentration data are available in the reviewed papers.

Six other reports are worth noting:

- Australia, 2005-2007 (FSANZ, 2010): 369 samples of domestically-produced fresh horticultural foods (lettuces, sprouts, strawberries, parsley, basil) were tested for STEC, *Salmonella* spp. and *Listeria* spp. *E. coli* O157:H7 were not detected but STEC were detected in one sample of sprouts collected at the end of production and one sample of parsley collected from the growing field. *Salmonella* Warrigul was detected in one sample of strawberries collected from the field. *L. monocytogenes* was detected in four samples (all strawberries; two sampled at the farm gate from the same packing shed, two sampled

at retail from the same grower). These bacteria were not detected on the 134 lettuce or two basil samples tested.

- Australia, 2006-2010, data from testing of wholesale fresh produce as part of verifying quality assurance and food safety programmes (FSANZ, 2011): *Salmonella* spp. were detected in 4/2,003 samples (salad mix, shredded lettuce, papaya and coriander), coagulase-positive *Staphylococcus* spp. were detected in 7/1,396 samples (including one sample of chives that had a concentration of $1.2x10^6$ CFU/g) and *L. monocytogenes* in 3/2,480 samples.

- The Netherlands, 2006-2007 (Wijnands *et al.*, 2014): Samples of raw produce entering two RTE vegetable producers, and samples of RTE products taken directly from these producers and from retail outlets were tested for *Salmonella* spp., *Campylobacter* spp., *E. coli* O157, and *L. monocytogenes*. Of the samples of raw produce, *Salmonella* spp. was detected in 6/1,860 (0.3%; endive, cucumber, lettuce), *Campylobacter* spp. was detected in 3/1,810 (0.2%; endive, lettuce), *E. coli* O157 was detected in 1/1,833 (0.1%; endive) and *L. monocytogenes* was detected in 1/1,860 (0.5%; curly endive). Of the samples of RTE salads from the producers and from retail, *Salmonella* spp. were detected in 1/1,902 (0.1%), but *Campylobacter* spp., *E. coli* O157 and *L. monocytogenes* were not detected (1,915, 1,911 and 1,932 samples tested for each pathogen, respectively). The concentrations in the positive samples were mainly between 0.02 and 0.06 MPN/g, except for *Salmonella* Typhimurium DT104 on iceberg lettuce (0.28 MPN/g) and on endive (>0.28 MPN/g).

- Japan, 1998-2008, data from the national food surveillance system (Hara-Kudo *et al.*, 2013): *Salmonella* spp. were detected in 2/1,315 cucumber, 2/1,758 lettuce, 7/4,848 sprout and 1/1,140 tomato samples, but were not detected in cabbage, Japanese white radish, long green onion or spinach samples (1,851 samples in total) nor in 1,464 "other fruits and vegetables". STEC were not detected in any of these samples (total 12,376 samples).

- USA, 2006-2007 (Ilic *et al.*, 2008): 1,356 samples of spinach entering and leaving two packing plants were tested for *Salmonella* spp., *Listeria* spp., *E. coli* O157:H7 and *Shigella* spp. *Salmonella* spp. were detected in 5/1,311 samples, all savoy (curly) spinach and all from different farms. One positive sample was unprocessed but the other four were processed (RTE). *L. monocytogenes* was detected in 3/409 samples (one unprocessed, two processed) at concentrations of <100 CFU/g, but all positive samples were from the same farm. *E. coli* O157:H7 and *Shigella* spp. were not detected.

- Germany, 2004 (Schwaiger *et al.*, 2011): 1,001 samples of raw produce sold loose from farms or supermarkets were tested for *Listeria* spp. and *Salmonella* spp. *L. monocytogenes* was detected on four samples but the report does not specify what the produce items were. The overall prevalence for *Listeria* spp. was 11/1,001 (1%), and this species was detected in all produce groups: Fruit vegetables (e.g., tomato, pepper, zucchini, cucumber), root vegetables (carrots celery, radish), salads (lettuce and spinach), bulbous vegetables (leek, garlic) and cereals. *S. enterica* (not serotyped) was detected on one garlic sample.

Overall, data for *L. monocytogenes*, *Salmonella* spp. and norovirus are readily available. These data show that *L. monocytogenes* and *Salmonella* spp. can both be detected on fresh fruits and vegetables, but *Salmonella* spp. are less likely to be found on fruits compared with vegetables (except for melons due to their ground contact), and are generally less prevalent when compared with *L. monocytogenes*. The data also indicate that norovirus is widespread on fruits and vegetables, but the viruses detected on the positive samples may not be infectious.

The survey data in TABLE 13 and above also indicate the potential for STEC, *Campylobacter* spp. and rotavirus to be present on fresh fruits and vegetables, although surveys have not often tested for the latter two pathogens. No recent surveys of Hepatitis A prevalence were located.

Shigella spp. and *Yersinia* spp. were not detected in the studies presented in TABLE 13, and recent survey data on these, and the other bacterial pathogens, are scarce for developed countries:[42]

- From 26 RTE vegetable salads purchased from retail in Greece, *Aeromonas* spp. was isolated from 16 (62%) (*A. hydrophila* was among the isolates), and two isolates of *Y. enterocolitica* were also recovered but the authors do not state the prevalence for this species (Xanthopoulos *et al.*, 2010).
- *A. hydrophila* were isolated from 11/151 (7%) RTE vegetable salads purchased from retail in Portugal, and in eight samples the concentration was $>10^5$ CFU/g (Santos *et al.*, 2012). The authors noted difficulties in distinguishing presumptive *Aeromonas* spp. from background microflora and that the prevalence could have been higher. *B. cereus* was also isolated from 15/66 samples (23%) at counts ranging <2.0-3.2 $\log_{10}$ CFU/g. *C. perfringens* were not detected.
- Presumptive *B. cereus* was detected in 44/48 (92%) samples of lettuce taken through food monitoring programmes (emetic strains were not detected) and in 15/27 (56%) samples of RTE vegetables and fruit (three were positive for emetic *B. cereus*) (Messelhäusser *et al.*, 2014). Presumptive *B. cereus* was also detected in 27/263 samples of fruits, vegetables and RTE products associated with foodborne illness in Germany, and emetic *B. cereus* was detected in nine of these positive samples.
- *C. botulinum* spores were detected in 1/128 samples of raw carrots and 1/188 samples of raw beans in France, and both were Type B (Sevenier *et al.*, 2012).
- *Shigella* spp. were not detected in 78 samples of fresh vegetables (lettuce, spinach, rocket, dill, parsley, green onion, green garlic, carrot and red cabbage) sampled from supermarkets and neighbourhood bazaars in Turkey (Cetinkaya *et al.*, 2008).
- *S. aureus* was detected in 11/137 lettuces purchased from restaurants in Spain, but was not detected in four melon and five coconut samples (detection limit 100 CFU/g) (Sospedra *et al.*, 2013).

Data on the prevalence of protozoan parasites in fruit and vegetables are also scarce and most surveys have been carried out in developing countries. Two recent surveys have been done in Canada:

- *Cyclospora* spp. were detected in 9/544 (1.7%) packaged leafy green vegetables by PCR, and *Cyclospora*-like oocyctes were identified by microscopy in five of these PCR-positive samples (Dixon et al., 2013). In the same set of samples, *Cryptosporidium* spp. were detected in 32/544 (5.9%) by PCR and confirmed in 23 by microscopy, and *Giardia* spp. were detected in 10/544 (1.8%) by PCR and confirmed in two by microscopy. The researchers were unable to estimate the viability of any of these parasites in this study. All of the PCR-positive leafy green samples were grown in Canada or the USA. There was no significant difference in the prevalence of protozoan pathogens between organic products (3/50) and non-organic products (48/494).

[42] Based on the United Nations assessment of which countries are considered developed, http://unstats.un.org/unsd/methods/m49/m49regin.htm (accessed 19 May 2015). This does not necessarily mean food safety systems are well developed in these countries.

- *Cryptosporidium* spp. were detected in one sample of organic spinach from a total of 157 samples from markets that included lettuces, green onions, spinach and strawberries (Bohaychuk *et al.*, 2009).

Giardia spp. and *Cryptosporidium* spp. have also been detected in salad vegetables collected from fields in Spain, and *Cryptosporidium* spp. were detected on vegetable samples from farmers' markets in Poland (Amoros *et al.*, 2010; Rzezutka *et al.*, 2010). *T. gondii* cysts were identified by PCR in 4/95 samples of fruits and vegetables from shops in markets in Poland (the positive samples were radishes (2), strawberries (1) and carrots (1)) (Lass *et al.*, 2009).

TABLE 13: Surveys of pathogens on fruit and vegetables overseas: Data summarised in recent review papers[1]

PATHOGEN	FOOD(S)	NUMBER OF SURVEYS EVALUATED	SURVEY COUNTRIES	PREVALENCE[2]	REFERENCE
Bacterial pathogens					
Campylobacter spp.	Vegetables	16	Malaysia, India, USA, Canada, various EU countries	Range ND-50%[3] ND in 7/16 surveys	(Verhoeff-Bakkenes *et al.*, 2011)
L. monocytogenes	RTE leafy salads, fresh-cut vegetables	34	Various European, UK and South American countries, Singapore, Hong Kong, Israel, Malaysia, South Africa, USA	Range ND-30% ND in at least one food in 16/34 surveys	(Hudson *et al.*, 2014)
	RTE fruit salads, fresh-cut fruit	6	Germany, Ireland, Malaysia, Spain, Switzerland	Range ND-19.5% ND in 4/6 surveys	(Hudson *et al.*, 2014)
	Sprouts	4	Australia, Norway, USA	Range ND-0.5% ND in 3/4 surveys	(Hudson *et al.*, 2014)
	Sprouts	10	Various EU countries	Range ND-7% ND in 6/10 surveys	(EFSA, 2011a)
Salmonella spp.	Bulb and stem vegetables, and carrots	13	Canada, Spain, USA, Mexico, UK	Range ND-8% ND in 11/13 surveys	(EFSA, 2014d)
	Berries	5	South Korea, Norway, Canada, USA	Range ND-0.7% ND in 4/5 surveys	(EFSA, 2014f)
	Tomatoes	11	Mexico, USA, UK, Canada, Japan	Range ND-7% ND in 6/11 surveys	(EFSA, 2014a)
	Sprouts	23	Various EU countries	Range ND-5.2% ND in 16/23 surveys	(EFSA, 2011a)
	Whole leafy green vegetables	24	Various EU countries, USA, Canada, Mexico, Singapore	Range ND-7% ND in 16/24 surveys	(EFSA, 2014c)
	Fresh-cut leafy green vegetables	33	Various EU countries, USA, Brazil, Singapore	Range ND-18%	(EFSA, 2014c)

				ND in 20/33 surveys	
	Melons and watermelons (whole)	19	Mexico, USA, Canada	Range ND-55% ND in 6/19 surveys	(EFSA, 2014e)
Shigella spp.	Scallions, green onions	1	Canada	ND	(EFSA, 2014d)
STEC	Sprouts	3	Various EU countries	ND	(EFSA, 2011a)
Yersinia spp.	RTE carrots	2	Spain, Finland	ND	(EFSA, 2014d)
Viral pathogens					
Norovirus[4]	Bulb and stem vegetables, and carrots	6	Egypt, USA, Malaysia, Turkey	Range 1.1-34%	(EFSA, 2014d)
	Berries	8	Various EU countries	Range ND-64% ND in 3/8 surveys	(EFSA, 2014f)
	Tomatoes	2	Belgium, Turkey	23%, 1%	(EFSA, 2014a)
	Leafy green vegetables	4	Canada, various EU countries	Range 0.8-21%	(EFSA, 2014c)
	Packaged leafy green vegetables, salad vegetables	2	Canada, Slovak Republic	54%, 8%	(EFSA, 2011d)
	Fruits and fruit salads, tomatoes	1	Belgium	46%	(EFSA, 2011d)
Rotavirus	Packaged leafy green vegetables	1	Canada	0.4%	(EFSA, 2011d)

[1] Surveys presented in the published review where ≤10 individual samples or lots of a particular food were tested, or surveys that were considered not applicable (e.g. green salad containing meat) were not included in this table.
[2] ND, not detected.
[3] High prevalence was either reported from developing countries or one 1998 UK report. The remainder were ND or <2%.
[4] Positive results usually indicate potential contamination with norovirus since most studies do not determine whether the viruses are viable.

A.3.2 Detection of pathogens in fresh juices overseas

Prevalence data for pathogens in fresh juices are scarce and most are from surveys in developing countries (e.g. (Gomez-Aldapa *et al.*, 2014; Islam *et al.*, 2010; Kim *et al.*, 2014)). *Salmonella* spp. and *E. coli* O157:H7 were not detected in 60 samples of bottled fresh squeezed orange juice sampled in Turkey (Bagci and Temiz, 2011). *Salmonella* spp. and *S. aureus* were detected in fresh orange juice prepared and served unrefrigerated in Spanish restaurants (Sospedra *et al.*, 2012).

A.3.3 Recalls overseas

Recalls are not necessarily linked to human infections but provide an indication of the frequency a hazard has been identified on a food.

For the period January 2008 to May 2015 there were eight recalls of fresh produce in Australia, of which five were for sprouts.[43] One of these recalls was for potential contamination with *Salmonella* spp. (in 2014) and the other four were for potential contamination with generic *E. coli*. Greek salad was also recalled for generic *E. coli* contamination in 2012. The final two recalls were both for frozen berries and the recalls were issued in February 2015. These recalls were associated with an outbreak of Hepatitis A linked to these products (see Section B.1.1).

European Member States are required to notify food recalls to the Rapid Alert System for Food and Feed (RASFF). EFSA has summarised RASFF data for foods of non-animal origin, for the period 2001-2011 (EFSA, 2013). Of the 904 RASFF records (including processed and dried products):

- 49 (5%) were for "leafy greens eaten raw as salads", contaminated with *Salmonella* spp. (33), *Campylobacter* spp. (6), *E. coli* (4), norovirus (2), *Listeria* spp. (1) and three recalls were associated with foodborne outbreaks;
- 24 (3%) were for tropical fruit and melons, contaminated with *Salmonella* spp. (16), HAV (2), *Bacillus* spp. (2), *E. coli* (1), *Staphylococcus* spp. (1), parasitic infection (1) and one recall was associated with a foodborne outbreak;
- 23 were for berries, contaminated with norovirus (16), calicivirus (2) and 5 were associated with foodborne outbreaks;
- 7 were for sprouted seeds (4 *Salmonella* spp., 3 *E. coli*);
- 5 were for tomatoes (1 HAV, 1 *Salmonella* spp., 3 foodborne outbreaks); and
- 4 were for tropical fruit (*Bacillus* spp., HAV, parasitic infection, *Staphylococcus* spp.).

There were also 53 records for mushrooms but it was not specified whether these were fresh or preserved (e.g. dried, under oil). Most of the mushroom records were for contamination with *Bacillus* spp., *Salmonella* spp. or *Clostridium* spp., and the presence of the latter suggests at least some of the products were preserved under oil or similar.

A search for the period January 2012-May 2015 located 35 RASFF "alert" notifications.[44] Of these, 20 were for norovirus or HAV in berries (these include multiple notifications by different countries for the products originating from the same country). Other notifications were for

[43] Recall data were kindly provided by FSANZ. FSANZ recalls can be viewed at http://www.foodstandards.gov.au/industry/foodrecalls/recalls/Pages/default.aspx (accessed 2 June 2014)

[44] https://webgate.ec.europa.eu/rasff-window/portal/?event=SearchForm&cleanSearch=1 (accessed 3 June 2015. Search: Type = food, Classification = alert, Hazard = pathogenic micro-organisms,

- *L. monocytogenes* in enoki mushrooms, vegetable mixes and fresh-cut vegetables;
- *Campylobacter* spp. in spring onions and mixed baby leaves;
- *Salmonella* spp. in baby spinach and rucola, sprouts, watermelons and frozen diced tomatoes; and
- *B. cereus* in sprouts.

One notification was also for a foodborne outbreak associated with cherry tomatoes but the pathogenic microorganism was not specified. These notifications were for products released onto the market. Additional notifications were also issued for border rejections or for "information for attention" and these included notifications for leafy vegetables (*Salmonella* spp., *L. monocytogenes*, *B. cereus*, HAV), sprouts (*B. cereus*, STEC, *L. monocytogenes*), fresh mushrooms (*Campylobacter* spp.), cherry tomatoes (STEC), celery (*Salmonella* spp.), lemon grass (*Salmonella* spp.), chillies (*Campylobacter* spp.) and berries contaminated with viruses.

The only notification located for juices was for acai berry juice contaminated with *B. cereus* (>15,000 CFU/g in one sample and diarrhoeal enterotoxin detected), but the notification did not specify if this was a pasteurised product.

notified between 01/01/2012 and 31/05/2015, Product Category = fruits and vegetables. Dried or preserved (e.g. in oil) products were not considered.

APPENDIX B: ADVERSE HEALTH EFFECTS – OVERSEAS INFORMATION

B.1 OUTBREAKS WHERE FRUIT, VEGETABLES, SPROUTS OR FRESH JUICES WERE IMPLICATED

Because the ranges of pathogens and foods considered in this document are so large we have not attempted to summarise all reported outbreaks since the 2008 Document. Instead, this section presents information from some summary reports and examples of outbreaks that were located from published works during the course of preparing this document.

A report published by the European Food Safety Authority in 2013 provides the most comprehensive summary of outbreak reports relevant to this document by summarising outbreaks from the EU zoonoses database and those reported in the scientific literature (EFSA, 2013).

The following foods relevant to this risk profile were the implicated vehicles in outbreaks reported to the EU zoonoses database for the period 2007-2011, where the evidence for this food being the vehicle of infection was considered "strong":[45]

- *B. cereus* intoxication: Lettuces (discussed further below).
- *C. perfringens* intoxication: Fresh mixed herbs used in buffet dishes.
- Salmonellosis: Fresh strawberry juice, watermelon, tomatoes, cut lettuce, baby spinach, cut rocket, lettuce leaves, onion, sprouts.
- Shigellosis: Baby corn, sugar peas, basil.
- *S. aureus* intoxication: Frozen beans, sprouts.
- Norovirus infection: Strawberries, raspberries, other berries, tomatoes, lettuces, onion.

In 2012 there were 39 outbreaks with strong evidence that were linked to vegetables (outbreaks linked to fruit are not reported separately) (EFSA and ECDC, 2014). The causative agent in 26% of these was viruses, 23% were caused by *Salmonella* spp., and illness from *Cryptosporidium* spp., *Bacillus* spp. and *Clostridium* spp. each caused 5% of these 39 outbreaks. In 2013 there were 37 outbreaks with strong evidence that were linked to vegetables (EFSA and ECDC, 2015).

During the period 2008-2012 there were some outbreaks in the EU that were notable due to the number of cases involved. The largest was an outbreak of STEC infection in 2011 caused by *E. coli* O104, a previously enteroaggregative serotype of *E. coli* that had acquired the ability to produce Shiga toxin (i.e. had become an STEC) yet did not have any of the other virulence markers typically associated with EHEC (Beutin and Martin, 2012; Frank et al., 2011). There were 4,321 cases associated with this outbreak, including 852 cases of HUS, and 50 people died (Buchholz et al., 2011). The majority of cases resided or had travelled in northern Germany, or had attended an event in France. Extensive investigations found the cause to be contaminated sprout seeds produced by a single supplier and mainly distributed in Germany (Buchholz et al., 2011). Some of the seeds were also distributed in France and fenugreek seeds were identified as the cause of the French outbreak (King et al., 2012). The

[45] The strength of the evidence related to an outbreak to be reported to EU level is based on an assessment of all available categories of evidence (i.e. descriptive, epidemiological or microbiological evidence) (EFSA 2013).

outbreak strain was not isolated from any seeds, but substantial evidence showed that a specific lot of fenugreek seeds imported from Egypt was the most likely vehicle of infection (EFSA, 2011c). Further information on sprout-associated outbreaks is included later in this section.

There have also been viral outbreaks in the EU that were notable due to the number of cases involved:

- 2010: **Lettuces** grown in France were linked to a cluster of 11 outbreaks of gastroenteritis in Denmark involving a total of 264 cases (n=264) (Ethelberg *et al.*, 2010). Most cases were infected by norovirus, and norovirus was also detected on lettuces. Enterotoxigenic *E. coli* O6:K15:H16 (not STEC), *S. aureus*, *C. perfringens* and *B. cereus* were also recovered from some of the outbreak cases, but not the lettuces. It appears that this is the outbreak associated with the *B. cereus* intoxication/lettuces combination reported on the previous page.

- 2012: An outbreak caused by imported frozen **strawberries** contaminated with norovirus involved 10,950 cases in Germany, of which 38 were hospitalised (EFSA and ECDC, 2014).

- 2013: Three multinational outbreaks of Hepatitis A were reported and **berries** were implicated in all of these (EFSA, 2014b; Gossner and Severi, 2014). The first outbreak, first reported in March in Denmark, involved 106 cases and was traced back by epidemiological studies to strawberries produced in Egypt and Morocco. Strawberries were also suspected among other fresh fruits consumed in the Red sea area in the second outbreak, which was first reported in Norway in April and involved 107 cases. There were 331 confirmed cases in the third outbreak, first reported in May in Germany. Hepatitis A, including the outbreak strain, was detected in frozen berries and berry products, particularly redcurrants from Poland and blackberries from Bulgaria, but a single point of contamination was not found.

And in 2012 there were two outbreaks caused by bagged **salads** contaminated with *Cryptosporidium* spp., affecting 264 people in Finland and 305 people in the UK (EFSA and ECDC, 2014).

Outbreaks associated with fresh vegetables and fruits in the USA have been summarised for the period 2004-2012 and compared to EU data for the same period (Callejón *et al.*, 2015). In the USA, 59% of the fresh produce outbreaks were of norovirus infection, and the majority of these were attributed to salad or leafy vegetables. Outbreaks of norovirus infection also dominated EU data (55% all fresh produce outbreaks), but berries were implicated more often than salad and leafy vegetables. Outbreaks of salmonellosis were the next most frequently reported aetiology, but these outbreaks were attributed to a number of different food vehicles. Leafy vegetables, salads and sprouts were commonly implicated in outbreaks of *E. coli* infection in both regions, but more so in the USA. There were eight outbreaks of cyclosporiasis reported in the USA but only three outbreaks of listeriosis (sprouts, melon). No outbreaks of clostridia intoxication or yersiniosis were reported in the USA during this period, and outbreaks of cyclosporiasis or giardiasis were not reported in the EU. Even when norovirus outbreaks are excluded, salad and leafy green vegetables were together implicated as the vehicles of infection more often than other food groups. A separate analysis of USA outbreaks attributed to fresh leafy vegetables for a wider period (1973-2012) also found that norovirus, STEC and *Salmonella* spp. were the most common causative pathogens associated with these vegetables (Herman *et al.*, 2015). A 2015 salmonellosis outbreak in the USA, caused by imported cucumbers contaminated with *Salmonella* Poona, involved 907 cases and lead to 204 hospitalisations and six deaths.[46]

[46] http://www.cdc.gov/salmonella/poona-09-15/index.html (accessed 13 April 2016).

Outbreaks associated with produce in Canada have been summarised for the period 2001-2009 (Kozak *et al.*, 2013). Of the 27 outbreaks analysed, salmonellosis accounted for nine and the implicated foods were sprouts (3 outbreaks), cantaloupe (2 outbreaks), cut fruit (2 outbreaks), cucumber and tomatoes. There were seven outbreaks of cyclosporiasis; basil was implicated in five of these and cilantro in the other two. Lettuce and spinach were implicated most in outbreaks of STEC infection, and contaminated baby carrots were the cause of one of the shigellosis outbreaks. The two Hepatitis A outbreaks were caused by infected food handlers.

There have also been some review papers focussing on specific foods:

- Raw **sprouts** have been implicated in numerous outbreaks and contaminated seeds are usually the cause. For the period 2008-2010 there were nine outbreaks of salmonellosis and one of listeriosis in the USA where sprouts were the implicated vehicle of infection (Dechet *et al.*, 2014). The largest of these involved 256 cases infected by *Salmonella* Saintpaul (8 hospitalised), and was caused by contaminated seeds sourced from Italy. In five of the salmonellosis outbreaks it was found that producers were not complying with FDA guidelines (soaking seeds in 20,000 ppm calcium hypochlorite solution and testing spent sprout irrigation water for pathogens). When 30 sprout-associated outbreaks reported on internet-based databases for the period 2000-2011 were summarised, all of the outbreaks were salmonellosis or STEC infection and most were caused by alfalfa sprouts (Yang *et al.*, 2013).

- Contamination by *Salmonella* spp. was the cause of all of the multistate outbreaks in the USA reported between 1973 and 2010 where raw **tomatoes** were the implicated vehicle of infection (Bennett *et al.*, 2015). On-farm failures were the use of surface water for irrigation and applying chemicals to tomato plants (seven outbreaks), presence of animals or their faeces in tomato fields or in adjacent wild animal habitats or pastures (four outbreaks), and location of tomato fields in low-lying, flood-prone areas (two outbreaks). Packing-house failures included inadequate tomato wash systems (seven outbreaks) and wild animals or their faeces inside the packing-house (three outbreaks).

- A review of outbreaks in the USA for the period 1973-2011 in which a single type of **melon** was implicated identified 34 outbreaks involving 3,602 cases, 322 hospitalisations and 49 deaths (including three miscarriages) (Walsh *et al.*, 2014). Cantaloupes were implicated in 56% of the outbreaks, watermelons in 38% and honeydew melons in 6%. Salmonellosis and norovirus infection were reported for 56% and 15% of the outbreaks, respectively.

- A review of outbreaks associated with **berries** during the period 1983-2013 identified 27 outbreak events and found that norovirus and hepatitis A virus were the causative agents in all but one event (Tavoschi *et al.*, 2015). When 13 norovirus outbreaks linked to imported frozen **raspberries** in Finland during 2009 were investigated, it was found that two contaminated batches of frozen raspberries were the likely source in six of these outbreaks, demonstrating how frozen raw berries can repeatedly cause outbreaks (Sarvikivi *et al.*, 2012). A recent outbreak of Hepatitis A in Australia caused by contaminated imported frozen berries involved 28 cases (last report as at 25 March 2015) (Anonymous, 2015).

In 2014 a case of listeriosis was linked to consumption of stonefruit in the USA, which was the first time listeriosis has been linked with this food (Jackson *et al.*, 2015). A second case of listeriosis was identified that was likely to be caused by consumption of fruit from the same supplier, but the evidence was not sufficient to recognise this event as an outbreak.

Further examples of outbreaks have been listed in TABLE 14.

Reports of foodborne outbreaks caused by *B. cereus* and *C. perfringens* did not provide enough detail to determine whether some of the food vehicles implicated (e.g. “salad”, “other”) were made from fresh fruits or vegetables (Bennett *et al.*, 2013; Grass *et al.*, 2013).

TABLE 14: Examples of outbreaks linked to fresh fruits or vegetables or fresh juices that were reported in the scientific literature from 2008

CAUSATIVE PATHOGEN	FOOD(S)	YEAR	COUNTRIES	NUMBER OF CASES[1]	EVIDENCE	FAILURES	REFERENCE
Bacteria							
C. jejuni	Raw peas	2008	Alaska	98	Strain isolated from peas	Contaminated with faeces from wild birds in growing area, inadequate sanitising	(Gardner *et al.*, 2011)
C. botulinum	Fresh carrot juice	2006	Canada, USA	6	*C. botulinum* toxin detected in unopened bottles after incubation at 35ºC for 6 days, and in remnants of juice from patients.	Inappropriate refrigeration allowing outgrowth and toxin production	(Sheth *et al.*, 2008)
L. monocytogenes 1/2a, 1/2b	Cantaloupe	2011	USA	147 (33 deaths, 1 miscarriage)	Case-case study. Strains isolated from fruit from patients' homes and supplier's packing shed	Inappropriate processing equipment, no chlorination of cantaloupe wash water	(Anonymous, 2012; CDC, 2012; Cosgrove *et al.*, 2011)
L. monocytogenes	Diced celery	2010	USA	10 (3 non-perinatal deaths)	Strain detected in bagged diced celery	Not identified	(Gaul *et al.*, 2013)
Salmonella Reading, *Salmonella* Newport	Salads	2008	Finland	106 (2 deaths)	Only shared food between outbreak clusters	Not identified	(Lienemann *et al.*, 2011)
Salmonella Saintpaul	Jalapeño and serrano peppers	2008	North America	1442	Case control studies, traceback investigation, strains isolated from peppers	Possibly irrigation with contaminated water	(Barton Behravesh *et al.*, 2011; Jungk *et al.*, 2008)
Salmonella Senftenberg	Fresh basil	2007	Europe, North America	32	Strain isolated in food	Not identified	(Elviss *et al.*, 2009; Pezzoli *et al.*, 2008)
Salmonella Litchfield	Papaya	2006/07	Australia	26	Strain isolated from whole and cut papaya from stores.	Untreated river water used to wash the fruit prior to packaging	(Gibbs *et al.*, 2009)

Salmonella Newport	Tomatoes	2002, 2005	USA	510, 72	Case control study, strain isolated from irrigation water	Use of contaminated irrigation water	(Greene *et al.*, 2008)
Salmonella Saintpaul	Cantaloupe	2006	Australia	115	Case control study, strain isolated from cantaloupe	Multiple failures including use of untreated water on RTE fruit, not using disinfectants properly.	(Munnoch *et al.*, 2009)
Salmonella Typhimurium, *Salmonella* Saintpaul	Fresh orange juice	2005	USA	152 Typhimurium 5 Saintpaul	Case control study, *S.* Saintpaul isolated from juice	Multiple hygiene failures at juicing premises.	(Jain *et al.*, 2009)
S. sonnei	Salad	2008	Austria	36 C 18 P	Cohort study	Contaminated by food preparer (salad preparer was carrier)	(Kuo *et al.*, 2009)
S. sonnei	Fresh basil	2011	Norway	46	Cohort study, traceback investigation	Not reported	(Guzman-Herrador *et al.*, 2011)
S. sonnei	Baby corn	2007	Australia, Denmark	227	Cohort study	Poor hygiene at packing house (Thailand)	(Lewis *et al.*, 2009)
S. sonnei	Raw carrot	2004	Multiple: Airline passengers departing Hawaii	47 C 116 P	Food histories, menu review	Faecal contamination of batch of carrots, hygiene failures and carrots stored at >10ºC at caterer premises	(Gaynor *et al.*, 2009)
S. aureus	Tomato	2011	Spain	42	Cohort study, *S. aureus* enterotoxins and cells detected in food	Contamination by asymptomatic food handler	(Solano *et al.*, 2013)
STEC: *E. coli* O145	Romaine lettuce	2010	USA	26 C, 7 P (3 HUS)	Strain isolated from unopened bag of lettuce, case control studies	Contamination of irrigation water with septic tank waste	(CDC, 2010; Crawford *et al.*, 2010)
STEC: *E. coli* O157:H7	Spinach	2006	USA	49 (9 HUS)	Strain isolated from unopened bags of spinach, case control study	Strain isolated from river water, cattle manure and wild pig faeces in and around the spinach growing site	(Wendel *et al.*, 2009)

Yersinia pseudo-tuberculosis O:1	Grated carrots	2006	Finland	>100	Cohort study, strain isolated from carrot distributor's storage facility	Not identified	(Rimhanen-Finne *et al.*, 2009)
Y. enterocolitica O:9	Bagged salad mix	2011	Norway	21	Case control study, *Y. enterocolitica* isolated from salads, but not serotype O:9	Not reported	(MacDonald *et al.*, 2011)
Protozoa							
C. cayetanensis	Basil	2005	Canada	200	Cyclospora isolated from food	Not reported	(Kozak *et al.*, 2013)
C. cayetanensis	Basil or garlic	2006	Canada	28	Epidemiological evidence	Not reported	(Kozak *et al.*, 2013)
C. cayetanensis	Sugar snap peas	2009	Sweden	12 C 6 P	Common food	Not identified (imported product, likely contaminated in source country)	(Insulander *et al.*, 2010)
Cryptosporidium hominis	Salad bar ingredients (carrots, peppers)	2005	Denmark	13 C 86 P	Case control study	Possibly contaminated water for holding carrots	(Ethelberg *et al.*, 2009)
T. gondii	Green vegetables (possibly escarole)	2009	Brazil	11	Case control study	Not reported	(Ekman *et al.*, 2012)
Viruses							
HAV	Pomegranate seeds used in raw frozen fruit blend	2012	Canada	6 C	Common exposure, HAV detected by PCR in food (genotype not confirmed)	Not identified (imported product, likely contaminated in source country)	(Swinkels *et al.*, 2014)
Rotavirus	Raw sliced cabbage	2012	Japan	6	Strain detected in asymptomatic food handler	Possibly contamination by food handler	(Mizukoshi *et al.*, 2014)

[1] C, confirmed cases; P, probable cases.

B.2 CASE CONTROL STUDIES INVESTIGATING FRUIT AND VEGETABLES AS A RISK FACTOR

TABLE 15 summarises examples of case control studies conducted in developed countries that considered fresh fruit or vegetable consumption as one of the risk factors for infection. From the studies where a statistically significant odds ratio was reported (marked in bold in TABLE 15), all but two studies found consumption of fruits and vegetables to be protective. The Ireland study found that consumption of lettuce was a risk factor for sporadic campylobacteriosis, second only to chicken when ranked by the population-attributable fraction. The authors suggested that, while contamination pre-harvest was a possibility, cross-contamination from meat during food preparation was also a potential contributing factor. The Finnish study identified imported fruits/berries as a risk factor for infection by *Y. enterocolitica* serotype 1A, a result which differed markedly from the other serotype/fresh produce combinations investigated in this study (all protective or not significant). The authors did not offer any possible reasons for this finding and a multivariate analysis was not carried out to identify whether this risk factor was independent from other risk factors investigated such as travelling abroad.

A meta-analysis of 38 case control studies considering campylobacteriosis found consumption of fruit and vegetables to be protective (Domingues *et al.*, 2012b). A meta-analysis of 34 case control studies considering salmonellosis found consumption of vegetables to be protective, but consumption of fruits was not a significant risk or protective factor (Domingues *et al.*, 2012a).

TABLE 15: Case control studies published since 2008 considering raw produce as a risk factor

TIME PERIOD	COUNTRY	INFECTION	RISK FACTOR	NUMBER OF PARTICIPANTS		REPORTING OF RISK FACTOR		ODDS RATIO (95% CONFIDENCE INTERVAL) BY:		REFERENCE
				CASES	CONTROLS	CASES	CONTROLS	UNIVARIATE ANALYSIS	MULTIVARIATE ANALYSIS	
2003-2004	Republic of Ireland, Northern Ireland	Campylobac-teriosis	Vegetables and fruit	197	296	178	281	0.5 (0.2-1.1)	NS	(Danis *et al.*, 2009)
			Lettuce	197	296	124	181	**1.6 (1.0-2.6)**	**2.6 (1.3-5.2)**	
			Prepared salad (other than lettuce, e.g. coleslaw)	197	296	71	147	**0.6 (0.4-0.9)**	**0.4 (0.2-0.8)**	
2001-2004	Australia	Listeriosis	Rockmelon/cantaloupe	112NP[5]	85NP[5]	50	32	2.1 (1.0-4.7)	NS	(Dalton *et al.*, 2011)
			RTE fruit salad	112NP	85NP	12	11	1.6 (0.5-4.5)		
			RTE other salad	112NP	85NP	3	16	0.2 0.0-0.9)		
2001-2004	Australia	Listeriosis	Rockmelon/cantaloupe	19P[5]	12P[5]	8	3	4.0 (0.2-236)	NS	(Dalton *et al.*, 2011)
			RTE fruit salad	19P	12P	3	1	2.0 (0.0-78)		
			RTE other salad	19P	12P	2	5	0.3 (0.0-4.3)		
2003-2007	Australia	STEC O157 infection	Consumption of raw vegetables	41	303	78	78	1.0 (0.5-2.6)	NS	(McPherson *et al.*, 2009)
			Consumption of homegrown fruit, vegetables, or herbs	41	299	22	39	0.4 (0.2-1.0)	**0.4 (0.2-0.95)**	
2003-2007	Australia	Non-O157 STEC infection	Consumption of raw vegetables	69	303	51	78	**0.3 (0.2-0.5)**	**0.3 (0.2-0.6)**	(McPherson *et al.*, 2009)
			Consumption of homegrown fruit, vegetables, or herbs	66	299	18	39	**0.3 (0.2-0.7)**	**0.4 (0.2-0.8)**	
2008-2012	The Netherlands	STEC O157 infection	Consumption of lettuce and other vegetables eaten raw	<10 y:[1] 39	1563[1]	72	65	1.4 (0.6-3.1)	NS	(Friesema *et al.*, 2014)
				≥10 y: 91		75	82	0.7 (0.4-1.1)	**0.5 (0.3-0.9)**	
2008-2012	The Netherlands	Non-O157 STEC infection	Consumption of lettuce and other vegetables eaten raw	<10 y:[1] 15	1563[1]	60	65	0.8 (0.3-2.5)	NS	(Friesema *et al.*, 2014)
				≥10 y: 63		73	82	0.6 (0.3-1.0)	NS	
2004	Sweden	*Y. enterocolitica* infection	Fresh juice	117[3]	339[3]	11	80	**0.4 (0.3-0.4)[4]**	**0.5 (0.2-0.9)**	(Boqvist *et al.*, 2009)

2006	Finland	Infection with *Y. enterocolitica* 3-4/O:3 or 2/O:9	Fruits/berries, domestic	52	128	37	105	0.6 (0.3-1.3)	NR	(Huovinen *et al.*, 2010)[2]
			Fruits/berries, imported	53	127	46	112	0.9 (0.3-2.4)		
			Root vegetables	49	129	35	100	0.9 (0.4-2.0)		
			Lettuce, cabbage	52	129	44	112	1.0 (0.3-3.0)		
			Onion, leek	50	130	35	98	0.8 (0.4-1.9)		
			Cucumber, tomato, pepper	53	130	49	127	0.2 (0.0-1.5)		
2006	Finland	Infection with *Y. enterocolitica* 1A	Fruits/berries, domestic	89	243	75	221	0.6 (0.3-1.3)	NR	(Huovinen *et al.*, 2010)[2]
			Fruits/berries, imported	95	239	91	210	**3.5 (1.2-10.5)**		
			Root vegetables	89	237	77	200	1.4 (0.7-2.9)		
			Lettuce, cabbage	96	240	84	229	**0.3 (0.1-0.8)**		
			Onion, leek	91	237	63	193	**0.5 (0.3-1.0)**		
			Cucumber, tomato, pepper	96	247	94	245	0.4 (0.0-5.1)		
2009-2010	Germany	Yersiniosis	Consumption of raw vegetables	571	1798	313	1268	**0.5 (0.4–0.7)**	**0.5 (0.4-0.6)**	(Rosner *et al.*, 2012)

NR, not reported; NS, not significant and values not reported.

[1] Divided into cases or controls aged <10 years or ≥10 years, however Friesema *et al*. (2014) do not provide the number of controls in each age group.

[2] Huovinen *et al.* (2010) also found that 'washing pre-washed roots' (root vegetables), 'washing vegetables other than roots' and 'washing pre-washed vegetables other than roots' were protective.

[3] Children aged 6 years or less.

[4] ORs reported are imputed values, which took into account partially-completed survey forms from cases and controls.

[5] NP, non-perinatal cases; P, perinatal cases.

B.3 RISK ASSESSMENTS AND OTHER ACTIVITIES

Two risk topics are applicable to fruit and vegetables; 1) assessments that consider RTE foods and 2) assessments that consider fruit and vegetables. Three risk assessments on *L. monocytogenes* in RTE foods were published prior to 2008 (EFSA, 2007; FAO/WHO, 2004; USFDA/USDA, 2003). These have not been updated so represent the most recent information available. In summary:

- USA (quantitative risk assessment): Considered raw vegetables, and raw/dried vegetables as a separate food groups and ranked these alongside 21 other RTE foods (USFDA/USDA, 2003). Both food groups were rated "low risk" on a per serving or per annum basis.[47]
- FAO/WHO (quantitative risk assessment, did not specifically consider the risk from fresh produce): Modelling predicted that nearly all listeriosis cases were the result of ingesting high numbers of *L. monocytogenes* (FAO/WHO, 2004).
- EU (scientific opinion, did not specifically consider the risk from fresh produce): Most listeriosis cases were due to consumption of RTE foods able to support *L. monocytogenes* growth and containing concentrations well above 100 CFU/g.

Assessments that consider fruit and vegetables are summarised in the following sections.

B.3.1 FAO/WHO

The FAO/WHO report on microbiological hazards in fresh fruits and vegetables was cited in the 2008 Document (FAO/WHO, 2008a). Three additional and relevant reports were published by FAO/WHO during 2008:

- Fresh leafy vegetables and herbs: Through considering data on fresh and fresh-cut leafy vegetables and herbs, the FAO and WHO identified a large number of risk factors that contribute to microbial contamination of these foods both pre- and post-harvest and listed risk management strategies to minimise the potential for contamination (FAO/WHO, 2008b).
- Viruses: After assessing scientific knowledge on viruses in food, the FAO and WHO concluded that norovirus and HAV on fresh produce were high priorities (FAO/WHO, 2008c). The main routes of contamination were through sewage-contaminated water, the use of human sewage as fertiliser and handling by infected food handlers.
- Parasites: Using multicriteria-based risk ranking, FAO and WHO concluded that *Cryptosporidium* spp./fresh produce, *G. duodenalis*/fresh produce and *C. cayetanensis*/fresh produce were ranked 5th, 11th and 13th respectively on a list of 25 parasite/food commodity pairs important on a global scale (FAO/WHO, 2014).

WHO has published a training resource for growers to help prevent contamination of food crops (WHO, 2012).

B.3.2 European Union

Partially in response to the 2011 outbreak of STEC infection in Germany, EFSA prepared a fast-track assessment of the exposure of the consumer to STEC through consumption of raw vegetables (EFSA, 2011b). Because information on the prevalence and quantity of STEC in vegetables both from surveillance and outbreak investigations were scarce, EFSA could not

[47] This document is currently being updated, http://www.fda.gov/Food/FoodScienceResearch/RiskSafetyAssessment/ucm247806.htm (accessed 16 June 2015).

estimate the relative exposure to humans from pre-harvest or post-harvest contamination of vegetables by STEC.

Using data from EU outbreaks between 2007 and 2011, the European Food Safety Authority (EFSA) estimated that foods of non-animal origin (FoNAO) were associated with 10% of all reported outbreaks, 26% of the cases, 35% of the hospitalisations and 46% of the deaths (EFSA, 2013). The proportions attributed to hospitalisations and deaths reduced to 8% and 5% respectively when data from the 2011 STEC O104 outbreak were removed.

Using a risk ranking model based on this set of outbreak data, which also considered the strength of association between a food/pathogen pair, the consequences of human disease and the probability of exposure, food/pathogen combinations were ranked according to how often they were linked to foodborne human cases originating from FoNAO in the EU (Da Silva Felicio *et al.*, 2015; EFSA, 2013). The top five food/hazard combinations relevant to this document were:

- First: *Salmonella* spp./leafy green vegetables eaten raw as salads;
- Second: *Salmonella* spp./bulb and stem vegetables, *Salmonella* spp./tomatoes, *Salmonella* spp./melons, pathogenic *Escherichia coli*/fresh pods, legumes or grain;
- Third: Norovirus/leafy green vegetables eaten raw as salads, *Salmonella* spp./sprouted seeds, *Shigella* spp./fresh pods, legumes or grain;
- Fourth: Norovirus/bulb and stem vegetables, Norovirus/raspberries, *Salmonella* spp./raspberries, *Salmonella* spp./leafy green vegetables mixed with other fresh FoNAO; *Shigella* spp./fresh herbs, pathogenic *Escherichia coli*/sprouted seeds, *Yersinia* spp./carrots;
- Fifth: Norovirus/tomatoes, norovirus/carrots, *Shigella* spp./carrots.

To follow up on this risk ranking exercise, EFSA has assessed several of these food/hazard combinations:

- *Salmonella* spp. and Norovirus in leafy green vegetables eaten raw as salads (EFSA, 2014c);
- *Salmonella* spp., *Yersinia* spp., *Shigella* spp. and Norovirus in bulb and stem vegetables, and carrots (EFSA, 2014d);
- *Salmonella* spp. and Norovirus in tomatoes (EFSA, 2014a);
- *Salmonella* spp. in melons (EFSA, 2014e); and
- *Salmonella* spp. and Norovirus in berries (EFSA, 2014f).

The risk factors identified for each of these food/hazard combinations were similar:

- Primary production: Presence of animals, application of contaminated organic amendments or water, contamination by equipment or workers. Processes which wet the external or edible portions of the crop close to harvest.
- Post-harvest processing: Contamination or cross-contamination via equipment, water (e.g. wash tanks) or food handlers. The risk of cross-contamination is reduced if disinfectants are properly used within the washing tank.
- Retail: Cross-contamination from other foods or surfaces, infected food handlers.

Additional risk factors for melons were fruit damage or cracking, contaminated ice and slow cooling when rinds are wet.

A recent quantitative risk assessment model for the European food chain estimated the risk of norovirus infection per serving of lettuce as $3x10^{-4}$ ($6x10^{-6}$–$5x10^{-3}$) and the risk of Hepatitis A jaundice per serving of lettuce as $3x10^{-8}$ ($7x10^{-10}$–$3x10^{-6}$) (Bouwknegt *et al.*, 2015). Contamination from workers' hands was the most important contribution to risk.

An earlier risk assessment of STEC and other pathogenic bacteria in seeds and sprouted seeds concluded (EFSA, 2011a):

- Sprouted seeds are RTE foods with microbial food safety concern due to the potential for certain pathogenic bacteria to contaminate the seeds and to grow during germination and sprouting, and to their consumption patterns (raw or minimally processed).
- *Salmonella* spp. and pathogenic *E. coli* (including STEC) are the most commonly reported bacterial pathogens causing outbreaks associated with the consumption of contaminated sprouts. Very low contamination levels of dry seeds (e.g. 4 MPN/kg) can cause sprout associated-outbreaks.
- *B. cereus*, *S. aureus*, *L. monocytogenes* and *Y. enterocolitica* have been implicated with sprout-associated outbreaks, although rarely.
- Large outbreaks involving sprouts (e.g. the outbreak in Germany 2011) illustrate the potential to cause major public health emergencies affecting previously healthy people and not limited to those considered particularly vulnerable to infections.
- Reliable methods for decontaminating all types of seeds or sprouted seeds are not currently available.
- The most relevant risk factors are those associated with agricultural practices during seed production, storage and distribution, e.g. contaminated irrigation water and/or manure, presence of birds and rodents in storage facilities, dust and soil particles.

EFSA have also studied the occurrence and control of foodborne viruses (EFSA, 2011d). Fresh produce is a source of viral infection in humans, but the importance of this relative to other sources of infection (including infected food handlers) is not known. It was recommended that food producers and handlers focus on preventive measures to avoid viral contamination rather than trying to remove/inactivate these viruses from food.

B.3.3 USA

The United States Food and Drug Administration (USFDA) has produced a risk ranking tool for fresh produce that ranks 53 commodity/pathogen pairs in terms of their public health risk (Anderson *et al.*, 2011).[48] The tool is based on data from reported outbreaks in the USA, prevalence studies and food consumption studies, but user inputs can determine the final ranking. After scenario and sensitivity analyses, the tool ranked EHEC/leafy green vegetables first, followed by *Salmonella* spp./tomatoes and *Salmonella* spp./leafy green vegetables. Leafy green vegetables often appeared among the top-ranked pairs.

USFDA Centre for Food Safety and Nutrition is developing a Quantitative Predictive Risk Assessment Model (QPRAM) that intends to predict and characterise risk from consumption of fresh produce as a result of behaviours and practices on farms and during the processing and consumption of crops.[49] The intention is for the tool to be used to optimise interventions to prevent contamination of fresh produce and to assist in trace-back investigations.

[48] The tool is available from http://foodrisk.org/exclusives/rrt/ (accessed 16 June 2015).
[49] http://www.fda.gov/food/foodscienceresearch/risksafetyassessment/ucm243439.htm (accessed 16 June 2015).

A survey of 226 fresh produce growers identified multiple practices that increased the risk of fresh produce being contaminated by pathogenic microorganisms (Harrison *et al.*, 2013). These included application of raw manures close to harvest and using water of uncertain quality.

B.3.4 Attribution studies

There have been several attribution studies published since 2008 based on human health surveillance data. This section summarises the results of only some of them, focussing on those that considered multiple pathogens and the EU and North American regions, and which reported attribution estimates for fresh produce.

Multiple countries, multiple pathogens (Greig and Ravel, 2009)

Foodborne outbreaks over the period 1988-2007 were collated from multiple sources and countries. From 498 outbreaks attributed to the food category 'produce', which includes fruits, vegetables and 'produce dishes', 38% were caused by *Salmonella* spp., 18% by norovirus and 15% by *E. coli*. In the next lowest group were *Cyclospora* spp. (6.5%), *Shigella* spp. (4.9%), Hepatitis A virus (4.5%) and *C. botulinum* (3.3%). All the other pathogenic bacteria, viruses and parasites were the cause of infection in <2% of the 'produce' outbreaks.

Europe, salmonellosis and campylobacteriosis (Pires et al., 2010)

Using outbreak data from the EU for the years 2005 and 2006, the following proportions of salmonellosis or campylobacteriosis outbreak cases and outbreaks were attributed to consumption of fruit and nuts, or vegetables (median values, numbers in brackets are 95% confidence intervals):

- Salmonellosis/fruit and nuts: 0.04% cases (0.00-0.15), 0.02% outbreaks (0.00-0.08)
- Salmonellosis/vegetables: 1.39% cases (0.48-2.85), 0.49 outbreaks (0.24-0.92)
- Campylobacteriosis/fruit and nuts: 0.098% cases (0.00-0.41), 0.11 outbreaks (0.00-0.48)
- Campylobacteriosis/vegetables: 0.11% cases (0.00-1.24), 0.18 outbreaks (0.00-0.80)

The approach included accounting for foods containing multiple ingredients and the values are much lower than those obtained in a more recent report based on USA data and described below, which only analysed single ingredient outbreaks.[50]

USA, multiple pathogens (IFSAC Project Team, 2015)

From a model of USA foodborne outbreak data for the period 1998-2012, using only outbreaks that could be attributed to a single food category, the estimates reproduced in TABLE 16 were produced for fresh produce. Notable observations from this analysis are that an estimated half of all domestically-acquired foodborne listeriosis cases were attributed to fruits (note the wide credibility interval), and 36% of all cases of *E. coli* O157 infection were attributed to vegetable row crops. While an estimated 18% of salmonellosis cases were attributed to seeded vegetables, the 90% credibility intervals show that the other produce categories are also important vehicles of infection. The estimated percentages of campylobacteriosis cases attributed to the produce groups were low which implies that non-produce food groups are more important sources of infection (an estimated 66% of cases were attributed to dairy). It is also notable that exposure of the population to sprouts is relatively minor considering other

[50] For a more recent summary of salmonellosis attribution, including attribution to fresh produce, see Pires S M, Vieira A R, Hald T and Cole D (2014) Source attribution of human salmonellosis: an overview of methods and estimates. Foodborne Pathog Dis; 11: 667-76..

food groups analysed (meats, dairy), yet an estimated 8% of salmonellosis and listeriosis cases were attributed to this food.

TABLE 16: The estimated proportions (and 90% credibility intervals) of domestically-acquired foodborne illnesses attributed to categories of fresh produce (data from the IFSAC Project Team report, 2015)

FOOD CATEGORY	*SALMONELLA* SPP. (n=597)	*E. COLI* O157 (n=170)	*CAMPYLOBACTER* SPP. (n=161)	*LISTERIA* SPP. (n=24)
Fruits	12% (8-16)	7% (3-12)	1% (<1-2)	50% (5-77)
Seeded vegetables	18% (13-25)	0	6% (1-13)	0
Sprouts	8% (5-12)	1% (<1-1)	0	8% (1-22)
Vegetable row crops	3% (1-6)	36% (26-46)	6% (2-11)	3% (<1-13)
Other produce	7% (3-11)	1% (0-2)	2% (<1-6)	0

APPENDIX C: OTHER PATHOGENS

During preparation of this document information was uncovered on other pathogens that may be associated with fresh produce in New Zealand.

C.1 HEPATITIS E VIRUS (HEV)

HEV is considered to be a travel-related illness in developed countries, but there is increasing evidence of locally-acquired HEV in these countries and undercooked or raw foods of animal origin have been reported as the suspected vehicles of infection (FAO/WHO, 2008c; Maunula *et al.*, 2013). HEV has been detected on fresh produce but the role of fresh produce in transmission of this virus to humans has not been investigated (Maunula *et al.*, 2013).

The following table is aligned with the pathogen tables included in Appendix A.1 and demonstrates how little is known about this virus. From the available information, contamination of fresh produce in New Zealand is most likely to occur through food workers. Hepatitis E does occur in New Zealand; in 2014 there were five cases notified of which two did not report travel overseas during the incubation period (Health Intelligence Team, 2015a).

Foodborne disease	Hepatitis E
Pathogenic strains	Classified as four distinct genotypes with one serotype. All genotypes are associated with human infections (Meng, 2011). HEV genotypes 1 and 2 are only found in humans. Genotype 3, a diverse cluster, affects humans and animals (notably swine, deer and rodents) worldwide and so has potential for zoonotic infections (Meng, 2011). Genotype 4 is also associated with human and swine but is currently mainly restricted to Asia. Genotypes 3 and 4 are thought to be less pathogenic than 1 and 2 (Teshale *et al.*, 2010). The genotypes currently circulating in human population in New Zealand is unknown. There are limited data on the seroprevalence in the New Zealand human population (Dalton *et al.*, 2007) and other countries.
Presence and survival in soil	Limited information on presence of HEV in soil. Only one study has been published to date, showing that HEV was detected in 4.9% soil samples (n=403) in India (Parashar *et al.*, 1998). No published data on the presence and concentration in New Zealand soils.
Presence and survival in water	**NZ**: HEV RNA was present in 4/109 (3.7%) water samples taken from two New Zealand rivers (Williamson *et al.*, 2011).
Presence and survival in manure	HEV infection is widespread in the pig population. **NZ**: The HEV antibody was present in the majority (90.9%, 20/22) of pig herds (Garkavenko *et al.*, 2001). Although pigs of all ages can excrete HEV, shedding is most frequently seen in pigs less than 4 months old. Recently infected pigs excrete viruses in faeces in large amounts for at least 3-4 weeks following infection (Pavio *et al.*, 2010). There is limited information on the presence and persistence of HEV in animal (i.e. pig) manure and none from New Zealand. One study described that HEV RNA was detected in concentrations up to 10^3 per 60 ml manure slurry samples collected from storage pits from 15/22 (68%) pig farms in Midwestern USA (Kasorndorkbua *et al.*, 2005).

Presence and survival in compost	Limited information. One Spanish study showed that while HEV was present in the raw pig manure, no HEV was detected (by PCR) in the final composted product (subjected to temperatures up to 65°C), showing that composting was effective in reducing the viral concentration (Garcia *et al.*, 2014). HEV genotypes 1 and 5 were only partially inactivated by temperatures between 45°C and 50°C, but fully inactivated at 60°C (Emerson *et al.*, 2005).
Excretion from asymptomatic humans	Incidence of asymptomatic infection and shedding is not fully understood but it is thought that most HEV infections are asymptomatic and excretion from asymptomatic individuals is likely. Most symptomatic infections occur in adults. Faecal shedding from humans usually commences around 5 days prior to clinical symptoms and persists for a further 2 to 3 weeks. Cases of chronic HEV are uncommon, occur mainly in immunocompromised individuals and involve genotype 3 (Aggarwal, 2011).

C.2 *CRONOBACTER* SPP. (PREVIOUSLY *ENTEROBACTER SAKAZAKII*)

Infections from *Cronobacter* spp. are usually caused by reconstituted powdered infant formula. The 2008 Document suggested the possibility of fresh produce being a vehicle of infection for this pathogen, based on data that showed its association with fresh produce and ability to grow on these foods. Reported infections of *Cronobacter* spp. are rare in New Zealand; only five cases of invasive disease have been reported since this disease became notifiable in 2005 (Health Intelligence Team, 2015a). It does not appear that *Cronobacter* spp. are a significant risk to human health in general, in New Zealand.

C.3 TYPHOIDAL SALMONELLAE

Humans are the exclusive reservoir for some serotypes of *Salmonella enterica* ssp. *enterica*, including Typhi, Sendai, and Paratyphi A, B or C (Gal-Mor *et al.*, 2014). These are called the typhoidal salmonellae and cause enteric fever in humans, also called typhoid or paratyphoid fever if caused by Typhi or Paratyphi, respectively. This is a systemic, life-threatening disease characterised by fever, chills, abdominal pain, swelling of the liver and spleen, spotty rash, nausea, headache, anorexia, dry cough, and diarrhoea or constipation.

The typhoidal salmonellae are endemic in developing countries. Cases of typhoid and paratyphoid fever are reported in New Zealand each year, but most reported living or travelling overseas during the incubation period. For example, in 2014 there were 42 cases of typhoid fever notified in New Zealand, of whom 33 reported overseas travel (Health Intelligence Team, 2015a). In the same year there were 21 cases of paratyphoid fever reported, of whom 19 reported overseas travel. The number of cases of typhoid fever notified in New Zealand has increased between 1997 and 2014. The 2014 rate was 0.9 per 100,000 population.

In developing countries, sewage-contaminated water is the main transmission route for typhoidal salmonellae, and the pathogens may be carried onto fresh produce by contaminated irrigation water (USFDA, 2012). In countries like New Zealand, where non-typhoidal salmonellae are not endemic, these pathogens are more likely to be transmitted by food or water contaminated by an infected person exhibiting symptoms, or by an asymptomatic carrier (Ministry of Health, 2012b). A small proportion (1-4%) of people infected with *S.* Typhi become asymptomatic carriers for more than 12 months, shedding *S.* Typhi in their faeces at concentrations of 10^6-10^{10} cells/g (Gal-Mor *et al.*, 2014). Asymptomatic shedding of *S.* Paratyphi A may be similar.

In 2011, a worker employed in the kiwifruit industry under the Recognised Seasonal Employer programme was diagnosed with typhoid fever. Public health officials believe the worker was infected before entering New Zealand. The industry responded by destroying kiwifruit the worker may have come into contact with and ensuring other workers were free from infection.[51]

C.4 METAZOAN PARASITES

From a list of 24 globally-important parasite/food commodity pairs identified and ranked through a multicriteria risk ranking exercise by FAO/WHO, 11 were parasites paired with fresh produce, including *Cryptosporidium* spp., *G. duodenalis* and *C. cayetanensis* (FAO/WHO, 2014).[52] *Echinococcus granulosus*/fresh produce and *Echinococcus multilocularis*/fresh produce were ranked 2nd and 3rd (c.f. *Cryptosporidium* spp./fresh produce was ranked 5th). *E. granulosa* causes hydatid disease and human infection is caused by ingestion of *E. granulosa* in the egg form, which is excreted by dogs, the definitive host (Eckert and Deplazes, 2004). New Zealand was declared provisionally free from hydatid disease in 2002 but occasional human cases of hydatid disease are still reported (Health Intelligence Team, 2015a; Ministry of Health, 2012a). *E. multilocularis* is not found in New Zealand (FAO/WHO, 2014).[53] The liver flukes *Fasciola* spp. (ranked 12th) are present in New Zealand and are associated with wet areas where the definitive snail host lives, and while this may be an issue for watercress, no cases of domestically-acquired human infection have been reported (Berger, 2015; McKenna, 2009; Mitchell, 1995). *Toxocara* spp. (ranked 20th) are roundworms present in New Zealand that are associated with domestic animals, and there is evidence that New Zealand people have been exposed (Zarkovic *et al.*, 2007). Human infections from the four other parasites are more associated with developing countries or tropical/subtropical areas.[54]

In summary, these metazoan parasites do not appear to be of concern for fresh produce available in New Zealand, but there are no New Zealand-specific data to confirm this.

C.5 TOXIGENIC FUNGI

Mycotoxins produced by spoilage fungi will not usually be a hazard for humans consuming whole or cut fruit since rotten parts are usually discarded. It is more likely that humans will be exposed to fungal toxins through fruit and vegetable juices. Three mycotoxins are particularly associated with fruit and vegetable juices (Cressey, 2014; Moss, 2008):

- Patulin: Produced by a number of fungal species, particularly *Penicillium expansum*, which is the cause of 'blue rot' or 'soft rot' in apples. Patulin was detected in apple juices sold in New Zealand in the most recent survey, done in 1997 (Stanton, 1998), and has also been detected overseas in juices from peaches, pineapples and oranges.
- Ochratoxin A: Produced by some *Aspergillus* species and *Penicillium verrucosum*. *Aspergillus carbonarius* is a cause of rot in grapes and ochratoxin A has been detected in grape juice overseas.
- *Alternaria* toxins (including tenuazonic acid) are produced by species of *Alternaria* fungi, which are principally associated with grains but have also been detected on fruit and

[51] https://www.zespri.com/companyinformation/newsroom/kiwifruit-contained-following-typhoid-case and http://www.nzherald.co.nz/nz/news/article.cfm?c_id=1&objectid=10726169 (accessed 30 June 2015).
[52] In addition, *Trypanosoma cruzi*/fruit juices was ranked 10th, but this parasite only occurs in the Americas (FAO/WHO 2014).
[53] Largely found in the Northern Hemisphere, see also http://www.cfsph.iastate.edu/Factsheets/pdfs/echinococcosis.pdf (accessed 17 June 2015).
[54] *Ascaris* spp., *Balantidium coli*, *Entamoeba histolytica* and *Trichuris trichiura*.

vegetables. Overseas, these toxins have been detected in juices from many fruits and vegetables including pipfruit, grapes, berries, tomatoes, carrots, citrus and stonefruit.

A recent Risk Profile examined mycotoxins in the New Zealand food supply and concluded that dietary exposure to these three mycotoxins was low (considering all foods, not just fruit and vegetables), and that the risk of adverse health effects from these mycotoxins was also low, although there are many data gaps (Cressey, 2014). There are no regulatory limits for these mycotoxins and control is achievable through Good Agricultural Practice and omission of fruit and vegetables with signs of rot from juicing processes.

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