Supporting document 2

Microbiological assessment of berries, leafy vegetables and melons

P1052 - Primary Production and Processing Requirements for Horticulture (Berries, Leafy Vegetables and Melons)

Executive summary

Fresh fruit and vegetables are important components of a healthy diet, and horticultural produce in Australia is considered safe and nutritious. However, in Australia and internationally, outbreaks of foodborne illness continue to be associated with the consumption of fresh horticultural produce. In order to minimise future outbreaks, it is important to understand the potential routes of contamination and which control measures can be implemented to help mitigate risks.

Food Standards Australia New Zealand (FSANZ) has undertaken an assessment of the microbiological food safety risks associated with fresh horticultural produce in Australia to guide decisions on appropriate regulatory and non-regulatory risk management measures. At the request of the then Australia and New Zealand Ministerial Forum on Food Regulation (the Forum – now the Food Ministers' Meeting), the assessment focused on high priority commodity sectors that have specific annexes within the Codex *Code of hygienic practice for fresh fruits and vegetables* (Codex 2017) that provide additional guidance for their hygienic production. As measures are already in place for seed sprouts and ready-to-eat, minimally processed fruits and vegetables, the scope of this assessment was limited to leafy vegetables, berries and melons.

The objective of the assessment was to identify and summarise the pathways of contamination, persistence and amplification of microbiological hazards throughout the primary production chain for leafy vegetables, berries and melons. The assessment also aimed to identify measures/controls that may minimise contamination of these commodities.

The assessment involved a qualitative through-chain analysis of the three horticultural commodity sectors. Due to the large variety and complexity of these sectors, a proxy approach was utilised. Representative products and associated hazards for each sector were selected to enable consideration of a range of cultivation and growing conditions, product types (including different surface types) and harvest methods.

Selected commodities included lettuce, parsley and spinach (leafy vegetables); blueberry, raspberry and strawberry (berries); and rockmelons and watermelons (melons). The scope of the pathogens associated with these commodities was restricted to the key bacterial pathogens—*Salmonella* spp., Shiga toxin-producing *Escherichia coli* and

Listeria monocytogenes—and the viral pathogens—hepatitis A virus and norovirus.
Based on the evidence reviewed in this report, the following risk factors represent important potential routes of microbial contamination for all the commodity sectors assessed:

- Incursion by wildlife and domestic animals
- Location of growing areas near or on land used for practices that may increase the likelihood of contamination, such as livestock production, as a wildlife habitat, urban or industrial waste
- The occurrence of extreme weather events, such as flooding or heavy rain, that could transfer pathogens to produce, fields, or irrigation water sources
- The application of untreated or insufficiently treated manure or compost amendments
- The use of contaminated water for irrigation, application of agricultural chemicals, and/or postharvest washing and sanitising
- Insufficient monitoring and application of postharvest washing and sanitisation systems for leafy vegetables and melons
- Contamination and cross-contamination due to poor worker and equipment hygiene, both at harvest and postharvest.

Comparative estimates of risk between or within the three commodity sectors were not possible due to the qualitative nature of this assessment, limited data availability, and the large number of potential exposure pathways considered for different pathogen/commodity combinations. However, commodity specific factors that may lead to increased risk were identified.

Additional risk factors identified for leafy vegetables include: the surface characteristics of produce with rougher surfaces; the growth of plants close to the ground; the potential for internalisation of pathogens into the edible part of the plant from contaminated seed, or from contaminated soil or water via the roots and/or cut or damaged parts of plants; field packing with no subsequent washing and sanitising; the potential for internalisation during vacuum cooling or postharvest washing; and the potential for growth of *E. coli*, *Salmonella* spp., and *L. monocytogenes* on whole and/or cut leafy vegetables.

Additional risk factors identified for berries include: rougher surface characteristics of strawberries and raspberries; growth of strawberries closer to the ground; the use of contaminated water for berries that require frost protection; the potential for internalisation of pathogens into the edible portion of the plant from contaminated soil via the roots; hand-picking and excessive handling; and the packing of berries with no washing and sanitising step.

Additional risk factors identified for melons include: the rougher surface of rockmelons compared to watermelons; the growth of melons close to the ground; the field packing of melons with no subsequent sanitisation step; the potential for internalisation of pathogens into rockmelons during postharvest washing; and the potential for growth of *Salmonella* spp. and *L. monocytogenes* on the rind and flesh of rockmelons and the flesh of watermelons.

There is significant variation in the production methods and combinations of risk factors on farm, within and between the commodity sectors. That variation influences the presence and persistence of pathogens and, therefore, the associated risk. The commonality across these commodity sectors is that horticultural produce is generally consumed raw, with little or no further processing, and there is currently no single step during primary production and processing that can ensure end product safety.

A range of control measures were identified during primary production, harvest and postharvest activities to minimise pathogen contamination of horticultural produce. These include applying good agricultural practices, good hygienic practices at harvest and postharvest, and controlling inputs through-chain (including the effective use of sanitisers when applied) and are applicable to the commodities assessed in this report, whether intended to be consumed raw or processed as a ready-to-eat food without a control step that significantly reduces the microbial load.

The safety of these commodities relies on a consistent and well managed through-chain, multi-hurdle approach to minimise risk. This includes managing inputs and responding to changes in the growing environment that could increase the likelihood of contamination. It is not possible to completely eliminate risk to consumers from fresh produce at the primary production level—the handling through to, and by, consumers must also seek to minimise risks. However, this assessment concludes that risk management required to ensure end product safety of these commodities, needs to begin on farm.

Acknowledgements

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1 Introduction

Fresh fruit and vegetables are important components of a healthy diet, and horticultural produce in Australia is considered safe and nutritious. However, outbreaks of foodborne illness continue to be associated with the consumption of horticultural products both in Australia and internationally.

FSANZ has previously undertaken work regarding the primary production and processing of horticulture as part of Proposal P1015[1] (*Primary Production & Processing Standard for Horticulture*). This Proposal was abandoned in 2014 in favour of non-regulatory measures.

However, in response to more recent outbreaks, the Australia and New Zealand Ministerial Forum on Food Regulation (the Forum) has since agreed that there is a need to reassess the food safety risk management of five horticulture commodity sectors including:

- Leafy green vegetables
- Berries
- Melons
- Ready-to-eat, minimally processed fruits and vegetables
- Sprouts.

The commodities represent those with commodity-specific annexes in the Codex Alimentarius Commission (Codex) *Code of Hygienic Practice for Fresh Fruits and Vegetables* (Codex 2017). In 2006, Codex requested the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) provide scientific advice to support the development of the commodity-specific annexes, and in 2007 the FAO and WHO ranked leafy vegetables and herbs as Priority 1 commodities, and berries, green onions, melons, sprouted seeds and tomatoes as Priority 2 commodities based on the following criteria:

- Frequency and severity of disease
- Size and scope of production
- Diversity and complexity of the production chain/industry
- Potential for amplification of foodborne pathogens through the food chain
- Potential for control
- Extent of international trade and economic impact.

The Codex *Code of Hygienic Practice for Fresh Fruits and Vegetables* addresses Good Agricultural Practices (GAPs) and Good Hygienic Practices (GHPs) that help control microbial, chemical and physical hazards associated with all stages of the production of fresh fruits and vegetables, from primary production to consumption. The commodity specific annexes include additional recommendations to cover hygienic practices specific to these high priority commodities.

In June 2018 the Forum Ministers requested that FSANZ reassess the need to amend the Australia New Zealand Food Standards Code (the Code) to enact a primary production and processing standard to manage food safety for these commodities.

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[1] Proposal P1015 – *Primary Production & Processing Standard for Horticulture*:
https://www.foodstandards.gov.au/code/proposals/Pages/proposalp1015primary5412.aspx

The scope of this assessment has however been limited to three of the five identified commodity sectors; namely leafy vegetables, berries and melons, since:

- Ready-to-eat minimally processed fruits and vegetables are covered by current food safety requirements in Chapter 3 of the Code; and
- The production and processing of seed sprouts is covered by Standard 4.2.6 *Primary Production and Processing Standard for Seed Sprouts*.

The primary objective of FSANZ when developing or reviewing food standards is '*the protection of public health and safety*'. FSANZ is also required to have regard to '*the need for standards to be based on risk analysis using the best available scientific evidence*'. The development and application of a Primary Production and Processing Standard for fresh horticultural produce is dependent on an analysis of the public health and safety risks, economic and social factors and current regulatory measures and industry practices.

FSANZ uses a number of methodologies to assess public health and safety risks, including risk profiling, quantitative and qualitative assessments and scientific evaluations. The methodology utilised depends on the purpose of the assessment and on the availability, quality and quantity of relevant data.

The microbiological assessment for this Proposal involved a qualitative through-chain analysis of selected high priority horticultural commodity sectors. When considering the overall necessity for a Primary Production and Processing Standard, the outcomes of this assessment will be considered by risk managers together with outcomes from the assessment of economic and social factors and current regulatory measures.

2 Objective of the assessment, risk assessment questions, scope, and approach

2.1 Objective

The objective of the assessment was to identify and summarise the pathways of contamination, persistence, survival, and amplification of hazards throughout the primary production chain for leafy vegetables, berries and melons. The assessment also aimed to identify measure/controls that may minimise contamination of these commodities.

2.2 Risk assessment questions

The risk assessment questions outlined below were addressed during this assessment:

Q1. What are the key risk factors associated with the primary production and processing of fresh ready-to-eat horticulture products that apply broadly to the sector?
> Q1a. What are the main risk factors and/or production activities contributing to contamination with microbiological hazards?
> Q1b. What measure/controls may have minimised contamination of produce?
> Q1c. What are the commodities most often implicated in fresh ready-to-eat horticulture product related foodborne outbreaks?

Q2. Which commodities, or commodity groups reflected in the Codex Code of Hygienic Practice for Fresh Fruits and Vegetables pose a higher microbiological food safety risk due to their intrinsic properties and/or production method? What additional measures/controls may reduce the food safety risk?

2.3 Scope of assessment

The scope of the assessment was limited to the commodities and hazards selected by the process described in Section 3.1 due to the large variety and complexity of the identified horticulture sectors. They include:

Leafy vegetables
Commodities: Lettuce (including iceberg, cos/romaine, butterhead, oak), parsley (including continental and curly-leaf), spinach
Hazards: *Salmonella* spp., Shiga toxin-producing *Escherichia coli* (STEC), *Listeria monocytogenes*

Berries
Commodities: Blueberry, raspberry, strawberry
Hazards: Hepatitis A virus (HAV), norovirus (NoV), STEC

Melons
Commodities: Rockmelon, watermelon
Hazards: *Listeria monocytogenes, Salmonella* spp.

The qualitative assessment of possible risk factors associated with primary production, harvest, and primary processing of the commodities were limited in scope to the following:

- Primary production, including animal intrusion, characteristics of the production environment, weather and extreme weather events, seeds and seedlings, soil and soil amendments, water for primary production and other preharvest factors that may influence contamination
- Harvest and field packing operations and hygiene
- Processing, including postharvest handling and hygiene control, postharvest water use, worker health and hygiene, cold chain, cleaning and sanitisation of equipment and facilities
- Postharvest washing and sanitising of produce, including currently available water-based sanitisers and excluding novel technologies.

Horticulture-related processing activities that are already covered by requirements in Standard 3.2.2 are out of scope for this assessment (Figure 1).
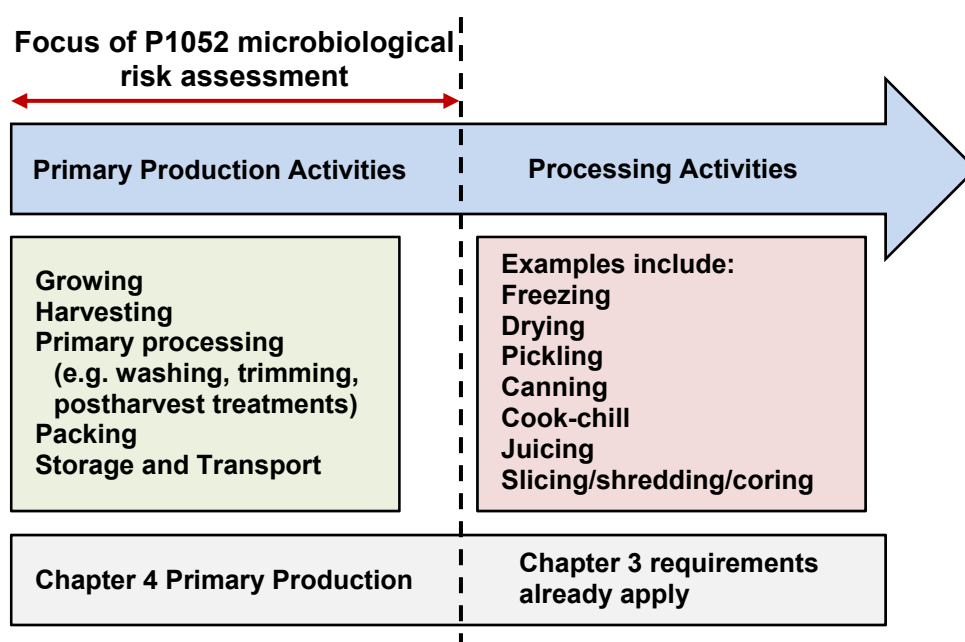
Figure 1. Schematic representation of the scope of the microbiological risk assessment for Proposal P1052

The scope of this work also does not include an assessment of the application of modified atmosphere packaging that could be applied to ready-to-eat, minimally processed fruits and vegetables[2]. Also out of scope are processes and risk factors that occur after fresh produce is released from the processing or packing shed, such as transport, cold chain maintenance to the consumer and processing/storing at retail, that are covered by requirements in Standard 3.2.2 in the Code, and consumer handling. While the important contribution of these aspects to end product safety are recognised and briefly discussed, the main objective was to identify the risk factors associated with the primary production, harvest, and postharvest activities identified above.

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[2] Relevant requirements in the Code pertinent to both Australia and New Zealand include general packaging requirements in Standard 1.1.1 (subsections1.1.1—10(10) and (11) (Packaging requirements) and maximum levels (MLs) for three packaging contaminants, regulated by subsection 1.1.1—10(5) and Standard 1.4.1 – Contaminants and natural toxicants. Details of maximum levels for specific foods are provided in Schedule 19 (sections S19—4 (metal contaminants) and S19—5 (non-metal contaminants). For Australia, Standard 3.2.2 – Food Safety Practices and General Requirements have requirements pertaining to food packaging. Standard 3.2.2 contains requirements for food businesses (including manufacturers, importers and retailers) regarding the safety of packaging.

2.4   A qualitative through-chain assessment approach

In Proposal P1015, FSANZ reviewed reports of foodborne illness associated with fresh horticultural produce from 1990–2011 to test assumptions about which commodities and risk factors were most likely to result in produce contamination and outbreaks of foodborne illness. That review identified that the main risk factors for the primary production and processing of fresh horticultural produce were the use of poor quality water (pre- and postharvest), faecal contamination by wildlife, and poor hygienic practices of workers through the supply chain. The review found that the commodity sectors most commonly involved in outbreaks included leafy vegetables, berries, melons, and minimally processed produce.

This assessment complements and builds on the assessment undertaken in Proposal P1015, by aiming to identify and summarise the scientific evidence of pathways of contamination, persistence and amplification of hazards throughout the primary production and primary processing chain. This assessment has also aimed to identify measures/controls that may minimise contamination of produce. In addition, recent outbreaks and prevalence data (2011-2019, since the P1015 assessment) and consumption characteristics for the in-scope commodities were summarised.

To guide the qualitative through-chain assessment, FSANZ has reviewed the information and data available for the in-scope commodities/hazards against similar factors considered by the FAO/WHO Expert Meeting in 2008 that addressed microbiological hazards associated with leafy vegetables and herbs (FAO/WHO 2008b).

Additionally, the assessment for this Proposal aimed to identify if the selected commodity sectors—or specific commodities within them—have commodity specific characteristics or production practices that make them more susceptible to contamination, compared to other commodities in the assessment, and subsequently require additional risk management measures.

Literature searches were undertaken to identify available evidence for the in-scope commodities and pathogens against the primary production, harvest, and primary processing activities considered to be in scope for this assessment (Appendix 1). The available data was then compiled and summarised for each commodity.

The qualitative nature of the assessment and scope meant it was not possible to provide comparative estimates of risk (likelihood and the severity of consequences) for the large number of individual risk factors, pathogens, and commodity combinations that were considered in this assessment. It was possible to identify risk factors, considering the intrinsic nature of the commodities, that may apply to different commodities and provide an indication of scenarios that may lead to increased levels of risk.

Variability and uncertainty are important parameters which influence the interpretation of the risk assessment and the outputs. Variability can relate to biological or other difference such as between consumers, strains of bacteria, or plant species. Uncertainty in the context of this risk assessment relates to the lack of knowledge concerning commodities, pathogens, and risk factors. The uncertainty for a given parameter can be reduced by collecting more data, but variability is an inherent characteristic which cannot be reduced, but can be described. When considering the uncertainty associated with the conclusions provided in this assessment the following qualitative categories were used that have previously been described elsewhere (European Food Safety Authority 2006).

Uncertainty category and interpretation

Low: There are solid and complete data available; strong evidence is provided in multiple references; authors report similar conclusions.

Medium: There are some but no complete data available; evidence is provided in small number of references; authors report conclusions that vary from one another.

High: There are scarce or no data available; evidence is not provided in references but rather in unpublished reports or based on observations, or personal communication; authors report conclusions that vary considerably between them.

Where uncertainty is low, confidence in the conclusions is considered high. Where there is medium uncertainty, confidence in the conclusions is considered medium. Where uncertainty is high, confidence in the conclusions is considered low.

3 Selection of proxies, and assessment approach

3.1 Selection of proxies

The Australian horticulture industry is very complex, with huge diversity in types of horticultural produce; variation in size and experience of producers and processors; geographic and climatic variability between production zones; and seasonality of produce. In order to assess the risks from horticultural produce in an efficient and effective manner, a proxy approach was utilised, focussing on a few specific representative products and associated hazards per horticulture sector that enabled consideration of relevant risk factors.

The three horticulture sectors included in the scope of the assessment are leafy vegetables, berries and melons. Specific products were selected to represent the variety of products within each sector. Differences in the properties of the produce (e.g. surface structure), growing conditions, and harvest and primary processing methods were taken into consideration when choosing the proxies.

3.1.1 Leafy vegetables (including leafy herbs)

Fresh leafy vegetables and herbs[3] include all vegetables and herbs of a leafy nature in which the leaf (and core) is intended to be consumed raw (FAO/WHO 2008a, 2008b). Examples are lettuce (all varieties), spinach, cabbages, chicory, leafy herbs (coriander, basil, parsley etc.) and watercress. Schedule 22 of the Code classifies leafy vegetables (including brassica leafy vegetables) as a large variety of edible plants with a high surface-to-weight ratio.

The main products from this category grown in Australia include cabbage, English spinach/silverbeet/kale, leafy Asian vegetables, leafy salad vegetables, lettuce and fresh herbs. The highest volume product in this category grown in Australia is lettuce (138,485 tonnes annually in 2017/2018) followed by cabbage (71,165 tonnes annually in 2017/2018) and leafy salad vegetables (56,297 tonnes annually in 2017/2018) (Hort Innovation 2019c). Parsley (including continental and curly-leaf) and coriander are the highest volume herbs produced. Production of leafy vegetables and herbs encompasses wide geographical and climatic conditions in Australia (Hort Innovation 2019c).

Leafy vegetables and herbs can be cultivated in either open fields or in protected cultivation systems, such as the soil-less hydroponic system; and products may be harvested by hand or mechanically. There can be wide variation in terms of inputs, location, farm size, productivity and target market. The variability that exists between production environments means that identification of hazards within a particular production system is critical to identifying and applying relevant and effective risk mitigation (FAO/WHO 2008b).

For leafy vegetables and herbs, food safety risks might be influenced by the production method, e.g. field versus greenhouse and soil versus hydroponics. Product surface/structure properties such as waxy versus absorbent and smooth versus crinkly are also relevant in terms of risk as is the degree of handling in the production and supply chain.

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[3] Consistent with FAO/WHO (2008b), the word *green* is excluded since 'some varieties may be colours other than green, and this term may be misleading and result in the exclusion of some varieties'.

Lettuce, baby spinach and leafy herbs such as parsley tend to be field grown in Australia, although there is a small, but growing, protected cropping and hydroponic production sector (R. Bennett, pers. comm.). Production encompasses wide geographic/climatic conditions (grown all over Australia) and there is variation in methods of water application (e.g. overhead/spray, trickle and furrow irrigation). Lettuce production encompasses dense head varieties such as iceberg through to cos/romaine, butterhead, oak and loose leaf varieties.

Few outbreaks in Australia have been directly attributed to a single fresh leafy vegetable or herb. Investigations of outbreaks due to salads or mixed foods typically do not identify individual components that might have introduced the hazard, nor where the hazard was introduced. NoV and *Salmonella* spp. are the main hazards involved in Australian outbreaks (OzFoodNet Annual and Quarterly reports 2003–2015[4]). The majority of NoV contamination occurs during food preparation (e.g. in restaurants) although there is some potential for NoV contamination during production or harvesting of fresh produce (Hall et al. 2014).

Australian data provided to inform the FAO/WHO (2008a) meeting identified *Salmonella* spp. and NoV as the principal hazards of concern for leafy vegetables. Considering data and submissions from many countries, the report also included enterohaemorrhagic *E. coli*, *Campylobacter*, *Shigella* spp., HAV, *Cyclospora cayatenensis*, *Cryptosporidium*, *Yersinia pseudotuberculosis* and *Listeria monocytogenes* as hazards of concern for these products.

Products and relevant hazards for inclusion in the FSANZ work

In order to consider a range of cultivation methods (e.g. hydroponic, field grown etc.), harvest methods, variety in product types, and taking into consideration the volume of product grown in Australia, we identified the following as proxies for fresh leafy vegetables and herbs:

- Lettuce (including iceberg, cos/romaine, butterhead, oak)
- Parsley (including continental and curly-leaf)
- Spinach

The hazards considered relevant in the Australian context are:

- *Salmonella* spp.
- STEC
- *Listeria monocytogenes*

3.1.2 Berries

Schedule 22 of the Code describes berries as being derived from a variety of perennial plants and shrubs having fruit characterised by a high surface-to-weight ratio, with the seeds often eaten along with the fruit. The term 'berries' can include, but is not limited to, strawberries, blueberries, raspberries, blackberries and mulberries. Raspberries, blackberries and their hybrid cultivars are members of the genus *Rubus* and are described collectively as rubus berries.

The main products from this category grown in Australia include blueberries, rubus berries (raspberries, blackberries, boysenberries etc.) and strawberries. The highest volume product in this category grown in Australia is strawberries (93,545 tonnes annually in 2017/2018), followed by blueberries (16,850 tonnes annually in 2017/2018) and raspberries (approximately 5,250 tonnes annually in 2017/2018) (Hort Innovation 2019b). Production encompasses wide geographical/climatic conditions (Hort Innovation 2019b).

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[4] OzFoodNet Reports: https://www.health.gov.au/internet/main/publishing.nsf/Content/cdna-ozfoodnet-reports.htm

In Australia most strawberries are grown on the ground in open fields, with only a small proportion grown in glasshouses or hydroponically (Strawberries Australia 2013). Blueberries and rubus berries are grown on bushes and canes, respectively, and are grown in the field or tunnels. Surface/structure properties (smooth/waxy versus 'hairy'; aggregated versus single drupe); the robustness of different types of berries to handling (mechanical versus hand harvesting; degree of postharvest handling) and decontamination steps (such as washing); and the growth habit of plants and hence proximity to soil (e.g. bush/cane versus ground) may influence the level of risk.

Outbreaks in Australia have been associated with imported mixed berries contaminated with HAV. The FAO/WHO (2008a) meeting identified HAV, NoV, *Cyclospora cayatenensis* and *Cryptosporidium parvum* as hazards of concern for these products. In addition, international berry outbreaks have also been associated with the bacterial pathogens STEC and *Salmonella* spp.

Products and relevant hazards for inclusion in the FSANZ work

In order to consider berries of different surface types (e.g. smooth versus aggregate), growing conditions (bush/cane versus ground) and taking into consideration the volume of product grown in Australia, we identified the following as proxies for berries:

- Blueberries
- Raspberries
- Strawberries

The hazards considered relevant in the Australian context are:

- HAV
- NoV
- STEC

3.1.3   Melons

Melons are classified in Schedule 22 (Foods and classes of foods) of the Code as fruiting vegetables belonging to the botanical family *Cucurbitaceae* (cucurbits). They include watermelon, rockmelon, honeydew melon, galia melon, charentais melon, Korean melon, hami melon and piel de sapo.

The highest volume product in this category grown in Australia is watermelon (170,039 tonnes annually in 2017/2018), followed by rockmelon (38,658 tonnes annually in 2017/2018) and honeydew (6,367 tonnes annually in 2017/2018) (Hort Innovation 2019b). Production encompasses wide geographical/climatic conditions (Hort Innovation 2019b).

Melon plants are grown as a ground vine (AMA 2019). The rough nature of the skin on many types of melon makes it difficult to remove any surface contamination, and it is also difficult to prevent soil and dust from getting onto the fruit and possibly contaminating it (FAO/WHO 2008a). Surface properties (netlike versus smooth) and some aspects of production (water use, protection from soil contamination) are the most relevant considerations affecting risk for melons.

Outbreaks in Australia have been linked to *Salmonella* spp. (2006, 2016) and *L. monocytogenes* (2010, 2018) in rockmelons. The FAO/WHO (2008a) meeting identified *Salmonella* spp., enterohaemorrhagic *E. coli* and NoV as hazards of concern for melons.

Products and relevant hazards for inclusion in the FSANZ work

In order to consider melons of different surfaces types (e.g. netlike versus smooth) and taking into consideration the volume of product grown in Australia, we identified the following as proxies for melons:

- Rockmelon (netted varieties)
- Watermelon

The hazards considered relevant in the Australian context are:

- *Listeria monocytogenes*
- *Salmonella* spp.

4 Outbreaks and prevalence of hazards in horticulture products

4.1 Australian outbreaks of foodborne illness associated with fresh horticultural produce

As part of Proposal P1015, FSANZ undertook a review of foodborne illness associated with fresh horticultural produce in Australia from 1990-2011. Similarly, for P1052 FSANZ has undertaken a review of scientific literature and technical reports published in the period 2011-October 2021 to identify Australian outbreaks of foodborne illness associated with fresh horticultural produce that occurred, or were reported, since the review undertaken for Proposal P1015. FSANZ also analysed outbreaks described in publicly available reports (see Table 1), such as those published by OzFoodNet—Australia's enhanced foodborne disease surveillance network—and food recall data (see Table 2), to provide a broader overview of the incidence of horticulture-related foodborne illness and microbial contamination of fresh horticultural produce in Australia.

The 1990-2011 review identified five fresh horticultural produce related outbreaks that occurred in Australia (and met strict inclusion search criteria) (FSANZ 2011) (see Table 1). Three of these outbreaks were associated with domestically produced product and two with imported product. For two of the outbreaks related to domestic product—rockmelon with *Salmonella* Saintpaul and papaya with *Salmonella* Litchfield—primary production food safety issues were identified that may have contributed to produce contamination, including the use of untreated or inadequately treated water and incorrect use of chemical disinfectants (Gibbs et al. 2009; Munnoch et al. 2009). The probable source of one of the outbreaks related to imported product—baby corn and *Shigella sonnei*—was poor sanitation at the packing and/or collection houses (Lewis et al. 2009). The source of contamination was not determined for the other outbreaks related to domestic and imported product.

Since 2011 there have been a number of outbreaks of foodborne illness associated with consumption of horticultural produce in Australia (see Table 1). Data on Australian outbreaks of foodborne illness associated with horticultural produce indicate *Salmonella* spp. and viruses (HAV and NoV) are responsible for the majority of recorded outbreaks. Leafy vegetables, berries and melons are the commodity sectors most often associated with these outbreaks. There was a lack of data from outbreak investigations regarding potential supply chain failure points for the majority of these outbreaks. However, the investigations into the outbreaks associated with melons identified some potential contributing factors. For example, the 2016 *Salmonella* outbreak attributed to rockmelons noted the insufficient monitoring and application of sanitiser and low general hygiene of the facilities (NSW Food Authority 2017). The investigation following the 2018 *L. monocytogenes* outbreak attributed to rockmelons concluded that site hygiene was generally adequate but extreme weather events, including dust storms and heavy rainfall, preceded the outbreak (NSW DPI 2018). The report concluded that the extreme weather events (heavy rainfall in December prior to harvest, followed by dust storms) could have been a significant contributing factor to the outbreak due to a potential increase of organic load and *L. monocytogenes* on the surface of rockmelons before harvest. The outbreak report stated that the sanitising step may not have been sufficient to remove all *L. monocytogenes* resulting in levels high enough to cause illness in the immunocompromised.

While the outbreak data suggest that few outbreaks related to horticulture produce are reported, there are limitations to outbreak data. Nearly half of the foodborne outbreaks identified in Australia have an unknown associated food vehicle (Astridge et al. 2011). Sources of foodborne illness are generally determined through epidemiological and/or microbiological associations in outbreak investigations. Critical in this process is the ability to identify an outbreak through the existing surveillance system to enable an investigation to then proceed. There are however challenges associated with identifying and attributing illness to a particular food, and include:

- Food recall biases when gathering food consumption histories (compounded by pathogens with long incubation periods, e.g. HAV)
- Time delays in recognition or notification of an outbreak, including:
  - the time taken for infected persons to seek medical treatment
  - obtaining stool samples
  - laboratory confirmation of the presence of pathogenic organisms
  - notification to public health authorities, and
  - identification and subsequent investigation of the outbreak
- Inability to trace food products to their source
- Reluctance of individuals to participate in investigations
- Long exposure windows for specific pathogens (e.g. *L. monocytogenes*)
- Inability to obtain representative food samples for analysis
- A lack of precision in, or suitable methods for, sample analysis and pathogen identification
- Immune status of the exposed population
- Food attribution in dishes with multiple food items
- The potential for variation in categorising features of outbreaks depending on investigator interpretation and circumstances.

Therefore, it is important to recognise that outbreak data are likely to only represent a small proportion of actual cases of foodborne illness, due to the reasons given above and as many people do not always seek medical attention for mild forms of gastroenteritis, medical practitioners do not always collect specimens for analysis, and not all foodborne illnesses require notification to health authorities[5]. Furthermore, some national data regarding the number of people affected was unavailable for more recent outbreaks.

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[5] A list of Australian national notifiable diseases is available at:
https://www1.health.gov.au/internet/main/publishing.nsf/Content/cdna-casedefinitions.htm#s

Table 1. Summary of Australian outbreaks associated with fresh and minimally processed horticultural produce

No	Commodity	Pathogen	Year	Imported or domestic	No. of cases	No. of deaths	Potential contributing factors	References
1	Rockmelon	*Salmonella* Saintpaul	2006	Domestic	100	0	Use of untreated or inadequately treated water, incorrect use of chemical disinfectants, temperature differential between fruit and wash water	(Munnoch et al. 2009)
2	Papaya	*Salmonella* Litchfield	2006	Domestic	26	0	Use of untreated river water, incorrect use of chemical disinfectants	(Gibbs et al. 2009)
3	Baby corn	*Shigella sonnei*	2007	Imported	12	0	Poor sanitation	(Lewis et al. 2009)
4	Tomato semi-dried	HAV	2009	Imported	563	0	Not reported	(Donnan et al. 2012)
5	Rockmelon and/or honeydew melon	*L. monocytogenes*	2010	Domestic	9	2	Not reported	(OzFoodNet 2010)
6	Fruit	NoV	2011	Domestic	15	0	Not reported	(OzFoodNet 2015)
7	Leafy vegetables, salads	*Salmonella* Anatum	2012	Domestic	15	0	Contaminated raw product, inadequate cleaning of equipment	(OzFoodNet 2015)
8	Salad	*Salmonella* Mississippi	2013	Domestic	36	0	Not reported	(OzFoodNet 2021b)
9	Leafy greens	*E. coli*	2014	Domestic	3	0	Cross-contamination from raw ingredients, ingestion of contaminated raw products	(OzFoodNet 2021b)
10	Fruit salad	NoV	2014	Domestic	9	0	Not reported	(OzFoodNet 2021b)
11	Salad	NoV	2014	Domestic	21	0	Food handler contamination, person-to-food-to-person transmission	(OzFoodNet 2021b)
12	Berries, mixed, frozen	HAV	2015	Imported	35	0	Ingestion of contaminated raw products	(OzFoodNet 2021b)
13	Salad	NoV	2015	Domestic	9	0	Person-to-food-to-person transmission	(OzFoodNet 2021b)
14	Mung bean sprouts	*Salmonella* Saintpaul	2016	Domestic	419	0	Ingestion of contaminated raw products	(OzFoodNet 2021a)
15	Leafy salad product, bagged	*Salmonella* Anatum	2016	Domestic	311	0	Ingestion of contaminated raw products	(OzFoodNet 2021a)

No	Commodity	Pathogen	Year	Imported or domestic	No. of cases	No. of deaths	Potential contributing factors	References
16	Rockmelon	*Salmonella* Hvittingfoss	2016	Domestic	144	1	Ingestion of contaminated raw products, inadequate monitoring and application of sanitiser, poor general facility hygiene	(NSW OzFoodNet 2017; OzFoodNet 2021a)
17	Berries, mixed, frozen	HAV	2017	Imported	4	0	Not reported	(DHHS Victoria 2017; SA Health 2017)
18	Rockmelon	*L. monocytogenes*	2018	Domestic	22	8	Existing sanitation processes were not adapted to account for extreme weather events	(NSW OzFoodNet 2018a)
19	Pomegranate arils, frozen	HAV	2018	Imported	30	1	Not reported	(NSW OzFoodNet 2018b)

4.2 Australian recalls of fresh horticultural produce due to microbial contamination

Food recalls are performed to remove unsafe food from the marketplace to protect public health and safety. Horticulture produce has been recalled in Australia due to contamination with pathogenic microorganisms, and this provides further evidence of the ability of pathogens to contaminate fresh produce though they are not always associated with outbreaks (Table 2).

The primary causes of fresh and minimally processed horticultural produce-related recalls were *Salmonella* spp. and *L. monocytogenes.* Other microbial pathogens associated with these recalls were *E. coli* and HAV. The main commodity sectors that were recalled were sprouts and mixed vegetables. The other sectors with multiple recalls were leafy vegetables, berries and melons

Table 2. Summary of Australian recalls associated with fresh and minimally processed horticultural produce (2011-2020)

Commodity	Microbial contaminant	Year	Imported or domestic	Associated with an Australian outbreak[6]
Leafy vegetables				
Pre-packaged salad leaves	*Salmonella*	2016	Domestic	Yes, outbreak no. 3
Loose baby spinach and mesculin lettuce	*Salmonella*	2016	Domestic	Yes, outbreak no. 3
Various leafy vegetables	*Salmonella*	2020	Domestic	No
Sprouts				
Sprouts (various)	*E. coli*	2011	Domestic	No
Alfalfa sprouts	*E. coli*	2012	Domestic	No
Mung Bean sprouts	*E. coli*	2012	Domestic	No
Sprouts salad	*Salmonella*	2014	Domestic	No
Mung Bean sprouts	*Salmonella* Saintpaul	2016	Domestic	Yes, outbreak no. 4
Mung Bean sprouts	*Salmonella*	2016	Domestic	No
Sprouts (various)	*Salmonella*	2018	Domestic	No
Alfalfa sprouts	*Salmonella*	2018	Domestic	No
Other/mixed vegetables				
Frozen carrot, sweetcorn and peas	*L. monocytogenes*	2018	Imported	No (associated with international outbreak)
Frozen mixed vegetables	*L. monocytogenes*	2018	Imported	No (associated with international outbreak)
Frozen mixed vegetables	*L. monocytogenes*	2018	Imported	No (associated with international outbreak)
Frozen peas and corn	*L. monocytogenes*	2018	Imported	No (associated with international outbreak)
Frozen mixed vegetables	*L. monocytogenes*	2018	Imported	No (associated with international outbreak)
Multiple frozen vegetable products	*L. monocytogenes*	2018	Imported	No (associated with international outbreak)

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[6] Refers to outbreaks listed in Table 1.

Commodity	Microbial contaminant	Year	Imported or domestic	Associated with an Australian outbreak[7]
Melons				
Rockmelon	*L. monocytogenes*	2018	Domestic	Yes, outbreak no. 18
Rockmelon	*Salmonella*	2016	Domestic	Yes, outbreak no. 16
Berries				
Frozen berries	HAV	2017	Imported	Yes, outbreak no. 17
Frozen berries (mixed and raspberries)	HAV	2015	Imported	Yes, outbreak no. 12
Frozen berries (mixed)	HAV	2015	Imported	Yes, outbreak no. 12
Other fruit				
Frozen pomegranates	HAV	2018	Imported	Yes, outbreak no. 19

4.3 International outbreaks of foodborne illness associated with fresh horticultural produce

A search for reports of international horticultural produce-associated outbreaks of foodborne illness from 2011-2019 was conducted using the EBSCO search engine to capture relevant studies from selected databases. Initial search results were subjected to two filtering steps, with articles describing 39 outbreaks meeting the search criteria (included in Appendix 2).

Outbreaks were most commonly caused by contamination with viruses (HAV and NoV), bacterial pathogens (particularly *Salmonella* spp., enterohaemorrhagic *E. coli* and *L. monocytogenes*) and enteric parasites (e.g. *Cyclospora cayetanensis*).

Commodity sectors most often associated with outbreaks were leafy vegetables (lettuces, bagged salads), berries, sprouts and melons.

Typically, these reports did not include robust analyses of the causes of the outbreaks—the specific production and processing practices that caused the contamination, or the relative contribution of potential sources of contamination. In the studies that did provide such evidence, the use of poor quality water for irrigation or application of crop protection chemicals; direct faecal contamination of produce growing in the field; and defects in facilities, hygiene, sanitation and process controls on farm, in processing facilities and along the supply chain were identified. These factors point to failures to implement, monitor and correct defects in GAP on farm and GHP postharvest.

4.4 Prevalence of pathogens in fresh horticultural produce

A search for scientific publications for the prevalence of hazards in horticultural produce from 2011-2019 was conducted using the EBSCO search engine to capture relevant studies from selected databases (Appendix 1). The products and relevant hazards are those described in Section 3. *Salmonella* spp. and *L. monocytogenes* prevalence data was extracted for the leafy vegetables and melon proxy groups. STEC prevalence data was identified for the leafy vegetables and berry proxy groups. Prevalence data for the two viruses—NoV and HAV— was extracted for the berry proxy group only. Initial search results were reviewed and assessed for relevance. Selected papers were then analysed and relevant information extracted and compiled (Appendix 3).

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[7] Refers to outbreaks listed in Table 1.

The *meta* library (Balduzzi et al. 2019) in R (R Core Team 2019) was used to determine a weighted prevalence estimate summary statistic using a random effect meta-analysis method. The results of the analysis for the prevalence of pathogens in samples collected in food businesses for each of the eight commodities (lettuce, spinach, parsley, rockmelon, watermelon, raspberry, blueberry and strawberry) are summarised in Table 3. Overall the prevalence of pathogens in each of the commodities ranged from 0.05% for HAV in raspberries to 2.9% for STEC in parsley.

Table 3. Mean percentage (%) of positive samples for pathogens in horticulture commodities

Commodity	*Salmonella*	STEC	*L. monocytogenes*	NoV	HAV
Leafy vegetables					
Lettuce	0.2% (33)	0.5% (9)	0.5% (15)	n.a.	n.a.
Spinach	0.06% (13)	0.6% (5)	1.7% (15)	n.a.	n.a.
Parsley	1.4% (4)	2.9% (3)	n.d.	n.a.	n.a.
Berries					
Raspberry	n.a.	n.d.	n.a.	2.6% (12)	0.05% (4)
Blueberry	n.a.	n.d.	n.a.	2.4% (5)	n.d.
Strawberry	n.a.	n.d.	n.a.	1.4% (8)	0.3% (4)
Melons					
Rockmelon	0.7% (6)	n.a.	0.7% (4)	n.a.	n.a.
Watermelon	n.d.	n.a.	n.d.	n.a.	n.a.

n.a. – not applicable; n.d. – no data; values in brackets are the number of surveys.

In interpreting the prevalence summary statistics in Table 3 it is important to note both the number of studies for each commodity:pathogen combination, the number of samples in each study and the heterogeneity between studies. For *Salmonella* spp. in lettuce, the commodity group with the highest number of prevalence studies, the estimated prevalence is 0.2% based on 33 individual studies. The most number of samples was from a US survey with 19244 results (Reddy et al. 2016) and the least, a survey by (Vital et al. 2014) with only 10 samples. *Salmonella* spp. prevalence for individual studies ranges from 0% (multiple studies) to 32% (Abatcha et al. 2018). The large variability between individual studies highlights potential for contamination of horticulture products during primary production and processing.

For this analysis, the literature for STEC-type prevalence studies was split in two groups: the culture based methods specific for *E. coli* O157:H7 and the predominately molecular based methods (e.g. PCR) for the presence of Shiga toxin genes (stx_1 and stx_2) in *E. coli* isolates. *E. coli* O157:H7 is one of many *E. coli* types that make up the STEC group which are capable of causing human illness. Reporting *E. coli* O157:H7 would therefore underestimate the prevalence of this important pathogen. Therefore, the results reported in Table 3 are for the STEC group using the PCR prevalence results. No prevalence data was identified for STEC in any of the berry types.

Overall, nearly 70% (90/130) of the identified prevalence studies included either lettuce or spinach. For all other commodities only NoV in raspberries had more than ten prevalence studies. Despite the difference in studies the statistical analysis does show that each of the bacterial and viral pathogens have been identified in the horticulture products.

5   Overview of commodity production systems

A summary of Australian production systems for the in-scope commodities is provided below.

5.1   Leafy vegetables primary production and processing

Australia produces a diverse range of leafy vegetables, supported by consumer demand for choice and convenience. Dense head lettuces include cos, iceberg and oak, and a variety of leafy salad vegetables including baby spinach and rocket. Leafy herbs include basil, chives, coriander, mint and parsley amongst others.

Leafy vegetables are produced in all Australian states, enabling year-round production and availability. In 2017/18 the annual production of leafy vegetables in Australia was 315,890 tonnes (Hort Innovation 2019c). Head lettuce is produced in the highest total volume. Leafy salad vegetables account for the highest total value.

In Australia there is estimated to be 1,000-2,000 leafy vegetable businesses, the majority of which are small (5-19 employees) or micro (up to 4 employees) sized businesses.

Most produce is distributed domestically. Washed, bagged, and ready-to-eat products are popular (Woodward 2018). In 2017/18, 70% of Australian households purchased head lettuce, 55% purchased leafy salad vegetables and 38% purchased leafy herbs (Hort Innovation 2019c).

A relatively small volume of head lettuce and leafy salad vegetables are exported[8]; minimal volumes are imported. There is no import or export of leafy herbs.

The on-farm activities and inputs for leafy vegetables are summarised in Figure 2.

Leafy vegetables are grown from seeds or seedlings purchased from seed suppliers and commercial nurseries. Some growers raise their own seedlings to transplant. Field preparation may include ploughing, forming soil beds, applying fertiliser and correcting soil pH. Seeds are sown directly into soil by hand or using a seed machine. Seedlings are planted by hand or with transplanting machines.

Side dressings of fertiliser, often heat-treated manure, are applied during growth. Water is supplied through drip, furrow or overhead irrigation. Crops are monitored and controlled for pests and weeds.

Leafy vegetables are generally harvested by hand, although mechanical harvesters are available for lettuces and baby spinach. Harvested products are collected in containers or crates. Some head lettuces are field trimmed, cored, packed into waxed cartons and palletised to be sent directly to market as a ready-to-wash product. Containers and crates are palletised and transported to packing sheds; refrigerated vehicles are used for long distances.

The activities and inputs at packing sheds for head lettuce and leafy salad vegetables (summarised in Figure 3) differ slightly from leafy herbs (summarised in Figure 4).

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[8] Fresh fruit and vegetables are prescribed products. The Export Control legislation defines the compliance requirements for export businesses, refer to the Department of Agriculture, Water and the Environment website.

Harvested head lettuce and leafy salad vegetables in crates, containers, or cartons are immediately cooled using forced air, vacuum or hydrovac cooling. They are then washed, sorted, trimmed and sanitised. This is followed by drying with air-drying systems or commercial spinners. Product is packed into plastic bags or sleeves, or bulk-packed into cartons or crates. Packed products are then palletised, shrink wrapped and stored in coolrooms before distribution.

Leafy herbs are also immediately cooled upon receipt. They are not commonly treated with sanitisers but are regularly sprayed with or dipped in water to prevent moisture loss. Leafy herbs are bunched, packed into plastic bags, punnets or plastic film, and then placed into waxed cartons. Unpackaged herbs may also be bulk-packed into styrofoam boxes or waxed cartons.

Some leafy vegetables (washed or unwashed) are sent for further processing. Processor activities are summarised in Figure 5. Most activities in processing factories use mechanical or robotic equipment. Products are washed, trimmed and sanitised. They are then dried in air-drying systems or spinners and packed into plastic bags. For some products (e.g. ready-to-eat leafy salads) the plastic bag is gas flushed (modified atmosphere packaging) before sealing. Packed products are palletised and stored in coolrooms before distribution.

From the packing shed, ready-to-wash or ready-to-eat leafy vegetables are transported under refrigeration to distribution centres, wholesale markets, or processors. Processors send processed products to distribution centres. The distribution centres dispatch products to retail stores, and wholesale markets to food service outlets. Consumers can purchase products directly from wholesale markets or retail outlets.
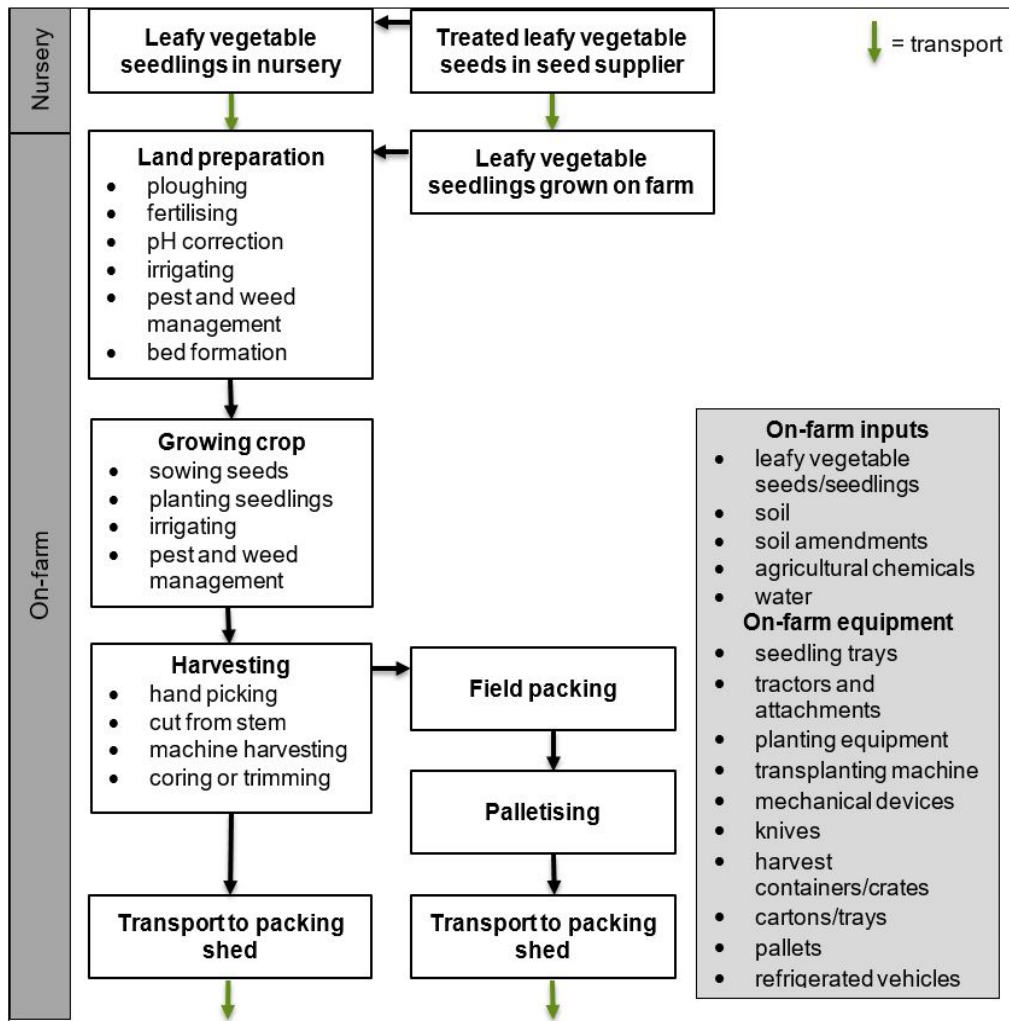
Figure 2. Generalised on-farm activities and inputs for leafy vegetable production in Australia. Diagram based on information in IFPA et al. (2006)
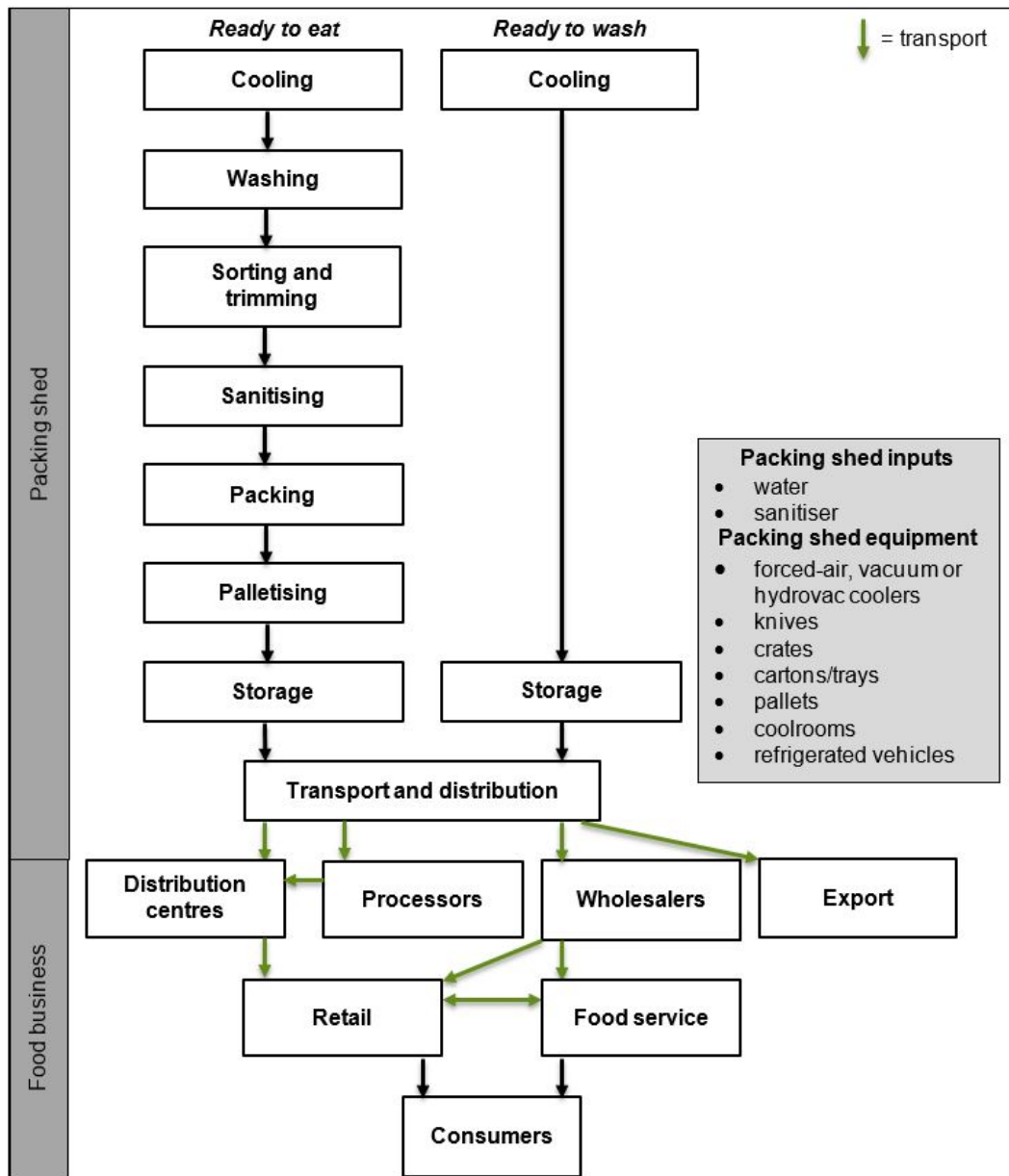
Figure 3. Generalised packing shed activities and inputs for head lettuce and leafy salad vegetable production in Australia. Diagram based on information in Lockrey et al. (2019).
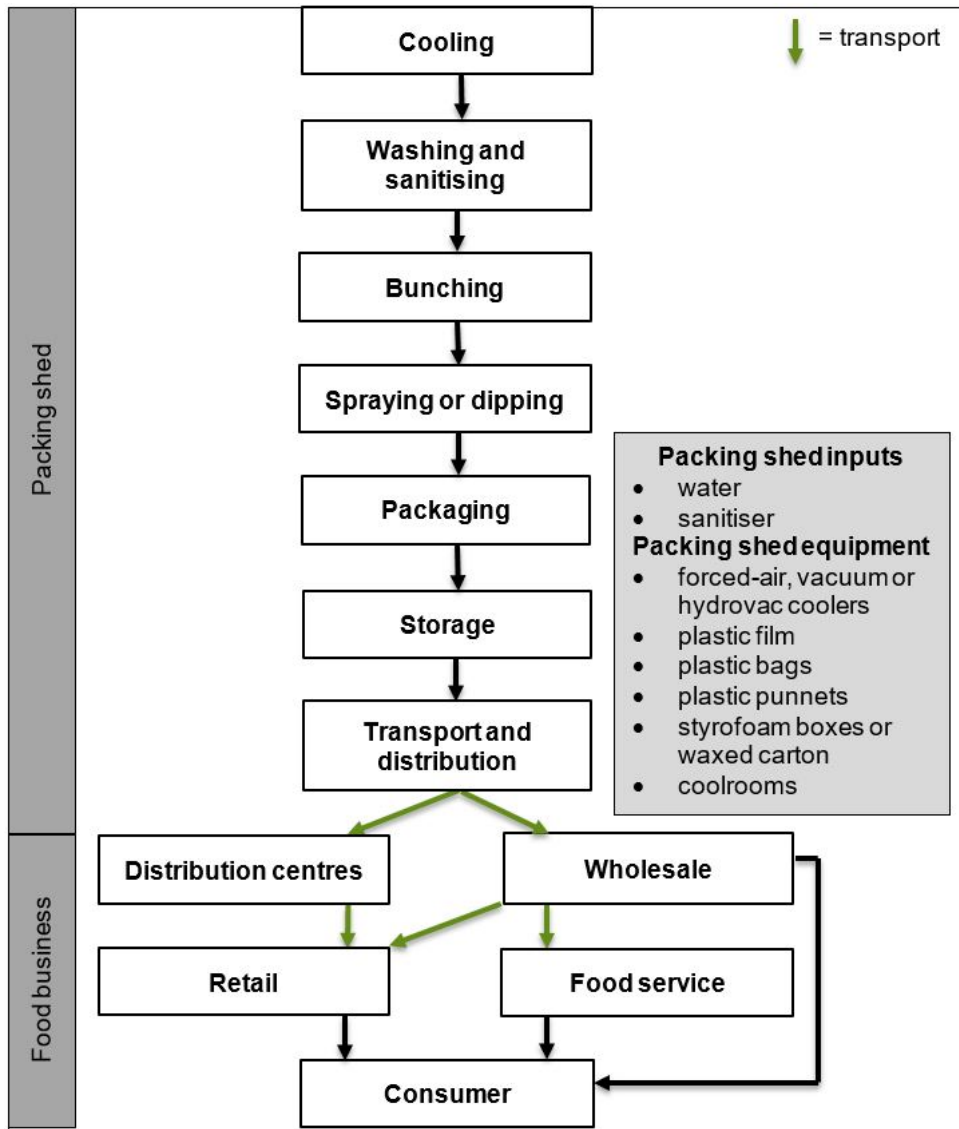
Figure 4. Generalised packing shed activities and inputs for leafy herb production in Australia. Diagram based on information in Lopresti and Tomkins (1997).
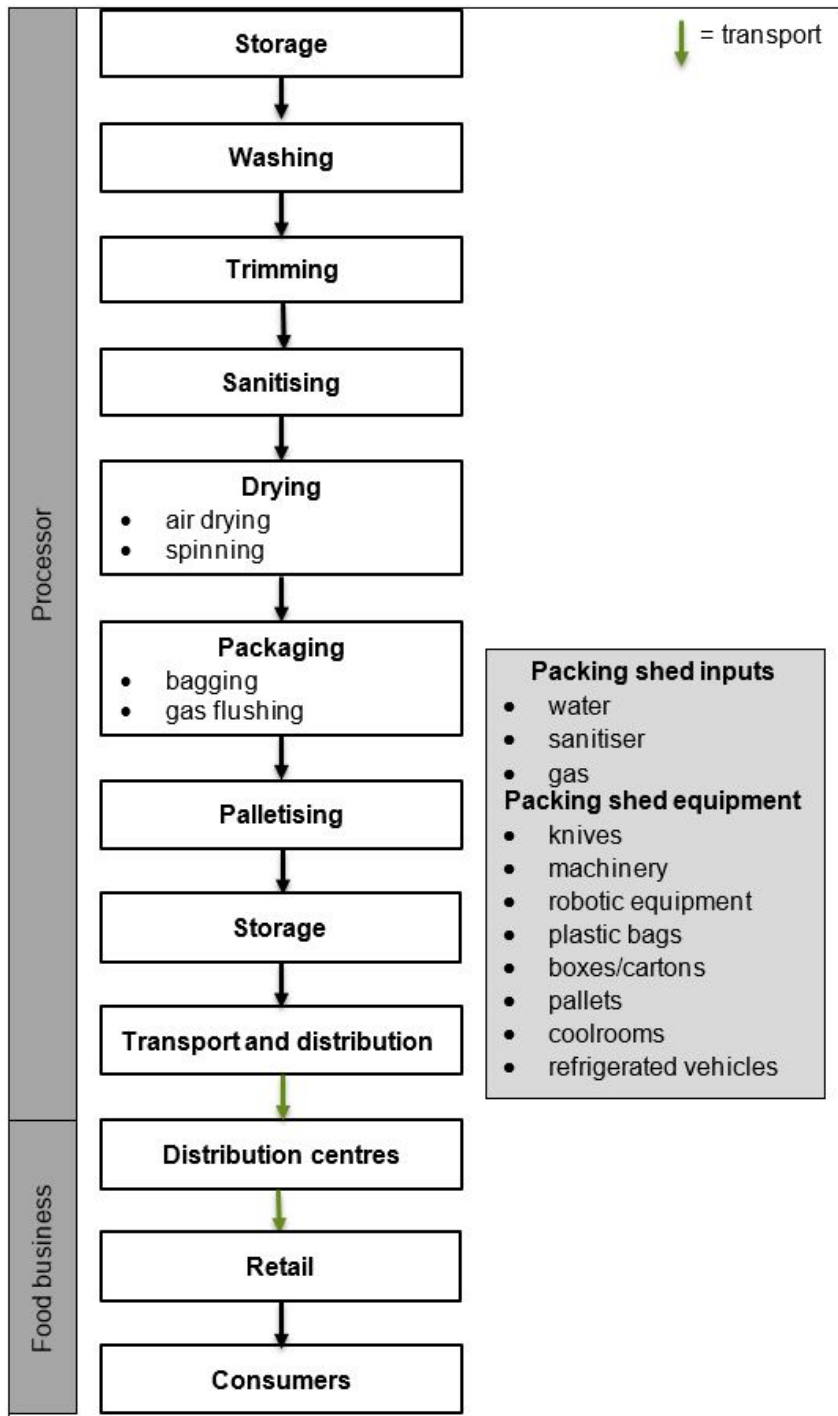
Figure 5. Generalised processor activities and inputs for leafy vegetable production in Australia. Diagram based on information in Lockrey et al. (2019).

5.2 Berry primary production and processing

The four major berries grown in Australia are blueberries, blackberries, raspberries and strawberries. Multiple varieties of each are produced. Boysenberry, loganberry, silvanberry, and youngberry are rubus hybrid cultivars grown in relatively minimal quantities.

Berries are grown in most Australian states, which enables year-round production. In 2017/18 the annual production of berries in Australia was 116,585 tonnes (Hort Innovation 2019b). Strawberry production accounts for 80% of total production. In Australia there is estimated to be 500-600 berries businesses. About half of these are medium sized businesses (20-199 employees), with most other businesses being small (5-19 employees) or micro (up to 4 employees) sized businesses.

The majority of Australian berry production enters the domestic market as fresh ready-to-eat product. Some 'pick your own' enterprises allow customers to harvest their own berries for purchase. A small proportion of production is exported as fresh or frozen berries[9]. There is minimal import of berries.

The main activities and inputs involved in berry production and supply are shown in Figure 6. As berries are a soft delicate fruit and are susceptibility to fungal decay, berries destined for the fresh market do not undergo a washing and sanitising step.

The overall production of the different berry types is similar but there are a few differences, as described below.

5.2.1 Strawberries

In Australia most strawberries are grown on the ground in open fields. Some strawberries are grown hydroponically, either in the field or in glasshouses.

Strawberry plants propagate by producing runners. Strawberry crops are grown from these strawberry runners, predominantly sourced from Victoria and Queensland. Commercial nurseries propagate runners under certification schemes. Field preparation includes ploughing soil into raised beds, which are covered with plastic (polythene) sheeting. Fumigants, herbicide, compost and other soil additives (e.g. lime to correct soil pH) may be used. Runners are manually or mechanically planted into the beds and in some cases are then covered with protective polythene tunnels (cloches).

Water is generally supplied by trickle or sprinkler irrigation. Strawberries are harvested manually into trays that are taken to on-farm packing sheds. Refrigerated vehicles are used for long distance transport to packing/processing sheds.

In the packing shed, harvested strawberries are quickly cooled by forced-air cooling systems. They are hand packed into punnets, stacked into cartons and stored in coolrooms before distribution. Fruit that is second or third grade may be packaged and frozen. Strawberries are sometimes sold directly to consumers from farm packing sheds (farm gate sales).

The majority of strawberries are sold domestically. Fresh product is transported under refrigeration to retail distribution centres and wholesalers for supply to food service and direct to consumers. Second and third grade fruit may be sent for further processing.

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[9] Fresh fruit and vegetables are prescribed products. The Export Control legislation defines the compliance requirements for export businesses, refer to the Department of Agriculture, Water and the Environment website.

5.2.2   Blueberries

In Australia, blueberries are traditionally grown in open fields (orchards), however, more recently they have also been grown under plastic tunnels. The general flow of activities involved in blueberry production and processing is included in Figure 6.

Blueberry plants are propagated from hardwood cuttings of disease-free mother plants and grown in open fields. Plants may be grown low to the ground (lowbush varieties) or up to three metres tall (highbush varieties). Field preparation may include the use of fertilisers, compost or other soil additives.

For field grown blueberries, plants are hand-planted into soil with compost or mulch applied. Netting may be used to protect plants from birds and also other wildlife. Water is applied to plants by trickle or micro-jet irrigation. Fertilisers can be applied directly to the base of plants, alongside them or through irrigation systems.

For blueberries grown under plastic tunnels, plants are hand-planted into substrate. The plastic tunnels protect the crop from birds and other wildlife. Water and nutrients are applied into the substrate.

The fruit are harvested mechanically or by hand, and transported to on-farm packing sheds. Refrigerated vehicles are used for long distance transport to packing sheds. Following harvest, blueberries are cooled using forced-air coolers. They are hand sorted and hand packed into retail punnets. Few operations have adopted machine packing. Punnets are packed into cardboard cartons and stored in coolrooms for distribution.

Most blueberries are sold domestically. Fruit is transported under refrigeration to retail distribution centres and wholesalers who supply to food service outlets and directly to consumers. Blueberries are also exported overseas. Some fruit is also sold on to food manufacturers/processors.

5.2.3   Raspberries

In Australia, raspberries are either grown in largescale open field plantations or under plastic tunnels. Raspberry plants are tall erect shrubs with woody stems (canes) and grow best in colder climates. Production activities for raspberries are based primarily on raspberry and blackberry production (Figure 6).

Raspberry plants are sourced through the Rubus Multiplication Scheme managed by Raspberries and Blackberries Australia. Growers may also propagate their own plants from established stock.

Raspberry plants are hand-planted and are positioned on trellises with wire or twine used to train plants to grow along them.

For field grown raspberries, weed control coverings used include polyethylene sheeting and polythene or natural mulch. Raspberry plants are not usually grown through polythene sheeting. Trickle or micro-jet irrigation systems are used and fertiliser may also be applied. The entire plantation may be covered with netting to protect it from birds and also other wildlife. Shade clothes are often also erected for sun protection.

For raspberries grown under plastic tunnels, plants are hand-planted into substrate. The plastic tunnels protect the crop from birds and other wildlife. Water and nutrients are applied into the substrate.

Most raspberry operations hand-pick and field-grade ripe berries, which are packed directly into retail punnets. Large operations may use mechanical harvesters. Produce is then taken to packing sheds prior to distribution. Refrigerated vehicles are used for long distance transport to packing sheds.

Following harvest, raspberries are cooled using forced-air coolers. Punnets are packed into cardboard cartons and stored in coolrooms before distribution. Berries may also be frozen before distribution.

Raspberries are distributed in refrigerated vehicles. Hand-picked berries are distributed to the fresh market. Most are taken to retail distribution centres, and to wholesalers who supply food service outlets and directly to consumers. Mechanically harvested berries are often sent for further processing.

Figure 6. Generalised flow of activities and inputs for berry production in Australia. Diagram based on information in DPIRD WA (2016a, 2016c, 2016d)

5.3　Melon primary production and processing

Australian melon production covers a wide range of geographical and climatic conditions, enabling year-round supply (Hort Innovation 2019a). The estimated growing area is 6,000-8,000 hectares (Hort Innovation 2018). In 2017/18 the annual production of melons in Australia was 215,519 tonnes (Hort Innovation 2019b). In Australia there is estimated to be 250-300 melon businesses. The majority of these are small sized (5-19 employees) and about a third are medium sized (20-199 employees) businesses.

Watermelon is the most common melon grown in Australia, accounting for nearly 80% of production. Most melons produced in Australia are sold on the domestic market as either fresh whole or fresh cut fruit. Some whole fruit is also exported[10] but only minimal amounts are imported.

Melons are a ground vine, broad-acre crop that requires large areas of land for production. All melon species in Australia are grown under field production systems involving similar practices. Figure 7 outlines on-farm and packing shed activities and inputs for melons. Key steps in the supply chain are described below.

Melon seeds or seedlings are sourced from seed suppliers or commercial nurseries. Before planting, field preparation may include use of fumigants, herbicides, fertilisers or soil additives. Planting of seeds or seedlings can be done manually or mechanically, either directly into soil or through polythene mulch. Plants are watered through trickle, furrow or overhead irrigation. Fertilisers are applied through side dressings or irrigation. Vines are trained during growth onto beds so that developing fruit remains on mulch. The fruit may be placed on plastic cups or pads, or hand-turned by workers, to prevent development of ground spot blemishes.

Melons are manually harvested by cutting or twisting fruit off the vine. Picked melons are placed into harvest bins directly or by conveyor belts, then transported to packing sheds. Refrigerated vehicles are used for long distance transport to processing/packing sheds.

On arrival at the packing shed, melons may be cooled before further processing. Muskmelons are then either 'dry dumped' or 'wet dumped'. Dry dumping involves placing fruit on conveyer belts that move through a water spray to remove soil, followed by sanitiser spray to reduce the level of surface microorganisms. Wet dumping involves immersing melons in large tanks filled with water containing sanitiser, then moving fruit along belts to be washed, scrubbed and further sanitised. They are then sprayed with or dipped in fungicide to prevent fruit rot. Following these treatments, the fruit is air-dried sometimes with the assistance of fans.

Melons are then sorted to remove undesirable fruit (e.g. overripe, misshapen) and to separate by colour and maturity. They are graded by size, packed into cartons or trays, palletised and stored in coolrooms. Some melons, particularly honeydew melons, may be ripened using ethylene gas before placing in cold storage. Watermelons generally do not receive any treatment and are often packed in field.

The distribution of melons can be complex, involving multiple businesses and business types. Melons are transported refrigerated to wholesale markets, distribution centres, processors, retailers or to the export market.

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[10] Fresh fruit and vegetables are prescribed products. The Export Control legislation defines the compliance requirements for export businesses, refer to the Department of Agriculture, Water and the Environment website.

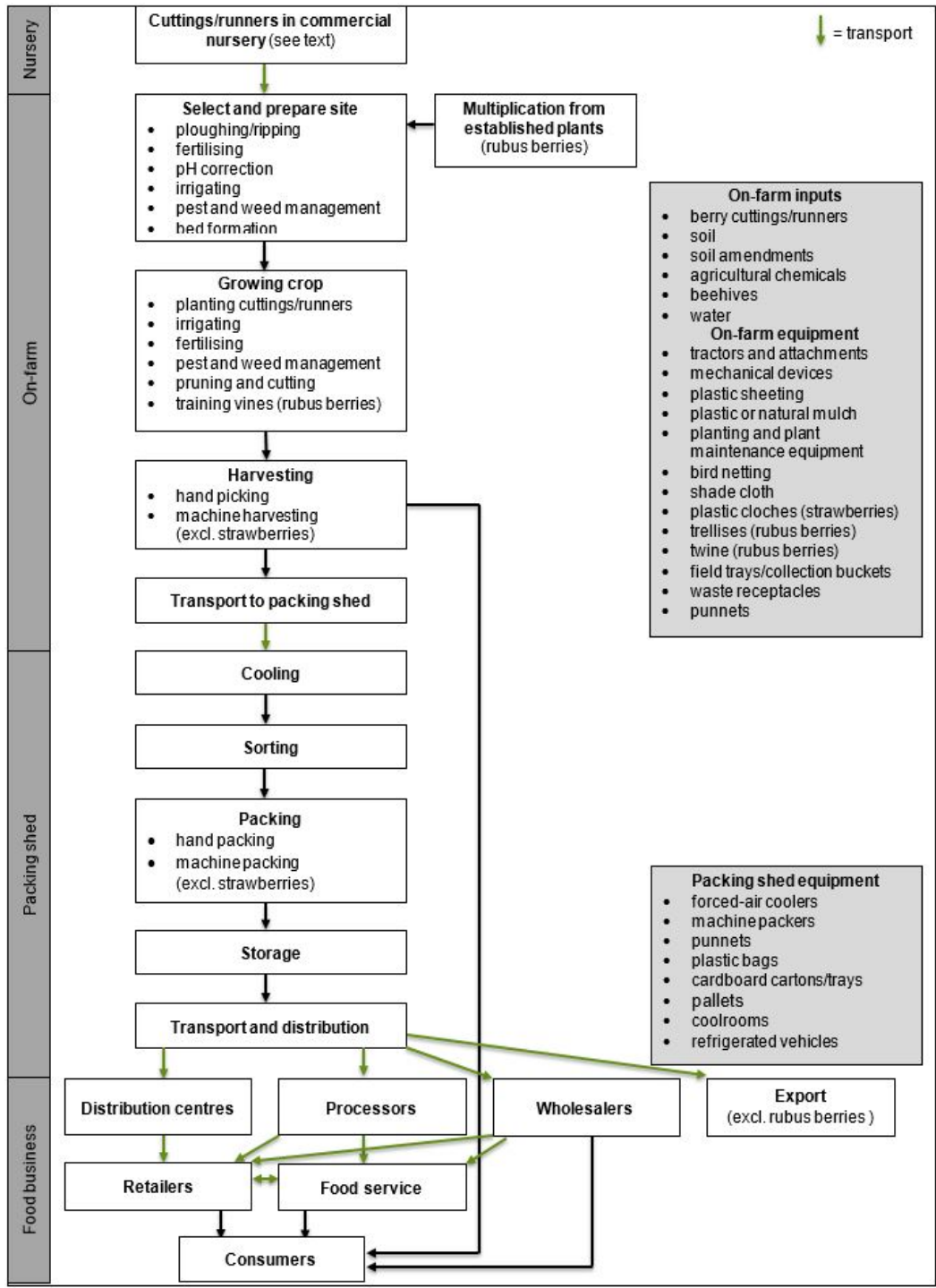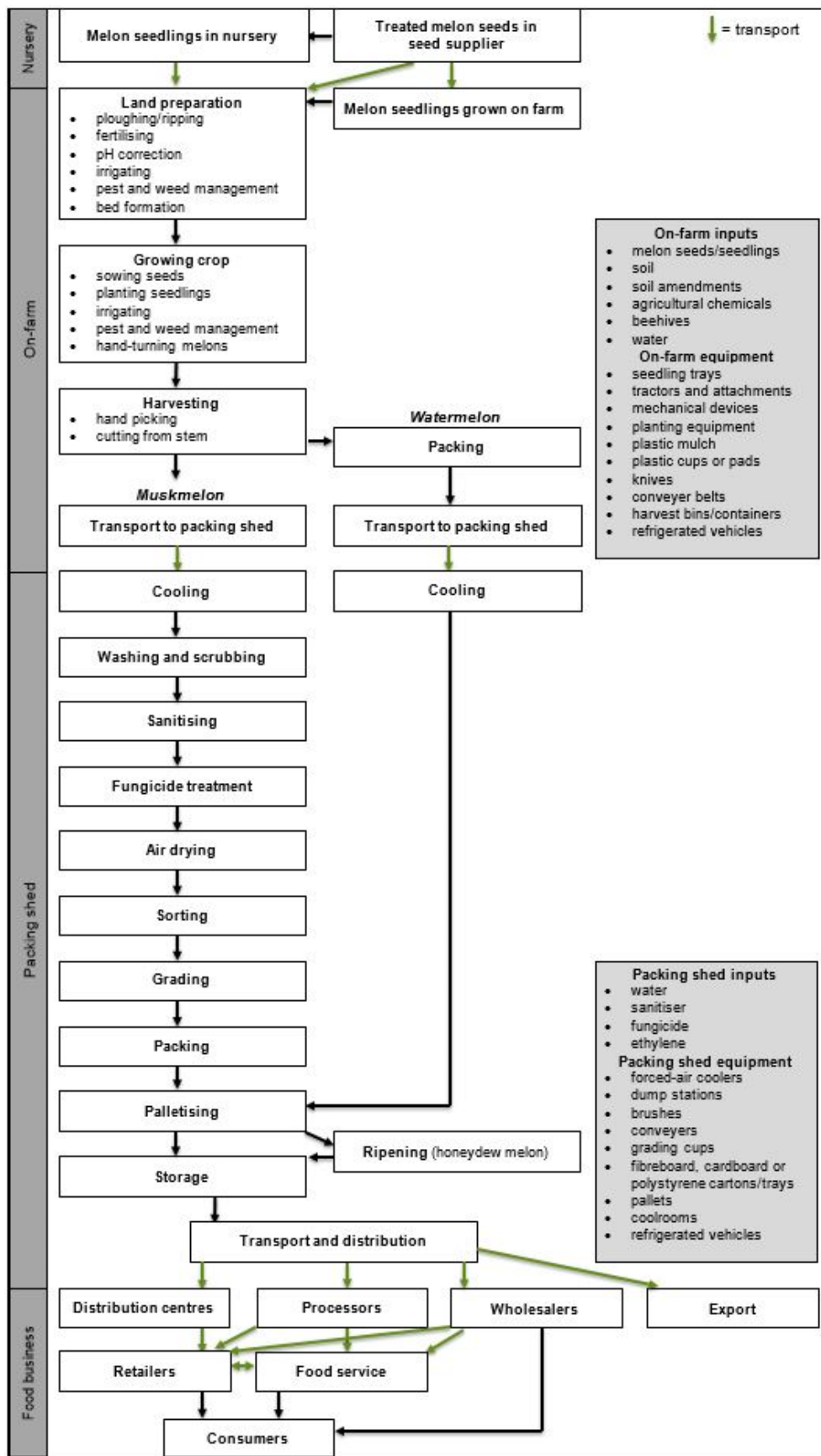Figure 7. Generalised flow of activities and inputs for melon production in Australia. Diagram based on information in NSW DPI (2019) and Wright et al. (1997)

6   Commodity consumption patterns

The proportion of the population that consume a particular food, the amount consumed, and the frequency of consumption are important considerations for exposure assessments in microbiological risk assessments. The data from the 2011-12 Australian National Nutrition and Physical Activity Survey (NNPAS)[11], have been summarised to provide an indication of the consuming populations and amount of the in-scope commodities consumed. However, a full exposure assessment for the large variety of primary production pathways, pathogens, and commodity combinations considered was beyond the scope of this assessment.

The data summarised represent the recall of a single day of consumption and, therefore, would not capture consumers who ate the commodities on a different day during the week or infrequently throughout the year. The number of respondents in each age group were 2,412 (2-16 years), 8,635 (16-69 years), and 1,107 (70+ years). Results are provided as the percentage of respondents reporting to consume the commodity, and the mean grams per day reported by respondents who consumed the commodity.

The consumption data for the commodity sectors and individual commodities is detailed in Appendix 4. However, due to the low number of respondents for some individual commodities, it is not appropriate to use the data to make assumptions of differences in risk between the individual commodities.

The survey results indicate that leafy vegetables as a sector were eaten by a larger proportion of respondents as mixed dishes[12] (that may or may not have been cooked reducing the likelihood for pathogens to be present at consumption), and a lower proportion ate leafy vegetables as is, with little or no further processing. The average amount consumed was generally less than 30g/day.

Berries were consumed by a larger proportion of people at lower amounts (approximately 5g/day) as part of a mixed dish that may or may not be cooked, and a lower proportion ate berries as a fresh piece of fruit, generally consuming 72g/day. Very few respondents reported consuming berries as fruit juice.

Melons were eaten by a similar proportion of people as mixed dishes or as a fresh piece of fruit. When eaten as is, approximately 200g/day was consumed by respondents in all age groups.

It should be noted that these data only represent a small proportion of the Australian population and it is likely that consumer habits may have changed significantly in the past decade.

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[11] 2011-12 Australian National Nutrition and Physical Activity Survey (NNPAS), a 1 day 24-hour recall survey of 12,153 respondents aged 2 years and above (with 64% of respondents (n=7735) undertaking a second 24-hour recall on a second non-consecutive day). The results in this report are derived using day 1 of data only. A respondent is counted as a consumer if the food was consumed on day 1 only. These data were weighted during the calculations undertaken in Harvest.

[12] 'Mixed dishes' represent those dishes that may combine multiple foods and may or may not be cooked.

7 Microbial hazard characteristics

Selection of microbial hazards of concern for this assessment was based on consideration of Australian and international data on causes of fruit- and vegetable-associated illness provided to inform the 2008 expert meeting (FAO/WHO 2008a). Australian prevalence and outbreak data since that time were also considered, to ensure that emerging hazards of particular relevance to the Australian production and processing environment were included in this report where appropriate.

FSANZ has previously undertaken detailed hazard characterisations of the in-scope microbial hazards included in this assessment (FSANZ 2018), and summaries of the characterisations are included in Appendices 6-10.

Hepatitis A
Hepatitis A (HAV) belongs to the Picornaviridae family of viruses. It is a small (25–28 nm) non-enveloped icosahedral virus with a single stranded RNA genome. HAV cannot multiply outside the host or grow in food. However the virus can survive in food and still be present at the point of consumption. The virus can also survive in the environment. HAV hosts are primarily humans and HAV infects the liver, with disease characterised by liver inflammation and the development of jaundice. This viral hazard is characterised in Appendix 6.

Listeria monocytogenes
Listeria monocytogenes is a Gram-positive, non-spore forming rod-shaped bacterium that is found throughout the environment. It has been isolated from domestic and wild animals, birds, soil, vegetation, fodder, water and from floors, drains and wet areas of food processing factories. *L. monocytogenes* is a bacterium that causes listeriosis, a disease that can have severe consequences for particular groups of the population. This bacterial hazard is characterised in Appendix 7.

Norovirus
Norovirus (NoV) belongs to the Caliciviridae family of viruses. It is a small (27–40nm) non-enveloped icosahedral virus with a single stranded RNA viral genome. NoV cannot multiply outside the host or grow in food. However the virus can survive in food and still be present at the point of consumption. The virus can also survive in the environment. Infection with NoV generally leads to symptoms of gastroenteritis, although asymptomatic infection can also occur. This viral hazard is characterised in Appendix 8.

***Salmonella* spp.**
Salmonella spp. are Gram-negative, non-spore forming rod-shaped bacteria belonging to the Enterobacteriaceae family. *Salmonella* spp. are carried by a range of domestic and wild animals and birds and have been widely isolated from the environment. *Salmonella* spp. are bacteria that cause salmonellosis, a common form of foodborne illness in humans. Outcomes from exposure to *Salmonella* spp. can range from generally mild symptoms to severe disease and can be fatal. This bacterial hazard is characterised in Appendix 9.

Shiga toxin-producing *E. coli*
E. coli are Gram-negative, rod-shaped bacteria belonging to the Enterobacteriaceae family. *E. coli* form part of the normal gut flora of humans and other warm-blooded animals. Although most *E. coli* are considered harmless, certain strains can cause severe illness, particularly Shiga toxin-producing *E. coli* (STEC).The major animal reservoir of STEC is ruminants, particularly cattle and sheep. This bacterial hazard is characterised in Appendix 10.

8 Commodity characteristics

The survival and growth of pathogens and therefore, the risk associated with pathogens and fresh produce, is influenced not only by the external surrounding environment but also the intrinsic properties of the produce. These include the water activity, pH, and availability of nutrients in the flesh or tissues and also properties of the external surface of the produce.

In this section we limit discussion to a description of the general structural characteristics of the in-scope commodities, structural characteristics that may influence pathogen attachment to the surface of the edible portion of the plant or rind for melons, and the potential for growth of bacterial pathogens (persistence for viral pathogens) on the surface and internal parts of the produce under controlled storage conditions where temperature is the main variable. Sections 9-12 include discussion of environmental and other risk factors that may influence the growth and internalisation of pathogens regarding the specific commodities during primary production.

8.1 Leafy vegetables

Lettuce, parsley and spinach leaves generally have a slightly acidic pH (in the range 5.4-6.8) (US FDA/CFSAN 2003), high water activity and typically contain up to 2% sugars, including fructose and glucose (and sucrose in parsley) (FSANZ 2019). Lettuce (*Lactuca sativa*) are annual or biennial plants grown for consumption of the leaves. There is great diversity in the size, shape, and leaf type. However, lettuce leaves are formed on short stems close to the ground, and the leaves form a dense head or loose rosette. The structure can also be described as a vase. The height of plants is generally less than 80cm. The leaves of lettuce plants can be curly or smooth. Spinach (*Spinacia oleracea*) are annual plants that produce edible leaves. The leaves are produced on a stem that can be 30cm tall, with smaller leaves at the top and larger leaves at the bottom of the stem. Parsley (*Petroselinum crispum*) are annual or biennial herbaceous plants that produce a clusters of compound leaves in the first season that are generally consumed fresh or dried. Leaves can be flat or curly depending on the cultivar and the plant generally grows to heights less than 80cm.

The attachment of pathogens to leafy vegetables is reliant on a complex combination of physical, chemical, and biological factors that may differ by commodity and cultivar. Surface characteristics such as topography, the presence of stomata, cuticular waxes, age of leaves, and surface roughness can influence bacterial attachment. Confocal microscopy shows that the surface of lettuce, spinach and rockmelon have the highest surface roughness compared to apples, tomatoes, and oranges, which had the least surface roughness (Lazouskaya et al. 2016). The antimicrobial activity associated with leaf tissue constituents—such as essential oils, phenols, alkaloids, unsaturated long chain aldehydes, and peptides—may also influence the attachment or growth of pathogens, particularly to damaged or cut leafy vegetables. The phenolic content extracted from leaves of lettuce, spinach, and parsley was reported to be 16.3, 20.6, and 98.7 gallic acid equivalents/g, respectively (Khalil and Frank 2010). Antimicrobial activity has also been attributed to peptides in spinach and to high ferrous sequestering activity in extracts of parsley (Posada-Izquierdo et al. 2016).

Factors influencing the attachment of *Salmonella*, *L. monocytogenes* and *E. coli* have been described for lettuce. *L. monocytogenes* and *E. coli* have been shown to preferentially attach to cut edges rather than to intact surfaces of lettuce (Boyer et al. 2011; Takeuchi et al. 2000). In one study, *Salmonella* Typhimurium attached equally to cut edges and intact surfaces, and the difference in attachment for *E. coli* and *L. monocytogenes* was only small (0.2–0.6 Log), with approximately 5 Log CFU/g attachment reported for all three pathogens (Takeuchi et al. 2000). Furthermore, *L. monocytogenes* has been demonstrated to attach to lettuce following 1 second of exposure to a 5 Log CFU/mL inoculum (Kyere et al. 2019), and similar results are reported for *Salmonella* spp. (Patel and Sharma 2010). Differences in attachment have been reported to depend on the serovar of *Salmonella* and the cultivar of lettuce (e.g. romaine vs iceberg) (Patel and Sharma 2010). The level of attachment of pathogens to lettuce leaves has also been shown to be affected by the presence of stomata, cuticular waxes, age of leaves, and surface roughness (Brandl and Amundson 2008; Ku et al. 2020; Lima et al. 2013).

Salmonella spp., *L. monocytogenes* and *E. coli* have been demonstrated to attach to both cut and whole spinach leaves (Engels et al. 2012; Ijabadeniyi et al. 2011). *Salmonella* spp. and *E. coli* were observed to attach on the surface of spinach, and preferentially attached to stomatal openings and cuticle cracks on the leaves (Neal et al. 2012). Furthermore, both pathogens were observed in the tissue of spinach, indicating that internalisation had occurred (Neal et al. 2012). Studies have identified that vein density and leaf age correlate with reduced recovery of *E. coli* from the surface of spinach leaves (Doan et al. 2020), implying surface roughness leads to increased attachment.

Limited data is available regarding the attachment of *L. monocytogenes*, *E. coli* or *Salmonella* spp. to parsley. However, all pathogens have been demonstrated to attach to both cut and whole parsley leaves (Duffy et al. 2005b; Khalil and Frank 2010; Lang et al. 2004).

The growth potential of bacterial pathogens on the edible portion of leafy greens can differ between intact surfaces, damaged surfaces, and extracts. While damaged leaves can provide access to moisture and nutrients for growth, leaves can also contain antimicrobial substances that can reduce or prevent growth. Moreover, each commodity has a carrying capacity for microorganisms that varies due to factors such as background microbiota and surface characteristics.

The growth potential of pathogens on damaged leaves and in extracts of lettuce, spinach and parsley has been reported in several studies. *E. coli* O157:H7 was unable to grow on damaged parsley or baby romaine lettuce during storage at 8°C for 3 days, but was able to grow by 1.2 Log CFU/leaf on damaged spinach under the same conditions (Khalil and Frank 2010). In that study, growth was observed on damaged parsley and spinach (~2 Log/leaf) held at 12°C for 3 days but, again, not on damaged lettuce. However, extracts of the same commodities all supported the growth of *E. coli* O157:H7 at 12°C for 12h. The authors concluded that leaf extracts are a poor model to simulate growth on damaged tissues. They speculated that the higher growth on damaged spinach leaves—compared to romaine lettuce leaves—may be due to the higher antioxidant activity in spinach reducing tissue oxidation and stimulating growth (Khalil and Frank 2010). In another study, *Salmonella* spp. and *E. coli* were inoculated into extracts of spinach, parsley and iceberg lettuce and held at 8, 10, 16 and 20°C. *Salmonella* and *E. coli* were able to grow in all extracts. However, growth was dependent on the temperature, pathogen, and type of produce (Posada-Izquierdo et al. 2016). The authors identified that spinach was more conducive to bacterial growth than parsley and lettuce, and speculated the difference was due to the different composition of the leaf tissues and the presence of natural antimicrobials. The potential for growth of *L. monocytogenes* on damaged leaves or extracts of the in-scope commodities is poorly documented in the literature.

The potential for growth on intact surfaces of fresh produce was recently reviewed for *L. monocytogenes* (Marik et al. 2019). Whilst growth was reported on the surface of intact spinach at temperatures of 4-20°C in two studies (Omac et al. 2015; Omac et al. 2018), Likotrafiti et al. (2013) reported no growth on intact lettuce at 10, 20, or 30°C), which contrasts with the reported growth on shredded or cut lettuce at these temperatures reported below. However, growth of *Salmonella* spp. and *E. coli* has been reported on intact lettuce leaves when exposed to warmer temperatures and available water on the surface of leaves (Brandl and Amundson 2008). *L. monocytogenes* was not observed to grow on intact parsley at 10, 20, or 30°C over 5 days (Likotrafiti et al. 2013). However, the initial concentration on the parsley was approximately 5.5 Log CFU/g, and this may have exceeded the carrying capacity of the leaves because the high level of *L. monocytogenes* and the presence of other microorganisms may have limited the available nutrients *L. monocytogenes* required for growth (Likotrafiti et al. 2013).

The growth of *Salmonella* spp., *E. coli*, and *L. monocytogenes* has been described for cut lettuce (Koseki and Isobe 2005a, 2005b; Oliveira Elias et al. 2018), and for whole baby spinach leaves (Omac et al. 2015; Omac et al. 2018; Puerta-Gomez et al. 2013). Furthermore, isothermal and non-isothermal growth models have been developed for the three pathogens on leafy vegetables, incorporating data for a variety of leafy greens (McKellar and Delaquis 2011; Mishra et al. 2017b). However, comparing models is difficult due to differences in the types of produce, pathogen strains, treatment of produce (e.g. cut or uncut), level of background microflora, and methods of inoculation.

While the growth of *Salmonella* spp. and *E. coli* are controlled at < 5°C, *L. monocytogenes* is able to grow at refrigeration temperatures. A growth rate of 0.021 Log CFU/h and a lag time of 60.1 hours has been estimated in cut lettuce at 5°C (Koseki and Isobe 2005a). However, at this temperature, reduced maximum population densities have been reported compared to growth at higher temperatures (Koseki and Isobe 2005a). At 10°C, growth rates and lag times have been estimated in cut lettuce for *L. monocytogenes* (0.05 Log CFU/h, 45.6h) (Koseki and Isobe 2005a), *Salmonella* (0.05 Log CFU/h, 24.6h) and *E. coli* (0.02 Log CFU/h, 2.1h) (Oliveira Elias et al. 2018). At 25°C, the predicted time for a 3 Log increase on cut lettuce was less than 20h for all pathogens.

Growth rates and lag times have been estimated at 10°C on spinach for *L. monocytogenes* (0.05 Log CFU/h, 45h; (Omac et al. 2015), which is similar to its growth on lettuce. However, in another study, no lag time was reported for *Salmonella* and *E. coli,* which both grew at 0.01 Log CFU/h in spinach stored at 10°C (Puerta-Gomez et al. 2013). At 30°C, the predicted time for a 3 Log increase on spinach was less than 7h for all pathogens.

Salmonella spp. have been demonstrated to increase by 2-3 Log within 7 days of storage at 25°C, and decrease when stored at 5°C on dip-inoculated parsley bunches (Duffy et al. 2005b). However, growth rates were not reported in the study. Limited declines (~1 Log) and persistence of *Salmonella* and *E. coli* on parsley stored at 4°C for 24 days has been reported (Hsu et al. 2006). However, no other reports of growth potential were identified for parsley.

The ability of pathogens to internalise in edible portions of leafy vegetables appears to be highly variable. Significant gaps exist in the evidence base, with studies principally focussed on lettuce (for all three pathogens) and *E. coli* O157:H7 (for all three crops). No studies report evidence of growth/multiplication of pathogens in intra- or inter-cellular spaces in the edible parts of leafy vegetables. Specific risk factors for pathogen internalisation are discussed in Section 9, and a brief summary is provided below.

Salmonella spp. have been demonstrated to readily internalise in roots and leaves of lettuce varieties at levels up to 2.5 Log/CFU/g, and to remain viable in internal spaces for prolonged periods (Ge et al. 2012; Standing et al. 2013; Zhang et al. 2016). However, there is an absence of data on internalisation of salmonellae in parsley or spinach.

E. coli O157:H7 strains have been shown to readily internalise into the roots of leafy vegetables, but appear to only rarely/sporadically translocate to the leaves (Erickson et al. 2014b; Sharma et al. 2009; Standing et al. 2013). In lettuce, internalised *E. coli* O157:H7 can remain viable for prolonged periods (Standing et al. 2013).

L. monocytogenes inoculated onto seeds of lettuce was found to internally colonise the germinated seedlings and persist until harvest (Shenoy et al. 2017). When applied to lettuce seedlings in contaminated irrigation water, *L. monocytogenes* was found to internalise in leaves of lettuce at levels up to 3.5 Log/CFU/g (Standing et al. 2013), and could be detected for up to 14 days post inoculation. However, there is an absence of data on internalisation of *Listeria* in parsley or spinach.

8.2   Berries

Berries are pulpy fruit with a high water content (85-92% depending on species), high sugar content and a soft skin. They are particularly susceptible to physical damage, and this provides conditions that can lead to increased microbial contamination (EFSA Panel on Biological Hazards 2014b). The pH of fresh berries ranges from 3.12-3.33 for blueberries, to 3.22-3.95 for raspberries and 3.0-4.1 for strawberries (Knudsen et al. 2001; US FDA/CFSAN 2003). Due to the low pH of berries they generally do not support the growth of bacterial pathogens, such as STEC. However if bacteria and viruses are present they can potentially persist on the surface of berries. Viral internalisation into strawberry fruit has also been reported.

Different varieties of berries grow on different styles of plants. Strawberry plants are grown close to the ground, while blueberries grow on a bush, and raspberries grow on canes so the fruit are elevated from the ground.

The surface characteristics vary between berry types. Blueberries have a smooth surface while raspberries and strawberries are aggregate fruit and have an irregular surface. Rougher or more irregular surfaces, and the presence of stigma on aggregate fruits, provide more sites for microbial harbourage and also enable better attachment (Bozkurt et al. 2020). An experimental study examined the adhesion of HAV and NoV onto the surface of strawberries and raspberries. After artificial inoculation of the berries with HAV or NoV GI it was estimated that up to 1% of viral particles adhered to the surface of raspberries and 0.1% to the surface of strawberries. Adhesion of NoV GII was much lower for both berries (Deboosere et al. 2012). In a study of 13 bacterial pathogens, including *E. coli* O157:H7, artificial inoculation of strawberries with ~5 Log CFU/g of each of pathogen, led to ~4 Log CFU/g attachment of *E. coli* O157:H7, and the other bacterial pathogens, to the strawberry surface (Gómez-Aldapa et al. 2018). Also, raspberries have a higher respiration rate than strawberries at room temperature, leading to a higher humidity in the microclimate of the raspberry surface. As such, viruses prone to desiccation may be more protected on raspberries compared to strawberries (Verhaelen et al. 2012).

There is evidence that berries do not support the growth of STEC. An experimental study by Gómez-Aldapa et al. (2018) showed that the level of *E. coli* O157:H7 on artificially inoculated strawberries decreased over time. On strawberries with ~4 Log CFU/g of attached *E. coli* O157:H7 there was a 3 Log reduction in *E. coli* O157:H7 levels over 15 days storage 22°C and a ~2.3 Log reduction over 15 days storage at 3°C.

Berries are easily damaged due to their high moisture content and soft skin. An experimental study by Nguyen et al. (2014) compared *E. coli* O157:H7 levels on artificially inoculated bruised and intact fresh whole strawberries and blueberries. During 7 days storage at 15.5°C, *E. coli* O157:H7 levels declined by an average of 1.5-1.8 Log and 1.5-1.6 Log for bruised and intact strawberries, respectively, and 0.7-1.6 Log and 1.6-1.9 Log for bruised and intact blueberries, respectively. There was no significant difference in the *E. coli* O157:H7 levels between bruised and intact fruit over the storage period.

However, bacterial pathogens can persist in the internal parts of berries, i.e. on the cut internal surface or internally within a whole berry. In an experimental study by Knudsen et al. (2001) cut or whole fresh strawberries were artificially inoculated with a five strain cocktail of *E. coli* O157:H7 (Log 7 CFU/sample) and stored at 5 or 24°C. The *E. coli* O157:H7 population remained relatively constant on the cut surface of strawberries when stored for 7 days at 5°C or for 2 days at 24°C. In comparison the *E. coli* O157:H7 levels declined on whole strawberries by 2 Log or ~0.5 Log when stored at 5°C (7 days) or 24°C (2 days), respectively. A study by Yu et al. (2001) artificially inoculated strawberries with different *E. coli* O157:H7 strains either internally (via syringe) or on the external surface (mean inoculation level of 4.36 Log CFU/g for both methods). After three days of storage at 5°C there was only a 0.52-0.69 Log reduction of *E. coli* O157:H7 populations internally within the berry compared with a 1.3-1.77 Log reduction on the surface of the berry. After 24 hour storage at 23°C there was a similar level of survival of *E. coli* O157:H7 between the surface and internally inoculated berries. Interestingly, one *E. coli* O157:H7 strain demonstrated a population increase of 0.24 Log and 0.52 Log on the surface and internally inoculated berries, respectively (it was not reported if these population increases were significant). This may be attributed to an increased acid tolerance of this strain, however, it was only a very short storage time. For the other *E. coli* O157:H7 strain there was a 0.66 Log and 0.51 Log reduction on the surface and internally inoculated berries, respectively.

There is evidence that viruses can persist on the surface of berries. In an experimental study by Leblance et al. (2019), blueberries were artificially inoculated with HAV ($10^{7.1}$PFU/ml) and stored at 4 or 21°C. There was no significant loss of infectivity of HAV on the fresh blueberries after 21 days storage at 4°C or 7 days storage at 21°C. Verhaelen et al (2012) had similar findings in raspberries and strawberries artificially inoculated with human NoV GII.4 (2×10^6 genomic copies) or human NoV GI.4 (8×10^6 genomic copies), with no loss in viral infectivity for either fruit when stored at 4°C for 7 days. However, when stored at 21°C for 3 days there was a 0.2 Log and 0.3 Log loss of infectivity of hNoV GII and hNoV GI, respectively on raspberries, and 0.5 Log and 1.2 Log loss of infectivity for hNoV GII and hNoV GI, respectively on strawberries. This suggests that NoVs are more persistent on raspberries compared to strawberries, which may be due to factors of the fruit matrix, such as the higher respiration rate of raspberries (described above). As berries destined for the fresh market do not undergo a washing and sanitising step, any viruses present on the berries will remain as there is no pathogen reduction step.

There is also evidence that viruses can internalise into berry fruit. An experimental study by DiCaprio et al. (2015) showed that NoV surrogates—murine NoV and Tulane virus—were able to internalise from the soil into strawberry plants and the strawberry fruit (see Section 9.5.1.2).

8.3   Melons

The internal flesh of rockmelons have a pH of 6.13-6.70, water activity of 0.97-0.99, and contain sugars including fructose, glucose, and sucrose. The total soluble content of rockmelons has been report to range from 10-15 ° Brix (EFSA Panel on Biological Hazards 2014c). Watermelon flesh is reported to have a lower flesh pH range of 5.18-5.60, total soluble solid content between 7-9 ° Brix, but a similar water activity to rockmelons (EFSA Panel on Biological Hazards 2014c). The flesh of both rockmelons and watermelons provides a favourable environment for the growth of *L. monocytogenes* and *Salmonella* spp*.* Rockmelon and watermelon are the fruit produced respectively from *Cucumis melo* and *Citrullus lanatus* that are species of annual plants that grow with weak stems that can be up to 3m long and trail along the ground. *Cucumis melo* includes a number of varieties of melons including both smooth and netted melons.

Rockmelon have a raised netted rind that make the surface rougher than un-netted varieties and may provide attachment sites and protection for microbial pathogens from sanitisers and other mitigation measures. The netting develops from the blossom end when melons are 10-12 days old and is formed by natural cracking on the rind that covers the entire fruit by the end of the fruit expansion stage. These cracks are then sealed by the formation of a thick raised cuticle (Keren-Keiserman et al. 2004). In contrast, watermelons have a comparatively smoother surface, and experimental quantification of surface roughness by scanning interferometry demonstrated the significantly smoother surface of watermelons compared to rockmelons (Kwon et al. 2018). Furthermore, the surface of lettuce, spinach and rockmelon had a similar highest surface roughness compared to apples, tomatoes, and oranges that had the smallest surface roughness when examined using confocal microscopy (Lazouskaya et al. 2016). While there is some evidence that suggest the surface roughness of rockmelons may reduce the efficacy of sanitisers directly compared to the smoother surface of watermelons (Kwon et al. 2018), the potential for pathogens to initially attach with a higher probability to rockmelons compared to watermelons is poorly documented in the literature.

However, studies have attributed significantly higher populations of bacteria, yeast, and mould observed on rockmelons compared to smoother surfaced melons to surface characteristics (Ukuku et al. 2019; Ukuku and Sapers 2007), and the differences in attached *Salmonella* spp. or *L.* monocytogenes inoculated on to the surface of rockmelons compared to watermelons or honeydew differed from 0-0.6 Log (Kwon et al. 2018; Ukuku et al. 2019; Ukuku and Sapers 2007). The surface characteristics of netted rockmelon may provide further protection slowing inactivation in the field environment, however, comparative studies of pathogen survival between watermelon and rockmelon were not identified.

There is evidence that *L. monocytogenes* and *Salmonella* spp. can grow both in the flesh and on the rind of rockmelons. A number of studies have investigated the growth rate of *L. monocytogenes* and *Salmonella* spp. at different temperatures on the flesh of melons and include a number of predictive models. Notably, the growth rates of *L. monocytogenes* (Danyluk et al. 2014; Penteado and Leitão 2004a) and *Salmonella* spp. (Golden et al. 1993; Li et al. 2013; Penteado and Leitão 2004b) have been shown to be similar for watermelon and rockmelon flesh despite the slight difference in pH. The comparison of a *L. monocytogenes* model for growth in cut melons (Danyluk et al. 2014) compared to that of *Salmonella* spp. (Li et al. 2013) showed that *L. monocytogenes* has faster growth rates at lower temperatures (4-20°C), but at temperatures 20-25°C *Salmonella* spp. growth outpaces *L. monocytogenes.* As noted by the authors, '*Salmonella* are generally considered to have a much higher probability of illness when low doses are ingested (FAO/WHO, 2002) compared to *L. monocytogenes* (FAO/WHO, 2004); the relatively lower growth rate on cut melons does not hinder the potential of *Salmonella* to cause more outbreaks and cases than *L. monocytogenes*'.

A comparison of two growth models demonstrated a high level of agreement predicting that a single cell of *L. monocytogenes* on melon flesh at room temperature (20-25°C) could grow quickly to levels that would have a high probability of causing illness in susceptible consumers (Bartlett et al. 2020; Danyluk et al. 2014; Fang et al. 2013). *Salmonella* spp. can also grow quickly to levels that have a high probability of causing illness at ambient temperatures (Li et al. 2013). In contrast, if melon was stored at 5°C, due to the effect of lower temperatures slowing microbial growth rates, it is predicted to take 2-3 weeks for a single cell of *L. monocytogenes* to grow to levels likely to cause illness in a susceptible consumer, and storage at this temperature or below should prevent the growth of *Salmonella* spp.

The available data in the literature suggest that growth on the rind of rockmelons is possible for *L. monocytogenes*, however, the results are less consistent than growth in the flesh (Salazar et al. 2017; Scolforo et al. 2017). Consequently, there is less agreement on the growth rates on the rind compared to on the flesh. Regardless, there is evidence that growth can occur at relatively high rates at optimal temperatures, particularly if rockmelons are not refrigerated, retain surface moisture, or develop condensation. Similarly, *Salmonella* spp. have been reported to grow on the rind of rockmelons stored at room temperature and 37°C (Annous et al. 2005; Beuchat and Scouten 2004). No data regarding the survival or growth of the pathogens on watermelon rind was identified.

9 Primary production environment risk factors

As for all fresh produce, the in-scope commodities are exposed to similar intentional on farm inputs, such as water, soil and amendments, seeds and seedlings and human activity, that may lead to contamination of produce (FAO/WHO 2008b). Fresh produce can also be exposed to similar unintentional risk factors, such as animal intrusion, climate, extreme weather events, and topographical features (FAO/WHO 2008b).

This section identifies and discusses the evidence of contamination pathways, persistence, survival or amplification of hazards that may influence the risk of contamination in the primary production environment. It also identifies controls or mitigation steps that may reduce or limit the risk.

9.1 Animals, wildlife, and livestock

Wild and domestic animals are known reservoirs of foodborne pathogens and their presence in the primary production environment can present a risk to the safety of fresh produce. Animals—including insects, birds, and reptiles—can directly contaminate crops via faecal waste, urine, hair/feathers or animal carcasses, but also indirectly via contamination of other inputs such as water and soil (FAO/WHO 2008b). Bird droppings and airborne contaminants (e.g. due to birds nesting around the packing area, nearby livestock or poultry production) may be a source of contamination (EFSA Panel on Biological Hazards 2014b).

9.1.1 Available data

There are a number of studies that have assessed the occurrence or prevalence of different pathogens associated with both wild and domestic animals and established that there are a variety of animal carriers of zoonotic pathogens such as STEC, *Salmonella* spp. and *L. monocytogenes* (FAO/WHO 2008b; Jay-Russell et al. 2012; Jay-Russell et al. 2014; Kilonzo et al. 2013).

In Australia, *Salmonella* spp. have been isolated from domestic pets, livestock and wildlife (Parsons et al. 2010; Scheelings et al. 2011; Simpson et al. 2018). STEC is most often associated with mammals and has frequently been isolated from Australian livestock (Barlow et al. 2015; Klein et al. 2010; Mellor et al. 2016) and a variety of wildlife with varying frequency (Gordon and Cowling 2003). Furthermore, STEC is also reported to be carried by Australian marsupials (Rupan et al. 2012). Surveys of *L. monocytogenes* from Australian animals is limited. However, this pathogen has been isolated from cattle (Klein et al. 2010), listeriosis outbreaks have occurred in sheep following floods or droughts (Eamens et al. 2003), and *L. monocytogenes* is expected to be carried by various wildlife as evident in overseas studies (FAO/WHO 2008b). These bacterial pathogens can also be shed in the faeces of both symptomatic and asymptomatic humans.

The host range of HAV is generally considered to be limited to humans and some non-human primates, but some other animals have recently been reported as hosts (Oliveira Carneiro et al. 2018). NoV is reported to infect humans and some animals and therefore indirect zoonotic transmission from animals may be possible (Bank-Wolf et al. 2010). Both viruses are shed in the faeces of humans, and unauthorised human access could present a potential risk to fresh produce safety, and to a lesser extent animal incursion.

The evidence specifically associated with the in-scope commodities and hazards is summarised below.

9.1.1.1 Leafy vegetables

There is strong evidence that outbreaks due to contaminated leafy vegetables and other horticultural products have been associated with the presence of sheep, cattle, feral swine and deer near or in growing fields (Jay et al. 2007; Laidler et al. 2013; Mikhail et al. 2018). For example, in an environmental survey of implicated agricultural land and waterways following a large outbreak of *E. coli* O157:H7 linked to bagged spinach from California, *E. coli* isolates from feral swine, cattle, surface water, sediment and soil were found to match the outbreak strain (CalFERT 2007; Jay et al. 2007). These findings showed that, while cattle may be the primary environmental reservoir of *E. coli* O157, interspecies transmission to other livestock and wildlife can broaden potential sources of contamination of leafy vegetables growing in nearby fields. It is also notable that Jay et al. (2007) found that the outbreak strain persisted in environmental samples up to 3 months after the initial outbreak.

In studies on experimental faecal contamination in vegetables plots, it has been observed that splashes from rain or irrigation water can transfer pathogens and faecal indicator organisms onto leafy vegetables up to at least 1.6 metres away from faecal deposits (Atwill et al. 2015; Jeamsripong et al. 2019; Weller et al. 2017). Wind and dust can also spread faecal contamination from off-farm sources. Hoar et al. (2013) observed airborne bacteria at least 6 metres from sheep grazing in grassy fields in California's Imperial Valley, and concluded that a buffer zone of 9 metres between grazing lands or domestic animals and the crop edge is adequate to minimise contamination of nearby crops. However, pathogens have been shown to be dispersed much further distances by wind and dust (see Section 9.2.1.1 for further discussion).

9.1.1.2 Berries

The risk of contamination from wild and domestic animals[13] can vary between the different berry types. For example, soil contamination with faeces is a particular risk for berries likely to have direct soil contact, e.g. strawberries (EFSA Panel on Biological Hazards 2014b). Most strawberries are grown in open fields and are located directly on the ground or on plastic mulch close to the ground. Conversely, raspberries and blueberries are grown slightly elevated from the ground and are generally grown under netting or plastic tunnels to protect them from birds and also other wildlife.

Wildlife incursion into open fields where berries are grown close to the ground, can lead to faecal contamination by wildlife. For example, there is evidence of an outbreak of *E. coli* O157:H7 in the US due to wildlife incursion into strawberry growing fields. Epidemiological evidence linked the outbreak to strawberry consumption, and the outbreak strain was isolated from deer faeces found in the growing fields (Laidler et al. 2013). Wildlife incursions into berry growing fields have also been documented in Australia (DEDJTR and FSANZ 2016).

An experimental study by Wu et al (2013) demonstrated that direct contact of blueberries with deer faeces artificially inoculated with non-pathogenic *E. coli* O157:H7 (final inoculum of 7 Log CFU/g) led to transfer of non-pathogenic *E. coli* O157:H7 to the fruit and which could persist for at least 72 hours (length of study).

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[13] Birds are classed as animals.

9.1.1.3 Melons

Watermelons and rockmelons in Australia are grown in large open fields directly on the ground or on plastic mulch close to the ground and can be damaged in the field. As such, melons can be exposed to direct and indirect contamination by domestic and wild animals and their faeces. Damaged fruit can also act as an attractant for animals. Due to the size of some growing areas fencing or netting are not practical for the management of animal or unplanned human incursion.

No experimental or observational evidence regarding the introduction of *L. monocytogenes* or *Salmonella* spp. via wild or domestic animals into melon fields in Australia was identified. However, wildlife feeding on and defecating in Mexican rockmelon growing orchards have been found to harbour *S. enterica* (Aguilar et al. 2005).

9.1.2 Conclusions, uncertainty, and data gaps

Incursion by wildlife and domestic animals are risk factors that are likely to apply broadly to the commodity sectors. There is a low level of uncertainty and a high level of confidence regarding this conclusion based on the available evidence for pathogen prevalence in Australian animals and the evidence reviewed for leafy vegetables and berries.

While the presence of animals can always be considered a hazard, incursion by animals close to harvest, with high density, or with high frequency is likely to present a higher risk for all commodities. As discussed in other sections, pathogens are generally reported to decline (but can persist) in the field environment following a contamination event. However, there is limited and variable data regarding the survival of pathogens associated with direct or indirect animal incursion. As such, there is a high level of uncertainty in regard to how environmental factors influence the survival and transmission of pathogens associated with animal incursion. There is also limited available data regarding the prevalence of foodborne pathogens on animals or in animal faeces relevant to Australia. Furthermore, data describing the density of wildlife, the frequency of ingress into fields, and the quantification of transmission from faeces or animals to fresh produce via different mechanisms such as soil or water are limited in the Australian context. International studies investigating these factors are more frequent for leafy vegetables but very little data is available for berries or melons.

It is plausible that those commodities that grow closer to the ground including lettuce, spinach, parsley, rockmelon, watermelon and strawberries may be at a greater risk of contamination via direct contact with faeces or soil affected by faeces compared to blueberries and raspberries. However, the indirect contamination from wind or water inputs affected by animal incursion or direct contamination from birds, climbing animals, or larger mammals would still present a risk to those higher growing commodities. Again, high uncertainty exists regarding how the distance away from the point of animal incursion may affect the likelihood of contamination. The lack of studies in this area means that there is also high uncertainty regarding the potential variability associated with how the type of intruding animal influences the likelihood of contamination and the extent that wildlife incursion will influence risk for particular commodities of melons and berries.

Whilst some studies reported above have attempted to model the complex interactions between wildlife populations, topography, climate, hydrology, and weather, these are limited in the literature (Mishra et al. 2017a).

Expert scientific reports concur with these conclusions and have identified animal incursion as important risk factors that may contribute to microbiological contamination of all of the in-scope commodities (EFSA Panel on Biological Hazards 2014a, 2014b, 2014c; FAO/WHO 2008b, 2011). It is noted that, in some cases, these risks may be better managed for those crops produced in more protected systems (e.g. greenhouse, tunnel, hydroponic or netted systems). However, these systems are not universally applied within any of the commodity sectors in Australia.

9.1.3   Mitigation measures

There is evidence for the efficacy of no-harvest zones near wildlife faecal contamination and buffer zones between crops and grazing animals for leafy vegetables to reduce the likelihood of microbial contamination of harvested produce (Hoar et al. 2013; Jeamsripong et al. 2019, 2019). These studies have suggested exclusion zones of 152.4 cm (5 ft) from animal faecal matter and 9.1 m (30 ft) between grazing lands or domestic animals and the crop edge can reduce the risk of contamination of leafy vegetables.

The mitigation recommendations provided by other agencies, organisations and peak industry bodies for limiting the risk from animal and human ingress are similar across the in-scope commodity sectors (EFSA Panel on Biological Hazards 2014a, 2014b, 2014c; FAO/WHO 2008b; FPSC A-NZ 2019; IFPA et al. 2006; NSW DPI 2019; UC Davis 2020) and include:

- Regular risk assessment including evidence of wildlife or pest incursion, and assessment of the proximity to known wildlife and pest reservoirs
- Monitor wildlife, pest, or human ingress regularly and particularly prior to harvest. If detected, decisions should be made about whether to harvest from affected areas
- Growing areas should be protected and maintained to deter wildlife intrusion using appropriate biological, cultivation, and physical and chemical pest control methods, and by limiting water and waste in the field
- Water sources and other inputs, such as soil amendments, should be protected from animal incursion
- Dissuasive feeding of animals
- Locate production areas to minimise the potential for animal ingress
- Use buffer zones, no harvest zones, and physical barriers/or to protect production area
- Use of co-management strategies
- Train staff to identify and report signs of animal/human incursion.

9.2   Characteristics of the production site

Characteristics of the production site—including prior and surrounding or adjacent land use, topology, hydrology, and climate—can influence the transfer of pathogens from the environment to crops (FAO/WHO 2008b). Famers may rotate and change crops grown on fields and new fields may be established. Prior land use including land used to cultivate a different crop; supplemented with soil amendments; irrigated in a manner inappropriate for the new commodity; or used for livestock production, as a wildlife habitat or for land fill for urban or industrial waste have been identified as risk factors that may contribute to the contamination of fresh produce (FAO/WHO 2008b).

Topological risk factors include the positioning of crops below contaminated areas where runoff, wind, dust or other environmental factors can transfer pathogens to fresh produce (FAO/WHO 2008b). Hydrological risk factors include rising water tables that can transfer human pathogens from areas of high density populations, and low lying areas holding stagnant water that could be contaminated and subsequently transferred to crops (FAO/WHO 2008b).

9.2.1 Available data

Different aspects of weather and climate have been shown to influence the transmission, survival and growth of microorganisms. Airborne microorganisms can exist both as free cells and with particulate matter with concentrations reported to vary from 1.96 Log to 8.11 Log CFU/m^3 per cubic meter of air. Similar landscapes have been shown to produce similar airborne microbiological profiles but local meteorological conditions—such as wind, temperature, and humidity—can affect the composition of airborne communities (Tignat-Perrier et al. 2019).

Rain or precipitation have been identified as important factors that can increase the likelihood of transfer of microorganisms to fresh produce via splash transference (Monaghan and Hutchison 2012; Strawn et al. 2013; Ward et al. 2015). Furthermore, high temperatures and high humidity are reported to increase the potential for growth of pathogens on produce (Park et al. 2012). However, UV radiation from sunlight has been reported to decrease pathogens in water, soil, and on the surface of fresh produce over time and is one of the factors that contributes to the reported general decline of pathogens on produce in the field (EFSA Panel on Biological Hazards 2014a; Williamson et al. 2017).

Environmental factors such as soil available water, soil type, temperature, and proximity to water sources and other uses of land—such as roads, urban development and pasture/hay grass—can affect the likelihood of contamination by microorganisms (Monaghan and Hutchison 2012; Strawn et al. 2013; Ward et al. 2015).

Topological and hydrological characteristics such as catchment topology, flow rate, and flow pathways have also been demonstrated to play a role in the transfer of microorganisms in agricultural settings (Abu-Ashour and Lee 2000; Murphy et al. 2015).

The evidence specifically associated with the in-scope commodities and hazards is summarised below.

9.2.1.1 Leafy vegetables

Prior land use can affect the likelihood of microbial contamination of leafy vegetable crops. In a study on factors affecting contamination with generic *E. coli*, 955 georeferenced spinach samples were collected over two growing seasons on 12 farms in two US states (Park et al. 2013b; Park et al. 2014). Amongst other factors, the study identified the absence of grazing and hay production in the field before spinach planting—along with other field hygiene factors (e.g. use of portable toilets and washing stations in the field, training staff/temporary workers to use portable toilets)—as significantly reducing the likelihood of contamination (OR = 0.06; 95%CI 0.01–0.30).

Nearby livestock operations can contaminate leafy vegetable crops through dispersal of pathogens by wind and dust over significant distances, particularly in drier landscapes. Yanamala et al. (2011) detected *Salmonella* spp. and generic and pathogenic *E. coli* on fresh spinach samples placed up to 46 metres from a cattle feedlot operation. Berry et al. (2015) investigated climatic factors that influenced the detection of generic *E. coli* and pathogenic *E. coli* O157:H7 on leafy vegetables (including spinach) grown in plots up to 180 metres from

a cattle feedlot. They concluded that drier and windier conditions significantly increased the probability of dissemination of the bacteria onto crops, a conclusion supported by routine detection of airborne *E. coli* at that distance.

While few studies have specifically investigated the contribution of topological and hydrological factors to microbial contamination of leafy vegetables, some preliminary findings indicate that these factors can affect food safety. In their multi-factor analysis of generic *E. coli* contamination of spinach crops, Park et al. (2013b) found that contamination was significantly more likely when the crop was located on sloped terrain. In analyses of irrigation water systems associated with the 2006 *E. coli* O157:H7 outbreaks associated with Californian lettuce and spinach, Gelting and colleagues (2011; 2015) considered potential routes of contamination and cross-contamination between sources of water for irrigation. They assessed interactions between groundwater and surface waters in the context of watershed topology, land use patterns and farm water management and irrigation practices. They noted that the typical siting of crops on flatter valley floors, with cattle and other pastures on surrounding hillsides, increased the potential for contaminated runoffs, especially during heavy rainfall events. They concluded that a systematic analysis of water sources, extending to sources beyond the farm gate, was necessary to limit the potential for contamination of irrigation water sources in situations where other land uses (e.g. cattle grazing, dairy farms) competed with crop production for resources.

Studies on the effects of seasonal and climatic conditions on pathogen prevalence, survival and growth on leafy vegetables demonstrate complex interactions between interdependent factors such as temperature, humidity and rainfall. For example, under conditions of minimum and maximum temperatures and daylight period typical for winter (June) in Salinas Valley, California, Tyagi et al. (2019) showed that survival of enterohaemorrhagic *E. coli* (EHEC) strains (serotypes O157 and O26) inoculated onto greenhouse lettuce is higher at 45% relative humidity (RH) than at 75% RH. However EHEC survival was not related to RH under temperature and photoperiod conditions typical of early autumn (March). The authors linked differences in survival to UV exposure and gene regulatory responses affecting transcription of osmotic and oxidative stress response and virulence genes. Liu et al. (2016) found that temperature was an important variable affecting presence and levels of generic *E. coli* on leafy vegetables (lettuce and spinach), modelling data from 562 samples of lettuce and spinach collected in 2011–2013 from 23 open-field farms in Belgium, Brazil, Egypt, Norway, and Spain. Amongst other significant farm management variables, the minimum temperature of the sampling day affected the likelihood of *E. coli* contamination of leafy vegetables, while the maximum temperature during the 3 days before sampling affected levels of *E. coli* found on the leafy vegetables.
Applying a quantitative microbial contamination model, Allende et al. (2017) assessed the impact of weather conditions (e.g. seasonality, solar radiation and rainfall) and other factors on *E. coli* contamination of open-field grown baby spinach at harvest in Spain. The model indicated that contamination levels were significantly affected by solar radiation intensity and rainfall—mostly due to soil splashing (Monaghan and Hutchison 2012)—and predicted higher *E. coli* prevalence and levels in winter than in spring. These findings are supported by the logistic regression modelling of Park et al. (2014), who looked at the effect of local weather variables (ambient temperature, precipitation, and wind speed) and other factors on generic *E. coli* on spinach in western and south-western United States. The model identified precipitation as the best single predictor of spinach contamination, with probability increasing with every mm increase in the mean amount of rain in the previous 29 days (OR = 3.5, 95% CI 1.7–7.3). In contrast, Weller et al. (2015) observed significantly higher likelihood of contamination of spinach with *Listeria* spp. (including *L. monocytogenes*) 24 hours after rain, but not at longer time-points (out to 6-8 days after a rain event). It is unclear if this difference reflects differences in experimental approach or persistence of *E. coli* and *Listeria* spp. on spinach leaves.

9.2.1.2 Berries

Studies on these risk factors specifically for the berry/hazard combinations were not identified.

9.2.1.3 Melons

Studies on these risk factors specifically for the melon/hazard combinations were not identified.

9.2.2 Conclusions, uncertainty, and data gaps

The risk factors associated with characteristics of the production site including prior and surrounding land use, topology, hydrology, and climate are likely to apply broadly to the commodity sectors, but may be better managed in some of the protected cropping systems. There is a low level of uncertainty and high level of confidence in this conclusion based on the general evidence for these factors to be associated with bacterial contamination and the available evidence for leafy vegetables.

If leafy vegetable, berry or melon growing fields are located downstream or at lower elevations than industrialised or heavily populated areas or land used to farm livestock, this could lead to a higher likelihood of contamination of produce. However, while this has been demonstrated in evidence discussed above for spinach and lettuce, and provides a medium level of uncertainty for these commodities, no direct evidence describing the contribution of site location to contamination of parsley, berries or melons was identified. As such, although it is plausible that these factors also apply to berries and melons, there is a high level of uncertainty due to the lack of available data. There is also a lack of data regarding the contribution of climate, hydrology, topology or geographical features to microbial contamination of berries and melons and a lack of understanding how the likelihood of contamination may vary between them.

If leafy vegetable, berry or melon fields are located near land used to farm livestock, this could lead to a higher likelihood of contamination of produce. However, whilst there is direct evidence in the literature for spinach, only circumstantial evidence is available to be considered for lettuce, parsley, berries and melons. As such, there is high uncertainty as to what extent the likelihood of contamination may vary between commodities.

It is generally accepted that there is a decrease in the risk associated with a contamination event due to environmental factors as the time before harvest is lengthened. The effect of time, climate, and weather factors on the survival of pathogens has been described more broadly in the literature for soil and water, and to some extent specifically for leafy vegetables. However, the effects of these factors are reported to be variable depending on the pathogen studied, the factors studies (e.g. precipitation, solar radiation, wind speed etc.), the type of study undertaken, if the study is laboratory or field based, and the geographical location of the field study. There is a lack of studies considering the complex interaction between these environmental factors, specifically in the Australian context, and how they may influence the likelihood of contamination or influence the rate of decline of pathogens in the field following a contamination event. Moreover, factors that may potentially lead to increased bacterial growth or declines in inactivation rates (e.g. fluctuations in rainfall, relative humidity, temperatures, and UV) on produce in the field are poorly documented. Also, due to the lack of data for parsley, berries and melons, there is high uncertainty to what extent environmental factors influence contamination and persistence of pathogens on these different commodities.

Previous expert scientific reports have also identified these as important risk factors for all in-scope commodities (EFSA Panel on Biological Hazards 2014a, 2014b, 2014c; FAO/WHO 2008b, 2011).

9.2.3 Mitigation measures

The recommendations regarding the risk factors related to the characteristics of the production site including prior and surrounding land use, topology, hydrology, and climate are similar across commodity sectors (EFSA Panel on Biological Hazards 2014a, 2014b, 2014c; FAO/WHO 2008b; FPSC A-NZ 2019; IFPA et al. 2006; NSW DPI 2019; UC Davis 2020) and include:

- Environmental risk assessments should be undertaken preplanting and within one week of harvest to assess on-site and surrounding off-site microbial, chemical or physical factors that may affect product safety in the field
- Production practices should be tailored to identified risks in each unique production environment
- Primary production should not occur in areas where it is likely that the presence of pathogens may represent an unacceptable risk of contamination to fresh produce
- If potential hazards are identified appropriate interventions such as buffer areas, ditches, physical barriers, and co-management strategies can be used to minimise the likelihood of transfer of microbial contaminates to crops.

9.3 Extreme weather events

Extreme weather events are considered to include unexpected, unusual, severe, or unseasonal weather. Extreme weather events include: dry periods and wind that cause large dust storms that disperse microorganisms; drought that can lead to ground compaction and decreased plant health leaving produce more susceptible to contamination; heavy rain or flooding that may submerge and contaminate crops; or severe frost, hail, or wind that can damage produce. Heavy rainfall or flooding can transfer pathogens from faecal waste, rooting vegetation, contaminated surround sites, soil, or waterbodies via splashing onto the crop, runoff or immersion of crops. Increased organic load from weather events in the form of soil or increased amounts of damaged produce entering washing systems can also inhibit the efficacy of some sanitisers (EFSA Panel on Biological Hazards 2014b; FAO/WHO 2008b; FDA 2011).

9.3.1 Available data

Flooding is one of the most common natural disasters internationally and is associated with increases of waterborne disease outbreaks (Boxall et al. 2009; Paterson et al. 2018). The microbial contamination of agricultural soils is shown to increase after flooding (Casteel et al. 2006; Castro-Ibáñez et al. 2015), and contamination from surrounding sites after heavy rain or flooding can also increase microbial contamination of produce and inputs such as irrigation water (Oliver et al. 2007). Drought and resulting soil compaction can lead to increased runoff from surrounding grazing areas and higher loads of contamination during heavy rain or flooding (EFSA Panel on Biological Hazards 2014a).

Dust can also act as a vector for microorganisms and present a contamination risk, as reviewed by Gonzalez-Martin et al. (2014) and Zhao et al. (2014). Frost protection is used on some crops during very cold weather and this technique could potentially introduce contamination if pathogens were present in the water source and able to persist during frost events (Gutierrez-Rodriguez and Adhikari 2018).

The evidence specifically associated with the in-scope commodities and hazards is summarised below.

9.3.1.1 Leafy vegetables

Leafy vegetables in Australia can be grown in a variety of geographical locations either in the field or with protected cropping. As such, crops can be exposed to a variety of extreme weather events.

It is well-established that flooding events increase the likelihood of microbial contamination of leafy vegetables. Castro-Ibáñez et al. (2015) reported high levels of coliforms and generic *E. coli* and prevalence of *Salmonella* spp. one week after a natural flooding event in lettuce fields in the south-east of Spain. There was a low prevalence of non-O157 EHEC, but no *E. coli* O157:H7 or *L. monocytogenes* were detected at that time point. All microbial contaminants had declined to normal or undetectable levels three weeks after the flooding, aside from *Salmonella* spp.—which were no longer detected after five weeks—and two sporadic detections of *L. monocytogenes* in the week three samples. In a multi-country study, Ceuppens et al. (2015) similarly found that flooding of fields increased the risk of detection of *Salmonella* spp. in leafy vegetables (lettuce, spinach and basil) when tested within one week of a flooding event.

Several studies have investigated the effects of pathogen load, water and heat stress on colonisation of leafy vegetables by *Salmonella* and *E. coli*, with variable results. Ge et al. (2012) found that levels of internalised *S.* Typhimurium in lettuce via root uptake from contaminated soil increased under drought conditions when soil levels were $\geq 10^8$ CFU/g, as well as under excess moisture conditions when soil levels were $\geq 10^9$ CFU/g. Internalised bacteria were detected in leaf tissue, but not in roots, with levels up to 2.5 log CFU/g under optimal irrigation conditions. In similar experiments, Zhang et al. (2016) evaluated internalisation, through root uptake, of *S.* Infantis in lettuce grown under different levels of water stress (no drought control, mild drought, and severe drought). At high inoculum levels—a single application to the soil of irrigation water containing 8 log CFU/mL three days prior to harvest—the detection frequency and level of internalisation of *Salmonella* from roots into lettuce leaves were higher in the severe drought group, possibly due to physical damage to the roots caused by drought stress. Also, Erickson et al. (2014b) found that internalisation of *E. coli* O157:H7 into roots of 4–6 week old lettuce, spinach and parsley plants was higher when the soil was saturated compared to when it was moistened—possibly reflecting greater survival of bacteria in the saturated soil than in the non-saturated soil. No significant differences in average levels of internalised *E. coli* O157:H7 was observed between spinach, lettuce and parsley roots. In all three, translocation of bacteria from roots to leaves was rare. A subsequent attempt by Erickson et al. (2014a) to maximize the exposure of germinating spinach and lettuce seeds to *E. coli* O157:H7 by increasing the moisture content of soil did not increase the degree of internalisation of pathogens. In contrast, Ge et al. (2014) found no effect of water stress on *S.* Typhimurium internalisation in lettuce two days after application of 2.3–6.3 Log CFU/plant to the leaf surface. Inoculum levels in that range also did not greatly affect the overall levels of internalisation, which were in the range 3.0–3.4 Log CFU/g by plate count of homogenised, surface-sterilised leaves. Again, no internalised bacteria were found in root tissue.

The work of Zhang et al. (2009a) supports earlier studies that imply that internalisation of *E. coli* O157:H7 is a rare event in lettuce plants, regardless of the heat or water stress the plants experience. In controlled environment experiments, heat stress during growth of lettuce did not promote or enhance internalisation of *E. coli* O157:H7—it was not detected on any leaf surfaces or in any surface-sanitised macerated leaf samples, and was only detected in one of 144 root samples.

9.3.1.2   Berries

In Australia berries can be grown in open fields where they are exposed to the elements, or they can be grown under plastic tunnels that protect the crop from the weather.

Frost protection can be used to protect crop losses during freezing temperatures. In Australia this technique is used by some strawberry growers (dependent on location) (DAF QLD 2014). As blueberries and raspberries are generally grown under plastic tunnels or netting, and these provide some level of protection, frost protection is not commonly used for these types of berries in Australia.

Frost protection generally involves overhead irrigation and uses large quantities of water. As such, growers generally depend on surface water sources to protect their crops (Conlan et al. 2018; DAF QLD 2014; Gutierrez-Rodriguez and Adhikari 2018). There is preliminary evidence that the use of contaminated water for frost protection of berries can lead to contamination of the berries. In a preliminary study by Cooney et al. (2016) strawberry plants were inoculated with *E. coli* and surrogates of *Salmonella enterica*, *E. coli* O157:H7 and *L. monocytogenes* (average inoculum of Log 4.5 CFU/mL) via the irrigation water used during frost protection. *E. coli* was detected 36 days post inoculation, while all surrogate populations were at or below the level of detection after day four post inoculation. In another preliminary study, Reed et al. (2018a) inoculated blueberry blossoms with generic *E. coli*, EcW778 (Log 5 CFU/mL), via the irrigation water used during freeze events. EcW778 was detected on blueberry samples at harvest 75 days post inoculation and on swabs of the harvester and from the packinghouse. There is also preliminary evidence that rain events can increase pathogenic bacteria levels in surface water used for irrigation of berries and/or frost protection (Carter et al. 2016).

Minor flood events in which the flood water does not contact the plants or fruit (grown in raised beds) has been shown not to significantly affect the microbiological contamination of strawberries (Delbeke et al. 2015). This was confirmed in an experimental study by Shiraz et al. (2020), in which strawberry beds were subjected to different level flood waters with varied concentrations of generic *E. coli* (10^2 CFU/L or 10^6 CFU/L). In this study, the level of the flood water (strawberry fruit submerged versus only the plant) did not influence the outcome, as generic *E. coli* was not detected on any fruit samples. However, generic *E. coli* was still detected in the soil after 48 hours in beds flooded with a higher microbial load. This implies that even when the edible portion of strawberries did not come in direct contact with floodwater, there was the potential risk for them to pick up contaminants from the soil for up to two days after floodwater receded (Shiraz et al. 2020).

9.3.1.3   Melons

In Australia, rockmelons and watermelons are grown in a number of geographical regions which can expose the fruit to diverse climates and growing environments that may influence the risk of microbial contamination.

The contamination of rockmelons by *Salmonella* spp. after heavy rainfall was observed in a field trial that inoculated an attenuated *S.* Typhimurium strain into furrow irrigation water applied to rockmelon fields (Lopez-Velasco et al. 2012). Following an unusual two day rain event, the attenuated *S.* Typhimurium strain was recovered from the rinds of 71% (15/21) of melons sampled from the centre of raised beds 43 days post inoculation. The authors attributed this to splash transference because in a trial the year before, with no rain event, only 21% (3/14) of rockmelons were contaminated 35 days post inoculation that had developed in contact with the soil in the furrows.

The outbreak investigation following the 2018 Australian outbreak of listeriosis associated with rockmelons identified that extreme weather events including dust storms and heavy rainfall preceded the outbreak (NSW DPI 2018). The report concluded that extreme weather events (heavy rainfall in December prior to harvest, followed by dust storms) could have been a significant contributing factor to the outbreak due to an increase of organic load and *L. monocytogenes* on the surface of rockmelons before harvest. The outbreak report stated that the combination of a 1 minute and 30-40 second pre-wash and scrubbing step followed by a 35 second sanitising step of 100ppm chlorine sprays and scrubbing may not have been sufficient to remove all *L. monocytogenes* contamination resulting in levels high enough to cause illness in the immunocompromised.

Given the reported limited efficacy of sanitisers (see Section 11.4) to remove pathogens from the surface of rockmelons under optimised conditions in laboratory experiments, the presence of increased pathogen and organic matter loads from weather events in the field and the subsequent harvesting of affected fruit may present a higher risk of contamination. This may not be able to be adequately managed by the adjustment of postharvest washing and sanitisation regimes alone.

9.3.2 Conclusions, uncertainty, and data gaps

The risk factors associated with the occurrence of extreme weather events apply broadly to leafy vegetable, berry, and melon commodity sectors but may be better managed in some of the protected cropping systems. There is a low level of uncertainty and high level of confidence in this conclusion based on the general evidence for these factors to be associated with the transfer of pathogens and the available evidence for leafy vegetables, berries, and melons. For viral pathogens, extreme weather events, such as flooding or heavy rain, that could transfer sewage to irrigation sources or fields are risk factors for all commodities.

Flooding, heavy rain, and dust storms represent a risk to all the commodities as they can increase the potential transfer of pathogens to the growing site or agricultural inputs. There is a low level of uncertainty and high level of confidence in this conclusion based on the general evidence for these factors to be associated with the transfer of pathogens and the available evidence for leafy vegetables, berries, and melons. Depending on the level of contamination, the type of event, and the timing of events before harvest, the risk may be greater for berries, watermelons, and leafy vegetables that are not washed or sanitised after harvesting. However, high organic loads on produce can reduce the efficacy of water based sanitisers that are applied to leafy vegetables and rockmelons. This can result in a limited reduction in risk, or even possibly a risk increase if cross-contamination is facilitated due to ineffective application of washing or sanitisers. Furthermore, it is possible that increased surface roughness of some commodities may aid attachment of pathogens and organic matter resulting in an increased risk for those commodities. However, comparable studies describing the variation in likelihood of contamination of the different commodities as a result of certain extreme weather events and potential differences between commodities based on surface characteristics are lacking. As such, there is high uncertainty regarding how the type of extreme weather event or commodity will influence the likelihood of contamination.

Extreme weather events that occur close to harvest are considered to pose an increased risk to leafy vegetables, berries and melons as pathogen levels are generally reported to decline in the primary production environment over time. However, there is great variation in the reported rates of declines of various pathogens in different media such as water or soil. There is a lack of studies considering the complex interaction between environmental factors, specifically in the Australian context, and how they may influence the likelihood of contamination or influence the rate of decline of pathogens in the field following an extreme weather event. Furthermore only a small number of studies for a few pathogens have

provided direct evidence of the potential for contamination following extreme weather events and provide limited data regarding subsequent survival on the surface of produce, soil or water that is affected. Therefore, there is a high uncertainty regarding how the length of time between a particular extreme weather event and harvest will influence the rate of decline of different pathogens in the field or on the surface of different commodities.

It is also plausible that there may be potential for internalisation as a result of flooding, heavy rain, or dust storms that involve contamination at high levels and that cover or submerge substantial amounts of the commodities. This assumption is based on extrapolating evidence discussed in sections that report the internalisation of pathogens into the edible portions of lettuce via roots (see Sections 9.5.1.1 and 9.6.1.1), internalisation via exposure to damaged areas of plants of rockmelons (see Section 9.5.1.3), or internalisation via submersion during washing for leafy vegetables and rockmelons (see Section 11.4.1). Therefore, there is high uncertainly and low confidence in this assumption as direct evidence of flooding or dust storms leading to internalisation of pathogens into the edible portions of plants is not available nor are studies that have investigated the potential. Also, there is a lack of data for spinach, parsley, watermelons and berries regarding submerged product. The internalisation of pathogens into leafy vegetables and strawberries has been reported via roots exposed to contaminated irrigation water or soil, but this has not been observed for rockmelons.

The use of contaminated water to protect against frost has been identified as a risk factor applying to berries or other crops that employ overhead irrigation to protect against frost. It is established by the preliminary evidence presented above and in Section 9.6, that the use of contaminated water applied to the surface of leafy greens, berries, or melons particularly close to time of harvest, will increase the risk of contamination of the edible parts of those commodities. However, there is variation and limited evidence regarding the subsequent survival, decline, or growth of pathogens on the surface of produce following the application of contaminated water, particularly in regard to freezing events.

Previous expert scientific reports have identified extreme weather events as important risk factors that can contribute to microbiological contamination of the in-scope commodity sectors (EFSA Panel on Biological Hazards 2014a, 2014b, 2014c; FAO/WHO 2008b, 2011).

9.3.3 Mitigation measures

Differing recommendations are provided regarding how commodities affected by flooding[14] should be handled following the event. Extreme weather events that occur close to harvest are considered the greatest risk, as pathogen levels have generally been reported to decline in the primary production environment over time. However, rates of decline can vary between pathogens and inputs such as soil or water (Castro-Ibáñez et al. 2015; EFSA Panel on Biological Hazards 2014a; Won et al. 2013b). This provides some evidence for recommendations that specify that, if a flood event affects the edible parts of fresh fruit and vegetables within a specified time before harvest, the produce should not be consumed as raw product.

Some recommendations specify testing of affected produce and enhancing postharvest washing or sanitisation. However, the use of sanitisers and washing alone to ensure product safety after these events is not recommended because these interventions have limited efficacy for removing pathogens from the surface of produce, and the efficacy of some sanitisers can be reduced by higher organic loads that may be deposited after flooding (see Section 11.4.1). There is no practical method to recondition the edible parts of commodities

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[14] Flooding is defined by the FDA as the flowing or overflowing of a field with water outside a grower's control. Pooled water (e.g. after rainfall) that is not reasonably likely to cause contamination of the edible portions of fresh produce is not considered flooding (FDA 2011).

affected by floodwater that is potentially contaminated with sewage, chemicals, heavy metals, pathogenic microorganisms or other contaminants to ensure product safety (FDA 2011).

In general, the mitigation recommendations provided by other agencies, organisations and peak industry bodies are similar across the in-scope commodity sectors (EFSA Panel on Biological Hazards 2014a, 2014b, 2014c; FAO/WHO 2008b; FPSC A-NZ 2019; IFPA et al. 2006; NSW DPI 2019) and include:

- Environmental risk assessments to be undertaken within one week of harvest to assess on-site and surrounding off-site microbial, chemical or physical factors that may affect product safety in the field
- Include extreme weather events and damage to crops in preplanting and preharvest risk assessments, and include factors that may affect subsequent washing and sanitisation efficacy, such as increased organic loads from flood, dust/soil or damaged produce
- Ensure good drainage of fields and use wind breaks where possible
- Limit cross-contamination via equipment or personnel between affected and non-affected fields.

Differing recommendations have been provided regarding produce handling after flooding:

- The Queensland Government advises that, if flooding or heavy rain occur, the harvest of melons and cucurbits should be postponed and decisions made regarding the need for enhanced postharvest washing or sanitisation (DAF QLD 2016)
- The Fresh Produce Safety Centre Australia & New Zealand's *Guidelines for Fresh Produce Food Safety 2019* recommend testing and use of a pathogen reduction treatment (e.g. washing with sanitiser) postharvest if flood water contacts the harvestable part of produce (FPSC A-NZ 2019)
- The FDA's Guidance for Industry: *Evaluating the Safety of Flood-affected Food Crops for Human Consumption 2011* (FDA 2011) states:
  'If the edible portion of a crop is exposed to flood waters, it is considered adulterated under section 402(a)(4) (21 U.S.C. 342(a)(4)) of the Federal Food, Drug, and Cosmetic Act and should not enter human food channels. There is no practical method of reconditioning the edible portion of a crop that will provide a reasonable assurance of human food safety. Therefore, the FDA recommends that these crops be disposed of in a manner that ensures they are kept separate from crops that have not been flood damaged to avoid adulterating "clean" crops'.
  For crops in which the flood waters did not contact the edible portion of the crops, the FDA recommends that growers should evaluate the safety of the crops for human consumption (FDA 2011)
- The European Commission recommends that:
  'Fresh fruit and vegetables for which the edible part has come into contact with flood waters close to harvest (less than two weeks) should not be consumed as raw product. If the flooding event takes place more than two weeks before harvest or if these products are processed, a case-by-case (site-specific) risk assessment should be performed' (European Commission 2017).

9.4   Seeds and seedlings

Seeds or seedlings can be acquired on or off farm from a variety of sources. If seeds or seedlings are contaminated this can lead to the introduction of contamination to a farm, retention of the contaminant by the seed/plant and potential transfer to the final produce (FAO/WHO 2008b).

9.4.1 Available data

Sprouted seeds are frequently implicated in cases of foodborne illness and, as mentioned previously, were not included in the scope of this assessment as Standard 4.2.6 *Production and Processing Standard for Seed Sprouts* of the Code[15] requires sprout processors to implement effective management of inputs and limit contamination during the primary production of seed sprouts.

The introduction of Standard 4.2.6 was supported by evidence that demonstrated the growth of *Salmonella* spp. and *E. coli* O157:H7 during the germinating process of sprout seeds (Charkowski et al. 2002; Fu et al. 2008; Gandhi et al. 2001; Howard and Hutcheson 2003; Liao 2008; Liu and Schaffner 2007; Montville and Schaffner 2005; Palmai and Buchanan 2002; Pao et al. 2005; Stewart et al. 2001a; Stewart et al. 2001b). Studies have also reported that *Salmonella* spp. and *E. coli* O157:H7 can become internalised in the tissue of seed sprouts during germination (Warriner et al. 2003), and that biofilms could form on the sprout surface (Fett 2000; Fett and Cooke 2003; Warriner et al. 2003). Moreover several studies have shown that levels of bacterial pathogens in spent irrigation water during the germinating process is strongly correlated to levels found in the contaminated seed sprouts (Howard and Hutcheson 2003; Johnston et al. 2005b; Liu and Schaffner 2007; Stewart et al. 2001b). Although care must be taken in extrapolating outcomes regarding the contamination of seed sprouts to other fresh produce, this indicates areas of potential concern for other crops raised from seeds and seedlings.

The available data for the in-scope commodities is summarised below.

9.4.1.1 Leafy vegetables

The production of seeds for leafy vegetables generally occurs in agricultural areas and involves the use of various agricultural inputs. Seeds are generally sowed directly into the soil, but the use of transplanted seedlings is also practiced by the industry. Seedlings are generally produced in greenhouses or tunnels. If exposed to contaminated inputs, seeds and seedlings maybe contaminated by microbial pathogens.

There is little information available about the prevalence and levels of pathogens on naturally contaminated seeds of leafy vegetables. In greenhouse and growth chamber experiments it has been found that pathogens can bind strongly to seeds of leafy vegetables; can persist through prolonged periods of seed storage; and, in some instances, can colonise internal tissues and the surface of seedlings during germination and early seedling growth.

Cui et al. (2017) found that the attachment of selected *Salmonella* spp. and EHEC strains to lettuce (and other vegetable) seeds increased in line with the level of pathogens they were exposed to, and reported the binding of up to ca. 5 log CFU/g bacteria on lettuce seeds. Significantly more bacterial cells attached to mechanically damaged seeds than to intact seeds, and fungicide treatment had little effect on the attachment of most bacterial strains to lettuce seeds. Erickson et al. (2014a) observed binding of 2.79 ± 0.32 Log CFU/g *E. coli* O157:H7 to spinach seeds soaked for 5 minutes in a 4 Log CFU/ml bacterial suspension and dried overnight at room temperature in a laminar flow hood.

Long-term survival of strains of *Salmonella* spp. and *E. coli* O157:H7 on lettuce seeds was described by van der Linden et al. (2013). Seeds inoculated with between 7.75 and 8.63 Log CFU/g (dry weight seed) were stored for up to two years in the dark at room temperature. After 48 weeks, contamination levels had decreased by 1.05 Log CFU/g

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[15] Standard 4.2.6 *Production and Processing Standard for Seed Sprouts:*
www.legislation.gov.au/Details/F2012L00023

(*S*. Thompson); 1.62 Log CFU/g (*S*. Typhimurium); and 4.31 Log and 4.70 Log CFU/g (for the two *E. coli* O157:H7 strains). After 2 years storage, high levels of contamination (7.35±0.06 Log CFU/g) remained on *S*. Typhimurium contaminated seeds, while *E. coli* O157:H7 could only be detected by enrichment (<1.3 Log CFU/g). *Salmonella* spp. could be recovered from every individual seed tested, while *E. coli* O157:H7 could only be recovered from 4–14% of the seeds, depending on the recovery method used.

Several studies have demonstrated the contamination of leafy vegetable seedlings grown from contaminated seed. Habteselassie et al. (2010) observed rapid colonisation of lettuce seedlings—particularly in the root zone—when seeds contaminated with a bioluminescent derivative of *E. coli* O157:H7 were germinated in agar plugs in a growth chamber. Initial bacterial levels of 6.49 Log CFU/g (seed) increased to 7.31 Log CFU/g in seedlings 7 days after seeding, and persisted for the 14 days of the experiment. Similarly, van der Linden et al. (2013) observed *S*. Typhimurium levels averaging almost 5 Log CFU/g on seedlings 3–11 days after germination from dehulled contaminated seeds that had been stored for two years. In the same set of experiments, seedlings from *E. coli* O157:H7 contaminated seeds carried up to ca. 4.4 Log CFU/g after germination. Cui et al. (2018) observed similar seedling contamination dynamics and loads, and noted bacterial strain and plant tissue type differences in adhesion and growth of *Salmonella* spp. and EHEC cells attached to lettuce seeds during germination on an artificial growth medium.
Internalisation of pathogens in seedlings grown from contaminated seeds appears to be dependent on the bacterial species and strain; plant species and cultivar; and possibly the levels of contamination on the seeds. Erickson et al. (2014a) detected no pathogen internalisation in spinach seedlings grown from contaminated seeds. However, *L. monocytogenes* has been shown to colonise lettuce seedlings—on the surface and internally—when present on seeds (Shenoy et al. 2017). When lettuce seeds were artificially contaminated for 30 minutes in 10^8 CFU/mL bacterial suspension, levels of *L. monocytogenes* reached ≥ 7.0 Log CFU/g three days post-germination—irrespective of lettuce cultivar or bacterial strain used—when grown in soft-top agar in greenhouse conditions. Levels remained ≥ 5.0 Log CFU/g for the entire period of cultivation, up to harvest (more than 60 days). However, in potting mix and soil growth media, contamination persisted for significantly shorter periods of time (up to 45 days in commercial potting mix). Internalised bacteria were found in both intra- and inter-cellular locations in all major tissue types in 20-day-old plants grown in commercial potting mix.

Some studies show that pathogens can persist on or in the edible portion of leafy vegetable seedlings for several weeks. Standing et al. (2013) observed the uptake, internalisation and persistence of *E. coli* O157:H7, *S*. Typhimurium and *L. monocytogenes* in lettuce seedlings watered daily with nutrient solution inoculated with 10^5 CFU/mL of the respective pathogens under laboratory conditions. Maximum average levels of pathogens in surface-sterilised leaves of seedlings were: 4.53 Log CFU/g (*E. coli* O157:H7); 3.46 Log CFU/g (*L. monocytogenes*); and 1.35 Log CFU/g (*S*. Typhimurium). *E. coli* O157:H7 and *S*. Typhimurium remained detectable after 28 days of seedling growth, while *L. monocytogenes* was not detected after more than 14 days. It is not clear, from the description of the experimental methods provided, whether internalisation occurred through roots, leaves or both. However, Wong et al. (2019) observed internalisation of a range of *Salmonella* serovars in lettuce seedlings inoculated by immersion of the leaves of the plants in cell suspensions. Laser scanning confocal microscopy showed that cells or cellular aggregates were located within stomata, in surface depressions adjacent to stomata, or on random leaf surface locations on seedlings that were successfully colonised. The ability to colonise and persist on seedlings over the five days of the experiment showed plant cultivar and *Salmonella* strain variability.

9.4.1.2 Berries

In Australia berries are generally propagated from vegetative propagules (e.g. strawberry runners or from cuttings). The plants can be propagated by the grower or purchased, and if exposed to contaminated inputs may introduce microbial pathogens.

Experimental or epidemiological information assessing the contamination of HAV, NoV or STEC in strawberry, blueberry or raspberry plants due to propagation and the potential for persistence of contamination and/or transfer to edible produce was not identified in the literature.

9.4.1.3 Melons

Rockmelons and watermelons in Australia are grown both from seeds and seedlings that can be purchased or produced in areas outside of the principal production fields, and if exposed to contaminated inputs may introduce microbial pathogens.

Experimental or epidemiological information assessing the contamination or persistence of *L. monocytogenes* or *Salmonella* spp. on rockmelon or watermelon seeds or seedlings and the potential for transfer to edible produce was not identified in the literature.

9.4.2   Conclusions, uncertainty, and data gaps

The evidence presented above indicates that pathogens can bind strongly to seeds of leafy vegetables; can persist through prolonged periods of seed storage; and, in some instances, can colonise internal tissues and the surface of seedlings during germination and early seedling growth. The internalisation of pathogens into seedlings grown from contaminated seeds appears to be dependent on the bacterial species and strain; leafy vegetable species and cultivar; and possibly the levels of contamination on the seeds.

It is possible that the internalisation and persistence in the edible part of the plant resulting from contaminated seed could represent a risk factor for some leafy vegetables. There is medium uncertainty and confidence in this conclusion based on the evidence available provided for leafy vegetables. However, due to a lack of studies investigating both the potential for contamination via the seed and subsequent internalisation into the fruits of berries and melons, it is not possible to determine whether this risk factor applies to these commodities. Notably, because blueberries, raspberries, and strawberries are rarely grown from seed, and potentially the distance required to translocate from seed to fruit during germination and subsequent growth of melons reduce the likelihood of internal contamination via the seed for these commodities. However, there is little information available determining the prevalence and levels of pathogens on naturally contaminated seeds of any commodity. As such, the exposure to pathogens could be due to the seed itself or attributed to contaminated inputs that then contact the seed; these other inputs are discussed in other sections.

9.4.3   Mitigation measures

No specific recommendations for the in-scope commodity sectors were identified. However, pertinent requirements do apply for seed sprouts, in recognition of the potential for foodborne pathogens present on seeds to survive and grow through the germination and seedling growth stages. Standard 4.2.6 *Production and Processing Standard for Seed Sprouts* of the Australia New Zealand Food Standards Code[16] requires sprout processors to:

- Comply with the general food safety management requirements.
- Not produce or process seed sprouts if the processor ought reasonably know or suspect that the seed is of a nature or in a condition that would make the seed sprouts unacceptable
- Take all reasonable measures to ensure inputs do not make the seed sprouts unacceptable
- Implement effective decontamination processes prior to sale or supply of seed sprouts
- Have one step forward and one step back traceability
- Not sell or supply seed sprouts for human consumption if the sprout processor ought reasonably know or reasonably suspect that the seed sprouts are unacceptable.

9.5   Soil, soil amendments, and fertilisers

Soil and organic soil amendments such as manure, human biosolids, compost, and plant biowastes may harbour and promote the survival of pathogens. Soil amendments[17] are often applied to improve soil health and increase the nutrients available for crops. Plant biowastes are regularly retilled back into the soil following harvest. The prevalence of pathogens in soil, the ability of the pathogen to persist in the soil, the amendment type, application method, application rate, application frequency, time between application and planting or harvest are identified as risk factors that may contribute to the transfer of pathogens to fresh produce (FAO/WHO 2008b).

9.5.1   Available data

Foodborne pathogens can survive in soil for extended periods (Avery et al. 2012). For example, *L. monocytogenes*, *Salmonella* spp*., E. coli*, and viruses have been reported to survive for up to 84 days (Locatelli et al. 2013), 180 days (Danyluk et al. 2008), 180 days (Nyberg et al. 2010) and 170 days (Rzezutka and Cook 2004), respectively. Although most pathogens are generally reported to decline in soil overtime, the potential for growth in soils has been reported for bacteria with addition of nutrients and moisture to soils (Danyluk et al. 2008), in the absence of soil microbiota (McLaughlin et al. 2011), and in some manure amended soils (Berry and Miller 2005).

Enteric pathogens have also been observed to survive in manure for weeks to years (Avery et al. 2012; EFSA Panel on Biological Hazards 2014a, 2014b, 2014c), and environmental factors have been shown to reduce their persistence including higher pH, temperatures, fibre levels, and aeration levels. The extended persistence of enteric pathogens in manure amended soils has also been reported to range for days to years (Avery et al. 2012; EFSA Panel on Biological Hazards 2014a, 2014b, 2014c). Pathogen persistence is reduced by

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[16] Standard 4.2.6 *Production and Processing Standard for Seed Sprouts:* www.legislation.gov.au/Details/F2012L00023

[17] Soil amendment: Physical, chemical and biological material added to the soil to improve the health, nutrition and crop productivity of the soil, e.g. inorganic fertilisers, manure and compost (NSW DPI 2019).

factors including higher temperatures, increased levels of native microflora, lower levels of available nutrients, and lower clay content (EFSA Panel on Biological Hazards 2014a).

L. monocytogenes has been reported to grow in soils—whether manure amended or not—in the presence of competing natural background microorganisms (Dowe et al. 1997). A recent meta-analysis that quantitatively assessed the effect of different environmental factors on the persistence and inactivation of *E. coli* in manure amended soils determined that temperature and the method of application were the most significant factors. Higher temperatures (>20°C) and incorporation into the soil resulted in extended persistence of *E. coli* compared to lower temperatures (0–10°C) and surface application in field studies (Tran et al. 2020). (Phan-Thien et al. 2020) showed that the persistence of *Salmonella enterica* in soil environments was significantly influenced by a range of individual and interacting environmental effects, including temperature, soil type and amendment addition. However, in the meta-analysis by (Tran et al. 2020), no significant factors were shown to contribute to the decline of *Salmonella* spp. in field studies, although the rate of decline was similar to that of *E. coli*—the average time for a 1 Log reduction at 10–20°C in manure amended soils was 10.50 days and 11.67 days, respectively. In their modelling of the risk from *E. coli* O157:H7 in fresh-cut cos lettuce, (Bozkurt et al. 2021) used a soil prevalence of 42% as an input of the model, based on recorded levels of *E. coli* in manure amended soil in Australia.

Plant material biowastes are often used as soil amendments and composts. Lemunier et al. (2005) assessed the persistence of *L. monocytogenes*, *E. coli*, and *Salmonella* spp. experimentally inoculated into composts composed of varying proportions of paper, cardboard, fruits, vegetables and green waste. Mature compost did not support the extended survival of *L. monocytogenes* and only limited survival of *E. coli*. However, extended survival of *S*. Enteritidis inoculated into mature compost was observed over three months, which indicates the importance of reducing the likelihood of recontamination of composts.

The use of human biosolids to amend soils or contamination by faecal pollution increases the risk from viruses including NoV and HAV. Enteric viruses have been reported to persist in the soil up to 100 days due to their resilience to environmental factors, but factors such as pH, soil type, and temperature can influence their persistence (Rzezutka and Cook 2004). NoV and HAV have also been reported to maintain infectivity in animal manure for 60 days (Wei et al. 2010).

The evidence specifically associated with the in-scope commodities and hazards is summarised below.

9.5.1.1 *Leafy vegetables*

In a number of studies, under experimental conditions in controlled environments and in the field, *E. coli*, *Salmonella* spp. and *L. monocytogenes* applied in contaminated soil and/or compost to the root zone have been observed to colonise the roots and edible parts of leafy vegetables, including internally in some cases. The incidence and degree of colonisation and internalisation reported is highly variable, and depends on the experimental set-up; soil characteristics; plant species and cultivar; and the bacterial species and strain. The type and microbiological quality of any soil amendments used and their time and method of application are also important factors affecting the risk of contamination of leafy vegetables. Key findings are summarised below.

Pathogens present in soils due to the application of contaminated soil can transfer to, and persist on or in, the edible parts of lettuce, spinach and parsley.

Various *Salmonella* serovars have been shown to be able to colonise roots (Arthurson et al. 2011; Fornefeld et al. 2018) and leaves (Arthurson et al. 2011; Honjoh et al. 2014; Nicholson et al. 2015; Zhang et al. 2016) of lettuce when present in soils. The capacity of salmonellae to internalise in the leaves after uptake through the roots appears to be limited and variable. Honjoh et al. (2014) found surface contamination, but did not detect internalisation in lettuce during a 10-week cultivation from seed in soils inoculated with up to 10^8 CFU/g *S*. Enteritidis. Ge et al. (2012) found that when soil was inoculated with high levels of *S*. Typhimurium ($\geq 10^7$ CFU/g soil), internalisation into lettuce via root uptake occurred. However, both Nicholson et al. (2015) and Zhang et al. (2016) found low levels (generally <2 Log CFU/g) of internalised salmonellae in leaves of lettuce plants grown in soils contaminated with 4.45 Log CFU/g and 5 Log CFU/g, respectively, of a number of serovars (Newport, Typhimurium, St. Paul, Montevideo, Infantis).

Low level colonisation of roots and leaves of the three in-scope leafy vegetables by *E. coli* O157:H7 is consistently reported (Erickson et al. 2014a; Mootian et al. 2009; Nicholson et al. 2015), but internalisation in edible parts of the plants appears to be relatively uncommon, limited to low levels, and dependent on water and soil conditions and plant age.

Erickson et al. (2014a) found that internalisation observed in growth chamber experiments—when spinach and lettuce seeds were germinated in soil containing ≥ 3.5 Log CFU/g *E.coli* O157:H7—was not replicated when seeds were germinated in the field; and also observed limited surface contamination in the field grown plants. In further growth chamber experiments, Erickson et al. (2014b) observed transient internalisation into roots and leaves of spinach plants at soil loads of *E. coli* O157:H7 as low as 4.2 Log CFU/g. At higher soil pathogen loads (ca. 7 Log CFU/g), similar average levels of internalisation of *E. coli* O157:H7 into roots of 4–6 week old lettuce, spinach and parsley plants were observed. In all three cases, internal translocation of bacteria from roots to leaves was rare.

Similar results were obtained by Mootian et al. (2009) for young lettuce plants (12 day post-germination) exposed to up to 10^4 CFU/g *E. coli* O157:H7 in soil. However, for mature plants (30 days) surface and internalised *E. coli* O157:H7 in lettuce leaves were detected by enrichment 15 days after exposure in soil to levels as low as 1–2 Log CFU/g. Nicholson et al. (2015) also observed prolonged internal colonisation of *E. coli* O157:H7 into the leaves of 6 week old lettuce plants, with 33% of plants containing up to 0.38 Log CFU/shoot 22 days after exposure to 5.11 CFU/g in soil. Conversely, only transient surface contamination and no internalisation in leaves was observed when lettuce seedlings were grown in soil contaminated with five strains of GFP-labelled *E. coli* O157:H7 (individually or together) at 3, 4 or 6 Log CFU/g, regardless of heat and soil moisture conditions (optimal or stressed), type of lettuce, or age of plants (Zhang et al. 2009a; Zhang et al. 2009b).

For *L. monocytogenes*, Honjoh et al. (2018) found leaf surface contamination but no internalisation in lettuce grown from seed in soils inoculated with 4–8 log CFU/g.

Also, soil characteristics were shown to affect adherence of *E. coli* O104:H4 to, and internalisation into, roots of lettuce grown under greenhouse conditions in soils contaminated with bacteria at 2.2×10^8 CFU/g soil (Eissenberger et al. 2020). Bacterial loads of 5.0×10^3 CFU/g and 2.6×10^4 CFU/g were detected in the roots of lettuce grown in diluvial sand or alluvial loam, respectively, after surface disinfection. However, no assessment was undertaken of the potential for bacterial translocation from the roots to the edible portion of the plants.

The use of insufficiently composted manure as fertiliser has been associated with increased *Salmonella* spp. prevalence in leafy vegetables in a multi-country survey (Ceuppens et al. 2015). However, evidence of *Salmonella* spp. colonisation and internalisation from experiments involving leafy vegetable cultivation in inoculated, compost amended or manure

amended soils is variable.

No contamination of leaves was observed in spinach grown from seed in cattle manure slurry amended soil inoculated with up to 6 Log CFU/g *S*. Weltevreden (Arthurson et al. 2011), or in edible parts of lettuce seedlings exposed to up to 6.5 Log CFU/g *S*. Typhimurium in soils amended with municipal sewage sludge or cattle manure (Fornefeld et al. 2018; Franz et al. 2005). However, Shah et al. (2019) observed low levels of S. Newport (<5 MPN/plant) in spinach plants grown in soil amended with inoculated heat-treated poultry pellets, and Islam et al. (2004b) detected an avirulent strain of *S*. Typhimurium on lettuce and parsley grown in soil fertilised with contaminated (to 10^7 CFU/g) poultry or bovine manure composts for up to 63 days and 231 days, respectively.

While internalisation was not assessed in either of the above cases, others have described *Salmonella* spp. colonisation. Murphy et al. (2016) found internalised *S*. Senftenberg in roots and leaves of lettuce plants up to 50 days after three week old seedlings were transplanted into peat growing media amended with contaminated (at 5 Log CFU/g wet wt) food waste derived compost or anaerobic digestate liquid. Also *S*. Newport was recovered at levels of between 75 and 1275 CFU per plant in six week old mini-cos lettuce grown from seed in cattle manure amended soil (inoculated at 10^7 CFU/g wet wt) (Klerks et al. 2007a; Klerks et al. 2007b). Bacterial cells were mainly present in the stem of the plants, between the root-stem transition point and the leaves.

Evidence of colonisation and internalisation of leafy vegetables by *E. coli* from experiments involving cultivation in inoculated, compost amended or manure amended soils is variable.

While it has been demonstrated that *E. coli* O157:H7 can persist for more than five months in manure amended soils planted with lettuce or parsley (Islam et al. 2004a), few studies describe surface contamination of the roots of leafy vegetable. Persistent colonisation of the rhizosphere (roots and closely associated soil) was observed by Habteselassie et al. (2010) in lettuce plants grown in cattle manure amended soil, but Franz et al. (2005) only observed colonisation of one of 24 washed root samples of lettuce plants grown on two types of soil amended separately with three types of cattle manure.

Internalisation of *E. coli* into the roots of leafy vegetables varies. In three week old lettuce seedlings transplanted into contaminated amended soils, internalisation of root-associated *E. coli* O157:H7 was observed for up to 50 days (Murphy et al. 2016). However, Sharma et al. (2009) found only sporadic internalisation in roots of baby spinach seedlings planted in soil amended with cattle faecal slurries. Also, Johannessen et al. (2005) reported no internalisation in lettuce roots 50 days after seedlings were transplanted into soil fertilised with bovine manure/urine slurry inoculated with ca. 10^4 CFU/g E. coli O157:H7.

Results of studies on *E. coli* colonisation of edible parts of leafy vegetables are also variable. Several studies show surface contamination of lettuce or parsley grown from seeds or transplanted seedlings in contaminated manure amended soils (Islam et al. 2004a; Jensen et al. 2013; Mootian et al. 2009; Oliveira et al. 2012; Solomon et al. 2002b). However, others have reported a lack of colonisation (Franz et al. 2005; Johannessen et al. 2005), or only transient colonisation (Habteselassie et al. 2010), in lettuce in similar experiments.

Internalisation of *E. coli* O157:H7 for up to 50 days in edible parts of lettuce grown in manure amended soils has been observed in some studies (Mootian et al. 2009; Murphy et al. 2016; Solomon et al. 2002b). However, Mootian et al. (2009) observed only transient internalisation in young plants, and no internalisation when older plants were exposed to up to 10^4 CFU/g *E. coli* O157:H7 in amended soils. Sharma et al. (2009) found only sporadic internalisation over four weeks in leaves of baby spinach seedlings planted in soil amended with cattle faecal slurries, and did not observe internalised GFP-labelled *E. coli* microscopically.

Few studies have examined the role of soil amendments in the colonisation of leafy vegetables by *L. monocytogenes*. Murphy et al. (2016) observed high prevalence of surface contamination of roots and leaves (up to 43% and 67%, respectively) with *L. monocytogenes* in lettuce seedlings transplanted into peat growing media amended with contaminated (at 5 Log CFU/g wet wt) food waste derived compost or anaerobic digestate liquid. No internalisation of *L. monocytogenes* was observed. Using *L. innocua* as a non-pathogenic surrogate for *L. monocytogenes*, Oliveira et al. (2011) observed contamination of lettuce leaves in seedlings transplanted into organic compost amended soil (6-7 Log CFU/g) under outdoor conditions. However, they did not assess if there was internalisation via root system, or if leaf contamination occurred by other means, such as by direct contact with soil or transfer by insects or other vectors.

9.5.1.2   Berries

Some types of berries, such as blueberries and raspberries, grow on bushes or vines so are elevated from the ground. These berries are also often grown under plastic tunnels and are planted into substrate (e.g. peat moss or coco peat) rather than soil, with nutrients added directly into the substrate. Other types of berries, such as strawberries, are generally grown on plastic or mulch close to the ground or directly on the ground. A field experiment found no significant difference in the persistence of generic *E. coli* on strawberries grown on different mulch types—straw and plastic (Généreux et al. 2015). Strawberries grown on straw mulch are in contact with straw and soil, which may lead to microbial contamination if pathogens are present in the soil or amendments, while plastic forms a barrier between the fruit and the soil.

International studies have been performed to investigate the presence of STEC in the soil of strawberry farms. In Norway STEC was detected in 1.3% (1/80) of soil samples collected in strawberry fields via PCR, however, this was not culture confirmed. Generic *E. coli* was detected in 38.8% (31/80) soil samples (Johannessen et al. 2015). In a South Korean study no generic *E. coli* or *E. coli* O157:H7 was detected in soil collected from tunnel style strawberry greenhouses (number of samples was not reported) (Yoon et al. 2010). In a US study of soil in blueberry fields, generic *E. coli* was detected at low levels (0.13-0.25 Log CFU/g), while STEC was not detected (0/48) regardless of fertilizer application over a two year period. The generic *E. coli* levels of post-fruit harvest soil samples were slightly increased, which was possibly due to the increased human activities and/or the environmental temperature in the late production season (Shen et al. 2020). In a similar study by Sheng et al (2019) generic *E. coli* was detected at low levels (<1 Log CFU/g) and STEC was not detected (number of samples was not reported), regardless of fertilizer application, in the soil of a US red raspberry field.

Experimental studies have shown that pathogens can persist in the soil of strawberry beds and internalisation of pathogens into strawberry plants from the soil can also occur. A study by Shaw et al. (2015b) showed that when strawberry plants were artificially inoculated with surrogates for *E. coli* O157:H7 (10^7 CFU/plant), the pathogen surrogates (other *E. coli* strains which did not differ in their growth and survivability compared to *E. coli* O157:H7) could survive for at least 15 weeks in the soil of strawberry beds under greenhouse conditions, even though contamination did not occur directly into the soil. DiCaprio et al. (2015) demonstrated that inoculation of the soil of strawberry plants with NoV surrogates (2×10^8 PFU/plant)—murine NoV (MNV-1) and Tulane virus (TV)—led to viral internalisation through the roots and dissemination of the virus to the fruit. After soil inoculation, 31.6% (12/38) and 37.5% (18/48) of the strawberry fruit harvested were positive for infectious MNV-1 or TV, respectively, over the 14 days of the study.

9.5.1.3 Melons

Rockmelons and watermelons in Australia are grown either on plastic mulch close to the ground or directly on the ground. This may lead to microbial contamination if pathogens are present in the soil or amendments. The variety of soil amendments used by the industry is likely to be diverse and can include amendments produced both on and off farm.

The prevalence of *Salmonella* in the soil of rockmelon fields internationally has been investigated. 8.3% (2/24) soil samples collected from Mexican rockmelon farms during harvest were positive for *Salmonella* spp. (Espinoza-Medina et al. 2006). 3.7% (2/54) of soil samples collected from US melon production fields were positive for *Salmonella* spp. (Dev Kumar et al. 2015).

L. monocytogenes has not been isolated from soil samples from rockmelon production fields in Mexico 0/38 (Heredia et al. 2016), Korea 0/18 (Park et al. 2013a), or the US 0/54 (Dev Kumar et al. 2015). No Australian data on the presence of *L. monocytogenes* in soil in melon fields was identified.

Salmonella spp. have been reported to persist and survive in the soil of experimentally inoculated rockmelon fields for at least 49 days (Lopez-Velasco et al. 2012). Similarly, preliminary evidence by Burris et al. (2018) reported the survival of a *Salmonella* serovar cocktail inoculated (8.4 Log CFU/root zone) into the soil of greenhouse grown vines for at least 20 days post inoculation. Data reporting the survival of *L. monocytogenes* in melon fields was not identified, however the bacterium is reported to survive in soil for up to 84 days (Locatelli et al. 2013). As these pathogens were reported to decline in the soil, the initial contamination load is an important consideration regarding the risk posed by contamination in soil.

The internalisation of *Salmonella* spp. into rockmelon flesh via the roots from contaminated soil has not been observed in preliminary studies. A *Salmonella* serovar cocktail (serovars Javiana, Newport, Panama, Poona and Typhimurium) inoculated into soil of rockmelon plants maintained in a greenhouse at high levels (8.4 log CFU/root zone) was found to translocate to the lower stem in 5% (2/40) of plants sampled seven days post inoculation. However, the subsequent internalisation of *Salmonella* to the mature fruit via contaminated soil was not observed (Burris et al. 2018).

There is preliminary evidence that different soil cultivation practices may influence the risk from pathogens in soil, with one study reporting that the use of different combinations of muscadine-pomace compost, mushroom compost, solarisation and cultivation of a mustard cover crop successfully inactivated *Salmonella* spp. experimentally inoculated into melon fields (Reed et al. 2018b).

An outbreak investigation in the US concluded that contamination of fresh rockmelon by *Salmonella,* that caused 261 illnesses and 6 deaths, originated in the production environment as the outbreak strain was found in soil samples on the implicated farm. The investigation hypothesised it was likely the contamination was introduced either from irrigation water or biological soil amendment (FDA 2014).

The evidence summarised below regarding the ability of pathogens to internalise into rockmelon flesh via wounds and the blossom has implications for food safety if these areas are exposed to pathogens. This could include via irrigation water or soil amendments applied in a way that contacts these areas, dust, or submersion in water (such as floodwater). The potential uptake of *Salmonella* spp. from the environment due to wounding of plants has been investigated. *Salmonella* was detected nine days after exposure to an attenuated *S.* Typhimurium strain (4 Log CFU/mL) at a site where the peduncle was slightly damaged

with a needle. Positive detections occurred in the peduncle (75%), adjacent vine tissues (25%) and the fruit mesocarp flesh (5%) from 20 samples (Lopez-Velasco et al. 2012). The percentages of positive tissues increased with increasing inoculum doses in subsequent experiments, and suggests that internalisation and transference can occur if a high enough inoculum can reach wound sites, but the ability to reach the internal fruit is dose dependent.

Preliminary evidence suggests that *Salmonella* spp. can internalise into rockmelon flesh via contamination of the flower blossom. A *Salmonella* serovar cocktail (serovars Javiana, Newport, Panama, Poona and Typhimurium) was inoculated onto the blossom of rockmelon plants maintained in a greenhouse at 4.4 log CFU/blossom and resulted 86% (12/14) of harvested rockmelons positive for *Salmonella* internalised in the flesh. In this study only inoculation of blossoms and not the inoculation of soil resulted in internalisation by *Salmonella* into melon flesh (Burris et al. 2018).

9.5.2 Conclusions, uncertainty, and data gaps

The contamination of soil or soil amendments are risk factors that apply broadly to the commodity sectors. There is a low level of uncertainty and high level of confidence in this conclusion, based on the general evidence for the presence and persistence of pathogens in soil and soil amendments, and the available evidence for leafy vegetables, berries, and melons.

Surface contamination via contaminated soil is a risk factor for all commodities. There is a low level of uncertainty and high level of confidence in this conclusion, based on the general evidence for the presence and persistence of pathogens in soil and soil amendments, and the available evidence for leafy vegetables, berries, and melons.

It is plausible that those commodities that grow closer to the ground—including lettuce, spinach, parsley, rockmelon, watermelon and strawberries—may be at a greater risk of contamination via direct contact with soil compared to blueberries and raspberries. However, indirect contamination, such as soil carried via wind or water, would still present a risk to those higher growing commodities. Studies directly assessing the potential for contaminated soil to transfer pathogens to the external portions of watermelons, raspberries, and blueberries were not identified. Moreover, studies assessing differences in levels of attachment by pathogens onto different commodities as a result of the intrinsic surface properties, such as surface roughness, are limited. However, it is plausible that those commodities with rougher surfaces would afford increased protection to pathogens from environmental factors and subsequent washing and sanitising.

Contamination of soil or application of contaminated soil amendments that occur close to harvest are generally considered to represent an increased risk. There is a low level of uncertainty and high level of confidence in this conclusion, based on the general evidence for the presence and persistence of pathogens in soil and soil amendments. However, the persistence, decline, or growth of pathogens contaminating soil or soil amendments is reported to vary greatly in the literature reviewed. Moreover, the variety of different methods used in studies can make comparisons difficult. For example, experimental studies in laboratory environments may not represent the complexity of the field environment, and field studies do not always examine the same environmental parameters that can influence persistence. In general, pathogens are reported to decline in soils but that rate of decline can be highly variable due to pathogen, environmental, and physical soil parameters.

Internalisation of pathogens contaminating soil via the roots to the edible portion of the plant is a risk factor that has been identified for leafy vegetables and strawberries. While there is medium uncertainty and confidence in this conclusion for leafy vegetables, high uncertainty exists regarding other commodities. Only a single study was identified for strawberries and rockmelons. No studies have investigated the potential for internalisation via roots from contaminated soil for watermelons, blueberries, and raspberries. It is likely that the larger distance required to translocate from the soil to the edible portion of melons, raspberries, and blueberries may reduce the risk of internalisation of pathogens via the roots for these commodities, but further studies are needed for confirmation.

At the low concentrations of pathogens generally reported in the field, the available data suggest that internalisation of pathogens from contaminated soil into the edible part of leafy vegetables via the roots has a low probability. However, higher concentrations associated with contamination events (such as the application of raw manure) present an increased risk. There is medium uncertainty and confidence in this conclusion for leafy vegetables. However, a variety of factors may influence the potential for internalisation of pathogens via soil including the concentration, pathogen species or strain, plant cultivar, plant age, plant physiology factors, and environmental factors such as soil moisture. Moreover, factors affecting the probability that the pathogens would be present in the edible portions at the time of harvest, such as the subsequent persistence or potential for growth of pathogens in the plant tissue, are poorly documented.

The density of pathogens in soil associated with leafy vegetables, berries and melons is generally reported to be low. There are very limited studies in the Australian context for these commodities. However, high densities of pathogens have been reported in soil internationally. It is likely that high levels of contamination can occur as sporadic events, with pathogens likely to be heterogeneously present in soil leading to difficulties in detection.

Previous expert scientific reports have identified soil and soil amendments as important risk factors that can contribute to surface and/or internal microbiological contamination of the in-scope commodities (EFSA Panel on Biological Hazards 2014a, 2014b, 2014c; FAO/WHO 2008b, 2011).

9.5.3 Mitigation measures

The majority of evidence for the efficacy of soil decontamination methods such as soil fumigation (Tadmor et al. 2005), solarisation (Antoniou et al. 1995), and steaming (Gilardi et al. 2014) has been described mainly for plant pathogens, and further research is required for foodborne pathogens such as *Salmonella* spp., *L. monocytogenes*, and *E. coli* (Gurtler 2017). However, all of these interventions reduce the natural populations of microbes in the soil, and it is hypothesised that the colonisation of the soil by the natural microbiota makes it harder for introduced bacteria to dominate. For example, the reduction of soil microbiota by fumigation has been shown to increase the persistence and survival of subsequently introduced *E. coli* (van Elsas et al. 2007). Soil biofumigation via the application of biomass amendments that release antimicrobial compounds has been investigated for inactivation of plant pathogens (Mazzola et al. 2001), but evidence relating to foodborne pathogens is limited. Some evidence is available for the inactivation of *E. coli* O157:H7 in soil using biochar amendments (Gurtler et al. 2014).

There is evidence that soil amendments that include animal manures treated using pasteurisation or composting can reduce levels of foodborne pathogens (Germer et al. 2010; Shepherd Jr et al. 2010), but only if undertaken under optimised, well controlled and monitored conditions that can be complicated to implement consistently, particularly on farm (Erickson et al. 2010; Erickson et al. 2015a, 2015b).

As indicated below, differing advice has been provided regarding the addition of untreated animal manures to crops of fresh produce. A recent meta-analysis that quantitatively assessed the effect of different environmental factors on the persistence and inactivation of *E. coli* and *Salmonella* spp. in manure amended soils determined that the median time for a 1 Log reduction was 7.62 days and 5.67 days, respectively, in the field conditions of temperatures from 10-20°C. While this median rate of inactivation suggests that *E. coli* and *Salmonella* spp. would die off within proposed exclusion times of 90 and 120 days, the authors indicated that the 95% upper CI predicted values of inactivation provide a more conservative estimation and indicate that the Log reduction in 120 days could be 8.18 and 4.74 Log CFU/g for *E. coli* and *Salmonella* spp., respectively (Tran et al. 2020). Thus, the initial contamination level of manure amended soils is an important variable to consider. The authors concluded that it is unlikely that risks from manure amendments with high loads of pathogens will be mitigated by a uniform exclusion period (Tran et al. 2020).

In general, the mitigation recommendations provided by other agencies, organisations and peak industry bodies are similar across the in-scope commodity sectors (EFSA Panel on Biological Hazards 2014a, 2014b, 2014c; FAO/WHO 2008b; FPSC A-NZ 2019; IFPA et al. 2006; NSW DPI 2019) and include:

- Minimisation of soil contact and the use of plastic mulch under crops
- Avoiding untreated animal manures
- Avoiding treated composts containing animal manures or poultry litter or, if treated soil amendments or compost are applied, documenting the source of the compost and its microbial quality test report or ensuring it is compliant with Australian Standard AS 4454-2012: Composts, soil conditioners and mulches or the Freshcare Compliant Compost Standard
- If compost or soil amendments are produced on farm, validating and monitoring the process to ensure microbial quality
- Maximising the time between any soil amendment application and harvest
- Implementing practices to minimise contamination of treated soil amendments
- Using crop-based manures and crop rotation to improve soil health.

Conflicting advice is provided regarding the addition of untreated animal manures:

- Commodity specific guidelines for leafy greens (IFPA et al. 2006) and rockmelons (NSW DPI 2019) advise that untreated or raw animal manure must not be used during the primary production of these commodities
- The Fresh Produce Safety Centre Australia & New Zealand's *Guidelines for Fresh Produce Food Safety 2019* advise to avoid using amendments with untreated animal manure for produce that may be eaten uncooked but also recommend a 90 day exclusion period between grazing or the application of soil amendments containing untreated animal manure and crop harvest for the in-scope commodities. No testing is required after the exclusion period, prior to use. It is noted that some standards mandate a longer exclusion period, e.g. the Fresh Salad Producers Group (A-NZ) voluntary Standard for Fertilisers and Soil Additives (manure is not applied within 365 days of harvest) and the Harmonised Australian Retailer Produce Scheme (HARPS) (FPSC A-NZ 2019)
- The Freshcare *Food Safety & Quality Standard Edition 4.2* requires liquid or foliar sprays derived from untreated manures that may contact the harvestable part of the crop, to have a 90 day exclusion period for produce that may be eaten uncooked and a 45 day exclusion period for all other produce. No testing is required after the exclusion periods, prior to use (Freshcare 2020)
- The GLOBALG.A.P. *Primary farm assurance: All farm base – Crops base – Fruit and Vegetables: Control points and compliance criteria* states that when raw animal manure is used, it should be incorporated into the soil at least 60 days prior to

harvest, and in the case of leafy vegetables raw manure should not be applied after planting. No testing is required after the exclusion period, prior to use (GlobalG.A.P. 2020)

- The US 7 CFR 205.203 *Soil fertility and crop nutrition management practice standard* requires raw animal manure to be composted unless it is incorporated into the soil at least 120 or 90 days prior to the harvest for product whose edible portion does, or does not, have direct contact with the soil, respectively (US GPO 2011).
- The *Food safety practices* of the California Leafy Green Products Handler Marketing Agreement recommends not to use or apply soil amendments that contain un-composted, incompletely composted or non-thermally treated animal manure to fields for lettuce and leafy vegetable production. If these materials have been applied to a field, a one year waiting period is recommended prior to producing leafy vegetables (California LGMA 2020).

9.6 Water

Water is an important source of contamination of fresh produce. For example, water used for irrigation can be a vehicle for viral, bacterial or parasitic pathogens. The use of contaminated irrigation water can increase the risk of exposure of roots, plants, and produce to pathogens (FAO/WHO 2008b; Uyttendaele et al. 2015).

The production of fresh produce relies on the use of water that can be obtained from a variety of different sources including surface water (e.g. streams, rivers, lakes and ponds), groundwater, rainwater, reclaimed water, or potable water sources. Water can be supplied through channels, irrigation ditches, or piping and can also be collected in basins, wells, or dams. Preharvest water use also includes application of farm chemicals to crops and the cleaning of field equipment (FAO/WHO 2008b; Uyttendaele et al. 2015). Use of agricultural chemicals, such as pesticides or fungicides mixed with contaminated water can also led to pathogen contamination of the crop (Miranda and Schaffner 2018).

The quality of agricultural water can be affected by the type of water source, upstream land use, rainfall levels and runoff, flooding, drought, biofilms in irrigation equipment, the topography and land use of the surrounding area, and animal activity in and around water sources (FAO/WHO 2008b).

9.6.1 Available data

The sources of agricultural water are generally associated with different levels of risk based on their likelihood to contain microbial pathogens. Municipal potable water is considered the best quality water, followed by groundwater, rainwater, surface water, and the least acceptable being wastewater (Leifert et al. 2008; Uyttendaele et al. 2015). Due to the generally acceptable quality and low cost of groundwater, this source of water is increasingly being used on horticultural crops (Uyttendaele et al. 2015).

Irrigation water is recognised as a potential vehicle for microbial pathogens in Australia. However, studies describing the prevalence of different foodborne pathogens in different types of water sources, and the environmental factors that may influence their prevalence, are limited. *E. coli* and faecal coliforms have been found in creeks that flow through areas of pristine land, farm land or mixed-use land, and in associated drinking water reservoirs in Australia (Miles et al. 2010; Thurman et al. 1998). In their modelling of the risk from *E. coli* O157:H7 in fresh cut cos lettuce, (Bozkurt et al. 2021) used a prevalence of 4.9% in irrigation water as an input of the model, based on recorded levels of *E. coli* in irrigation water in Australia.

A variety of *Salmonella* serovars have been isolated from different water sources during outbreak investigations including agricultural water sources in Western Australia (Gibbs et al. 2009), Queensland, and the Northern Territory (Munnoch et al. 2009). Recent sampling of various types of agricultural water sources in NSW also detected *Salmonella* spp. and *E. coli* in water and sediment (van Ogtrop 2018). Comparatively, very limited information is available for *L. monocytogenes*, though it has been detected in dam water in Australia (McAuley et al. 2014).

The contamination of water sources by NoV or HAV is generally the result of wastewater leakage or overflow. NoV is shed in large numbers in the faeces of both symptomatic and non-symptomatic carriers and is commonly present in wastewater. A high genotypic diversity of NoV has been reported in wastewater in NSW and Victoria (Lun et al. 2018). HAV is less common than NoV in Australia, though the presence (or absence) of the virus in Australian waters or wastewater is poorly documented.

Extended survival times in autoclaved river water have been reported for *Salmonella* spp. (>100 days), *E. coli* O157:H7 (>100 days)*,* and NoV (up to 60 days) (Ibrahim et al. 2019). *L. monocytogenes* was reported to survive 120 days in river water (Budzińska et al. 2012), and HAV survived for up to 56 days in sterile groundwater (Sobsey et al. 1986). Mean viral inactivation rates in tap water, polluted river water, unpolluted river water, and ground water have been reported to all be less than 1 Log per day, indicating that survival and persistence in these water sources could be prolonged depending on the initial concentrations (Rzezutka and Cook 2004).

However, survival and persistence of pathogens in agricultural waters depends on multiple factors, such as the pathogen, potential for biofilm formation, temperature, pH, salt, dissolved oxygen concentration, nutrient availability, interaction with other microorganisms, and exposure to UV light radiation (Liu et al. 2018). Therefore, generalised survival times should be interpreted with caution. Furthermore, limited evidence is available regarding the potential for growth of foodborne pathogens in different agricultural water sources.

Whereas irrigation water contacting the edible parts of fresh produce can be minimised by using drip irrigation, the contact of pesticides/fungicides with the crop is desired or unavoidable for their successful application (Verhaelen et al. 2013a). The growth and persistence of *Salmonella enterica* was found to be supported by some pesticide formulations depending on the type of water used for reconstitution and the temperature of storage (Lopez-Velasco et al. 2013). Similarly, Dobhal et al. (2014) concluded that pesticides and fungicides should be tested for their ability to support pathogen survival or growth prior to application on fresh produce to understand the risks associated with their application, based on their inconsistent observations of inactivation, persistence, or growth of *E. coli* or *Salmonella* spp. in a variety of pesticides. NoV does not multiply outside the human host, but has been observed to remain infectious when contaminated water is mixed with a variety of pesticides over a 2 hour study period (Verhaelen et al. 2013a). Therefore, the use of viral or bacterial contaminated water to reconstitute pesticides can represent a risk of microbial contamination if applied to fresh produce.

The evidence specifically associated with the in-scope commodities and hazards is summarised below.

9.6.1.1   *Leafy vegetables*

There is a strong link between the presence of *Salmonella* spp., *E. coli, L. monocytogenes* and other pathogens in water used for irrigation and application of agricultural chemicals and the contamination of leafy vegetables (Ceuppens et al. 2014). Water source strongly influences the level of risk (Jung et al. 2014), with *Salmonella* spp. associated with the use of

surface water as the irrigation water source and STEC more often associated with the use of collected rainfall water (Ceuppens et al. 2015; Jung et al. 2014). Spray/mist application of contaminated water can directly deposit pathogens on the edible portions of the crop (Erickson et al. 2019b) or cause splashing from contaminated soils (Honjoh et al. 2014), while drip, furrow and flood irrigation methods can introduce pathogens to the root zone. In either case, there is potential for internalisation of pathogens in the edible parts of the plants. Contaminated irrigation water has been implicated in large outbreaks of foodborne illness associated with consumption of leafy vegetables (spinach and lettuce) (FDA 2018; Gelting et al. 2011; Gelting et al. 2015). An FDA–led environmental assessment on a 2018 outbreak of *E. coli* O157:H7 associated with romaine lettuce from the Yuma growing region of Arizona and California determined that contaminated canal water used for the application of crop protection chemicals was the most plausible route of crop contamination (FDA 2018). The report noted that plants might have been rendered more susceptible to microbial colonisation due to leaf damage caused by a freeze event that preceded aerial application of pesticides in the weeks before harvest.

Specific experimental evidence of the ability of pathogens in irrigation water to contaminate leafy vegetables is summarised below.

Salmonella Typhimurium, present in spray irrigation water at 10^5 CFU/mL, has been shown to persist on lettuce and parsley plants for significant periods of time: 63 days and 231 days, respectively, in a field trial (Islam et al. 2004b). Applied at similar levels (5.3-5.8 Log CFU/mL in spray irrigation water) to lettuce plants in a growth chamber, a mix of *Salmonella* serovars Enteritidis and Newport was found to rapidly and durably colonise (>85% of plants at day 12) and internalise in leaf tissue (35 of 48 plants 1 hour after application; 21 of 48 plants after 24 hours) (Erickson et al. 2019b). When applied at 10^8 CFU/mL in sub-irrigation water three days prior to harvest of lettuce plants grown in sandy soil in a greenhouse, *S*. Infantis internally colonised leaves of 29% of plants (Zhang et al. 2016). No internalised bacteria were found in leaves of lettuce grown in loamy soil, or when present at 10^5 CFU/mL in the irrigation water.

E. coli O157:H7 present in irrigation water applied to soil or leaves has been shown to colonise and persist on edible parts of leafy vegetables for significant periods of time—and to internalise under some conditions.

Several studies have demonstrated the ability of *E. coli* O157:H7 to colonise lettuce leaf surfaces when applied in irrigation water to the soil surface or into the root zone (Habteselassie et al. 2010; Mootian et al. 2009; Oliveira et al. 2012; Solomon et al. 2002a; Solomon et al. 2002b). Experimental conditions—including the level of *E. coli* applied to the plants and the period between inoculation and sampling—differ significantly between these studies. But all demonstrate high prevalence of contamination (40–82%) persisting for between >5 days and 9 weeks after exposure. Internalisation of *E. coli* O157:H7 was observed in up to 16% of plants by Mootian et al. (2009) and 10% of plants by Solomon et al. (2002a). However, Zhang et al. (2009a) saw no internalisation in lettuce plants exposed to 6 Log CFU/g by sub-surface watering of sandy loam soil.

Results of studies applying *E. coli* O157:H7 contaminated irrigation water (at levels of 5 Log CFU/mL or higher) directly to lettuce or spinach leaf surfaces are highly variable (Alam et al. 2014; Bezanson et al. 2012; Erickson et al. 2019b; Islam et al. 2004a; Oliveira et al. 2012; Patel et al. 2010; Solomon et al. 2002a; Zhang et al. 2009b). However, they demonstrate persistence for between 2 and 22 weeks, usually at high prevalence. For example, Islam et al. (2004a) reported levels of around 1 Log CFU/g in lettuce and spinach plants in a field trial 77 and 177 days, respectively, after a single irrigation treatment with water containing 5 Log CFU/mL of a GFP-labelled, *stx*⁻ strain of *E. coli* O157:H7. Internalisation of E. coli O157:H7 in leaf-irrigated lettuce was observed in 30–100% of plants

by Solomon et al. (2002a), depending on experimental conditions. However, Zhang et al. (2009b) saw no internalisation in lettuce plants inoculated at 6 log CFU/plant directly onto leaf surfaces.

A study by Zhang et al. (2016) observed differential effects of *Salmonella* contamination in irrigation water and soil type in colonisation and internalisation of a *Salmonella* Infantis strain in lettuce. No internalisation of *S.* Infantis into lettuce was observed when irrigation water contained 5 Log CFU/mL. However, when irrigation water contained 8 Log CFU/mL *S.* Infantis, internalisation occurred in 29% (7/24) of plants grown in sandy soil, while no internalisation was observed in plants grown in loamy soil (0/24). It should be noted that the 8 Log CFU/mL *S.* Infantis level used in this study is higher than what would be anticipated to occur.

Few studies have assessed the persistence of *L. monocytogenes* in leafy vegetables due to irrigation with contaminated water, and none have demonstrated internalisation of the pathogen.

In a three year longitudinal field trial, Guévremont et al. (2017) found *L. monocytogenes* in only one of 288 lettuce samples irrigated with pig and cattle manure contaminated water or aerated pond water, indicating low prevalence and/or environmental persistence under the experimental conditions. In a study of risk factors for *L. monocytogenes* contamination, Weller et al. (2015) found only two of 334 positive spinach leaf samples. Since the survey identified 86/1092 positive soil samples and 33/52 positive surface water samples, it implies that transfer of the pathogen to spinach is either rare or short-lived.

In lettuce planted in soil contaminated to 8 log CFU/g *L. monocytogenes* and further contaminated by soil surface irrigation every two weeks for ten weeks with $2x10^{10}$ CFU in water, Honjoh et al. (2018) found 3/12 samples above the limit of detection, and no internalisation. Due to the experimental setup, they could not rule out direct contamination of the leaves with contaminated soil. When *L. monocytogenes* was applied by direct spray inoculation of leaves, contamination was detected 7 days later only when the inoculum was greater than 1,600 CFU/plant. In a further experiment, 4 or 6 Log CFU *L. monocytogenes* directly surface spotted onto the main vein of leaves could be detected up to 6–12 days, with higher inoculum and/or leaf surface damage prolonging the survival period.

9.6.1.2   Berries

Surface water, such as dams and rivers, are commonly used for irrigation of berries in Australia. The different types of berries utilise varied irrigation methods. For strawberries, overhead irrigation is commonly used to establish young plants and to cool plants in hot weather. Drip irrigation can be used in addition to overhead irrigation for young plants to promote root establishment. Once strawberry plants are established, drip irrigation is routinely used (DPIRD WA 2016b; Hort Innovation 2019a). Raspberries are commonly irrigated via drip or mini-sprinklers (Menzies and Brien 2002). The majority of blueberries use drip irrigation, although in some parts of Australia micro-jet sprinklers are also used (DPIRD WA 2016a; Wilk et al. 2009). The water used for agrochemicals may vary across the industry from surface water to potable water.

Fungicides and pesticides are often applied to berry fruit just before harvest to prolong shelf life. Insecticides can also be applied, for example to raspberries that are to be mechanically harvested (this harvesting technique can led to insect contamination of harvested fruit). The application of agrochemicals onto the fruit can introduce pathogens if contaminated water is used for reconstitution (Verhaelen et al. 2013a). The withholding period of some agrochemicals used in berry production in Australia is very short. For example, blueberries, rubus berries (raspberries) and strawberries can be harvested after three days, one day or

immediately (no withholding period), respectively, after treatment with certain fungicides to prevent grey mould (APVMA 2017; DPIRD WA 2015). Under such circumstance, if contaminated water is used to apply agrochemicals then pathogen die-off on the produce surface may not have occurred during the short withholding period.

STEC and human NoV strains have been detected internationally in irrigation water collected from strawberry farms, while HAV was not detected. In a Belgian study, STEC was detected (via RT-PCR) in 11/78 (14.1%) of irrigation water samples (bore water and ponds), with culture isolates (STEC O26) obtained from 2/78 (2.6%) of the irrigation water samples (Delbeke et al. 2015). In Norway, 10/16 (62.5%) of irrigation water samples were presumptive positive for STEC (via PCR), but none were culture confirmed. Generic *E. coli* was detected in all 16 irrigation water samples (Johannessen et al. 2015). NoV was detected in 3/23 (13%) or irrigation samples collected in the Czech Republic, while HAV was not detected in any samples (Dziedzinska et al. 2018). In European and South Korean studies NoV GI was not detected (0/56) and detected in 2/3 samples of irrigation water, respectively, and NoV GII was detected in 2.6% (2/56) and 2/3 or irrigation water samples, respectively. HAV was not detected in the irrigation water collected in the European (0/56) or South Korean (0/3) studies (Maunula et al. 2013; Shin et al. 2019).

Contaminated irrigation water was the suspected source of contamination for some international HAV and NoV outbreaks in berries. For example, the 2002 New Zealand HAV outbreak associated with raw blueberries was suspected to have been caused by faecally polluted groundwater or an infected food handler. This was because the implicated orchard only had pit latrines and no running water and there had been high rainfall during the harvest season. However, ground water samples were not tested for the presence of HAV (Calder et al. 2007). Similarly, the source of contamination of the 2012 German NoV outbreak linked to strawberries was not determined, but was hypothesised to be due to contaminated water as several different NoV genotypes were detected in the strawberries (Bernard et al. 2014).

There is no evidence of pathogen internalisation into berry plants via contaminated water.

9.6.1.3   Melons

In Australia, different water sources are used for the irrigation of melons with surface water including dams, streams, and rivers the most common. The methods of irrigation also vary across the industry with above surface or sub-surfaces drip irrigation commonly used, and furrow irrigation and overhead sprinklers used less commonly by the industry (NSW DPI 2019).

Only limited evidence of environmental testing of water sources[18] used in Australia for the production of melons was available. *Salmonella* Chester was isolated from a small number of water samples, 11.8% (2/17), taken from untreated irrigation channel water destined to be treated and used in the production of Australian melons. In contrast, *L. monocytogenes* was not isolated from the same agricultural water samples (NSW DPI 2020).

There is international evidence that various water sources used for rockmelon irrigation can be contaminated with *Salmonella* spp. *Salmonella* spp. were detected in 4/17 (23.5%) irrigation water samples taken from drippers and furrows and 1/11 (9.1%) ground water samples taken after filtration from Mexican rockmelon farms (Espinoza-Medina et al. 2006). *Salmonella* spp. have been isolated from water samples on US melons farms taken from 9/70 (12.8%) irrigation water source samples (including river, aquifer or underground), 1/15 (6.7%) tank water samples, 2/25 (8.0%) irrigation water samples delivered in the field by drip or irrigation channel, and from water from a pipe used to deliver irrigation water to the

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[18] Not associated with outbreak investigations.

field (Castillo et al. 2004). These isolates included *Salmonella* serovars linked to three large outbreaks associated with melons in the US. *Salmonella* spp. were also isolated from 4/15 (26.7%) water source samples including well, canal, or dam water, and 1/15 (6.7%) irrigation water samples delivered in the field by drip or irrigation channels in Mexican melon fields (Castillo et al. 2004). 25% (3/12) of water samples taken from US melon fields were positive for *Salmonella* spp. but not for *L. monocytogenes* (Dev Kumar et al. 2015).

L. monocytogenes was not isolated from irrigation water samples taken from rockmelon farms in Korea (0/6 samples) (Park et al. 2013a), Mexico (0/38 samples) (Heredia et al. 2016), or the US (0/12 samples) (Dev Kumar et al. 2015).

There is evidence that *Salmonella* contaminated water can be a source of exposure to vines, fruit rind, and roots of melon plants, but internalisation via the roots to the internal flesh of rockmelons has not been observed. An attenuated *Salmonella* Typhimurium strain was applied via either furrow irrigation (2.12-3.01 Log CFU/mL) or subsurface drip irrigation (4.17-5.89 Log CFU/mL) to rockmelon and honeydew plants at the initial flowering stage. The use of contaminated water for furrow irrigation lead to contaminated soil in furrows and the edge of beds, but not in the centre beds or around the root zone of melon plants. The authors concluded this was due to a lack of lateral transfer of *Salmonella* through the soil profile. However, *Salmonella* was detected in the rhizosphere and soil near the roots of drip irrigated plants. Despite high recovery in the rhizosphere following irrigation with contaminated water, internalisation of *Salmonella* in mature harvested melons was not detected in the study (n=485 melons). Conversely, contamination by *Salmonella* was detected on the rind of fruit that developed in contact with the soil of furrow irrigated melons at 41 days post inoculation. However, the rind of drip irrigated melons was not tested. The authors concluded that this evidence suggests it is unlikely that contaminated irrigation water will lead to internalisation by *Salmonella* into the fruit due to a lack of evidence of long range transport from root to fruit, but transfer to the rind via contact with soil or splash transfer is a concern (Lopez-Velasco et al. 2012).

Outbreak investigations in Australia detected a variety of *Salmonella* serovars in agricultural water sources following the 2006 multi-state outbreak of *Salmonella enterica* serovar Saintpaul associated with the consumption of rockmelon (Munnoch et al. 2009). Although the outbreak strain was not isolated from water sources, *Salmonella* spp. were detected in bore, channel, and river water samples, and the use of untreated water to irrigate and wash melons were identified as important risk factor for contamination in both jurisdictions.

9.6.2   Conclusions, uncertainty, and data gaps

The use of contaminated water for irrigation or the application of agricultural chemicals is a risk factor that applies broadly to all commodities. There is a low level of uncertainty and high level of confidence in this conclusion, based on the general evidence for the presence and persistence of pathogens in water sources, and the available evidence for leafy vegetables, berries, and melons. For viral pathogens, the use of sewage-contaminated water for irrigation or the application of agricultural chemicals are risk factors that apply to all commodities.

Water contacting the edible parts of plants due to irrigation or agrochemical use close to harvest is considered to present an increased risk of contamination for all commodities. There is a low level of uncertainty and high level of confidence in this conclusion, based on the general evidence for the presence and persistence of pathogens in water sources, and the available evidence for leafy vegetables, berries, and melons. The transfer of pathogens contaminating irrigation water to the surface of leafy vegetables is well documented in the literature above, while only limited studies were available for rockmelons. Data for watermelons and all berry commodities are lacking. Therefore, while the use of contaminated water is a major risk factor for all of these commodities, there is considerable uncertainty

regarding any variation in the density, persistence, or decline of pathogens on the surface of produce for all commodities in the field associated with the use of contaminated water.

The application of water via drip as opposed to overhead irrigation is generally considered to reduce the likelihood of contamination of the edible portions of all commodities. There is a low level of uncertainty and high level of confidence in this conclusion based on the evidence available. Contaminated water applied by either method can still contaminate the soil. As described above in Section 9.5, pathogens can persist for extended periods of time in soil. Moreover, there is evidence for some commodities for the internalisation of pathogens via soil (see Section 9.5 above) and via water.

Internalisation of pathogens from contaminated water is a risk factor that has been identified for leafy vegetables, but evidence does not support this conclusion for rockmelons and no data is available for berries. The internalisation of pathogens contaminating irrigation water via the roots has been demonstrated for lettuce, spinach, and parsley. The evidence available varies, with internalisation via sub-irrigation water into the soil or via leaf irrigation only occurring in some studies or particular conditions (e.g. soil type). Evidence for rockmelons suggests that internalisation via the roots to the edible portion of the fruit is unlikely to translocate further than the roots even at high inoculum levels. However this finding is based on a single study. Moreover, no studies investigating the potential for internalisation of pathogens in contaminated water via the roots were identified for any berry commodity, although internalisation of NoV surrogates via inoculated soil was reported for strawberries in a single study (see Section 9.5.1.2). Therefore, there is high uncertainty regarding the potential for internalisation via contaminated water into the edible portion of melons and berries. As previously mentioned, it is likely that the larger distance required to translocate from the soil to the edible portion of melons, raspberries, and blueberries may reduce the risk of internalisation of pathogens via the roots for these commodities but studies are needed for confirmation.

At the low concentrations generally reported in the field, the available data suggest that internalisation of pathogens from contaminated water into the edible part of leafy vegetables via the roots has a low probability, but high concentrations associated with contamination events would present an increased risk. There is medium uncertainty and confidence in this conclusion for leafy vegetables. However, a variety of factors may influence the potential for internalisation of pathogens via irrigation water applied to soil, including the concentration, pathogen species or strain, plant cultivar, plant age, plant physiology factors, and environmental factors such as soil moisture. Moreover, factors affecting the probability that the pathogens would be present in the edible portions at the time of harvest, such as the subsequent persistence or potential for growth of pathogens in the plant tissue, are poorly documented.

The prevalence or concentration of foodborne pathogens in Australian water sources and contributing environmental factors are poorly documented. The contamination of water sources by foodborne pathogens is also likely to be transient and this leads to difficulties in determining baseline contamination levels, identifying factors that may increase pathogen loads, or linking outbreaks to contaminated water sources. Further studies are required that quantify foodborne pathogens in Australian water sources and investigate environmental factors such as runoff and flooding that may increase pathogen loads. Furthermore, studies quantifying pathogens in contaminated irrigation water and their subsequent transfer, persistence, and decline in the field needs to be further elucidated, particularly for berries and melons in the Australian context. How different surface characteristics of the produce may influence contamination via water is also unknown.

Previous expert scientific reports have also identified these as important risk factors that can contribute to microbiological contamination of the in-scope commodities (EFSA Panel on Biological Hazards 2014a, 2014b, 2014c; FAO/WHO 2008b, 2011).

9.6.3   Mitigation measures

Water reservoirs used for crop irrigation can be contaminated with indicator and pathogenic microorganisms. In order to minimise the risk of illness linked to contaminated produce, Water quality guidelines have been established by several international government agencies and industries bodies that set generic *E. coli* and/or faecal coliforms criteria for irrigation water used on food crops which vary between <1 and <1000 CFU/100 mL, the application of which has been reviewed recently (Uyttendaele et al. 2015). However, evidence for the utility of testing irrigation water for generic *E. coli* or total faecal coliforms (TFC) as indicators for the presence of pathogens such as *Salmonella* and *L. monocytogenes* is variable (Castro-Ibáñez et al. 2015; Falardeau et al. 2017; Gu et al. 2013; Holvoet et al. 2014b; Shelton et al. 2011; Truchado et al. 2018; Won et al. 2013a). Statistical correlations tend to be weak to moderate, at best. Although it is generally agreed that there is a higher probability of detection of pathogens when higher levels of *E. coli* and/or TFC are found, there is variability in suggested cut-off points for determining acceptability of irrigation waters (Castro-Ibáñez et al. 2015; Falardeau et al. 2017; Holvoet et al. 2014b; Lopez-Galvez et al. 2014; Shelton et al. 2011; Truchado et al. 2018).
There is some evidence that increasing the time between cessation of irrigation and harvest can reduce the likelihood of bacterial pathogens on leafy vegetables and the risk of illness from their consumption due to the effects of UV radiation, drying, and microbial competition (Alam et al. 2014; Fonseca et al. 2011; Moyne et al. 2011; Ottoson et al. 2011). There is also evidence that the risk from surface contamination of fresh produce is reduced by the use of subsurface irrigation that reduces the wetting of the edible portion of produce compared to sprinkler irrigation (Rock et al. 2019).

The mitigation recommendations provided by other agencies, organisations and peak industry bodies are similar across the in-scope commodity sectors (Codex 2017; EFSA Panel on Biological Hazards 2014a, 2014b, 2014c; FAO/WHO 2008b; FPSC A-NZ 2019; IFPA et al. 2006; NSW DPI 2019) and include:

- Water used for irrigation should not contain microbial contaminants at levels that may adversely affect the safety of fresh produce
- Undertake regular risk assessment of water sources; assess the potential for cross-contamination between water sources under normal and high rainfall/flood conditions; and ensure microbial quality of water is appropriate for its intended use
- Regularly test water sources and undertake appropriate treatment of water sources; maintain appropriate testing records; and increase testing if animal incursion, extreme weather or climatic factors affect water sources
- Implement actions to prevent contamination
- Record water sources used for irrigation and the site of irrigation
- Apply appropriate timing to cessation of irrigation of crops before harvest
- Use drip rather than overhead irrigation methods to limit contact with the produce
- Water used for the application of water-soluble fertilizers, pesticides and agricultural chemicals in the field and indoors should be of the same quality as water used for direct contact irrigation and should not contain microbial contaminants at levels that may adversely affect the safety
- Maintain and properly store equipment used to hold or distribute water, to limit microbial or pest contamination.

9.7 Production systems

There are a variety of primary production practices used to cultivate fresh produce commodities in Australia. Examples include organic farming practices, hydroponics, field crops and protected cropping. The inherent differences in these practices may influence the likelihood for contamination by microbial pathogens and the potential differences in microbial quality of these crops as a result of these practices are considered below.

9.7.1 Available data

It is generally recognised that protected cultures (those not grown in completely enclosed greenhouse environments but grown under some sort of cover) can reduce damage caused by poor weather conditions, animals and birds. For example, soil-less, protected cultures of strawberries, which are frequently grown above ground level, may be less likely to be exposed to contamination with pathogens from adjacent land in both outdoor and protected cultivation than other production systems (EFSA Panel on Biological Hazards 2014b).

The potential differences in microbial contamination risk factors between open fields and greenhouse (or protected cropping) has been investigated to some extent.

Pathogens were more often detected in irrigation water samples from open field farms (46.7%, 21/45) than from greenhouses used for lettuce production (12.0%, 9/75). The authors concluded that the open field production was more prone to faecal contamination possibly due to increased external sources, but noted that in general the greenhouse productions implemented more measures to manage risks (Holvoet et al. 2014a).

The difference in microbiological quality of commodities produced under organic or conventional farming systems is poorly documented, particularly in Australia. Some evidence suggests that, in general, organic produce may be more susceptible to faecal contamination with one study reporting indicator *E. coli* prevalence of 1.6% (46/476) and 9.7% (2/129) from a variety of produce sampled on organic and conventional farms respectively. Moreover, organic farms using manure or compost aged less than 12 months had an *E. coli* prevalence 19 times greater than if older materials were used (Mukherjee et al. 2004). However, other studies did not identify a significant difference in the microbial quality of produce between the two production methods, and concluded that fresh produce produced by both types of methods can be contaminated by microbial pathogens if the microbial quality of inputs are not managed (Kuan et al. 2017; Oliveira et al. 2010).

The survival and growth of in-scope pathogens in hydroponic nutrient solutions has been reported. For example, *Salmonella* spp. and *E. coli* O157:H7 survived and grew (1-6 Log CFU in 24 hours) in common hydroponic fertilizer solutions (Shaw et al. 2016). Furthermore, *E. coli* O157:H7 has been found to survive and proliferate in soil-substitute and hydroponic production systems if contaminated seed was used, and proliferation was enhanced in the hydroponic system (Xiao et al. 2015).

The evidence specifically associated with the in-scope commodities and hazards is summarised below.

9.7.1.1 Leafy vegetables

In Australia, leafy vegetables can be produced using a variety of methods including conventional and organic farming, field or protected cropping, and hydroponics.

There is mixed evidence regarding the potential for difference in the microbial quality of leafy vegetables produced by conventional or organic methods. For example, *Salmonella* spp. or *E. coli* O157:H7 were not detected in a variety of vegetables (including lettuce) sampled at retail markets in South Korea, in 2011-12, and no significant difference in overall microbiological quality as assessed by levels of aerobic mesophiles, coliforms and generic *E. coli* were identified between conventionally grown and 'environmentally friendly' products, (Ryu et al. 2014). In contrast, Szczech et al. (2018) analysed aerobic mesophilic bacteria, yeasts and moulds, *Enterobacteriaceae*, coliforms and *E. coli* in lettuce, and other horticultural produce from organic and conventional production systems in Poland during 2010-2014, and determined that organic lettuce harboured significantly more bacteria than conventional lettuce (mesophilic 6.7 and 6.4 Log CFU/g, coliforms 1.8 and 1.4 Log CFU/g; *Enterobacteriaceae* 2.5 and 1.9 Log CFU/g; *E. coli* 0.5 and 0.1 Log CFU/g for organic and conventional, respectively). It was concluded that the fertilization system practiced in organic farms—the application of animal manures both composted and not composted—may deteriorate the sanitary quality of the produce. Another study assessed microbiological quality of 354 samples of leafy vegetables (including spinach and lettuce) from organic and conventional production systems in Korea by detecting indicator organisms (aerobic bacteria, coliforms, and *E. coli*) and pathogens (*S. aureus*, *E. coli* O157:H7, *L. monocytogenes*, *Bacillus cereus*, and *Salmonella* spp.). All pathogens except *Salmonella* spp. were detected, but the study found that farming type, at most, only slightly affects the hygienic quality of leafy vegetables (Tango et al. 2014).

There is limited evidence of differences in the microbial quality of leafy vegetables grown in soil or hydroponic soil-less systems. In one study, the levels of mesophilic bacteria, lactic acid bacteria and total coliforms on lettuce grown in soil-less hydroponic systems were reported to be significantly lower than on lettuce grown in soil, with the authors concluding that the hydroponic system was more effective in controlling microbial contamination (Selma et al. 2012). However, comparative analysis of differences in both background microflora or the occurrence of pathogens between these systems is lacking. Evidence for the potential of internalisation and attachment by pathogens to leafy vegetables if present in hydroponic systems is well documented in the literature (Koseki et al. 2011; Kyere et al. 2019; Riggio et al. 2019; Sharma et al. 2009).

9.7.1.2   Berries

In Australia the majority of strawberries are grown in open fields, with limited hydroponic production. Blueberries and raspberries are generally grown in protected cropping under polythene plastic tunnels or netting. No Australian studies were identified that evaluated differences in the microbiological hazards between different production methods.

Internationally, a Belgian study of strawberry producers found that the different cultivation methods for strawberries—grown in soil in the field or plastic tunnels versus grown in substrate in greenhouses or plastic tunnels—did not significantly affect the prevalence of STEC contamination of strawberries, with no STEC detected on strawberries regardless of the cultivation method (Delbeke et al. 2015). STEC was detected in 8.3% of substrate samples (2/24) (both samples culture confirmed as STEC O26) but was not detected on swabs of plastic foil covering the soil (0/24) (Delbeke et al. 2015).

9.7.1.3   Melons

Although melons are generally grown in the field, protective cropping for high value specialty melons using greenhouses or polyethylene tunnels has been investigated in Australia (Jovicich and Wiggenhauser 2015). However, no evidence specifically evaluating differences in hazards for these systems were identified.

9.7.2 Conclusions, uncertainty, and data gaps

There is limited evidence available to be able to determine if the method of production significantly influences the likelihood of contamination for any of the in-scope commodities. As such, there is high uncertainty as to how different production methods can influence the likelihood for contamination. However, the microbial quality of agricultural inputs will be a significant determining factor regarding the risk associated with any production system.

9.7.3 Mitigation measures

The microbial quality of inputs in any production system need to be managed to ensure end product safety, whether that be:

- Water in the field or in greenhouse, hydroponic or other protected cropping system, or
- Soil or amendments in the field or substrates in soil-less cropping systems.

10 Harvest and field packing risk factors

The methods of harvesting fresh produce are diverse and can be achieved mechanically or by hand. Some produce will be packed in the growing field with or without minor processing (e.g. removing outer leaves) and other produce will be transported to packhouses (either on farm or off farm) for further postharvest processing. Risk factors for microbial contamination or cross-contamination in the field during harvesting and field packing include the initial level of contamination of fresh produce, the level of handling by workers, the health and hygiene practices of the workers, the types and cleanliness of tools and equipment used, and level of contact with the field environment during harvest and transport (Codex 2012; FAO/WHO 2008b).

10.1 Available data

Contamination of produce can occur at any point during production, but the increased handling and use of equipment at harvest increase the likelihood of contamination (EFSA Panel on Biological Hazards 2014a, 2014b). Contact with the mechanical equipment used to harvest produce has been identified as a potential mechanism of transferring contaminated faeces or soil to large amounts of produce (Jay et al. 2007). Furthermore, the excessive application of force either by hand or by machine harvesting is demonstrated to bruise or crack fresh produce (Hussein et al. 2020; Li and Thomas 2014), and can increase the likelihood of microbial contamination and subsequent growth.

The handling of fresh produce in the field can also lead to contamination by both bacterial and viral pathogens carried by workers (Bozkurt et al. 2020). Even if gloves are worn during harvesting, insufficient hand washing can contaminate gloves when they are put on (Monaghan and Hutchison 2016). Cross-contamination of fresh produce via workers' hands can potentially occur between contaminated and uncontaminated produce in the field (Verhaelen et al. 2013b).

The ability of pathogens to attach and persist on the surface of equipment used during harvesting such as harvester blades, knives, containers, and other contact surfaces of fresh produce is well documented in the literature (Aryal and Muriana 2019; Bonsaglia et al. 2014; Oliveira et al. 2014; Patel et al. 2011).

The evidence available specifically for the in-scope commodities is summarised below.

10.1.1   Leafy vegetables

In Australia lettuce is harvested and trimmed in the field by hand (see Section 5.1). Knives are used to cut the lettuce from the root and to remove outer leaves. The trimmed lettuce is generally then transported to a packing shed for further postharvest processing. In some cases the lettuce is field packed. Baby spinach is machine harvested in Australia and then transported to the packing shed for postharvest processing. Parsley destined for the fresh market is hand harvested using knives or clippers. The parsley is generally field packed, with the plants being bunched together prior to cutting and then a rubber band fastened around the stalks of the bunch. Some parsley is transported to the packing shed for postharvest processing.

The prevalence of *Salmonella* spp. internationally on lettuce in the field has been reported to range from 0-1.85% (Ceuppens et al. 2014; Kilonzo-Nthenge et al. 2018; Niguma et al. 2017; Telmoudi and Hassouna 2015). *Salmonella* spp. have also been detected on 1.1% (1/92) samples of spinach in the field in the US (Kilonzo-Nthenge et al. 2018). Internationally

L. monocytogenes has not been detected on lettuce in the field in Tunisia (n=150) (Telmoudi and Hassouna 2015) or Korea (n=20) (Song et al. 2019). *E. coli* O157:H7 has also not been detected on lettuce in the field in Brazil (n=54) (Ceuppens et al. 2014), in the US (n=115) (Kilonzo-Nthenge et al. 2018) or in Korea (n=20) (Song et al. 2019), or on spinach (n=92) or parsley (n=102) in the field in the US (Kilonzo-Nthenge et al. 2018). No Australian data on the prevalence of *L. monocytogenes*, *Salmonella* spp. or STEC on baby spinach, lettuce and parsley in the field was identified in the literature.

Cross-contamination of leafy vegetables has been demonstrated from scissors and knives used for trimming and coring (McEvoy et al. 2009; Taormina et al. 2009; Yang et al. 2012), and lettuce harvesting tools have been shown to transfer pathogens to several lettuce heads after becoming contaminated.

An experimental study by McEvoy (2009) found *E. coli* O157:H7 was transferred from an artificially contaminated coring knife ($2x10^5$ cells) to iceberg lettuce heads, with >3 Log CFU/g *E. coli* O157:H7 recovered from the cored lettuce. A single contaminated coring knife contaminated at least nineteen lettuce heads. When contaminated cored lettuce were subsequently incubated at 30°C (simulated field conditions), the *E. coli* O157:H7 population increased by >2 Log over 8 hours. When stored at 5°C (refrigerated temperature), there was no significant change in the *E. coli* O157:H7 population on the lettuce.

Yang et al (2012) also considered the potential for contamination of lettuce via field-coring harvesting knives artificially contaminated with *E. coli* O157:H7 (inoculation via dipping into soils containing up to 10^5 MPN/g soil). The type of soil (sandy vs clay), the soil's water content, the level of contamination and amount of blade contact with edible parts of the lettuce influenced transfer. Repeated transfer occurred on three sequentially cut lettuce heads when the blade was contaminated with 10^4 CFU/g in clay soil (25% water content) when the lettuce was cut into the edible portion, but no transfer occurred when the stem was cut with no contact with the edible portion.

Chlorinated water washing was shown to reduce, but not eliminate, contamination on lettuce harvesting tools. Field coring devices were artificially inoculated with *E. coli* O157:H7 (via dipping in soil containing up to 6.57 log CFU/g) and then immersed in chlorinated water (200 µg/mL total chlorine). Washing the contaminated device in chlorinated water was more efficient than water, but the contaminated blade still transferred *E. coli* O157:H7 to up to ten consecutively processed lettuce heads (Taormina et al. 2009).

International studies have investigated the occurrence of *Salmonella* spp. and *E. coli* on the hands of workers and harvesting equipment in lettuce fields. No *Salmonella* spp. or *E. coli* O157:H7 were detected on swabs of workers hands (n=18) or lettuce transport boxes (n=18) on Brazilian lettuce farms (Ceuppens et al. 2014). Also, no *E. coli* was detected on swabs of harvesting scissors, harvesting crates or farmer's hands (number of samples not reported) on romaine lettuce farms in the Philippines (Espigol et al. 2018).

E. coli O157:H7 and *Salmonella* spp. can adhere to stainless steel surfaces, such as the blades of spinach harvesters, and form biofilms (Patel et al. 2011; Ryu and Beuchat 2005; Ueda and Kuwabara 2007). Experimental studies have shown that total hypochlorite concentrations of up to 200 µg/mL did not eliminate *E. coli* O157:H7 or *Salmonella* Enteritidis biofilms from stainless steel surfaces (Ryu and Beuchat 2005; Ueda and Kuwabara 2007). Patel et al. (2011) showed that treatment of an artificially inoculated spinach harvest blade with a bacteriophage cocktail led to a ~4 Log reduction of five *E. coli* O157:H7 strains after two hours. Also, under experimental conditions there is no significant difference in the adherence of *E. coli* O157:H7 to new versus rusty spinach harvester blades.

An experimental study has examined the efficacy of acetic acid and chitosan to reduce the prevalence of *Salmonella* spp. and *E. coli* O157:H7 on leafy vegetables when applied prior to harvest. The vegetables were artificially inoculated with the bacterium (3-6 Log CFU/ml of each strain) and later treated with acetic acid (0.3-0.7%) - chitosan (0.1-0.3%). The response to the treatment varied depending on the type of leafy vegetable, cultivar, pathogen and concentration of acetic acid. For lettuce, acetic acid-chitosan treatment led to a significant reduction in the prevalence of *E. coli* O157:H7 and *Salmonella* spp. for four of five lettuce cultivars. For spinach, the treatment only had a significant effect on one of three cultivars for *Salmonella* spp. and no effect for lettuce. Acetic acid concentrations of 0.4%-0.7% were more effective, while addition of chitosan to the acetic acid treatment had no effect (Erickson et al. 2019a).

10.1.2 Berries

In Australia the majority of berries are hand-picked (see Section 5.2), particularly if the product is destined for the fresh market. As blueberries ripen at different times on the bush, these are harvested multiple times over several weeks. Blueberries are generally hand-picked, particular for the initial pickings, with mechanical harvesting sometimes used for later pickings. Most raspberries are hand-picked, although mechanical harvesting can also be used. Strawberries are always hand-picked, with strawberry beds picked over regularly to harvest the ripe berries. The stems of strawberries are removed in the field using either a thumbnail or mechanical device. Berries destined for the fresh market can be packed directly in the field or first transported to the packhouse.

Internationally the prevalence of HAV and NoV on berries in the field has been reported to range from 0-0.8% and 0-1.3%, respectively. HAV was detected on 0.8% (1/120) of strawberries collected from the farm during harvest season in South Korea, while neither NoV GI (0/120) or NoV GII (0/120) were detected (Shin et al. 2019). NoV was detected in 1.3% (2/156) of field strawberries collected in the Czech Republic, while HAV was not detected (Dziedzinska et al. 2018). STEC has not been detected on strawberries in the field in Belgium (0/72), Norway (0/80) or the US (0/82) (Delbeke et al. 2015; Johannessen et al. 2015; Kilonzo-Nthenge et al. 2018). No Australian data on the prevalence of STEC, NoV and HAV on blueberries, raspberries and strawberries in the field was identified in the literature.

International studies have isolated NoV and HAV from strawberry farm workers. A study in the Czech Republic detected NoV on 1/171 (0.6%) of swabs of workers hands, while HAV was not detected (Dziedzinska et al. 2018). Conversely a South Korean study detected HAV on 1/27 (3.7%) of the harvesters' glove samples, but did not detect NoV GI (0/27) or NoV GII (0/27) (Shin et al. 2019). In other studies, such as Maunula et al. (2013), neither HAV, NoV GI or NoV GII were detected on swabs of picker's hands/gloves (n=113) on European berry farms.

Experimental evidence has confirmed the transfer of pathogens (*E. coli* O157:H7 and human NoV) between artificially inoculated gloved hands and berries (Sharps et al. 2012; Shaw et al. 2015a; Verhaelen et al. 2013b). In a study by Shaw et al. (2015a) *E. coli* O157:H7 was applied to pig skin (10^4 CFU/cm^2), which was then secured to gloves, and the inoculated gloves used to pick 100 strawberries. The first ten strawberries to be picked had a higher *E. coli* O157:H7 count (mean of Log 2.10±1.78 CFU/g per strawberry), while the 50-100th picked strawberries had lower counts (mean of Log 1.37±1.53 - Log 1.67±1.04 CFU/g per strawberry). The transfer rates decreased from 38.14% (first strawberry) to 29.13% (100th strawberry). Sharps et al. (2012) examined the level of transfer of human NoV GI and GII and murine NoV (MNV-1) from artificially inoculated gloves (~ 10^6 genome copies of each virus) to blueberries and raspberries. The average percentage of transfer under wet conditions for NoV GI.3b, NoV GII.4 and MNV-1 for blueberries was 59%, 60% and 70%, respectively, and for raspberries was 33%, 26% and 20%, respectively. When the viral

inoculation was left to dry for 30 minutes prior to contact the average percentage of transfer was <10% for all viruses on either fruit. In a study by Verhaelen et al. (2013b) gloves were inoculated with human NoV GI.4 ($7x10^8$ genomic copies), NoV GII.4 ($2x10^8$ genomic copies), MNV-1 ($4x10^8$ genomic copies) and human adenovirus (hAdV-2) ($2x10^8$ genomic copies) and left to dry for 2 hours. The estimated level of transfer from gloves to raspberries and strawberries was mostly ≤1%. Transfer from produce to gloves was also examined in this study, with raspberries artificially inoculated with the same quantity of virus (the raspberries were sliced in half to allow more consistent inoculation). The level of transfer from raspberries to gloves (estimated at 3-40% between viruses) was higher than transfer from gloves to berries, adding to the likelihood of viral transfer due to cross-contamination from contaminated produce via workers hands. Also, the low level of transfer to the berries is generally characterised by a larger amount of berries being contaminated at low contamination levels (compared to high transfer proportion). These low level contamination levels, even though likely to cause disease, may not be detectable using present virus detection protocols and the possible heterogeneous distribution of virus particles in food batches (Verhaelen et al. 2013b).

International NoV and HAV berry outbreaks have been potentially linked to pickers in the field (Bozkurt et al. 2020). For example, a series of NoV outbreaks in Denmark associated with imported frozen raspberries were suspected to be caused by several independent contamination events including during harvest by infected farm workers, faecally contaminated irrigation water and/or during processing by an infected worker (Falkenhorst et al. 2005).

10.1.3   Melons

Rockmelons in Australia are harvested by hand, generally with the use of knives, and transported to a packing shed for further postharvest processing (see Section 5.3). Although more common in other countries, field packing of rockmelons in Australia is rarely undertaken. Watermelons in Australia are generally harvested with the use of knives and packed in field by hand.

The prevalence of *Salmonella* spp. on rockmelons in the field has been reported internationally ranging from 0 – 55%. *Salmonella* spp. were detected on 45% (9/20) of rockmelons via culture method and 11/20 (55%) via PCR from composite rinse samples comprising five field rockmelons collected from four farms in Mexico (Gallegos-Robles et al. 2009). 26% (9/35) of 25g rind/flesh samples taken from rockmelons at harvest on five Mexican farms were positive for *Salmonella* spp. via PCR method (Espinoza-Medina et al. 2006). 1/400 (six US farms) and 0/75 (three Mexican farms) swabs of 100cm^2 rind area of rockmelon rinds in the field were positive for *Salmonella* spp. (Castillo et al. 2004).

L. monocytogenes has not been detected on rockmelons in the field in the US (0/36 25g enriched rind samples) (Johnston et al. 2005a), Korea (0/18 25g rind samples), or Mexico (0/106 rockmelon rinse samples filtered then enriched) (Heredia et al. 2016). Furthermore, *L. monocytogenes* (n = 125) and *Salmonella* spp. (n = 64) were not isolated from rockmelon sampled from fields in NSW using a surface rinse (NSW DPI 2020).

The survival of *Salmonella* spp. on the rind of rockmelons in the field has been experimentally investigated. Attenuated *Salmonella* Poona inoculated onto field grown rockmelons at 12 days old was able to survive on the rind for at least 24 days after inoculation, although populations did decline significantly by approximately 3 Log CFU/3 cm^2 by 9 DPI (Gautam et al. 2014). An attenuated *Salmonella* strain survived for at least ten days on the rind of 30% (12/40) of rockmelons when applied in the field at inoculum levels of >6 Log CFU/mL, and for at least two days but less than ten days on 80% (4/5) rockmelons when inoculated at 4 Log CFU/mL (Lopez-Velasco et al. 2012).

The occurrence of *Salmonella* spp. and *L. monocytogenes* on the hands of workers in melon fields has been investigated overseas. *Salmonella* spp. were not detected in 24 surface samples from the hands of workers harvesting rockmelons on five commercial Mexican rockmelon farms analysed by both PCR and a standard method (Espinoza-Medina et al. 2006). Similarly, *L. monocytogenes* was not isolated from samples taken from the hands of workers during harvest on Mexican rockmelon farms (Heredia et al. 2016).

There is preliminary experimental evidence that *L. monocytogenes* and *Salmonella* spp. transfer to or from melons may be influenced by glove type and transfer rates are higher for rubber gloves (Pfuntner et al. 2017; Pfuntner et al. 2018). A study quantified *Salmonella* spp. transfer from contaminated gloves (rubber, nitrile, cotton) to rockmelons and vice versa assessing different contact times (5-20 seconds) and pressures. The test scenarios were inoculated rockmelon to clean glove and inoculated glove to clean rockmelon using a dry inoculum of *Salmonella* mixed with sand (10^6 CFU/g). Transfer coefficients were calculated and there were no significant difference in transfer rates between contact times for both scenarios. There was a positive association with pressure and transfer. Rates of *Salmonella* spp. transfer were significantly higher for inoculated rockmelon to glove than inoculated glove to rockmelon. Rubber glove transfer rates were significantly higher than cotton or nitrile gloves under both scenarios at the highest pressure (Pfuntner et al. 2017). The same experiment preformed with *L. monocytogenes* demonstrated transfer rates from a contaminated rockmelon to glove or contaminated glove to rockmelon was low using a dry inoculum. Transfer rates from inoculated glove material to rockmelon were highest for rubber gloves and lowest for nitrile gloves, regardless of contact time or pressure (Pfuntner et al. 2018).

Preliminary evidence was demonstrated for the novel use of octenidine dihydrochloride (OH) applied as a preharvest spray to reduce *Listeria* spp. on the surface of whole melons. Inoculated melons in the field were sprayed with 0.1% OH or 0.2% OH. Reductions of 1.0 and 1.5 Log CFU/cm^2 were observed for 0.1% and 0.2% OH, respectively, on day zero, and approximately 2 Log on 28 days post inoculation (Keelara et al. 2015).

10.2   Conclusions, uncertainty, and data gaps

Contamination and cross-contamination due to poor worker and equipment hygiene are risk factors that apply broadly to all commodities at harvest. There is a low level of uncertainty and high level of confidence in this conclusion based on the general evidence, and the available evidence for leafy vegetables, berries, and melons.

Contamination and cross-contamination due to poor worker and equipment hygiene are risk factors that apply broadly to all commodities at harvest. There is a low level of uncertainty and high level of confidence in this conclusion based on the general evidence, and the available evidence for leafy vegetables, berries, and melons**.** The experimental evidence cited above has demonstrated the potential for cross-contamination of pathogens by workers during manual harvesting of lettuce, parsley, rockmelons, strawberries, blueberries, and raspberries both through glove contact and the use of harvesting equipment, such as knives. Furthermore, field coring of lettuce can expose nutrients and experimental studies have indicated the potential for growth of *E. coli* O157:H7 on cored lettuces. Although contact with the mechanical equipment used to harvest produce has been identified as a potential mechanism of transferring contaminated faeces or soil to large amounts of produce, there is limited direct evidence in regards to leafy vegetables or berries that may be mechanically harvested. However, the experimental evidence available that describes the ability of bacterial pathogens to adhere to and from biofilms on surfaces commonly used during the harvesting of leafy vegetables and melons, i.e. knives or cutting blades, supports the conclusion that if equipment is not maintained in a hygienic manner that the risk of

contamination will increase, particularly if produce is damaged and exposed to contaminated equipment. Moreover, experimental evidence indicates that attachment and persistence may be enhanced on soiled and wet equipment surfaces as opposed to clean and dry surfaces. However, the evidence reviewed above is from experimental studies, rather than in the field, so there is high uncertainty regarding the level of cross-contamination that occurs in field during harvesting of leafy vegetables, berries and melons in Australia.

Leafy vegetables, berries, or watermelons packed in the field that are contaminated may, in some cases, present an increased risk compared to those that are subsequently washed and sanitised under optimised conditions (see Section 11.4 below on washing and sanitising). There is a low level of uncertainty and a high level of confidence that the use of sanitisers and disinfectants in wash water can provide modest reductions of pathogens on the surface of in-scope commodities but only if wash systems are well monitored and optimised. Due to the intrinsic nature of berries being soft and susceptible to fungal and microbial deterioration, especially if excess water is present, they are not generally washed or sanitised after harvest. Similarly, watermelons and some leafy vegetables (parsley and lettuce) packed in field do not undergo further postharvest processing. However, the actual level or difference in risk to consumers would be dependent on a variety of factors including the level of pathogen contaminating the products, potential for die-off or growth on the product, the efficacy of specific interventions, the maintenance of the cold chain, and consumer handling. For example, watermelons and rockmelons are not eaten whole as opposed to berries and, therefore, an infectious dose of the pathogen would need to be transferred to, or, already be present in the flesh, to cause illness. The ability of pathogens to be transferred from the rind to the flesh during the cutting of melons is, however, well documented in the literature.

For hand-picked rather than mechanically picked produce, the likelihood of transfer of pathogens from workers increases with increased handling. The likelihood of contamination is increased if handled excessively by workers who are infected by, or, carrying pathogens, whether they are symptomatic or asymptomatic. Experimental evidence has demonstrated that pathogens can be transferred from gloves to berries and rockmelons. However, there is limited data to quantify the potential for transfer of bacterial pathogens from workers to leafy vegetables. Outbreaks associated with contamination of berries by NoV and HAV have been potentially linked to pickers in the field. However, evidence of infected individuals causing outbreaks is limited for all commodities. International studies have also isolated NoV and HAV from hand swabs and harvester's gloves on strawberry farms, though similar surveillance on Australia farms was not identified for any commodity. No literature was identified that investigated pathogen contamination on gloves used during the hand harvesting of parsley, raspberry, blueberry or watermelon. In Australia, food safety management advice recommends that workers who are ill and can potentially contaminate produce should notify management, and not harvest or pack produce until fully recovered (FPSC A-NZ 2019; Freshcare 2020). However, this is subject to workers notifying management of their illness. As such, there is high uncertainty as to the extent that workers influence the likelihood of contamination of the in-scope commodities, particularly for NoV in the Australian context. HAV incidence in Australia is comparatively very low and mainly associated with overseas acquired cases compared to NoV (OzFoodNet 2018).

The prevalence or concentration of foodborne pathogens on leafy vegetables, berries or melons at harvest in Australian are poorly documented. International studies indicate a low prevalence of *Salmonella* spp. on lettuce (0-1.85%) and spinach (1.1%). Also, *L. monocytogenes* was not detected on lettuce and *E. coli* O157 was not detected on lettuce, spinach or parsley in the limited number of available studies. There is considerable variance in the prevalence of *Salmonella* spp. reported in the field for rockmelons (1-55%), and, comparatively, a limited number of studies have not isolated *L. monocytogenes*. No prevalence data were identified for watermelons. A low prevalence of viruses (0-1.3%) has been reported for strawberries in the international literature and the prevalence associated

with blueberries and raspberries is poorly documented. Furthermore, it can be difficult to directly compare the results of prevalence data as there are a variety of methods and levels of detection applied in the literature for the in-scope commodities and pathogens (see Section 4.4 for more discussion).

10.3   Mitigation measures

Farm management and hygiene practices—e.g. absence of grazing and hay production in the field before spinach planting; use of portable toilets and washing stations in the field; training staff/temporary workers to use portable toilets—have been identified as protective factors against contamination with generic *E. coli* (a proxy measure for pathogen contamination) (Park et al. 2014).

Although hand-sanitisers are useful against bacteria, they are relatively ineffective against human viruses, such as NoV. Handwashing and/or the use of clean gloves is recommended (DEDJTR and FSANZ 2016).

The mitigation recommendations provided by other agencies, organisations and peak industry bodies for limiting the risk from animal and human ingress are similar across the in-scope commodity sectors (EFSA Panel on Biological Hazards 2014a, 2014b, 2014c; FAO/WHO 2008b; FPSC A-NZ 2019; IFPA et al. 2006; NSW DPI 2019) and include:

- Undertake environmental risk assessments within one week prior to harvest to assess factors that may affect contamination of the produce
- Ensure robust traceability by labelling bins, boxes or other containers used in the field
- Ensure no contact of picking bins and harvesting aids with soil
- Where machine harvest is undertaken, ensure the use of well-designed equipment and establish scheduling and recording of maintenance and cleaning, including verification of the processes
- Where hand harvesting is undertaken, ensure harvest employees are well trained in harvest methods that minimise microbial risk, such as culling damaged/diseased produce; applying personal and equipment hygiene practices; recognising and reporting risk factors; and verify compliance with food safety policies
- Ensuring sick staff do not work
- If water is used, ensure it does not become a source of contamination
- Transport produce immediately to packhouses for cooling
- Provide appropriate sanitary facilities for workers in the field
- Regularly clean and sanitise harvest equipment
- Do not use harvest equipment to transfer waste.

Codex recommendations specific for leafy vegetables state that any water used during harvesting should be clean or preferably potable where there is direct contact between the water and edible portions of the leafy vegetables (Codex 2017).

Codex state safe handling, transport and storage practices and immediate cooling of berries occurs after harvesting. If pre-cooling is undertaken growers should use potable water for ice and hydro-coolers. For manual harvest considerations, over handling berries can cause damage and excessive temperatures during harvesting can decrease quality that can affect food safety due to fruit damage and juice leakage. A harvest supervisor should be appointed at all times to ensure that harvesters use proper hand-washing and follow procedures not to harvest wet, bruised and/or damaged fruits. Berries on the ground should not be harvested unless they are processed with a microbiological inactivation step. All workers should be trained in safe handling, transport and storage practices to ensure that berries are immediately cooled after harvesting. Preference should be given to field packing berries into

consumer-ready containers that will not be washed after harvest (e.g. strawberries) so as to minimize the possibility of microbial contamination through additional handling steps (Codex 2017).

It is noted that some countries require testing of pickers for HAV in the berry industry. However, Australia generally has a relatively low incidence of HAV, with a notification rate in 2019 of 1.0 cases per 100,000 population (246 cases) (NNDSS 2020). In Australia pickers are not routinely tested for HAV. As noted above, in Australia workers who are ill and can potentially contaminate produce should notify management, and not handle produce until fully recovered, as per food safety management advice (FPSC A-NZ 2019; Freshcare 2020).

11 Packhouse and postharvest processing risk factors

This section covers the activities that occur postharvest in packhouses, with washing and sanitising of produce considered in a separate section (see Section 11.4).

11.1 Packhouses and postharvest processing

The postharvest processing methods of fresh produce are diverse and can increase microbial risks. Fresh produce that is contaminated in the field can enter the processing environment and contaminate food contact surfaces and subsequently cross-contaminate uncontaminated produce upon entry. The microbial quality of water or ice used throughout processing also presents a risk if contaminated with pathogens or allowed to settle in processing areas (FAO/WHO 2008b). Furthermore, if produce is damaged during processing this can lead to a higher load of organic matter, soiling of contact surfaces, increased access to nutrients for contaminating pathogens, and may assist internalisation. As nearly all fresh produce undergoes some processing they may be exposed to workers, different contact surfaces, water, and processing aids in the processing environment. As such, Good Manufacturing Practices (GMPs) and Good Hygiene Practices (GHPs), must be implemented consistently to manage microbial risks in the processing environment (FAO/WHO 2008b).

11.1.1 Available data

A lack of consistent compliance with and implementation of GMP, GHP, and food safety management systems will represent a risk factor for increased microbial contamination for all of the in-scope commodities (EFSA Panel on Biological Hazards 2014a, 2014b, 2014c).

Bacterial pathogens including *L. monocytogenes, Salmonella* spp.*,* and *E. coli* have been observed to form biofilms on equipment surfaces used in the harvesting and postharvest processing of fresh produce (Aryal and Muriana 2019; Bonsaglia et al. 2014; Oliveira et al. 2014). The cleanliness and type of contact surfaces is known to influence the formation of biofilms, with biofilms more likely to be formed on rougher surfaces where nutrients and water may be trapped due to the surface being harder to clean (Lim et al. 2020).

Food handlers can contaminate produce during processing, for example while sorting and packaging produce (Verhaelen et al. 2013b). More frequent handling of fresh produce during postharvest processing can increase the risk of contamination via the faecal-oral route for some bacterial pathogens and particularly viruses such as NoV and HAV (EFSA Panel on Biological Hazards 2014b). Moreover, viral pathogens do not form biofilms and are unable to multiply outside the human host, but NoV and HAV have been reported to persist and remain infective on different food contact surfaces for weeks (Bae et al. 2014; Lamhoujeb et al. 2009; Mormann et al. 2015).

The evidence specifically associated with the in-scope commodities and hazards is summarised below.

11.1.1.1 Leafy vegetables

In Australia leafy vegetables are generally cooled[19] on arrival at the packing shed (see discussion on cooling below). Some product is trimmed and packed in the field. Other product, such as baby spinach, is sorted by hand, with undesirable leaves removed at the packhouse. Once cooled, leafy vegetables are generally washed, undergo sorting/trimming (as required), sanitised, dried and packed. Product can be packed into plastic bags or sleeves or bulk-packed into cartons, crates or boxes and is then stored in coolrooms until distribution.

Traceability systems vary throughout the industry. Product that is packed in plastic (e.g. bagged or wrapped) can contain information about the supply chain. However, some products, such as bagged loose leaf product, can be co-mingled prior to packing. Product that is bulk-packed can contain information on the packing boxes.

Cooling of leafy vegetables can be done using cool rooms or via other systems such as vacuum cooling. Although vacuum cooling efficiently reduces the temperature of produce down from field temperature (e.g. from 28°C), the negative pressure applied during vacuum cooling changes the structure of lettuce tissue, such as stomata, suggesting a possible mechanism of internalization by pathogens (EFSA Panel on Biological Hazards 2014a; Li et al. 2008). In an experimental study lettuce was incubated at 28°C to mimic field temperature and then artificially inoculated with a derivative of *E. coli* O157:H7 prior to the vacuum cooling process. There was a higher level of *E. coli* O157:H7 internalization into lettuce tissue that had undergone vacuum cooling (5.4%) compared with the control group (4.2% internalisation). Also, under vacuuming conditions there was deeper penetration of *E. coli* O157:H7 within the lettuce tissue (Li et al. 2008).

E. coli O157:H7 can form stable, viable biofilms on polystyrene and stainless steel surfaces in the postharvest processing environment and transfer to leafy vegetables at high levels (Adator et al. 2018). An experimental study assessed the ability of 14 STEC strains to form biofilms on polystyrene and stainless steel. Biofilm formation was dependent on temperature, at 10°C all 14 strains formed weak biofilms on both surfaces, whereas at 25°C the strains formed stronger biofilms. STEC remained viable within dry-surface biofilms on both surfaces for at least 30 days. For 13 of the 14 strains, STEC was transferred from biofilms formed on polystyrene to lettuce within two minutes contact time (transfer of up to 6.35 Log CFU/g). The transfer rate was lower from stainless steel, with 8 of the 14 strains transferred to lettuce (up to 1.45 Log CFU/g). In general STEC transfer to fresh lettuce from biofilms decreased as the biofilms aged.

An experimental study by Buchholz et al. (2012) demonstrated the transfer of *E. coli* O157:H7 from leafy vegetable to equipment during simulated small-scale commercial processing. Lettuce and baby spinach were artificially inoculated with a cocktail of four *E. coli* O157:H7 strains and contained up to 6.2 Log CFU/g (lettuce) and 2.5 Log CFU/g (baby spinach) at the time of processing. *E. coli* O157:H7 was transferred from lettuce to up to 0.53%, 0.93%, 0.48%, 0.19% and 0.15% of surfaces on the shredder, conveyor belt, centrifugal drier, flume tank and shaker table, respectively. For baby spinach the level of transfer was lower, with *E. coli* O157:H7 transferred to up to 0.05% and 0.01% of surfaces on the centrifugal drier and flume tank, respectively.

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[19] FPSC A-NZ (2019) recommends cooling of produce post-harvest, but does not provide a target temperature. FDA (2008) recommends holding cut melons and any other fresh-cut product determined to need temperature control for safety at ≤5°C, but acknowledges that this is quality-focused.

The survival of *E. coli* O157:H7 and *Salmonella* spp. on stainless steel surfaces soiled with leafy vegetable juices was examined by Posada-Izquierdo et al. (2013). Food-grade stainless steel was inoculated with different vegetable juices containing 10^7-10^8 CFU/cm^2 of *E. coli* O157:H7 or *Salmonella* spp. Both microorganisms had reduced survival when parsley or spinach juice was present compared to lettuce juice, with both bacteria only detected up to 48 hours post inoculation in the spinach and parsley juice, while *E. coli* O157:H7 and *Salmonella* spp. could be detected up to 192 or 168 hours post inoculation in lettuce juice. This shows that there is variation in the persistence of *E. coli* O157:H7 and *Salmonella* spp. on stainless steel surfaces depending on the type of leafy vegetable juice present.

Limited data was identified in the literature on the prevalence of STEC, *Salmonella* spp. or *L. monocytogenes* on lettuce, baby spinach and parsley in packing facilities. A study by Espigol et al. (2018) detected *E. coli* on swabs of trimming scissors, worker's hands, plastic holding crates and dryers during postharvest processing of lettuce in the Philippines (number of samples not reported), however, the *E. coli* was not typed to the strain level. Holvoets et al. (2012) did not detect *E. coli* on swabs of food handler's hands or gloves (n=18) in Belgian leafy vegetable processing facilities, but did detect *E. coli* on 16.7% (3/18) of swabs of conveyor belts, 27.8% of weighing units (5/18) and a parsley sample (1/3). Neither *L. monocytogenes* nor *Salmonella* spp. were detected at the packing facilities, including no detection on lettuce and parsley, however the sample numbers were very small (n=3 for lettuce and parsley).

The level of surface contamination of processing equipment after cleaning has been investigated in fresh cut vegetable (including lettuce) production plants in Finland. Samples were collected at different stages along the processing line. The total aerobic microbial contamination was highest on a centrifuge basket of lettuce and belt of cutter equipment. This shows that surfaces such as the inside of machines and cutters used for postharvest leafy vegetable processing may need more effective cleaning as surfaces could potentially develop biofilms (Lehto et al. 2011).

The 2001 Australian *Salmonella* outbreak associated with lettuce was linked to inadequate cleaning of post harvesting processing equipment used to shred the lettuce. This demonstrates that poorly cleaned and maintained equipment can harbour pathogens and provide a reservoir of contamination (FAO/WHO 2008b; Stafford et al. 2002).

11.1.1.2 Berries

In Australia, there is variation within the industry regarding the packing of berries destined for the fresh market. Some product is field packed and then cooled once it reaches the packhouse, while other product is first transported to the packhouse for cooling prior to being hand sorted and hand packed. As such, sorting and removal of damaged product can occur in the field or at the packhouse. The packed product is stored in coolrooms until distribution. Strawberries and raspberries are sent to market as soon as possible due to their short shelf-life, whereas blueberries have a longer shelf-life.

Traceability varies across the industry. As berries destined for the fresh market are sold in small plastic punnets which are marked with the company name, product can be traced back to this level. However, some companies are supplied by over 100 growers so more sophisticated traceability systems are required to trace back to individual farms.

As described above (in Section 10.1.2), experimental evidence has confirmed the transfer of pathogens (*E. coli* O157:H7 and human NoV) from artificially inoculated gloved hands to berries (Sharps et al. 2012; Shaw et al. 2015a; Verhaelen et al. 2013b). It has also been shown that human NoV can be transferred from fomites to berries, as may occur from postharvest surfaces and equipment (Sharps et al. 2012). In an experimental study,

stainless steel coupons were artificially inoculated with human NoV GI and GII and murine NoV (MNV-1) (~ 10^6 genome copies of each virus) and clean gloves first touched the contaminated stainless steel before touching blueberries and raspberries. Under wet conditions the average percentage of transfer of NoV GI.3b, NoV GII.4 and MNV-1 to blueberries was 24%, ~20% and <10%, respectively, and to raspberries was 38%, 50% and <10%, respectively. When the viral inoculation was left to dry for 30 minutes prior to contact the average percentage of transfer was <10% for all viruses on either fruit (Sharps et al. 2012).

No Australian data, and very limited international data, was identified in the literature on the prevalence of STEC, NoV or HAV in blueberry, raspberry and strawberry packing facilities. A European study did not detect HAV, NoV GI or NoV GII (n=24) from swabs taken of conveyor belts in berry processing plants (Maunula et al. 2013).

A study by Gazula et al. (2019) used indicator organisms (faecal coliforms and enterococci) to evaluate the hygiene conditions of blueberry packing lines in the US. Various parts of the packing line was swabbed in the morning, middle of the day and at the end of the day (the packing facilities sanitised daily either at the beginning or end of the day). The morning samples had the lowest incidence of faecal coliforms and enterococci, with higher incidences recorded for the middle and end of the day samples. This indicates the packing line surfaces were contaminated during the packing processes.

11.1.1.3   *Melons*

In Australia, rockmelons are sometimes cooled on arrival at the packing shed. During processing they may come into contact with various surfaces and are handled by workers when packing into cartons or trays. The point that undesirable fruit is removed is variable across the industry with some removing fruit before washing and sanitising and others removing fruit at the packing table.

Traceability systems differ throughout the industry but include the recording of batch numbers and packers in many cases. Rockmelons are palletised and stored in cool rooms. In contrast, watermelons generally do not receive any postharvest treatment and are packed in field.

The prevalence of *Salmonella* spp. has been investigated both on the rind of postharvest rockmelons and in the postharvest environment, indicating likely pathways of contamination to some extent with varying results. In Australia, recent surveys conducted[20] in the melon postharvest environment on three farms in NSW detected *L. monocytogenes* in one boot swab (1/12). The bacterium was not detected from conveyor belt, roller, wall, packing table, cleaning equipment, or drain swabs (0/67). *L. monocytogenes* was also not detected in the rinse of 40 unwashed and 148 washed rockmelons (NSW DPI 2020).

Salmonella spp. were isolated by enrichment and culture method from <1% of rockmelons sampled before washing (1/150) or after washing (1/150) (by swabbing 100cm^2 of rind) on six US farms (Castillo et al. 2004). *Salmonella* spp. were not isolated from the hands of workers packing and grading rockmelons (0/45), or from conveyor belts in contact with washed melons (0/30), but was isolated from 4% (2/50) of surfaces and 0.8% (2/250) packed rockmelons swabbed in the cool room. In the same study, similar prevalence was also reported for postharvest melons and surfaces from Mexican farms and no difference in levels of contamination were observed between the countries. The authors concluded that irrigation water was not the primary source of contamination due to a lack of relatedness between *Salmonella* isolated from irrigation samples (see Section 9.6.1.3) and from the surface of

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[20] Surveys not associated with outbreaks.

melons determined by PCR and DNA fingerprinting (Castillo et al. 2004). Furthermore, the isolates from the cool room and on the melons did not show significant relatedness. The authors noted that more extensive studies were required to confirm links between potential sources and contamination of postharvest melons. Moreover, the sensitivity of the swabbing method may be less than other sampling methods (Espinoza-Medina et al. 2006).

Duffy et al. (2005a) recovered *Salmonella* spp. from 3% (3/100) rockmelons following washing and not from melons in the field (0/100) or other environmental samples, and suggested that contamination occurred postharvest or that washing may have reduced attachment of *Salmonella* spp. present in the field and resulted in easier identification by the surface swab method used. Similarly, 3.3% (3/90) (Johnston et al. 2005a), and 0/26 (Johnston et al. 2006) of rockmelons (25g rind/flesh) sampled from the postharvest environment were positive for *Salmonella* spp. by enrichment and culture methods of *Salmonella* spp*.* in the US.

Due to the low prevalence generally reported for *L. monocytogenes* there is little data on the pathways of contamination, but positive detections are more often reported in the post-processing environment. *L. monocytogenes* was not isolated from rockmelons sampled immediately after washing (0/3), immediately after rinsing (0/3), from the conveyor belt after rinsing (0/18), or from the box before distribution (0/18) on US farms and packing sheds (Johnston et al. 2005a). Similarly, no positive samples were reported for rockmelons sampled from conveyor belt after washing or the wash tank (0/36), or from the box before distribution (0/6) (Johnston et al. 2005a; Johnston et al. 2006). Sampling undertaken by the US FDA at 17 commercial rockmelon producers and/or packers in the US identified eight sites which were negative for *Listeria* spp. on produce and environmental samples, and eight sites where produce and environmental samples were positive for *Listeria* spp. One site returned two positive samples for *L. monocytogenes* from conveyor belts (FDA 2015).

Due to the generally low prevalence of pathogens on fresh produce, microbial indicators have been used to determine if microbial postharvest contamination is influenced by product type or production step. Research indicates that rockmelons support higher microbial loads than other produce, and microbial loads increase from the field to packing shed. For example, Duffy et al. (2005b) demonstrated that *E. coli* was isolated more frequently from rockmelons (13%) sampled in the field when compared to oranges (0%) and parsley (1%). *E. coli* was also more frequently isolated in the packing shed after sorting and washing for all produce, but was always more prevalent on rockmelons. Indicator levels were shown to increase significantly from the field through to packing on rockmelons while they remained relatively constant for cilantro and parsley. Similar results were also obtained in a later study showing increases in microbial levels during packing of rockmelons (Johnston et al. 2006). A more recent study also determined that general indicator levels and prevalence increased from the field to the packing facility, and that the levels were similar for jalapenos and tomatoes but higher for rockmelons (Heredia et al. 2016). As with other studies, the authors attributed these differences to the intrinsic characteristics of rockmelons having a rough netted rind that may facilitate bacterial attachment in comparison to the smooth waxy cuticle of tomatoes and jalapenos that may impede attachment (Johnston et al. 2005a). Experimental evidence has shown that if *L. monocytogenes* or *Salmonella* spp. enters the packing environment it is able to persist on postharvest processing materials and persistence is promoted on soiled materials. Commercially available materials including roller conveyors (polyvinyl chloride), conveyor belts (polyvinyl chloride, polyurethane and nitrile rubber), and brush filaments (nylon and polyethylene) support attachment of *L. monocytogenes*, but higher attachment was observed for nylon (3.4-4.4 Log CFU/cm^2 respectively) and polyethylene (4.0 Log CFU/cm^2) brushes. All materials soiled with rockmelon flesh/rind extract supported the persistence of *L. monocytogenes* at relatively constant concentrations throughout the 14 day trial but clean materials decreased from 4.5 Log CFU/mL to below the level of detection by day 10 (polyvinyl chloride and polyurethane conveyor belt), and

day 5 (rubber nitrile conveyor belt). *L. monocytogenes* declined but not to below the level of detection by day 14 on clean nylon brushes and foam padding. The type of contaminated surface also significantly affected the probability that a melon would be contaminated. The percentage of melons contaminated by rolling across conveyor belt material of polyvinyl chloride (52%), polyurethane (43%), and rubber nitrite (49%) was significantly lower than foam pads (78%) inoculated with 2.5 Log CFU/mL *L. monocytogenes* (Nyarko et al. 2018). Similarly, experimental evidence has demonstrated that *Salmonella* spp. and *L. monocytogenes* can persist in the environment and form biofilms on a variety of food contact surfaces relevant to the Australian industry in the presence of low concentrations of both rockmelon peel and flesh extracts (Abeysundara et al. 2017; Abeysundara et al. 2018).

As reviewed previously by FSANZ, poor sanitary conditions of the postharvest environment were identified during investigation of melon associated outbreaks including the 2016 Australian *Salmonella* outbreak (NSW Food Authority 2017), 2012 US *Salmonella* outbreak (FDA 2014), 2011 US listeriosis outbreak (FDA 2012), and the 2006 Australian *Salmonella* outbreak (Munnoch et al. 2009).

11.2   Conclusions, uncertainty, and data gaps

Contamination and cross-contamination of produce due to poor worker and equipment hygiene during packing and postharvest are risk factors that apply broadly to all commodities. There is a low level of uncertainty and high level of confidence in this conclusion based on the general evidence, and the available evidence for leafy vegetables, berries, and melons.

The main postharvest risk factors for all commodities are contamination and cross-contamination of produce due to poor worker and equipment hygiene. There is a low level of uncertainty and high level of confidence in this conclusion based on the general evidence, and the available evidence for leafy vegetables, berries, and melons**.** Experimental evidence has shown that if pathogens enter the processing environment they are able to persist on postharvest processing materials and in some cases persistence can be promoted on soiled materials. There is experimental evidence supporting this conclusion for all commodity sectors. However, there is some variation in the persistence of certain pathogens on postharvest surfaces, and in the presence of different leafy vegetable juices.

If workers are sick or handle contaminated produce during postharvest operations the likelihood of contamination and cross-contamination is increased for all commodities. Although only limited direct evidence for the potential for workers to contaminate or cross-contaminate produce was identified for the various commodities at postharvest, this potential is well documented at harvest (see Section 10). As such, there is high uncertainty as to the extent that workers influence the likelihood of contamination of the in-scope commodities, particularly in the Australian context.

The use of vacuum cooling for leafy vegetables that are contaminated or the use of contaminated water during the process are risk factors that have been identified for leafy vegetables. There is medium uncertainty and confidence in this conclusion based on the available evidence for lettuce**.** Experimental evidence has been reported for the combination of *E. coli* and lettuce, and identified that the negative pressure applied during vacuum cooling can potentially facilitate internalisation due to changes in the structure of the lettuce tissue that can provide protection for pathogens from subsequent sanitisation. However, no data was available to determine if this risk factor also applies to spinach, parsley, or berries. Melons in Australia are currently cooled by forced air, cold rooms, or hydro-cooling.

11.3   Mitigation measures

Poor sanitary conditions in the postharvest environment have been associated with outbreaks of foodborne illness due to consumption of horticultural produce (CDC 2012). Pathogens can contaminate the postharvest environment, transferring between produce and postharvest surfaces and equipment. Persistence of pathogens on postharvest surfaces and equipment is enhanced on fouled vs clean surfaces. Workers hands and gloves can also facilitate cross-contamination during sorting and packing activities. These risk factors apply broadly across commodity sectors.

Mitigation and controls

The mitigation recommendations provided by other agencies, organisations and peak industry bodies are similar across the in-scope commodity sectors (EFSA Panel on Biological Hazards 2014a, 2014b, 2014c; FAO/WHO 2008b; FPSC A-NZ 2019; IFPA et al. 2006; NSW DPI 2019) and include:

- Risk assessments should be undertaken regularly and one week prior to harvest to identify risk factors that may influence postharvest practices or contamination
- All personnel at packhouses should receive basic training in food safety risks, personal hygiene, and health reporting requirements
- Guidance on the appropriate number and location of toilet and handwashing facilities equipped with potable water, paper towels and hand sanitisers should be followed, and hand sanitiser should not replace hand washing
- Packhouses should be designed to prevent cross-contamination—particularly the movement of contaminants from earlier to later process steps—and pest infestation, minimise airborne contamination, and facilitate adequate drainage
- Equipment sanitation procedures should be validated and must ensure microbial loads are lowered
- Facility and equipment sanitation practices should be documented and implemented in accordance with standard GMPs and GHPs to prevent contamination and cross-contamination
- Damaged equipment like roller brushes and cracked flaps or matting, which can act as harbourage sites for pathogens and contaminate produce intermittently, should be replaced and maintained appropriately
- A well-designed environmental monitoring program can reduce the risk of pathogens colonising the processing environment and subsequently contaminating produce
- HACCP-based food safety programs should be implemented
- Drinking quality water should be used for all postharvest operations
- Staff in the packing house should wear appropriate personal protective equipment, such as gloves.

11.4   Washing and sanitising produce

Washing with potable water and the application of sanitisers to remove pathogens from the produce surface has been recognised as an important contributor to the reduction of risk from pathogens on fresh produce. However, if not applied correctly, washing and sanitising can represent a significant risk factor that can lead to extensive contamination of produce (FAO/WHO 2008b).

In this section, the term disinfectant refers to the agents applied to process wash water to avoid cross-contamination. Sanitisers refers to the agents applied to wash water to reduce the level of microorganisms on the surface of fresh produce.

11.4.1   Available data

The washing step of fresh produce has been identified as a potential pathway for the introduction of pathogens and cross-contamination of fresh produce making proper water management a critical point in the postharvest processing (Holvoet et al. 2012). It is generally reported that washing fresh produce with water alone will reduce pathogens on the surface of fresh produce by <1 Log and if disinfectants are not used to maintain the microbiological quality of water the likelihood of cross-contamination is significantly increased (FAO/WHO 2008b).

Water based disinfectants and sanitisers commonly used by the fresh produce industry include aqueous chlorine (hypochlorite), aqueous chlorine dioxide, peroxyacetic (peracetic) acid (PAA), electrolysed water, and aqueous ozone. These different chemicals have advantages and disadvantages from efficacy, practicality, economic, environmental, and exposure perspectives that have been reviewed extensively in other publications (Goodburn and Wallace 2013; Miller et al. 2013; Parish et al. 2003; Ramos et al. 2013; Suslow 1997). In the following discussion, only the efficacy of these interventions is considered.

The use of disinfectants is recommended primarily to maintain water quality during the washing process and to reduce cross-contamination, but more recently disinfectants have also been employed as sanitisers with the aim to reduce pathogens on the surface of produce. The efficacy of disinfectants and sanitisers to achieve these different aims is dependent on a number of factors including: the type of sanitiser; the commodity; the prior processing of the commodity (e.g. whole versus cut); the target pathogen; water quality; methods of application; pH; organic loads from dirt or damaged/cut produce; temperature; concentration and contact times.

The efficacy of chlorine is greatly limited by the pH, organic load, and temperature of the wash water. Levels above or below pH 6-7.5 reduce the amount of hypochlorous acid available and, therefore, its effectiveness to inactivate bacteria (Suslow 1997). High organic loads in the wash water from cut produce, dirt, damaged produce, or other sources will reduce the efficacy of chlorine. This is because the organic matter reacts with and uses up the hypochlorous acid, therefore reducing the amount available to inactivate pathogens. Low temperatures also limit the efficacy of chlorine. Automated monitoring of these parameters and auto-dosing of chlorine and additional acids are essential to maintain efficacy in the presence of high organic loads (van Haute et al. 2013).

In general, the reactivity of disinfectants with organic matter has been reported in the order of PAA < chlorine dioxide < free chlorine < ozone (van Haute et al. 2015a). PAA and aqueous chlorine dioxide have become more popular as they are less reactive with organic matter, less affected by pH, and retain their efficacy at low temperatures commonly used in the postharvest washing of leafy vegetables. Research has demonstrated that PAA combined with lactic acid is an effective disinfectant option if organic loads are high and refresh rates of water are low, and can maintain the microbial quality of wash water by automatic monitoring of the disinfection residual of PAA and pH and auto-dosing of PAA and acids (van Haute et al. 2015a). Recent research has also demonstrated that chlorine dioxide can be an effective wash water disinfectant when organic loads are higher and when applied with continuous monitoring and application (Banach et al. 2018; van Haute et al. 2017). However, hydrogen peroxide has slow water disinfection kinetics and is quickly consumed making it ineffective at maintaining the microbiological quality of wash water with an organic load typical of fresh cut vegetables (van Haute et al. 2015b).

The target pathogen can also influence the efficacy of disinfectants and sanitisers, as different species and strains may be inherently more or less susceptible as chemicals can have different modes of action (van Haute et al. 2013). For example, a higher susceptibility to chlorine has been observed for *E. coli* compared to *L. monocytogenes* (Park et al. 2004; van Haute et al. 2013). It is also widely hypothesised that the surface characteristics of fresh produce can reduce the efficacy of sanitisers. It has been experimentally demonstrated that produce with rougher surfaces can reduce the efficacy of sanitisers, and this has been attributed to rougher surfaces promoting attachment and providing increased protection from interventions such as sanitisers (Fransisca and Feng 2012; Palma-Salgado et al. 2020; Wang et al. 2009).

The efficacy of disinfectants and sanitisers is generally considered to increase with increasing concentrations and contact times. However, fresh produce industries are limited in the concentrations and contact times that can be applied, as increases in these parameters can negatively reduce the quality and shelf life of produce. Furthermore, residual concentrations of disinfectants and sanitising chemicals on fresh produce are required to meet Maximum Residue Limits defined in standards both in Australia and overseas, and this can also limit the selection of concentrations and contact times applied by fresh produce sectors.

The evidence specifically associated with the in-scope commodities and hazards is summarised below.

11.4.1.1   *Leafy vegetables*

FAO/WHO (2008b) stated that there is a possibility of internalisation by pathogens due to the contraction of tissues of warmer fruit and vegetables when exposed to a negative temperature differential, i.e. when washed in cooler water. The effect of differentials between wash water and produce temperature for whole-lettuce heads has been reported. Lettuce heads pre-stored at 23°C and washed in water at 4°C resulted in significantly higher water absorption than if the lettuce was pre-stored at 5°C (Palma-Salgado et al. 2014). Unfortunately, the potential for the internalisation of pathogens was not investigated in that study, but as reported in previous sections, pathogens can internalise via damaged or cut leafy greens (Golberg et al. 2011; Saggers et al. 2008; Takeuchi and Frank 2000).

The ability of pathogens to internalise into whole and cut leafy vegetables during washing has been reported, and the influence of postharvest storage on internalisation has been investigated to some degree. When whole baby spinach leaves were washed for 2 minutes in wash water containing 6.5 Log CFU/mL *S.* Typhimurium, approximately 3 log CFU/g internalised into the spinach leaves (Gómez-López et al. 2013). The authors did not observe a significant decrease in the level of internalisation as a result of exposure to illumination or temperature differentials between the produce and the wash water. In contrast, exposing the baby spinach to relative humidity of 74% compared to 99% for 44 hours at 12°C before washing produced a significant reduction in the levels of internalisation which the authors attributed to increased stomatal closure at the lower relative humidity. Similarly, internalisation of *Salmonella* spp. has been reported during the washing of parsley but temperature differentials did not increase internalisation when parsley stored at 25°C was washed for 3 minutes with water at 5, 25, or 35°C with an inoculum level of 6 Log CFU/mL (Duffy et al. 2005b). The relevance of these studies to commercial scenarios may be limited as high inoculum levels were used that are unlikely to be encountered in commercial scenarios and no sanitiser/disinfectants was used in the studies. Regardless, the results indicate that disinfectants used in wash water can limit cross-contamination and the potential for internalisation in leafy vegetables, as water based sanitisers are not effective in eliminating internalised pathogens (Niemira 2007; Niemira 2008; Shynkaryk et al. 2015; van der Linden et al. 2016).

Currently available water based disinfectants are effective in significantly reducing pathogens in wash water. The use of appropriate disinfectants can reduce the likelihood of cross-contamination of leafy vegetables by effective inactivation of pathogens in wash water if applied and monitored under optimised conditions (Banach et al. 2017; Banach et al. 2020; Davidson et al. 2017; Jung et al. 2017; López-Gálvez et al. 2009; van Haute et al. 2015a; van Haute et al. 2015b; van Haute et al. 2017). For example, Lopez-Galvez et al. (2010; 2009) evaluated the potential for cross-contamination of cut lettuce by *E. coli* during processing with a pre-wash without sanitisers followed by washing with sanitisers. *E. coli* cells were able to attach in under a minute in the presence of high organic loads, and the subsequent sanitising wash for one minute with either 3ppm ClO_2 or 100ppm chlorine did not significantly reduce contamination levels on the surface of the cut lettuce (i.e. <1.3 Log CFU/g). However, the sanitisers were able to significantly reduce the levels of *E. coli* in the wash water to below the level of detection. The authors concluded that any step involving the immersion of produce in water should include a disinfection agent.

Similarly, Davidson et al. (2017) used a pilot scale processing line where inoculated (*E. coli* O157:H7 6 Log CFU/g) and un-inoculated lettuce was shredded, washed in a flume tank with and without 50ppm PAA or mixed peracid for 90 seconds with different levels of organic load, and dried using a shaker table and centrifuge. The efficacy of the sanitisers on inoculated lettuce was not affected by organic load and reduced the levels of *E. coli* O157:H7 on the lettuce by 0.97-1.74 Log CFU/g. However, although higher reductions of *E. coli* O157:H7 were observed in the wash water treated with the same disinfectants (>5 Log CFU/mL after 22 minutes of processing), *E. coli* O157:H7 was detected in almost all of the samples of three subsequent un-inoculated lettuce batches that were processed. Also, recent studies at semi-commercial and industrial scales have determined that target concentrations of 3-5ppm ClO_2 (Banach et al. 2018), and 75ppm PAA (Banach et al. 2020) in wash water cannot prevent the attachment and cross-contamination of shredded lettuce by *E. coli* added directly to the wash water (6 Log CFU/mL). However, these sanitisers did reduce the probability that cross-contamination would occur compared to if no sanitiser was used. Cross-contamination can still occur even with the addition of chemical disinfectants to the wash water, and indicates the importance of minimising contamination at the field level.

The studies evaluating the efficacy of sanitisers to remove pathogens from the surface of fresh produce use considerably diverse methods, and, therefore, only limited conclusions can be drawn from results between studies. The results from studies that have assessed the efficacy of sanitisers between the specific in-scope commodities of the leafy vegetable sector are inconsistent. Aqueous ozone (0.5-1.5ppm applied for 3 minutes) was demonstrated to be significantly more effective for removing *S*. Typhimurium on whole leaf lettuce (1.2-3.1 Log reduction) than parsley (0.3-2.6 Log reduction), and the authors suggested this is because lettuce leaves are smoother and more uniform than parsley leaves that may provide more attachment sites and protection for bacterial cells (Sengun 2013). In contrast, larger Log reductions were generally reported for the reduction of *E. coli* O157:H7, *Salmonella* spp. and *L. monocytogenes* on the surface of parsley (1.6-4.0 Log reductions) than cut lettuce (1.2-1.9 Log reductions) treated with 200ppm chlorine for 5 minutes, though statistical comparisons between the commodities were not undertaken (Lang et al. 2004). In another study, similar Log reductions of *E. coli* and *L. innocua* were reported for cut lettuce (2.1-2.9 Log reduction), spinach (1.7-2.7 Log reduction), and parsley (2.2-3.0 Log reduction) treated with either aqueous ozone (12ppm) or chlorine (100ppm) for 15 minutes (Karaca and Velioglu 2014). As such, there is insufficient evidence to determine if the efficacy of currently used sanitisers differ significantly as to influence the level of risk between lettuce, spinach and parsley. As mentioned previously, efficacy will be dependent on multiple factors such as the commodity, the prior processing of the commodity (e.g. cut or whole), the pathogen, the sanitiser, and the conditions and method of sanitiser application.

Water based sanitisers used at industry-relevant concentrations and contact times (that do not adversely affect sensory properties of produce) are limited in efficacy for removing pathogens from the surface of leafy vegetables and, in general, achieve reductions of <3 Log CFU (Karaca and Velioglu 2014; Lapidot et al. 2006; Niemira and Cooke 2010; Omac et al. 2017; Rahman et al. 2010; Rahman et al. 2016; Thi-Van et al. 2019; van der Linden et al. 2016). Studies reporting the efficacy of sanitisers to remove *L. monocytogenes*, *Salmonella* spp. and *E. coli* from the surface of the in-scope leafy vegetables are summarised in Appendix 11.

The use of disinfectants are mainly applied to decrease the likelihood of cross-contamination via the wash water but, when used as sanitisers, have limited efficacy against pathogens that are already on the surface of leafy vegetables entering the wash water. Therefore, disinfectants or sanitisers alone cannot be relied upon to ensure end product safety which will require a consistent through-chain approach to risk management.

11.4.1.2   Berries

Berries for consumption as raw produce are generally packed directly in the field and do not normally undergo washing or sanitising steps. This is because berries are highly susceptibility to fungal deterioration, which is promoted by the presence of water, and will shorten the shelf life of these products (Gazula et al. 2019; Yu et al. 2001). However, berries destined for freezing or further processing are generally subjected to washing and/or sanitising.

The potential for internalisation of viral or bacterial pathogens during washing of berries is poorly documented in the literature. The limited efficacy of commonly used water-based sanitisers to remove or inactivate NoV and HAV on the surface of berries has been demonstrated and studies are summarised in Appendix 11.

It is likely that the same limitations of disinfectants and sanitisers applied to leafy vegetables and melons will apply to berries. As such, the use of these interventions alone cannot ensure end product safety.

11.4.1.3   Melons

The Australian rockmelon industry uses a variety of methods to clean and sanitise the outside of whole melons during postharvest processing. Melons can be pre-cooled before either dry dumping or wet dumping. Melons are sometimes sprayed with or dipped in fungicide to prevent fruit rot. Following these treatments, the fruit is air-dried sometimes with the assistance of fans and cooled.

Rockmelon temperatures in the field before harvest are reported to reach 43.2°C internally and 49°C externally (Macarisin et al. 2017), and the temperature differential between warm fruit and cool dump tank water has been suggested as a cause of microbial internalisation (FDA 2017). Furthermore, temperature differentials between warm fruit and cool dump tank water was suggested as a potential route of contamination by *Salmonella* spp. into rockmelons following an FDA outbreak investigation (FDA 2013). A variety of bacterial pathogens have also been detected in the internal fruit of melons at wholesale (Esteban-Cuesta et al. 2018), but only limited studies have investigated the potential for internalisation of pathogens during the washing of melons postharvest. Webb et al. (2015) surface inoculated whole rockmelons with five strains of *L. monocytogenes* on the rind (8 Log CFU/mL) and stem scar (7 Log CFU/mL) and then the rockmelons were treated by sanitising in a dump tank (20-22°C) simulation for 8 minutes. However, both inoculated melons that were dump tanked and not dump tanked were positive for internalised *L. monocytogenes* following validated steam sanitising of the outside of the melons before

analysis. The authors suggested that passive diffusion of the liquid inoculum and not the process of dump tanking was responsible for internalisation. In another study, Macarisin et al. (2017) pre-warmed rockmelons (two cultivars either clipped or full slip) to 42°C or 18°C then immersed them in water at 6 or 18°C containing 4 or 6 Log CFU/mL with a three strain cocktail of *L. monocytogenes* for 30 minutes. A dye was used to visualise water internalisation that entered mainly via the stem scar and could reach the calyx of the fruit. *L. monocytogenes* was able to internalise into flesh with and without a temperature differential, but warmer fruit entering cooler water did show increased internalisation.

Experimental evidence suggests that the efficacy of sanitisers currently applied at industry relevant contact times (i.e. <2 minutes) may be limited to <3 Log CFU for removing *L. monocytogenes* or *Salmonella* spp. from the surface of whole rockmelons (Bartlett et al. 2020). These studies are summarised in Appendix 11. It should be noted, however, that these studies were conducted under laboratory conditions, mostly without the simulation of commercial brushing or the addition of organic matter in the form of soiling or damaged fruit that may influence the efficacy of some sanitisers. Therefore, although brushing of melons may increase efficacy of sanitisers, in actual scenarios, increased levels of organic matter and other microorganisms entering the system may in some cases limit the efficacy.

Laboratory and pilot commercial scale experiments have indicated that hot water has a high relative efficacy for removing *Salmonella* spp. on the rind of whole rockmelons with reductions up to 6 Log CFU being reported (Appendix 12). However, an increased risk of re-contamination has been demonstrated and is most likely due to thermal damage to the exterior of the produce (Solomon et al. 2006; Ukuku 2006). Similarly, experimental results for *L. monocytogenes* have reported reductions of >3 Log CFU for water applied at 80°C for 5 minutes (Appendix 12). Annous et al. (2013) also determined that while the reductions in *Salmonella* spp. following hot water immersion were high, the treatment was still not able to completely remove *S.* Poona from the surface of all melons, and, in another study, lower temperatures of 65°C or 75°C did not produce reductions greater than 1.2 or 3.3 Log CFU/cm^2 respectively (Solomon et al. 2006). Ukuku (2006) concluded that while hot water had a higher efficacy than chlorine, the potential risk for recontamination for melons treated with hot water is higher than that for 200ppm chlorine for 2 minutes, and 96°C water should therefore only be used for fruit destined to be processed into fresh cut melon immediately.

The ability of *L. monocytogenes* and *Salmonella* spp. on the rind of rockmelons to transfer to the flesh during cutting is well documented (Ukuku et al. 2004; Ukuku and Fett 2002; Ukuku and Sapers 2001), and experimental evidence has shown that the use of sanitisers on inoculated rockmelons can reduce the incidence of *Salmonella* spp. and *L. monocytogenes* in subsequently prepared fresh cut pieces. Greater reductions in the transfer of *Salmonella* spp. from inoculated rockmelon rind to flesh in treated versus non-treated melons has been reported for 1000ppm chlorine and 5% hydrogen chloride applied for 5 minutes (Ukuku and Sapers 2001); 2.5% and 5% hydrogen peroxide for 5 minutes (Ukuku 2004); and hot water (70 or 97°C) or 5% hydrogen peroxide (70°C) for 1 minute (Ukuku et al. 2004). Similar results for the reduction in transfer of *L. monocytogenes* has been reported for a number of sanitisers (Ukuku et al. 2005; Ukuku et al. 2012; Ukuku et al. 2016; Ukuku and Fett 2002). Notably, for both pathogens, the contact time for application of these sanitisers was for longer than generally used by the Australian industry on whole rockmelons (i.e. <2 minutes).

The efficacy of some sanitisers has been reported to be reduced on rockmelons compared to other fresh produce due to their rougher surface. Rodgers et al. (2004) reported that shredded lettuce and sliced apples inoculated with *L. monocytogenes* had the longest Log reduction times compared to whole rockmelons, apples, and lettuce when treated with ozone (3ppm), chlorine dioxide (3ppm and 5ppm), chlorinated trisodium phosphate (100ppm

and 200ppm chlorine), or peroxyacetic acid (80ppm) applied for up to 5 minutes. Of the whole fruit tested, rockmelons consistently had longer Log reduction times for *L. monocytogenes* for all six sanitisers when compared to whole lettuce and whole strawberries. Similarly, Singh et al. (2018) reported that reductions in *S.* Typhimurium inoculated onto the surface of whole rockmelons were consistently lower than those for whole blueberries washed with either acidic or near neutral electrolysed water, 100ppm chlorine, 2% lactic acid, or PAA (45, 85, and 100ppm) for 5 minutes at 5°C.

The limited available evidence suggests that differences in surface texture between watermelons and rockmelons may influence the efficacy of sanitisers. Comparative studies of the efficacy of currently used sanitisers on rockmelons and watermelons are lacking. One study that reported no differences in the efficacy of commonly used sanitisers for removing pathogens from the surface of rockmelons and watermelons unfortunately did not use appropriate statistical analyses because the decision to pool data for the two commodities were not justified and distorted the findings presented, reducing the reliability of their interpretation (Svoboda et al. 2016). However, Kwon et al. (2018) attributed the significantly reduced efficacy of novel steam treatments to inactivate *E. coli* O157:H7, *S.* Typhimurium and *L. monocytogenes* on rockmelons compared to watermelons to surface roughness which was demonstrated to be significantly higher for rockmelons using a 3D surface profiler. Similarly, the negative correlation between surface roughness and sanitiser efficacy has been reported in other studies (Fransisca and Feng 2012; Ringus and Moraru 2013; Wang et al. 2009)**.** It is generally expected that fruits with rougher surfaces, such as rockmelons present a greater risk due to the increased potential for attachment of pathogens on the rougher rind surface that provides greater protection from sanitisers.

A number of studies have investigated the efficacy of novel sanitisers on rockmelons. However, as many of these are unlikely to be used by the industry in the immediate future (due to cost, requirements for approvals and the requirement for further development to commercial scale) they are not considered in scope for this assessment.

Poor or potentially inadequate sanitation of postharvest melons was identified during investigations of melon associated outbreaks including the 2018 Australian *L. monocytogenes* outbreak (NSW DPI 2018), 2016 Australian *Salmonella* outbreak (NSW Food Authority 2017), 2012 US Salmonella outbreak (FDA 2014), 2011 US *L. monocytogenes* outbreak (US Department of Justice 2013), and the 2006 Australian *Salmonella* outbreak (Munnoch et al. 2009).

11.4.2   Conclusions, uncertainty and data gaps

The risk factors associated with inadequate washing and sanitiser application will apply broadly to all commodities that undergo these processes. There is a low level of uncertainty and high level of confidence in this conclusion based on the general evidence, and the available evidence for leafy vegetables, berries, and melons. For viral pathogens, the use of sewage-contaminated water for postharvest washing and sanitising are risk factors that apply to all commodities.

The likelihood for cross-contamination is increased during processes using water that is untreated or does not contain a disinfectant to maintain microbial quality throughout the process. There is a low level of uncertainty and high level of confidence in this conclusion based on the general evidence, and the available evidence for leafy vegetables and melons. The evidence above for lettuce and rockmelons indicate that currently available water based disinfectants can be highly effective in significantly reducing pathogens in wash water. However, although the likelihood of cross-contamination can be reduced if disinfectants are applied and monitored under optimised conditions, pathogens are not eliminated. However, due to the limited evidence available for spinach and parsley, and the great variety of

differences in the study methods for the available data, there is uncertainty as to how the likelihood of cross-contamination may differ between commodities. Moreover, reducing the likelihood of cross-contamination is dependent upon the optimisation of washing process that vary greatly between and within commodity sectors.

Water based sanitisers used at industry-relevant concentrations and contact times (that do not adversely affect sensory properties of produce) are generally limited in efficacy for removing pathogens from the surface of produce and, in general, achieve reductions of <3 Log CFU. There is a low level of uncertainty and high level of confidence in this conclusion based on the general evidence, and the available evidence for leafy vegetables and melons. There is experimental evidence available for all commodity sectors. However, there is a large variation in the methods employed in available studies and the majority of studies for melons are undertaken in laboratory conditions, where there are more studies that assess pilot or industry scale systems for leafy vegetables. There are many differences in the methods of inoculating produce, the type of sanitisers applied, concentrations, contact times, method of sanitiser application, levels of organic matter, the target pathogen and strain, and temperature of both commodity and sanitiser solution.

The limited comparative studies available suggest that sanitisers may be less effective at reducing pathogens on cut leafy vegetables compared to whole produce, and rockmelons compared to smoother melons such as watermelons. There is a medium level of uncertainty and confidence in this conclusion based on the general evidence, and the available evidence for leafy vegetables and melons. Results vary across studies and these comparative studies are limited in number. Assessing the difference in efficacy between studies and, particularly the effect of intrinsic characteristics such as surface roughness, is difficult due to the differences in methods employed in the studies reviewed above. There are many differences in the methods of inoculating produce, types of sanitisers applied, concentrations, contact times, method of sanitiser application, levels of organic matter, the target pathogen and strain, and temperature of both commodity and sanitiser solution. Moreover, while more recent studies that closely represent industry practices have been undertaken for leafy vegetables, the majority of studies for melons or berries are still undertaken at the laboratory scale and not always with industry relevant concentrations and contact times.

If factors critical to the application of disinfectants and sanitisers are not monitored, these processes can fail and instead result in increased risk due to cross-contamination of produce or limited to no reduction in pathogens on the surface of produce. There is a low level of uncertainty and high level of confidence in this conclusion. The efficacy of disinfectants and sanitisers is dependent on the recognition and management of a number of other factors. These factors can include pH, organic loads from dirt or damaged/cut produce, temperature, concentration, and contact time. The importance of these factors to sanitiser efficacy is well documented in the literature.

There is the potential for internalisation of pathogens into leafy vegetables and melons during washing. There is a medium level of uncertainty and medium level of confidence in this conclusion based on the general evidence, and the available evidence for leafy vegetables and melons. The ability of pathogens to internalise into whole and cut leafy vegetables during washing has been reported. Furthermore, there is evidence that *L. monocytogenes* has been shown to internalise into whole rockmelons via dump tank wash water with and without a temperature differential, though internalisation was slightly enhanced with a temperature differential between warmer fruit and cooler water. Although the evidence of internalisation is limited, pre-cooling of melons before washing and sanitising may reduce the likelihood of internalisation, and will also limit the potential for growth of pathogens during storage before processing. However, there is high uncertainty regarding the likelihood of internalisation of pathogens into watermelons and berries via wash water due to lack of evidence. It is noted that watermelons and berries generally do not undergo a postharvest washing and

sanitisation step unless they are undergoing further processing (e.g. freezing/fresh cut). The lack of this additional hurdle to further reduce the potential for contamination can increase the risk associated with these products if contamination occurs before or at harvest. The relevance of these studies to commercial scenarios may also be limited, as general high inoculum levels were used that are less likely to be encountered if GMPs and GAPs are implemented. Sanitisers were also not applied in the studies.

Sanitisers and disinfectants should be used in wash water to reduce the likelihood of contamination and the potential for internalisation into leafy vegetables and melons, as water based sanitisers are not effective in eliminating internalised pathogens. However, the use of disinfectants and sanitisers alone is insufficient to ensure end product safety, which is, instead, reliant upon a consistent through chain approach to risk management. There is a low level of uncertainty and high level of confidence in this conclusion. Overall, the body of evidence indicates that washing with sanitisers can be an important additional hurdle to reduce the likelihood of contamination, if applied correctly. However, the use of currently available water based sanitisers alone—even under optimised conditions—cannot completely remove the risk of cross-contamination, and only offers limited efficacy for removing pathogens from the surface of produce.

11.4.3   Mitigation measures

The mitigation recommendations provided by other agencies, organisations and peak industry bodies for limiting the risk from animal and human ingress are similar across the in-scope commodity sectors (EFSA Panel on Biological Hazards 2014a, 2014b, 2014c; FAO/WHO 2008b; FPSC A-NZ 2019; IFPA et al. 2006; NSW DPI 2019) and include:

- Risk assessment at harvest is required to determine if sanitiser efficacy might be compromised by increased organic load, produce damage or extreme weather events
- Ensure that appropriate concentrations and contact times of approved sanitisers are applied to reduce the likelihood of cross-contamination
- Auto-dosing and regular monitoring of water quality parameters and sanitisers is required to maintain efficacy
- Remove damaged product prior to sanitiser application to limit cross-contamination and reduce organic loads
- Evaluate the process design and ability to optimise for product viability. For example, evaluating specific product wash water disinfectant demand and product-to-water volume ratios; assessing use of filtration systems to remove sand or soil from water during processing; and assessing when water should be changed or added.

There are some specific recommendations regarding washing and sanitising for melons:

- Melons should be pre-cooled rapidly in cool rooms, preferably by forced air cooling, before and after washing and sanitising to limit pathogen growth and to ensure that water temperatures are higher than the internal temperatures of melons so as to minimise the risk of water infiltration (Codex 2017; NSW DPI 2019)
- Dry dumping is suggested over wet dumping (Codex 2017; NSW DPI 2019)
- If wet dumping occurs, water should be single use drinking quality and the full submersion of melons in colder dump tank water should be minimised or avoided, so as to reduce the likelihood of water infiltration (Codex 2017; NSW DPI 2019).
- If hot water treatments are used as an alternative to postharvest chemical fungicide treatments, it is recommended that the water temperature and treatment duration should be controlled, monitored and recorded (Codex 2017)
- A rinse step with sanitiser should be applied to rockmelons (NSW DPI 2019).

12 The cold chain

Processes and risk factors that occur after fresh produce is released from the processing or packing shed, such as transport, further cold chain maintenance to the consumer, processing/storing at retail and consumer handling or activities covered by Chapter 3 of the Code are not in scope for this assessment. The main objective of this assessment was to provide an indication of risk factors associated with the primary production, harvest, and postharvest activities discussed above. However, a brief discussion is included below on the importance of the cold chain that begins on farm. Maintenance of the cold chain can extend shelf life of fresh produce but can also prevent or limit the rate of microbial growth on produce.

12.1 Leafy vegetables

The cold chain for leafy greens requires maintenance from after harvest of the raw material, through to and at retail sale and also by the consumer. Lettuce, parsley, and spinach that is both cut and uncut can potentially support the growth of *E. coli*, *Salmonella* spp., and *L. monocytogenes,* but the growth of *E. coli* and *Salmonella* spp. can effectively be controlled if leafy vegetables are stored below 5°C and the growth rate of *L. monocytogenes* can be significantly reduced (Barlow et al. 2015; Duffy et al. 2005b; Khalil and Frank 2010; Omac et al. 2015; Omac et al. 2018; Posada-Izquierdo et al. 2016; Puerta-Gomez et al. 2013; Sant'Ana et al. 2012). Therefore, it is recommended to chill leafy vegetables below 5°C soon after harvest and through chain to limit microbial growth, noting that some commodities are not chill tolerant and will need to be stored at reduced temperatures that do not damage the produce and release nutrients that may support the growth of pathogens (Codex 2017).

12.2 Berries

Strawberries, raspberries, and blueberries have a low flesh pH that would not usually support the growth of bacterial pathogens (EFSA Panel on Biological Hazards 2014b). They are pulpy fruits with high moisture and sugar content and a soft skin, and are easily damaged (EFSA Panel on Biological Hazards 2014b; US FDA/CFSAN 2003). There is evidence that bacterial pathogens can persist on the surface of berries, and that persistence may be enhanced on cut surfaces of berries (Knudsen et al. 2001). There is evidence for the persistence of viruses on berries during storage (Leblanc et al. 2019; Verhaelen et al. 2012). It is recommended to chill berries immediately after harvest and through chain (Codex 2017).

12.3 Melons

There is the potential for *L. monocytogenes* and *Salmonella* spp. to multiply on the rind of rockmelons particularly if there is excess surface moisture, but no studies have investigated the potential for growth on the surface of watermelons (Annous et al. 2005; Beuchat and Scouten 2004; Salazar et al. 2017; Scolforo et al. 2017). If rockmelons or watermelons are damaged or cut this can provide access to the nutrients of the flesh that can increase the growth potential of these pathogens. *Salmonella* spp. can grow quickly to levels that may cause illness in the general population when left at room temperature, and *L. monocytogenes* can also quickly reach levels that has a high likelihood of causing illness in the susceptible population (Bartlett et al. 2020; Danyluk et al. 2014; Fang et al. 2013; Li et al. 2013). However, *Salmonella* spp. can effectively be controlled if melons are stored below 5°C and the growth rate of *L. monocytogenes* can be significantly reduced. It is hypothesised that increased surface roughness can enhance bacterial attachment and

potential for growth on the surface of produce, and watermelons have been shown to have a significantly smoother surface than rockmelons (Kwon et al. 2018). It is recommended to chill melons immediately after harvest, before processing, after processing, through chain, and by consumers to reduce the potential for growth of bacterial pathogens. All pre-cut melons should be packaged and refrigerated immediately and maintained below 5°C through chain and by consumers (Codex 2017).

13 Summary of conclusions

Significant variation in the production methods and combinations of risk factors on farm, within and between the commodity sectors influence the presence and persistence of pathogens and, therefore, the associated risk. The commonality across the in-scope commodity sectors is that they are generally ready-to-eat foods, consumed raw, with little or no further processing (except whole melons, which need to be cut), and there is no single step during their primary production and processing that can ensure end product safety.

The safety of these commodities relies on a consistent and well managed through-chain, multi-hurdle approach to minimise risk (Mogren et al. 2018). This includes managing inputs and responding to changes in the growing environment that could increase the likelihood of contamination. It is not possible to completely eliminate risk to consumers from fresh produce at the primary production level, and the handling through to the consumer must also seek to minimise risks. Moreover, consumer handling can also introduce hazards and increase risk from these products, particularly if the cold chain is not maintained. This assessment affirms that the path to ensuring the end product safety for these commodities begins on farm.

13.1 Key risk factors that apply broadly to all commodities

Based on the best available evidence reviewed in this report, there is a low level of uncertainty and high level of confidence that the following risk factors represent important potential routes of microbial contamination for all the commodity sectors assessed:

- *Incursion by wildlife and domestic animals*

Contact with animal reservoirs of microbial pathogens can lead to direct contamination of fresh produce via faecal waste, urine, hair or animal carcasses, but also indirectly via contamination of other inputs such as water and soil. While the presence of animals can always be considered a hazard, incursion by animals close to harvest, with high density, or with high frequency is likely to present a higher risk for all commodities.

- *Location of growing areas near or on land used for practices that may increase the likelihood of contamination, such as livestock production, as a wildlife habitat, urban or industrial waste*

Prior and surrounding land use—including land used to cultivate a different crop; supplemented with soil amendments; irrigated in a manner inappropriate for the new commodity; or used for livestock production, as a wildlife habitat or for land fill for urban or industrial waste—have been identified as risk factors that may contribute to the contamination of fresh produce.

- *The occurrence of extreme weather events, such as flooding or heavy rain, that could transfer pathogens to produce, fields, or irrigation water sources*

Flooding, heavy rain, and dust storms represent a risk to all the commodities, as they can increase the potential transfer of pathogens to the growing site or to agricultural inputs such as irrigation water. For viral pathogens, flooding or heavy rain may transfer sewage to irrigation sources or fields. Extreme weather events that occur close to harvest may present an increased risk, as pathogen levels are generally reported to decline in the primary production environment over time. There may also be the potential for internalisation as a result of flooding, heavy rain, or dust storms that involve contamination at high levels and that cover or submerge substantial amounts of the commodities.

- *The application of untreated or insufficiently treated manure or compost amendments*

Manure is a known reservoir of a number of foodborne pathogens. Manure-based soil amendments and composts that are not treated, or are insufficiently treated, can increase the risk of surface contamination of fresh produce, particularly if no further sanitising or processing is undertaken. Contamination of soil or application of contaminated soil amendments that occur close to harvest are generally considered to represent an increased risk.

- *The use of contaminated water for irrigation, application of agricultural chemicals, and/or postharvest washing and sanitising*

The use of contaminated water for irrigation or the application of agricultural chemicals can contaminate both the surface of fresh produce and the surrounding soil. Water contacting the edible parts of plants due to irrigation or agrochemical use close to harvest is considered to present an increased risk of contamination for all commodities, particularly if no further sanitising or processing is undertaken. For viral pathogens, the use of sewage-contaminated water for irrigation or the application of agricultural chemicals is a risk factor that apply to all commodities.

For produce that can be washed or sanitised, the likelihood for contamination or cross-contamination is increased during postharvest washing using water that does not contain a disinfectant to maintain microbial quality throughout the process. Water based sanitisers used at industry-relevant concentrations and contact times (that do not adversely affect sensory properties of produce) are generally limited in efficacy for removing pathogens from the surface of produce and, in general, achieve reductions of <3 Log CFU.

- *Insufficient monitoring and application of postharvest washing and sanitisation systems for leafy vegetables and melons*

The efficacy of disinfectants and sanitisers is dependent on the recognition and management of a number of other factors. These factors can include pH, organic loads from dirt or damaged/cut produce, temperature, concentration, and contact time. The importance of these factors to sanitiser efficacy is well documented in the literature. If factors critical to the application of disinfectants and sanitisers are not monitored, these processes can fail and instead result in increased risk due to cross-contamination of produce or limited to no reduction in pathogens on the surface of produce.

- *Contamination and cross-contamination due to poor worker and equipment hygiene, both at harvest and postharvest.*

Increased handling and use of equipment at harvest or postharvest increase the likelihood of contamination of produce. If personnel or equipment hygiene is not maintained, viral and bacterial pathogens can contaminate and cross-contaminate fresh produce.

Risk mitigation regarding personnel or equipment hygiene is particularly important for produce that is not subject to further washing or sanitising (or some other risk reduction step) before final packing. However, even produce that is washed and sanitised can be subsequently exposed to equipment or personnel.

13.2 Mitigations

The mitigation options that have been identified in the literature vary depending on the produce type and method of production. In general, the following summarises the mitigation measures broadly recommended for all the commodity sectors covered in this assessment, whether intended to be consumed raw or processed as a ready-to-eat food without a control step that significantly reduces the microbial load:

- GAP, GHP and GMP must be implemented consistently, through-chain, to manage the diverse microbial risks relevant to fresh produce

- To manage the risk from animal incursion, the potential for both indirect and direct faecal contamination of produce needs to be reduced

- The farm environment and surroundings should be assessed prior to planting and close to harvest to identify risks that may significantly reduce the microbiological safety of fresh produce. Primary production should not occur in areas where it is likely that the presence of pathogens may represent an unacceptable risk of contamination to fresh produce

- The occurrence of extreme weather events should be included in risk assessments prior to harvest, including an assessment of factors that may affect subsequent washing and sanitisation efficacy, such as increased organic loads from flood, dust/soil or damaged produce

- The potential for contamination of the surface of fresh produce via contaminated water or soil amendments should be reduced and the quality of agricultural water and soil amendments determined to be of appropriate microbiological quality for its intended use

- Risk assessment at harvest is required to determine if sanitiser or disinfectant efficacy might be compromised by increased organic load, produce damage or extreme weather events. Appropriate concentrations, contact times, and other critical parameters should be applied and monitored to maintain maximum efficacy

- Sanitisers and disinfectants should be used in postharvest wash water to reduce the likelihood of contamination, cross-contamination, and internalisation into leafy vegetables and melons, as water based sanitisers are not effective in eliminating internalised pathogens. Sanitisers cannot be relied upon for end product safety due to their limited ability to inactivate pathogens on the surface of produce

- Employees should be well trained in harvest and postharvest methods that minimise microbial risk, such as culling damaged/diseased produce; applying personal and equipment hygiene practices; and recognising and reporting risk factors. Compliance with food safety policies should be verified

- The potential for contamination from equipment or surfaces that fresh produce contacts during harvest and postharvest should be minimised through the implementation of appropriate hygienic practices.

13.3   Commodity-specific risk factors and mitigations

This assessment has identified risk factors that may lead to increased risk associated with specific commodities and additional mitigation measures.

13.3.1   Leafy vegetables

Foodborne outbreaks involving leafy vegetables have the potential to affect a greater proportion of the Australian population compared to berries or melons. Leafy vegetables were reported to be consumed by a larger proportion of Australians, across all age groups, in the NNPAS[21], when compared to berry and melon consumption (see Section 6 and Appendix 4). The number of people that are likely to be affected by the consumption of contaminated leafy vegetables is dependent on a number of factors, including the prevalence and level of contamination of pathogens on the product at consumption.

The prevalence or concentration of foodborne pathogens on leafy vegetables during primary production in Australia is poorly documented, but is generally reported to be low for all commodities and pathogen combinations. However, high levels of contamination can occur sporadically, due to the risk factors identified in this assessment. Detection of these events is challenging, since pathogens are likely to be only transiently and heterogeneously present in the environment. The limitations of end-point testing for assurance of safety are widely recognised in the literature. Hence, the end product safety of leafy vegetables is heavily reliant on a through-chain approach to risk management.

There are also additional risk factors associated with leafy vegetables that require additional risk mitigation measures, including production practices; intrinsic properties of products; and product/pathogen interactions.

The surface characteristics of some leafy vegetables with rougher surfaces or vase like characteristics may provide enhanced attachment and protection from subsequent washing and sanitising. This may leave these commodities more susceptible to contamination from risk factors such as animal intrusion, water, soil, soil amendments, and extreme weather events. It has been recommended that these leafy vegetables should be irrigated by a method that minimises wetting of the edible portion of the plant, to limit the potential for microbial attachment and survival (Codex 2017). There is some, limited evidence that sanitisers may be less effective at reducing pathogen levels on rough surface leafy vegetables (and also for cut leafy vegetables compared to whole produce). There is also the potential for internalisation of pathogens into leafy vegetables during washing and sanitising. Therefore, the proper application and monitoring of disinfectants and sanitisers is of particular importance to reducing the risk for these commodities.

Leafy vegetables grown close to the ground may be at greater risk of contamination via direct contact with faeces or contaminated soil. In addition, there is potential for internalisation and persistence of pathogens in the edible portion of leafy vegetables. This risk is increased in the presence of higher pathogen concentrations associated with contamination events such as the application of raw manure or contaminated water. Control measures that reduce the likelihood of contamination from soil or water contacting the seeds, roots or leaves reduce these risks. However, no specific risk mitigation measures beyond those described above (Section 13.2) were identified.

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[21] 2011-12 Australian National Nutrition and Physical Activity Survey (NNPAS), a 1 day 24-hour recall survey of 12,153 respondents aged 2 years and above (with 64% of respondents (n=7735) undertaking a second 24-hour recall on a second non-consecutive day). The results in this report are derived using day 1 of data only. A respondent is counted as a consumer if the food was consumed on day 1 only. These data were weighted during the calculations undertaken in Harvest.

The end product safety of field-packed leafy vegetables is heavily reliant on risk mitigation during primary production. In addition to the risk mitigation steps identified in Section 10.3, Codex recommends that any water used during harvesting should be clean (and preferably potable) where there is direct contact between the water and edible portions of the leafy vegetables (Codex 2017).

The use of vacuum cooling for leafy vegetables that are contaminated or the use of contaminated water during the process can potentially facilitate internalisation of pathogens, providing them protection from subsequent sanitisation. The use of water that is clean and potable during cooling can reduce the risk of internalisation. However, reducing the risk is also dependent on implementation of risk mitigation during primary production to reduce the likelihood of contamination prior to vacuum cooling.

Postharvest washing of leafy vegetables introduces a risk of cross-contamination of product. The use of sanitisers and disinfectants in wash water can reduce cross-contamination and provide modest reductions of pathogens on the surface of leafy vegetables. However, this is only achieved if wash systems are optimised and well monitored.

Leafy vegetables have intrinsic properties that support the growth of bacterial pathogens. The use of temperature control from harvest through to consumption can reduce the risk associated with bacterial growth. Codex recommends that these products should be cooled as soon as possible after harvest to 1-5°C, and that the cold-chain be maintained though to consumption (Codex 2017). It is noted, however, that some types of leafy vegetables are chill-sensitive, and may require slightly higher holding temperatures for retention of quality.

13.3.2   Berries

Of the three commodity groups, berries had the second highest reported consumption in Australia across all age groups after leafy vegetables (see Section 6 and Appendix 4). Fresh strawberries eaten raw had the highest reported consumption, followed by blueberries and raspberries. While foodborne outbreaks have only been associated with imported berries in Australia, the lack of outbreaks attributed to Australian berries does not equate to a lack of risk. However, there is a paucity of Australian data on the prevalence and levels of pathogens either on berries during primary production or associated with agricultural inputs.

In general, a low prevalence of viruses has been reported for strawberries in the international literature, but the prevalence associated with blueberries and raspberries is poorly documented. As with leafy vegetables, the presence of pathogens in the Australian berry primary production environment is assumed to be low, resulting in lowered general risk. However, as is evident from foodborne outbreaks associated with berries internationally, contamination events can occur as sporadic events—with pathogens present transiently and heterogeneously in the production and processing environment—leading to difficulties in detection. Risk factors identified in the primary production environment in Australia are relevant to berry production, and represent a risk to berry food safety.

Due to the intrinsic nature of berries, they are not generally washed or sanitised before packing. Therefore, the reduction of risk associated with berries is dependent on consistent risk mitigation during primary production to reduce the likelihood of contamination. Additional risk factors that can lead to contamination of berries were identified, and may require additional risk mitigation measures.

The rougher or more irregular surfaces of strawberries and raspberries may provide more sites for microbial attachment and protection in comparison to other commodities with smoother surfaces, such as blueberries. However, studies quantifying the difference in surface roughness of berries are limited, and there remains uncertainty about how this affects the likelihood of contamination of the different commodities.

Strawberries may be at a greater risk of contamination via direct contact with faeces or contaminated soil, as they grown closer to the ground compared to blueberries and raspberries. However, indirect contamination such as via wind or water inputs affected by animal incursion or direct contamination from birds, climbing animals, or larger mammals still present a risk to those higher growing commodities. The risk mitigation measures to reduce the contamination of the surface of berries from animal intrusion, soil amendments and irrigation water outlined in sections 9.1.3, 9.5.3, and 9.6.3 are pertinent to these commodities. In addition, Codex recommends that berry growers use production practices (e.g. site selection, wind breaks) to minimize the contact of berries with airborne contaminants and to limit contact with soil, animal droppings, soil amendments (including natural fertilizers) or irrigation water (Codex 2017).

The use of contaminated water for frost protection via overhead sprinklers could transfer pathogens to berries. The risk can be reduced by mitigation measures identified in Section 9.6.3. Codex recommends that clean or potable water should be used for berry primary production (Codex 2017).

Internalisation of pathogens contaminating soil via the roots to the edible portion of the plant is a potential risk factor that has been identified for strawberries. However, the relevance of internalisation in the context of berries during primary production has high uncertainty, as it is not known to what extent viral or bacterial pathogens can persist in the fruit, which is not normally conducive to the growth of bacterial pathogens. The implementation of mitigation measures that reduce contamination of inputs contacting the roots or leaves of plants would reduce the risk of internalisation.

Hand-picking of berries can increase the risk associated with viral contamination from symptomatic or asymptomatic workers. NoV and HAV have low infectious doses, and an infected worker can potentially contaminate a large quantity of berries, especially if the berries are excessively handled (see Appendices 6 and 8). However, there is a paucity of evidence on food handler contamination of berries in the primary production and processing environment in Australia, leading to a high degree of uncertainty in the level of risk posed.

General recommendations regarding the handling of berries at harvest are provided in Section 10.3. Codex specifically recommends minimising handling and sorting (e.g. by field packing fresh berries in consumer ready containers); and worker training in good hygienic practices during pre-harvest, harvest and postharvest activities, including transport and storage (Codex 2017). In addition, Codex recommends a harvest supervisor is appointed at all times; berries on the ground should not be harvested unless they are processed with a microbiological inactivation step; and immediate cooling of berries after harvesting and through distribution. If pre-cooling is undertaken, potable water is recommended for ice and hydro-coolers.

13.3.3   Melons

Melons have a slightly lower reported consumption across all age groups than leafy vegetables and berries in Australia (see Section 6 and Appendix 4). However, the mean serving size of melons was much larger (approximately 200 g/day) compared to leafy vegetables (approximately 25 g/day) or berries (approximately 75 g/day) consumed raw. As with leafy vegetables and berries, there are very limited data regarding the prevalence or levels of pathogens on rockmelons or watermelons during primary production, particularly in Australia. In general, the prevalence of pathogens in the Australian melon primary production environment is assumed to be low. However, outbreaks of foodborne illness in Australia and internationally have been linked to the melon primary production environment. Contamination can occur sporadically—with pathogens present transiently and heterogeneously in the environment—leading to difficulties in detection. Moreover, melons have characteristics that may predispose them to a higher risk of contamination that require additional risk mitigation during primary production.

The rougher surface of rockmelons may provide enhanced attachment and protection for pathogens from subsequent washing and sanitising compared to smoother surfaced watermelons. However, watermelons are still susceptible to surface contamination, and the adhesion of pathogens to the surface has been demonstrated. Melons may be at a greater risk of contamination via direct contact with faeces, or soil affected by faeces, due to their proximity to the grown when grown in the field. Therefore, reduction of contamination of the surface of melons—from animal intrusion or application of contaminated soil amendments or irrigation water—is relevant to all melons, particularly those with rougher surfaces (see sections 9.1.3, 9.5.3, and 9.6.3). Codex also recommends preventing or minimising contact between melons and soil, soil amendments, and irrigation water—including avoiding overhead irrigation, particularly for netted rind melons. Subsurface or drip irrigation presents the least risk of contaminating melon surfaces, but pooling water should be avoided (Codex 2017).

Watermelons may present an increased risk compared to those commodities that are subsequently washed and sanitised under optimised conditions. The level of risk from un-sanitised watermelons will be dependent on the prevalence of pathogens entering the primary production system and the steps taken during primary production to reduce contamination. While postharvest washing with sanitisers might reduce the risk for watermelons, studies on its efficacy are lacking.

Stem scars may provide a route of entry of foodborne pathogens, if present, into the edible portion of melons. Codex recommends implementation of appropriate handling practices to minimise stem scar and rind infiltration of pathogens into the edible portions of melon flesh, including during washing operations, storage and transport. The length and temperature of storage of melons should be determined depending on the stage of maturity of the melons at harvest (Codex 2017).

There is the potential for internalisation of pathogens into rockmelons during washing. *L. monocytogenes* has been shown to internalise into whole rockmelons via dump tank wash water with and without a temperature differential, though internalisation was slightly enhanced with a temperature differential between warmer fruit and cooler water. Although the evidence of internalisation is limited, pre-cooling of melons before washing and sanitising may reduce the likelihood of internalisation, and will also limit the potential for growth of pathogens during storage before processing. To minimise the risk of water infiltration, Codex recommends that dump tank water temperature should be higher than the internal temperature of melons; the full submersion of melons in colder dump tank water should be minimized or avoided; and the time melons remain in dump tank water should be minimised (Codex 2017).

The use of sanitisers in wash water can provide modest reductions of pathogen levels on the surface of melons. The efficacy of sanitisers in removing pathogens from the rougher surface of rockmelons may be reduced compared to produce with smoother surfaces. Hence, the proper application and monitoring of disinfectants and sanitisers is of particular importance to reducing the risk for rockmelons. Specific mitigations additional to those provided in Section 11.4.3 were not identified for melons.

Rockmelons have been demonstrated to support the growth of bacterial pathogens both on the rind and on the flesh. No evidence for the growth of bacterial pathogens on the rind of watermelons were identified. The flesh of watermelons and rockmelons support the growth of bacterial pathogens. Temperature control after harvest can reduce the potential for internalisation during washing and sanitising and prevent or slow the growth of pathogens on the flesh or on the rind of melons. Forced-air cooling can reduce the risk of water infiltrating into the melon, but may also spread contamination if equipment is not cleaned and disinfected regularly (Codex 2017).

14 Limitations

The qualitative nature of the assessment and scope meant it was not possible to provide comparative estimates of risk (likelihood and the severity of consequences) for the large number of individual risk factors, pathogens, and commodity combinations that were considered in this assessment. There is a paucity of Australian data regarding the concentration or prevalence of pathogens associated with the risk factors and the commodities throughout primary production and primary processing that would be required for a quantitative assessment.

There are limitations to the literature review process that identified the publications that were reviewed as evidence and the basis of conclusions for this assessment. The literature search used defined search strings using logical tests ('Boolean operators') that were defined by the risk assessment team to search bibliographic databases. The literature compiled for previous assessments by FSANZ were also considered. However, it is possible that some authoritative documents may not have been identified if these publications were not in the databases searched or did not match with the search strings. Furthermore, relevant publications in languages other than English may not have not been identified or included.

Detailed analysis of the publications highlighted a number of issues limiting the interpretation of the prevalence data, including representativeness, sample size, sample description and analytical methodology.

Most prevalence studies use a 'convenient sampling' approach. This sampling method is used for exploratory research with samples collected which are readily accessible and easy to obtain. A common setting for the sampling is supermarkets or farmers markets. Samples are potentially subject to bias e.g. multiple samples are collected from the same source and are not truly random. In addition, these studies often have a limited number of samples collected which are not sufficient to detect low pathogen prevalence. An alternative to convenient sampling is 'objective sampling'. Objective sampling uses a planned approach to select random samples which are statistically representative of the population. Only a few examples of this types of survey were found in the literature review. These surveys often have a larger sample size.

Inconsistent sample description was most notable for prevalence studies related to 'leafy greens' and especially lettuce. The food names mentioned in the published studies were extracted and consistently described using the EFSA FoodEx2 classification system. Where particular varieties of lettuce (e.g. cos, oak, iceberg etc.) were provided the names were matched to the appropriate FoodEx2 classification. However, in many cases the use of a generic 'lettuce' or uninformative descriptors (e.g. green) meant that samples were classified to a higher less specific classification of *Lettuce (generic)*. As a result, no sub-group analysis for pathogen prevalence between lettuce varieties was attempted.

Analytical methodology, especially culture based vs molecular techniques such as PCR makes interpretation of prevalence studies challenging. The lack of consistency in the type and number of PCR primers used for pathogens between studies is problematic. In this report prevalence data for *E. coli* O157:H7 was separated from the PCR data which captures the broader STEC group, of which *E. coli* O157:H7 is one member. The STEC group is notable for the presence of the stx_1 and stx_2 Shiga toxin genes.

Pathogen concentration studies, especially for bacterial pathogens, is absent. Studies that quantified bacterial loads on horticulture products are limited to indicator microorganisms such as the total count or generic *E. coli*. Evidence for the use of indicator microorganisms as proxies for the presence of pathogens is weak.

Potential for microbes displaying antimicrobial resistance (AMR) associated with horticultural produce is an emerging issue that can present increased risks to human health (Codex 2011). A limitation of this assessment is that the risk factors that may facilitate AMR in microbes associated with horticulture products were not considered. These risk factors may include the direct use of antimicrobials to control microbial plant disease (e.g. use of streptomycin against fire blight in apples[22]), the use of animal manures, and the use of irrigation water shared with the food animal industry (Australian Government Department of Health 2018). A review of published and grey literature on AMR in food[23], commissioned under *Australia's First National Antimicrobial Resistance Strategy 2015–2019*[24] noted that there is very limited or no AMR data for horticulture. The available data that has assessed the presence of AMR in Australian horticultural products reported 80% of *E. coli* (n=15) isolated from strawberries were resistant to ampicillin (Kurtböke et al. 2016), and 5% of *E. coli* (n=7) isolated from retail lettuce was resistant to one or more antimicrobials tested and multi-drug resistance to four antimicrobials was observed in two isolates (Barlow and Gobius 2008). As such, it is recommended that a dedicated risk assessment or risk profiling regarding AMR is undertaken for the Australian horticultural sector.

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[22] FSANZ risk assessment on antibiotic use in New Zealand apples: www.foodstandards.gov.au/publications/Pages/applerisk/fsanzriskassessmento5163.aspx

[23] Review of published and grey literature on the presence of antimicrobial resistance in food in Australia and New Zealand: https://www.amr.gov.au/resources/review-published-and-grey-literature-presence-antimicrobial-resistance-food-australia-and

[24] Responding to the threat of antimicrobial resistance: Australia's first national antimicrobial resistance strategy 2015-2019: https://www.amr.gov.au/resources/national-amr-strategy

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Appendices

Appendix 1 – Literature search strategy

Literature searches were performed using the EBSCO search engine. Search terms were combined in the following format (commodity specific term 1 OR commodity specific term 2…) AND (pathogen specific term 1 OR pathogen specific term 2…) AND (prevalence/risk factor term 1 OR prevalence/risk factor term 2…). For commodity and pathogen specific terms refer to Table A1-1, and for prevalence and risk factor specific terms refer to Table A1-2. Searches were performed separately for each commodity for prevalence and each individual risk factor.

Due to the weaker evidence base for berries and melons, additional searches were performed—refer to Table A1-3 for the additional search terms.

Table A1-1. Commodity and pathogen specific search terms

Commodity	Commodity specific terms	Pathogen specific terms
Leafy vegetables	Lettuce Spinach Baby spinach Parsley	Salmonella Salmonellae Salmonellosis Escherichia coli E. coli Listeria Monocytogenes Listeriosis Listeriae
Berries	Blue berr* Blueberr* Raspberr* Strawberr*	Escherichia coli E. coli Hepatitis A Hep A HepA HAV Norovirus NoV
Melons	Rockmelon Rock melon Cantaloupe Canteloupe Cantalope Muskmelon Persian melon Spanspek Sweet melon Sweet rough-skinned melon Watermelon Melon	Salmonella Salmonellae Salmonellosis Listeria Monocytogenes Listeriosis Listeriae

Table A1-2. Prevalence and risk factor specific search terms

Prevalence	Animals, wildlife and livestock	Characteristics of the production site	Extreme weather events	Seed and seedlings	Soil, soil amendments, and fertilisers	Water†	Harvest and field packing	Packhouse and post-harvest processing
Prevalence Survey Contamination Occurrence	Wildlife Animal Livestock Cattle Bird Feral pigs Deer Rodent Reptiles Amphibians Poultry Feces	Topograph Climat* Hydrolog* Weather Climate change Climate-change Winter Summer Autumn Spring	Flood* Flood water Dust Storm* Hail* Rain* Extreme weather Cyclone Extreme heat Extreme cold Wind Extreme temperature Frost Drought	Seed* Crop Crop selection Crop rotationVariet* Varietal Non-traditional Traditional	Soil Soil amendment Amendment Fertilizer Organic waste Manure Manure-amended Compost Human biosolid Plant biowaste Bioremediation	Wastewater Irrigat* Irrigation water Water irrigation Water source Water treatment Well Channel Canal Rainwater Reclaimed water Groundwater	Harvest* Field Pick	Post-harvest Process* Clean* Trim* Coring Wash* Sanitiz* Dry* Pack*

† additional water search: (pesticid* OR fungicid*) AND (water OR water quality OR water source)

Table A1-3. Additional search terms for specific commodities

Commodity(ies)	Additional risk factor terms
Berries	Hydroponi* Substrate Peat Tunnel*
Berries and melons	Surviv* Persistence Incidence Handling Attachment Coloniz* Exposure Grow* Predict* Transfer Transmission Biofilm Risk Hazard Damage Internaliz* Contaminat* Control Intervention Mitigation Management Microbiolog* Cropping intervals HACCP GMP GHP Water Treatment Monitor* Agricultural practices Intrinsic Practice Hygiene Staff Personnel Worker Source

Appendix 2 – Summary of international outbreaks associated with fresh and minimally processed horticultural produce

Commodity	Pathogen	Year	Location/s	Commodity origin	No. of cases (No. deaths)	Epidemiology comments[1]	Supply chain failure[2]	References
Leafy vegetables								
Lettuce, romaine	*Escherichia coli* O145	2010	USA	USA	31	E, L	n.d.	(Taylor et al. 2013)
Lettuce, romaine	*E. coli* O157:H7	2011	USA	USA	58	E	n.d.	(Slayton et al. 2013)
Basil	*Shigella sonnei*	2011	Norway	Israel	46	E	n.d.	(Guzman-Herrador et al. 2011; Guzman-Herrador et al. 2013)
Lettuce	*E. coli* O157:H7	2012	USA	USA	17 (2)	E	n.d.	(Marder et al. 2014)
Spinach & leafy vegetables, bagged	*E. coli* O157:H7	2012	USA	USA	33	L	n.d.	(CDC 2012b)
Coriander	*Cyclospora cayetanensis*	2013	USA	Mexico	270	E	n.d.	(Abanyie et al. 2015)
Leafy vegetables/ bagged salads	*C. cayetanensis*	2013	USA	Mexico	227	E	n.d.	(Buss et al. 2016)
Watercress	*E. coli* O157:H7	2013	UK	UK	28	E, L	Suspected contamination from nearby cattle via irrigation water.	(Jenkins et al. 2015; Launders et al. 2013)
Leafy vegetables/ bagged salads	*Salmonella* Coeln	2013 - 2014	Norway	Imported	26	E	n.d.	(Vestrheim et al. 2016)
Leafy vegetables/ bagged salads	*Listeria monocytogenes*	2015 - 2016	USA, Canada	USA	19 (1) in USA 14 (3) in Canada	E, L	Multiple defects in facilities, hygiene, sanitation and process controls in the produce processing facility.	(Public Health Agency of Canada 2016; Self et al. 2016; Self et al. 2019)
Lettuce, green coral	Norovirus	2016	Denmark	France	412	E, L	n.d.	(Müller et al. 2016)
Rocket	*E. coli* (EPEC & non-O157 STEC)	2016	Finland	Denmark	237	E, L	n.d.	(Kinnula et al. 2018)

Commodity	Pathogen	Year	Location/s	Commodity origin	No. of cases (No. deaths)	Epidemiology comments[1]	Supply chain failure[2]	References
Lettuce, romaine	*E. coli* O157:H7	2018	USA, Canada	USA	210 (5) in USA 8 in Canada	L	Outbreak strain identified (by WGS) in canal water used to irrigate and dilute crop protection chemicals applied by aerial and land-based sprays on farms identified in trace back.	(CDC 2018b; FDA 2018a; Public Health Agency of Canada 2018)
Lettuce, romaine	*E. coli* O157:H7	2018	USA, Canada	USA	62 in USA 29 in Canada	L	E. coli O157:H7 closely related (by WGS) to outbreak strain found in sediment in a water reservoir on a farm identified in trace back.	(CDC 2019b; FDA 2019; Public Health Agency of Canada 2019)
Packaged salad (romaine lettuce and carrots)	*C. cayetanensis*	2018	USA	USA	511	L	n.d.	(CDC 2018a; FDA 2018b)
Spinach	*Yersinia enterocolitica*	2019	Denmark, Sweden	Italy	57	E	n.d.	(Espenhain et al. 2019)
Sprouts								
Fenugreek sprouts	*E. coli* O104:H4	2011	Germany & 15 other countries	Egypt (seeds)	4075 (>50)	E, L	n.d.	(Buchholz et al. 2011; EFSA 2011; Foley et al. 2013; Frank et al. 2011a; Frank et al. 2011b; King et al. 2012; WHO 2011)
Alfalfa sprouts	*Salmonella* Cubana	2012	USA	USA	19	L	Multiple defects in facilities, hygiene, sanitation and process controls in the sprout growing facility.	(FDA 2012)
Mung bean sprouts	*L. monocytogenes*	2014	USA	USA	5 (2)	L	Multiple defects in facilities, hygiene, sanitation and process controls in the sprout processing and packaging facility.	(FDA 2015)

Commodity	Pathogen	Year	Location/s	Commodity origin	No. of cases (No. deaths)	Epidemiology comments[1]	Supply chain failure[2]	References
Alfalfa sprouts	*Salmonella* Muenchen, *Salmonella* Kentucky	2015–2016	USA	USA	26	L	Contaminated seed the likely source.	(CDC 2016d; FDA 2016)
Other vegetables								
Celery	*L. monocytogenes*	2010	USA	USA	10 (5)	E,L	Multiple defects in facilities, hygiene, sanitation and process controls in the produce processing facility.	(Gaul et al. 2013)
Tomatoes	*Salmonella* Strathcona	2011	Denmark	Italy	43 (1)	E	n.d.	(Muller et al. 2016)
Vegetables, various, frozen	*L. monocytogenes*	2013–2016	USA	USA	9 (1)	L	n.d.	(CDC 2016b)
Cucumber	*Salmonella* Newport	2014	USA	USA	275 (1)	E	n.d.	(Angelo et al. 2015)
Cucumber	*Salmonella* Poona	2015	USA	Mexico	907 (6)	E, L	n.d.	(CDC 2016c; Laughlin et al. 2019)
Peas, sugar snap	*C. cayetanensis*	2015	Canada	Guatemala	45	E	n.d.	(Whitfield et al. 2017)
Frozen corn (possibly other frozen vegetables)	*L. monocytogenes*	2015-2018	Austria, Denmark, Finland, Sweden, UK	Hungary	47 (9)	L	Persistent contamination at the processing plant, despite cleaning and disinfection.	(EFSA 2018a, 2018b)
Melons								
Watermelon	*Salmonella* Typhimurium	2009	New Zealand	New Zealand	18	E	Multiple defects in facilities, hygiene, sanitation and process controls by the watermelon grower/seller.	(McCallum et al. 2010)
Watermelon	*Salmonella* Newport	2011-2012	England, Wales, Northern Ireland, Scotland, Ireland, Germany	Brazil	63 (3)	E, L	n.d.	(Byrne et al. 2014)

Commodity	Pathogen	Year	Location/s	Commodity origin	No. of cases (No. deaths)	Epidemiology comments[1]	Supply chain failure[2]	References
Rockmelon	*Salmonella* Typhimurium, *Salmonella* Newport	2012	USA	USA	261 (3)	L	Multiple defects in GAP, facilities, hygiene, sanitation and process controls on farm and in product distribution.	(CDC 2012a; FDA 2013)
Berries								
Blueberries	*Salmonella* Newport	2010	USA	USA	6	E	n.d.	(Miller et al. 2013)
Raspberries, frozen	Norovirus	2010 - 2011	Denmark	Serbia	242	E, L	n.d.	(Muller et al. 2015)
Strawberries, fresh	*E. coli* O157:H7	2011	USA	USA	15 (2)	E	Contamination by deer faeces; failures in GAP on farm.	(Laidler et al. 2013)
Strawberries, frozen	Norovirus	2012	Germany	China	~11,000	E, L	n.d.	(Bernard et al. 2014; Made et al. 2013)
Strawberries, frozen	Hepatitis A	2012 - 2013	Denmark, Finland, Norway, Sweden	Egypt, Morocco	106	E	n.d.	(Gillesberg Lassen et al. 2013; Gossner and Severi 2014; Nordic Outbreak Investigation Team 2013)
Berries, mixed, frozen	Hepatitis A	2013 - 2014	Italy & 9 other countries	Suspected: Poland, Bulgaria	>1400	E, L	n.d.	(EFSA 2014; Guzman-Herrador et al. 2014; Guzman-Herrador et al. 2015; Scavia et al. 2017; Severi et al. 2015; Wenzel et al. 2014)
Strawberries, frozen	Hepatitis A	2016	USA	Egypt	143	L	n.d.	(CDC 2016a)
Raspberries/ blueberries, mixed, frozen	Hepatitis A	2017	Netherlands	Bulgaria	14	E	n.d.	(Mollers et al. 2018)
Strawberries, frozen	Hepatitis A	2018	Sweden, Austria	Poland	34	E, L	n.d.	(Enkirch et al. 2018)

Commodity	Pathogen	Year	Location/s	Commodity origin	No. of cases (No. deaths)	Epidemiology comments[1]	Supply chain failure[2]	References
Other fruit								
Mamey	*Salmonella* Typhi	2010	USA	Guatemala	12	E	Multiple defects in facilities, hygiene, sanitation and process controls in the produce processing facility.	(Loharikar et al. 2012)
Papaya	*Salmonella* Agona	2011	USA	Mexico	106	E, L	n.d.	(Mba-Jonas et al. 2018)
Pomegranate arils	Hepatitis A	2012	Canada	Egypt	8	E, L	n.d.	(Swinkels et al. 2014)
Apples, caramel apples	*L. monocytogenes*	2014–2015	USA	USA	35 (7)	E, L	n.d.	(CDC 2015, 2019a)
Papaya	*Salmonella* (multiple serotypes)	2016–2017	USA	Mexico	244	E, L	Produce prepared, packed or held under insanitary conditions.	(Hassan et al. 2019)

[1] E—epidemiological study, L—laboratory confirmed link between outbreak strain and implicated commodity or farm
[2] n.d.—not determined

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Appendix 3 – Summary of prevalence studies for pathogens in fresh and minimally processed horticultural produce sampled from food businesses

Commodity	Pathogen	Country	Positive samples	Total samples	Reference
Leafy vegetables					
Lettuce	*Salmonella* spp.	Brazil	0	30	Brandao et al (2014)
Lettuce	*Salmonella* spp.	Brazil	0	30	Brandao et al (2014)
Lettuce	*Salmonella* spp.	Brazil	0	20	Maffei et al (2013)
Lettuce	*Salmonella* spp.	Brazil	0	20	Maffei et al (2013)
Lettuce	*Salmonella* spp.	Brazil	0	20	Maffei et al (2013)
Lettuce	*Salmonella* spp.	Brazil	0	20	Maffei et al (2013)
Lettuce	*Salmonella* spp.	China	1	22	Ni et al (2018)
Lettuce	*Salmonella* spp.	Honduras	1	71	Maradiaga et al (2015)
Lettuce	*Salmonella* spp.	Italy	0	18	Terio et al (2017)
Lettuce	*Salmonella* spp.	Italy	0	67	Terio et al (2017)
Lettuce	*Salmonella* spp.	Japan	2	1758	Hara-Kudo et al (2013)
Lettuce	*Salmonella* spp.	Japan	0	419	Koseki et al (2011)
Lettuce	*Salmonella* spp.	Malaysia	2	25	Abatcha et al (2018)
Lettuce	*Salmonella* spp.	Malaysia	8	25	Abatcha et al (2018)
Lettuce	*Salmonella* spp.	Philippines	12	50	Vital et al (2014)
Lettuce	*Salmonella* spp.	Philippines	3	10	Vital et al (2014)
Lettuce	*Salmonella* spp.	Portugal	0	38	Santos et al (2012)
Lettuce	*Salmonella* spp.	Saudi Arabia	0	15	Al-Holy et al (2013)
Lettuce	*Salmonella* spp.	South Korea	0	55	Tango et al (2014)
Lettuce	*Salmonella* spp.	South Korea	0	63	Tango et al (2014)
Lettuce	*Salmonella* spp.	South Korea	0	30	Tango et al (2018)
Lettuce	*Salmonella* spp.	South Korea	0	30	Tango et al (2018)
Lettuce	*Salmonella* spp.	Switzerland	0	142	Althaus et al (2012)

Commodity	Pathogen	Country	Positive samples	Total samples	Reference
Lettuce	*Salmonella* spp.	Thailand	8	40	Niyomdecha et al (2016)
Lettuce	*Salmonella* spp.	The Netherlands	2	565	Wijnands et al (2014)
Lettuce	*Salmonella* spp.	Turkey	0	62	Gunel et al (2015)
Lettuce	*Salmonella* spp.	Turkey	0	62	Gunel et al (2015)
Lettuce	*Salmonella* spp.	USA	0	69	Korir et al (2016)
Lettuce	*Salmonella* spp.	USA	1	30	Liu & Kilonzo-Nthenge (2017)
Lettuce	*Salmonella* spp.	USA	0	24	Liu & Kilonzo-Nthenge (2017)
Lettuce	*Salmonella* spp.	USA	10	19244	Reddy et al (2016)
Lettuce	*Salmonella* spp.	USA	1	3310	Zhang et al (2018)
Lettuce	*Salmonella* spp.	USA	3	5548	Zhang et al (2018)
Lettuce	STEC	Argentina	3	267	Gonzalez et al (2017)
Lettuce	STEC	Canada	0	27	Wood et al (2015)
Lettuce	STEC	Canada	0	24	Wood et al (2015)
Lettuce	STEC	Canada	0	17	Wood et al (2015)
Lettuce	STEC	China	3	41	Li et al (2016)
Lettuce	STEC	Italy	0	255	Santarelli et al (2018)
Lettuce	STEC	Italy	0	196	Santarelli et al (2018)
Lettuce	STEC	Turkey	3	30	Ozpinar et al (2013)
Lettuce	*L. monocytogenes*	Brazil	3	152	Sant'ana et al (2012)
Lettuce	*L. monocytogenes*	Italy	0	255	Santarelli et al (2018)
Lettuce	*L. monocytogenes*	Italy	0	196	Santarelli et al (2018)
Lettuce	*L. monocytogenes*	Italy	0	18	Terio et al (2017)
Lettuce	*L. monocytogenes*	Italy	0	67	Terio et al (2017)
Lettuce	*L. monocytogenes*	Malaysia	1	14	Jamali et al (2013)
Lettuce	*L. monocytogenes*	South Korea	2	55	Tango et al (2014)
Lettuce	*L. monocytogenes*	South Korea	4	63	Tango et al (2014)
Lettuce	*L. monocytogenes*	South Korea	0	30	Tango et al (2018)

Commodity	Pathogen	Country	Positive samples	Total samples	Reference
Lettuce	*L. monocytogenes*	South Korea	1	30	Tango et al (2018)
Lettuce	*L. monocytogenes*	Spain	0	27	Moreno et al (2012)
Lettuce	*L. monocytogenes*	Switzerland	5	142	Althaus et al (2012)
Lettuce	*L. monocytogenes*	USA	0	69	Korir et al (2016)
Lettuce	*L. monocytogenes*	USA	0	52	Scheinberg et al (2017)
Lettuce	*L. monocytogenes*	USA	2	3310	Zhang et al (2018)
Lettuce	*L. monocytogenes*	USA	8	5548	Zhang et al (2018)
Spinach	*Salmonella* spp.	Czech Republic	0	21	Vojkovska et al (2017)
Spinach	*Salmonella* spp.	India	7	60	Mritunjay & Kumar (2017)
Spinach	*Salmonella* spp.	Italy	0	10	Terio et al (2017)
Spinach	*Salmonella* spp.	Japan	0	536	Hara-Kudo et al (2013)
Spinach	*Salmonella* spp.	South Korea	0	55	Tango et al (2014)
Spinach	*Salmonella* spp.	South Korea	0	63	Tango et al (2014)
Spinach	*Salmonella* spp.	South Korea	0	30	Tango et al (2018)
Spinach	*Salmonella* spp.	South Korea	0	30	Tango et al (2018)
Spinach	*Salmonella* spp.	USA	0	69	Korir et al (2016)
Spinach	*Salmonella* spp.	USA	8	43	Li et al (2017)
Spinach	*Salmonella* spp.	USA	0	33	Liu & Kilonzo-Nthenge (2017)
Spinach	*Salmonella* spp.	USA	0	52	Roth et al (2018)
Spinach	*Salmonella* spp.	USA	0	25	Roth et al (2018)
Spinach	*Salmonella* spp.	USA	3	5325	Zhang et al (2018)
Spinach	STEC	Argentina	0	19	Gonzalez et al (2017)
Spinach	STEC	China	0	19	Li et al (2016)
Spinach	STEC	India	2	60	Mritunjay & Kumar (2017)
Spinach	STEC	RSA	1	90	Du Plessis et al (2017)
Spinach	STEC	USA	23	6719	Feng & Reddy (2014)
Spinach	*L. monocytogenes*	Brazil	1	11	Sant'ana et al (2012)

Commodity	Pathogen	Country	Positive samples	Total samples	Reference
Spinach	*L. monocytogenes*	Czech Republic	1	21	Vojkovska et al (2017)
Spinach	*L. monocytogenes*	India	8	60	Mritunjay & Kumar (2017)
Spinach	*L. monocytogenes*	Italy	0	10	Terio et al (2017)
Spinach	*L. monocytogenes*	South Korea	4	55	Tango et al (2014)
Spinach	*L. monocytogenes*	South Korea	4	63	Tango et al (2014)
Spinach	*L. monocytogenes*	South Korea	0	30	Tango et al (2018)
Spinach	*L. monocytogenes*	South Korea	0	30	Tango et al (2018)
Spinach	*L. monocytogenes*	Spain	1	18	Moreno et al (2012)
Spinach	*L. monocytogenes*	USA	1	69	Korir et al (2016)
Spinach	*L. monocytogenes*	USA	0	43	Li et al (2017)
Spinach	*L. monocytogenes*	USA	2	52	Roth et al (2018)
Spinach	*L. monocytogenes*	USA	0	25	Roth et al (2018)
Spinach	*L. monocytogenes*	USA	1	46	Scheinberg et al (2017)
Spinach	*L. monocytogenes*	USA	5	5325	Zhang et al (2018)
Berries					
Raspberries	Norovirus	Belgium	1	12	Keuckelaere et al (2015)
Raspberries	Norovirus	Belgium	6	70	Keuckelaere et al (2015)
Raspberries	Norovirus	Belgium	4	10	Stals et al (2011)
Raspberries	Norovirus	China	11	120	Gao et al (2019)
Raspberries	Norovirus	China	0	124	Gao et al (2019)
Raspberries	Norovirus	China	13	120	Gao et al (2019)
Raspberries	Norovirus	Czech Republic, Poland and Serbia	0	60	Maunula et al (2013)
Raspberries	Norovirus	Czech Republic, Poland and Serbia	0	39	Maunula et al (2013)
Raspberries	Norovirus	Europe	0	536	Li et al (2018)
Raspberries	Norovirus	France	27	162	Loutreul et al (2014)
Raspberries	Norovirus	UK	7	310	Cook et al (2019)

Commodity	Pathogen	Country	Positive samples	Total samples	Reference
Raspberries	Norovirus	UK	10	274	Cook et al (2019)
Raspberries	Hepatitis A	Czech Republic, Poland and Serbia	0	60	Maunula et al (2013)
Raspberries	Hepatitis A	Czech Republic, Poland and Serbia	0	39	Maunula et al (2013)
Raspberries	Hepatitis A	Europe	0	536	Li et al (2018)
Raspberries	Hepatitis A	Turkey	20	240	Incili et al (2019)
Blueberries	Norovirus	Australia	0	14	Hodgson (2015)
Blueberries	Norovirus	China	12	120	Gao et al (2019)
Blueberries	Norovirus	China	0	108	Gao et al (2019)
Blueberries	Norovirus	China	8	120	Gao et al (2019)
Blueberries	Norovirus	Europe	2	126	Li et al (2018)
Blueberries	Hepatitis A	Australia	0	14	Hodgson (2015)
Blueberries	Hepatitis A	Europe	0	126	Li et al (2018)
Strawberries	Norovirus	Australia	0	138	Hodgson (2015)
Strawberries	Norovirus	Belgium	6	20	Stals et al (2011)
Strawberries	Norovirus	Brazil	0	12	Marti et al (2017)
Strawberries	Norovirus	China	64	300	Gao et al (2019)
Strawberries	Norovirus	Czech Republic	1	70	Dziedzinska et al (2018)
Strawberries	Norovirus	Czech Republic, Poland and Serbia	0	21	Maunula et al (2013)
Strawberries	Norovirus	Europe	1	918	Li et al (2018)
Strawberries	Norovirus	France	4	32	Loutreul et al (2014)
Strawberries	Hepatitis A	Australia	0	138	Hodgson (2015)
Strawberries	Hepatitis A	Brazil	2	12	Marti et al (2017)
Strawberries	Hepatitis A	Czech Republic, Poland and Serbia	0	21	Maunula et al (2013)
Strawberries	Hepatitis A	Europe	1	918	Li et al (2018)

Commodity	Pathogen	Country	Positive samples	Total samples	Reference
Melons					
Rockmelon	*Salmonella* spp.	Germany	2	147	Esteban-Cuesta et al (2018)
Rockmelon	*Salmonella* spp.	Germany	1	147	Esteban-Cuesta et al (2018)
Rockmelon	*Salmonella* spp.	Honduras	1	35	Maradiaga et al (2015)
Rockmelon	*Salmonella* spp.	UK	0	870	Willis et al (2016)
Rockmelon	*Salmonella* spp.	USA	9	16	Li et al (2017)
Rockmelon	*Salmonella* spp.	USA	14	16169	Reddy et al (2016)
Rockmelon	*Salmonella* spp.	USA	2	1075	Zhang et al (2018)
Rockmelon	*L. monocytogenes*	Germany	0	147	Esteban-Cuesta et al (2018)
Rockmelon	*L. monocytogenes*	Germany	0	147	Esteban-Cuesta et al (2018)
Rockmelon	*L. monocytogenes*	UK	51	870	Willis et al (2016)
Rockmelon	*L. monocytogenes*	USA	1	16	Li et al (2017)
Rockmelon	*L. monocytogenes*	USA	0	1075	Zhang et al (2018)

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Appendix 4 – Commodity Consumption[25]

Consumption by commodity sector

To provide an indication of the overall consumption for the commodity sectors, the consumption data has been summarised for those leafy vegetables eaten 'as is' (i.e. fresh or raw), eaten combined fresh and raw 'as salads', and also eaten as part of a 'mixed dishes'. This is because not all leafy vegetables, melons, and berries produced in Australia will be consumed fresh, some will be cooked or mixed with other products and processed in a variety of ways. 'Mixed dishes' represent those dishes that may combine multiple foods and may or may not be cooked[26]. Cooking will reduce the likelihood of pathogens being present when consumed and therefore the risk. As such, to understand the potential risk associated with fresh produce it is important to understand what proportions of commodities are consumed as is or are potentially undergoing some type of processing that could provide a reduction in risk.

Leafy vegetable consumption has been summarised for leafy vegetables eaten as is, eaten as salads and from mixed dishes.

For berries, consumption has been summarised for berries eaten as is (this includes both fresh and frozen berries), from mixed dishes (a list of the mixed dishes is provided in Appendix 5), and as juices. Juices may or may not undergo a pathogen reduction step depending on if they are made in the home with fresh or frozen berries or produced commercially where heat and/or pressure may be used in production to reduce the likelihood of contamination by pathogens.

Melon consumption has been summarised for melons eaten as is and from mixed dishes as no other category had sufficient responses to be included.

The percentage of respondents reporting to consume products from the commodity sectors as is (i.e. fresh or raw) as well as from mixed dishes, as salads (leafy vegetables only), or as juices (berries only), and the percentage of respondents only reporting to consume products from the commodity sectors as is (i.e. fresh or raw) are presented in Figure A4-1. The mean daily amounts consumed for respondents reporting to consume products from the commodity sectors are presented in Figure A4-2.

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[25] The 2011 – 2012 Australian Nutrition and Physical Activity Survey was part of the 2011 – 2013 Australian Health Survey. The results presented are derived from one day of dietary recall data and do not indicate the frequency of consumption. Refer to ABS (2014) Australian health survey: Nutrition first results - Foods and nutrients, 2011-12. Australian Bureau of Statistics, Canberra. https://www.abs.gov.au/ausstats/abs@.nsf/Lookup/4364.0.55.007main+features12011-12.

[26] Not all 'mixed dishes' are cooked or processed. An example of different mixed dishes is provided for berries in Appendix 5.

Leafy vegetable commodity sector consumption

The types of leafy vegetables included in the survey that were eaten as is included; Lettuce, cos, raw; Lettuce, iceberg, raw; Lettuce, mignonette, raw; Lettuce, raw, not further defined; Parsley, continental, raw; Parsley, curly, raw; Parsley, not further defined, raw; Rocket, raw; Mixed leafy greens, for salad recipes, lettuce, spinach & rocket, raw; Silverbeet, fresh or frozen, raw; Spinach (fresh, raw); and Mixed vegetables, fresh or frozen, raw.

The types of leafy vegetables included in the survey that were eaten as salads included; Chinese cabbage (Pe-tsai, Celery cabbage, Pak-tsai); Lettuce, cos, raw; Lettuce, head; Rucola (Arrugula, Rocket salad, Roquette); and Spinach. The list of mixed dishes including leafy vegetables is not provided as it includes dishes that may or may not be cooked or processed.

Respondents that reported eating fresh leafy vegetables as is, as a salad, and from mixed dishes were greater than 50% in each age group ((2-16 year group (51%), 17-69 years (69%), 70+ years (57%)) (Figure A4-1 A). The mean daily amount consumed was similar across the age groups 17-69 years (30g/day), and 70+ years (24g/day)), and lowest for the 2-16 year group (17g/day) (Figure A4-2 A).

The percentage of respondent consuming leafy vegetables as part of a salad were greatest 17-69 years (20%), 70+ years (17%), followed by 2-16 year group (11%). The mean daily amount consumed highest in the 17-69 years (25g/day) followed by 70+ years (19g/day)), and lowest for the 2-16 year group (14g/day) (data not shown).

Leafy vegetables only eaten as is were consumed by the greatest percentage of respondents in the 17-69 years (17.4%) group, followed by 70+ years (15.1%) and 2-16 years (10.1%) (Figure A4-1 B). The mean daily amount consumed was similar across the age groups (2-16 year group (21g/day),17-69 years (29g/day), and 70+ years (28g/day)) (Figure A4-2 B).

The survey results indicate that leafy vegetables were eaten by a large proportion of respondents mainly as mixed dishes (that may or may not have been cooked reducing the likelihood for pathogens to be present at consumption), and a lower proportion ate leafy vegetables as is with little or no further processing. The average amount consumed was generally less than 30g/day.

Figure A4-1. The percentage of respondents reporting to consume products from the commodity sectors as is (i.e. fresh or raw) as well as from mixed dishes, as salads (leafy vegetables only), or as juices (berries only) (**A**) and the percentage of respondents only reporting to consume the products as is (**B**).

Berry commodity sector consumption

The berry types reported in the survey to be consumed as is included; fresh and frozen Raspberry, Blueberry, Strawberry, Blackberry; and fresh Cranberry and Mulberry. Example lists of types of mixed dishes reported to be consumed and include a type of berry are provided in Appendix 5.

The percentage of respondents that reported eating berries as is, as mixed dishes, or as fruit juice were highest for the 2-16 year group (36%), followed by 70+ years (29%), and 17-69 years (26%) (Figure A4-1 A). The mean daily amount consumed was similar across the age groups (2-16 year group (30g/day), 17-69 years (32g/day), and 70+ years (31g/day)) (Figure A4-2 A).

The percentage of respondents that reported eating berries only as mixed dishes was highest for the 2-16 year group (28%), followed by 70+ years (23%), and 17-69 years (20%). The mean daily amount consumed was similar across the age groups ((2-16 year group (4g/day),17-69 years (5g/day), and 70+ years (5g/day)).

The percentage of respondents that reported eating berries only as juice was low for all age groups (2-16 year group (3%), (70+ years (1%), (17-69 years (1%)). The mean daily amount consumed differed slightly across the age groups ((2-16 year group (2g/day),17-69 years (8g/day), and 70+ years (0.3g/day)).

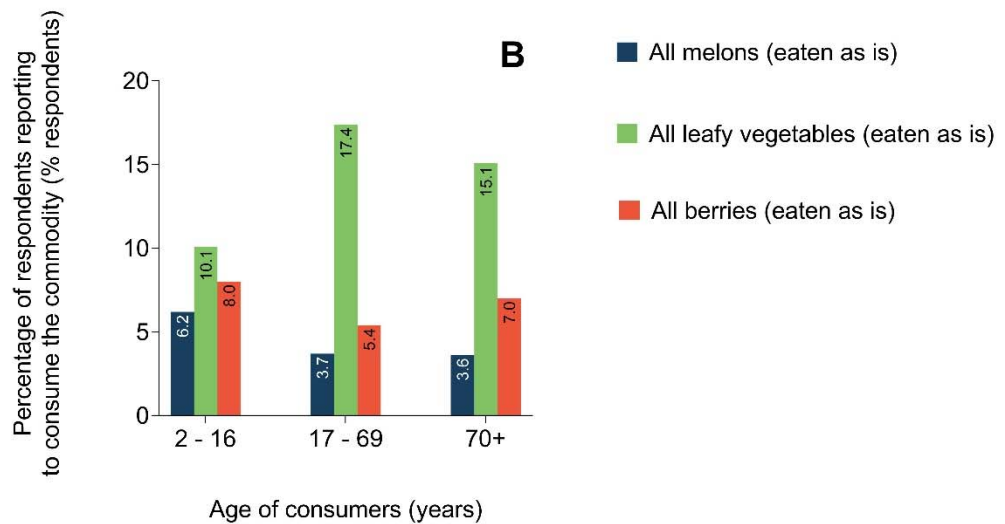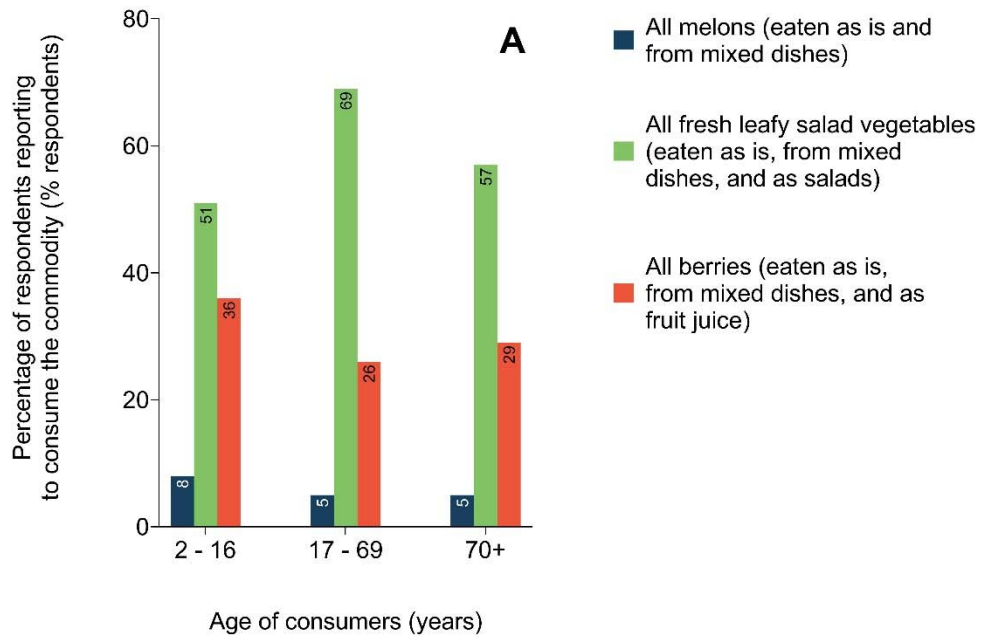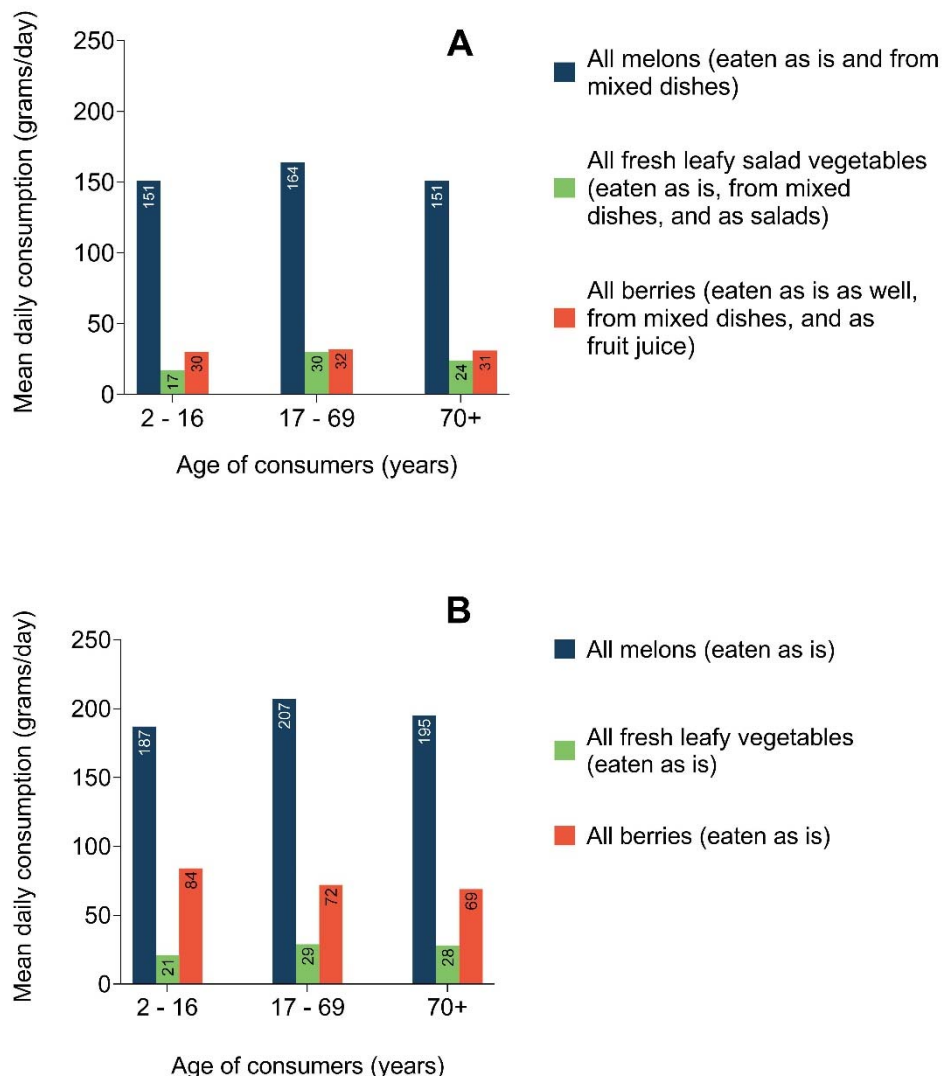Figure A4-2. The mean daily amounts consumed for respondents reporting to consume products from the commodity sectors as is (i.e. fresh or raw) as well as from mixed dishes, as salads (leafy vegetables only), or as juices (berries only) (**A**) and the mean daily amounts consumed for respondents only reporting to consume the products as is (**B**).

Fresh and frozen berries eaten as is (i.e. as a fresh piece of fruit) were consumed by the greatest percentage of respondents in the 2-16 year group (8.0%), followed by 70+ years (7.0%), and 17-69 years (5.4%)(Figure A4-1 B). The mean daily amount consumed differed slightly across the age groups (2-16 year group (84g/day), 17-69 years (72g/day), and 70+ years (69g/day)) (Figure igure A4-2 B).

Berries were consumed by a large proportion of people at lower amounts (approximately 5g/day) as part of a mixed dish that may or may not be cooked, and a lower proportion ate berries as a fresh piece of fruit, generally consuming 72g/day. Very few respondents reported consuming berries as fruit juice.

Melon commodity sector consumption

The four types of melons reported in the survey eaten as is included; honey dew, skin not further defined, peeled raw; rockmelon (cantaloupe), peeled, raw; watermelon, peeled, raw; and melon, peeled, raw, not further defined. The list of mixed dishes including melons is not provided as it includes mixed dishes that may or may not be cooked or processed.

Respondents that reported eating melons as is, as well as where it is consumed as part of a mixed dish were less than 10% for each age group ((2-16 year group (8.0%), followed by 17-69 years (5.4%), and 70+ years (5.1%)) (Figure A4-1 A). The mean daily amount consumed was similar across the age groups (2-16 year group (151g/day),17-69 years (164g/day), and 70+ years (151g/day)) (Figure A4-2 A).

All melons eaten as is were consumed by the largest percentage of respondents in the 2-16 year group (6.2%), followed by 17-69 years (3.7%), and 70+ years (3.6%) (Figure A4-1 B). The mean daily amount consumed was similar across the age groups (2-16 year group (187g/day),17-69 years (207g/day), and 70+ years (195g/day)) (Figure A4-2 B).

Melons were eaten by a similar proportion of people as mixed dishes or as a fresh piece of fruit, and when eaten as is approximately 200g/day was consumed by respondents in all age groups.

Consumption by commodity

To provide an indication of the overall consumption for the individual commodities, the consumption data has been summarised for the leafy vegetable commodities eaten as is, and eaten combined fresh and raw as salads. For berries, data for fresh and frozen commodties eaten as is has been summarised. Melons eaten as is are also summarised However, due to the low number of respondents for some particular commodities, it is not appropriate to use the data to make assumptions of differences in risk between the individual commodities.

The percentage of respondents reporting to consume the specific in scope commodities are presented in Figure A4-3, and the mean daily amount consumed for these commodities is present in Figure A4-4.

Lettuce, spinach, and parsley consumption

Lettuce eaten consumed as is was consumed by a larger percentage of respondents, across all age groups (2-16 (8.5%), 17-69 (14.2%), 70+ years (10.9%)), compared to parsley (2-16 (0.5%), 17-69 (0.4%), 70+ years (1.6%)) and spinach (2-16 (0.3%), 17-69 (1.0%), 70+ years (0.2%)) (Figure A4-3 C). The mean daily amount consumed was similar across the age groups for each commodity with the largest mean consumption in each commodity being 26g/day lettuce (17-69 years), 6g/day parsley (70+ years), and 20g spinach (17-69 years) (Figure A4-4 C). Spinach was reported to be consumed by more respondents in foods described as salads (2-16 (10.4%), 17-69 (19.1%), 70+ years (16.8%)), and at similar mean amounts (2-16 (1g/day), 17-69 (2g/day), 70+ years (1g/day)).

Strawberries, blueberries and raspberry consumption

For berries eaten as is, fresh strawberries were consumed by a higher percentage of respondents (2-16 (6.5%), 17-69 (3.1%), 70+ years (4.1%)) across all age groups compared to frozen strawberries, and fresh or frozen blueberries and raspberries. Reported percentage consumption of all these other commodities was <1.4% in all age groups (Figure A4-3 B). Mean consumption of fresh strawberries was similar across the age groups (2-16 (80g/day), 17-69 (77g/day), 70+ years (83g/day)). However, more variation was reported for the other commodities (Figure A4-4 B).

Watermelon and rockmelon consumption

Watermelon eaten as is was consumed by a larger percentage of respondents compared to rockmelon in the 2-16 year (4.9 vs 1.4%) and 17-69 year (2.7 vs 1.2%) groups, but similar percentages of consumption was reported in the 70+ year group for watermelon (1.9%) and rockmelon (2.1%) (Figure A4-3 A). The mean daily amount consumed was similar across the age groups for both commodities and ranged from 141g/day to 209g/day (Figure A4-4).
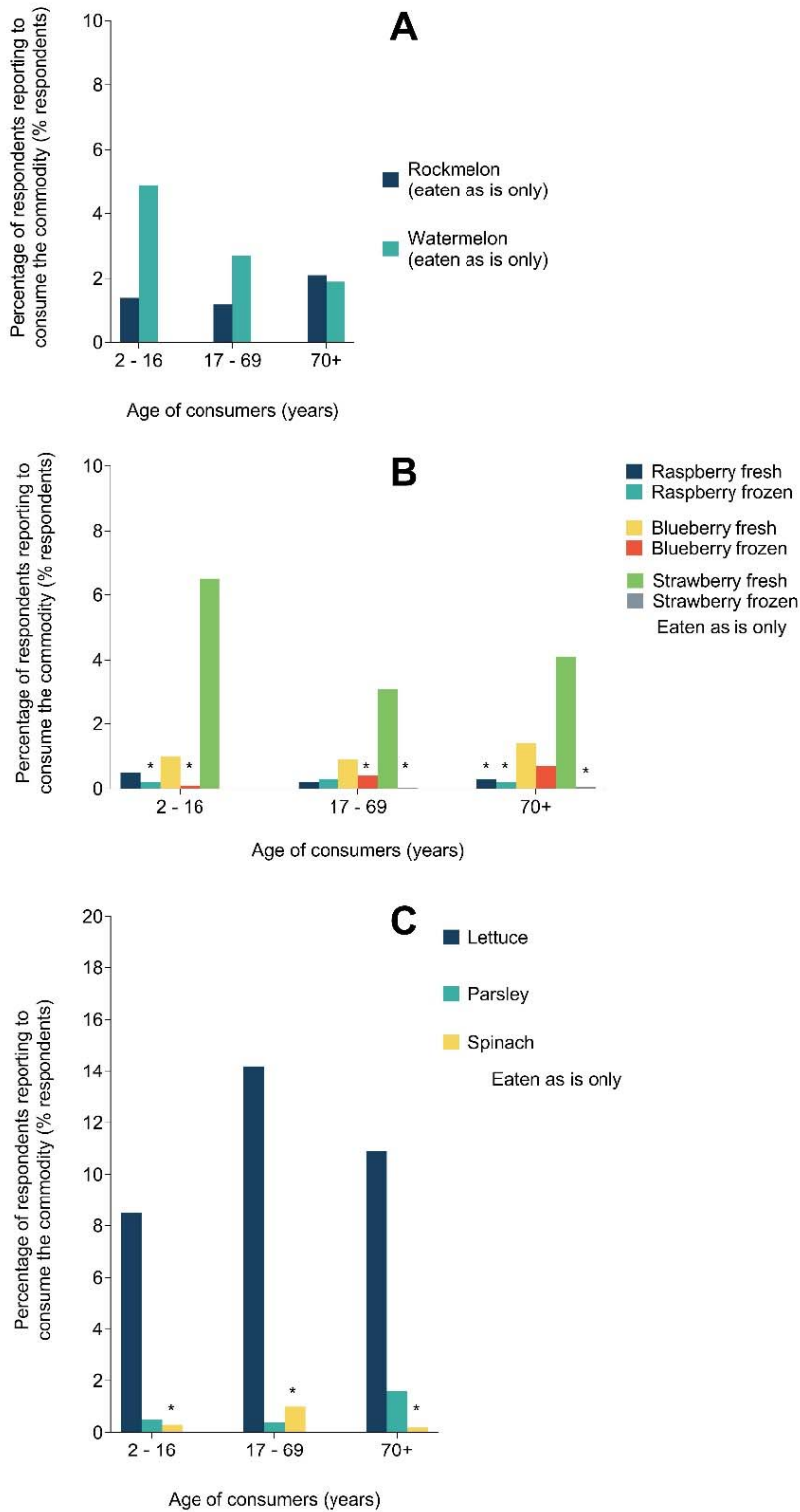
Figure A4-3. The percentage of respondents reporting to consume specific commodities as is (i.e. fresh or raw) for melons (**A**), berries (includes both fresh and frozen) (**B**), and leafy vegetables (**C**).
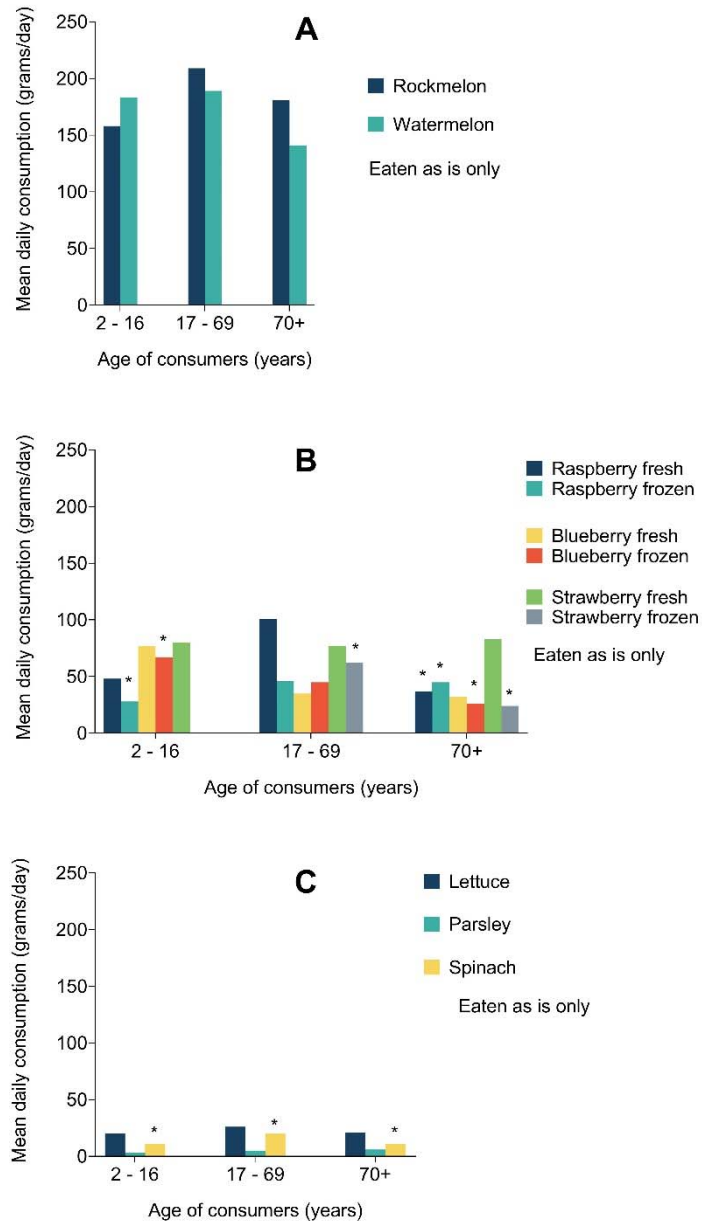
Figure A4-4. The mean daily consumption for respondents reporting to consume specific commodities as is (i.e. fresh or raw) for melons (**A**), berries (includes both fresh and frozen) (**B**), and leafy vegetables (**C**).

Appendix 5 – Example list of 'mixed dishes' reported to be consumed including blueberries, raspberries, and strawberries from the 2011-12 Australian National Nutrition and Physical Activity Survey (NNPAS)[27]

Blueberries
Iced tea, homemade, unsweetened
Juice, apple & forest fruits, commercial
Juice, fruit, commercial, not further defined
Juice, commercial, not further defined
Smoothie, fruit juice based, added berries
Smoothie, fruit juice based, added mixed fruit
Smoothie, fruit juice based, added mixed fruit & nuts or seeds
Juice, apple & berries, home squeezed
Juice, fruit & vegetable blend, apple, berries, beetroot & carrot, commercial
Mineral water, non-citrus flavoured, regular
Bagel, fruit, commercial
Bagel, fruit, commercial, toasted
Bun, sweet, with fruit (other than sultanas), uniced
Bun, sweet, with fruit (other than sultanas), iced
Bun, sweet, with fruit (other than sultanas) & nuts, iced
Bun, sweet, with mock cream & jam, uniced
Bun, sweet, not further defined
Breakfast cereal, whole wheat, small biscuit, with berries, added vitamins B1, B2, B3 & folate, Ca & Fe
Breakfast cereal, whole wheat, small biscuit, with fruit paste, added vitamins B1, B2, B3 & C & Fe
Breakfast cereal, mixed grain (rice & wheat), flakes, berries, added vitamins B1, B2, B3, B6 & folate, Ca, Fe & Zn
Breakfast cereal, mixed grain (wheat & corn), flakes, berries & sultanas, added vitamins A, B1, B2, B3, B6, E & folate, Ca, Fe & Zn
Breakfast cereal, mixed grain (wheat & oat), flakes, berries, added vitamins B1, B2, B3 & folate & Fe
Breakfast cereal, mixed grain (wheat, oat & corn), flakes & clusters, pomegranate & berries, added vitamins A, B1, B2, B3, B6, E & folate, Ca, Fe & Zn
Breakfast cereal, mixed grain (wheat, rice & oat), flakes, dried fruit, added vitamins B1, B2, B3 & E & Fe
Breakfast cereal, mixed grain (wheat, corn, rice & oat), clusters, sultana & berries, added vitamins B1, B2, B3, E & folate, Ca & Fe
Breakfast cereal, mixed grain (wheat, corn, rice & oat), flakes, berries, added vitamins B1, B2, B3, E & folate, Ca & Fe
Breakfast cereal, mixed grain, commercial, not further defined
Breakfast cereal, not further defined
Biscuit, sweet, not further defined
Biscuit, sweet, jam-filled, homemade from basic ingredients, fat not further defined
Biscuit, sweet, sandwich, cream & jam filling, commercial
Biscuit, sweet, cream assorted, commercial, not further defined

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[27] 2011-12 Australian National Nutrition and Physical Activity Survey (NNPAS), a 1 day 24-hour recall survey of 12,153 respondents aged 2 years and above (with 64% of respondents (n=7735) undertaking a second 24-hour recall on a second non-consecutive day). The results in this report are derived using day 1 of data only. A respondent is counted as a consumer if the food was consumed on day 1 only. These data were weighted during the calculations undertaken in Harvest.

Biscuit, sweet, marshmallow filling, chocolate-coated, commercial
Biscuit, sweet, chocolate, commercial, not further defined
Cake or cupcake, sponge, plain, commercial, uniced, filled with jam
Cake or cupcake, sponge, plain, commercial, uniced, filled with jam & cream
Cake or cupcake, sponge, plain, homemade from basic ingredients, uniced, filled with jam &/or cream
Cake or cupcake, sponge, plain, uniced, filled with fruit & cream
Cake or cupcake, sponge, chocolate flavoured, commercial, uniced, filled with jam & cream
Cake, lamington, filled with jam &/or cream
Cake or cupcake, berry, undefined fat, uniced
Cake or cupcake, berry, reduced fat, commercial, uniced
Cake, cupcake or muffin, berry, prepared from dry mix, undefined fat, uniced
Cake, friand, commercial, uniced
Cake, friand, homemade from basic ingredients, uniced
Muffin, cake-style, berry, commercial, uniced
Muffin, cake-style, berry, homemade from basic ingredients, uniced
Muffin, cake-style, commercial, not further defined
Cake or cupcake, berry shortcake, undefined fat
Slice, coconut, with jam, commercial
Slice, coconut, with jam, homemade from basic ingredients, fat not further defined
Slice, meringue, with jam, homemade from basic ingredients, fat not further defined
Slice, not further defined
Pie, sweet, apple & berry, from frozen, baked
Pie, sweet, mixed berry, homemade
Tart, jam, commercial
Tart, jam, homemade
Crepe or pancake, berry, homemade from basic ingredients
Waffle, berry, fresh, homemade from basic ingredients
Doughnut, jam filled, sugar coated
Doughnut, jam filled, with chocolate icing
Mixed berry, cooked
Blueberry, canned in syrup
Fruit salad, apple, banana & berries, homemade
Fruit salad, apple, banana, berries & other fruit, homemade
Fruit salad, apple, berries & orange, homemade
Fruit salad, banana, berries, melon & other fruit, homemade
Fruit salad, banana, berries & stone fruit, homemade
Fruit salad, banana, berries, mango & other fruit, homemade
Fruit salad, berries & pineapple, homemade
Fruit salad, berries, cherries, watermelon or other red fruit, homemade
Fruit salad, berries, mango, stone fruit & other fruit, homemade
Mixed berry, dried
Crumble, apple & berry, baked, homemade
Souffle, fruit
Yoghurt, natural or Greek, high fat (approx. 6%), added berry pieces
Yoghurt, berry pieces or flavoured, regular fat (approx. 3%)
Yoghurt, berry pieces or flavoured, with added fruit juice, reduced fat (approx. 2%)

Yoghurt, natural or Greek, regular fat (approx. 4%), added berry pieces
Yoghurt, berry flavoured, regular fat (approx. 3%), added cereals
Yoghurt, berry pieces or flavoured, reduced fat (1%)
Yoghurt, tropical fruit or fruit salad pieces or flavoured, with added fruit juice, reduced fat (approx. 2%)
Yoghurt, vanilla flavoured, reduced fat (2%), added berry pieces
Yoghurt, berry pieces or flavoured, low fat (<0.5%)
Yoghurt, berry pieces or flavoured, low fat (<0.5%), intense sweetened
Yoghurt, drinking style, fruit flavoured, reduced fat (1%)
Yoghurt, berry pieces or flavoured, regular fat (approx. 3%), added omega-3 polyunsaturates
Yoghurt, berry pieces or flavoured, reduced fat (1%), added fibre
Yoghurt, berry pieces or flavoured, low fat (<0.5%), intense sweetened, added fibre
Yoghurt, dessert flavoured, low fat (<0.5%), intense sweetened, added fibre
Yoghurt, not further defined
Ice cream, all flavours, homemade from basic ingredients
Yoghurt, frozen, berry flavoured, regular fat
Cake, cheesecake, biscuit base, fruit flavoured cream cheese topping, homemade from basic ingredients
Trifle, homemade from basic ingredients
Smoothie, cows milk, all flavours, added berries
Smoothie, cows milk, all flavours, added mixed fruit
Smoothie, cows milk, all flavours, added mixed fruit & egg
Smoothie, cows milk, all flavours, added mixed fruit, guarana & herbal extracts
Smoothie, cows milk, all flavours, added mixed fruit & nuts or seeds
Smoothie, non-dairy base, all flavours, added mixed fruit
Yoghurt, soy based, berry flavoured, regular fat (approx. 3%)
Jam, mixed berry, regular
Jam, not further defined
Jelly, sugar sweetened, all flavours, prepared, added berries
Bar, muesli or snack, plain or with 10% dried fruit
Bar, muesli or snack, plain or with 10% dried fruit, added vitamins B1, B2, B3, folate & Fe
Bar, muesli or snack, plain or with 10% dried fruit, high fibre, added vitamins B1, B2, B3, B6, E, & folate, Fe & Zn
Bar, muesli or snack, with 10% dried fruit & 5% nuts
Bar, muesli or snack, with 10% dried fruit & 10% nuts
Bar, muesli or snack, with 10% dried fruit & 45% nuts, chocolate-coated
Bar, muesli or snack, with 10% dried fruit & 60% nuts
Bar, muesli or snack, with 10% dried fruit & 60% nuts, yoghurt-coated
Bar, muesli or snack, with 15% dried fruit & 25% nuts, added vitamins B1, B2, B3, C & folate, Fe, & Zn
Bar, muesli or snack, with 20% dried fruit & 5% nuts
Bar, muesli or snack, with 20% dried fruit & 20% nuts, chocolate base
Bar, muesli or snack, with 30% dried fruit & 30% nuts
Bar, muesli or snack, gluten free, with 20% dried fruit & 20% seeds
Bar, muesli or snack, made from breakfast cereal with dried fruit
Bar, muesli or snack, not further defined
Bar, muesli or snack, plain or with 10% dried fruit, chocolate-coated
Bar, muesli or snack, plain or with 10% dried fruit, yoghurt-coated

Bar, muesli or snack, plain or with 10% dried fruit, yoghurt-coated, added vitamins B1, B2, B3, folate & Fe
Bar, muesli or snack, with 15% dried fruit & 15% nuts, yoghurt-coated
Bar, muesli or snack, with 30% dried fruit, yoghurt-coated, added vitamins B1, B2, B3, folate & Fe
Lolly, fruit flavoured, chewy
Wine cooler, wine & fruit juice blend, all flavours
Raspberries, Red, Black
Iced tea, homemade, unsweetened
Juice, apple & forest fruits, commercial
Juice, fruit, commercial, not further defined
Juice, commercial, not further defined
Smoothie, fruit juice based, added berries
Smoothie, fruit juice based, added mixed fruit
Smoothie, fruit juice based, added mixed fruit & nuts or seeds
Juice, apple & berries, home squeezed
Juice, fruit & vegetable blend, apple, berries, beetroot & carrot, commercial
Cordial, apple & berry, regular, stronger than recommended dilution
Cordial, apple & berry, regular, recommended dilution
Cordial, apple & berry, regular, weaker than recommended dilution
Cordial, apple & berry, intense sweetened or diet, recommended dilution
Cordial base, apple & berry, regular
Cordial base, apple & berry, intense sweetened or diet
Mineral water, non-citrus flavoured, regular
Bun, sweet, with fruit (other than sultanas), uniced
Bun, sweet, with fruit (other than sultanas), iced
Bun, sweet, with fruit (other than sultanas) & nuts, iced
Bun, sweet, with mock cream & jam, uniced
Bun, sweet, not further defined
Breakfast cereal, whole wheat, small biscuit, with berries, added vitamins B1, B2, B3 & folate, Ca & Fe
Breakfast cereal, whole wheat, small biscuit, with fruit paste, added vitamins B1, B2, B3 & C & Fe
Breakfast cereal, mixed grain (rice & wheat), flakes, berries, added vitamins B1, B2, B3, B6 & folate, Ca, Fe & Zn
Breakfast cereal, mixed grain (wheat & corn), flakes, berries & sultanas, added vitamins A, B1, B2, B3, B6, E & folate, Ca, Fe & Zn
Breakfast cereal, mixed grain (wheat & oat), flakes, berries, added vitamins B1, B2, B3 & folate & Fe
Breakfast cereal, mixed grain (wheat, oat & corn), flakes & clusters, pomegranate & berries, added vitamins A, B1, B2, B3, B6, E & folate, Ca, Fe & Zn
Breakfast cereal, mixed grain (wheat, rice & oat), flakes, dried fruit, added vitamins B1, B2, B3 & E & Fe
Breakfast cereal, mixed grain (wheat, corn, rice & oat), clusters, sultana & berries, added vitamins B1, B2, B3, E & folate, Ca & Fe
Breakfast cereal, mixed grain (wheat, corn, rice & oat), flakes, berries, added vitamins B1, B2, B3, E & folate, Ca & Fe
Breakfast cereal, mixed grain, commercial, not further defined
Breakfast cereal, not further defined
Biscuit, sweet, not further defined
Biscuit, sweet, jam-filled, commercial
Biscuit, sweet, jam-filled, reduced fat, commercial

Biscuit, sweet, jam-filled, homemade from basic ingredients, fat not further defined
Biscuit, sweet, marshmallow filling, commercial
Biscuit, sweet, sandwich, cream & jam filling, commercial
Biscuit, sweet, cream assorted, commercial, not further defined
Biscuit, sweet, marshmallow filling, chocolate-coated, commercial
Biscuit, sweet, chocolate, commercial, not further defined
Cake or cupcake, sponge, plain, commercial, uniced, filled with jam
Cake or cupcake, sponge, plain, commercial, uniced, filled with jam & cream
Cake or cupcake, sponge, plain, homemade from basic ingredients, uniced, filled with jam &/or cream
Cake or cupcake, sponge, plain, uniced, filled with fruit & cream
Cake or cupcake, sponge, chocolate flavoured, commercial, uniced, filled with jam & cream
Cake, lamington, filled with jam &/or cream
Cake or cupcake, berry, undefined fat, uniced
Cake or cupcake, berry, reduced fat, commercial, uniced
Cake, cupcake or muffin, berry, prepared from dry mix, undefined fat, uniced
Cake, friand, commercial, uniced
Cake, friand, homemade from basic ingredients, uniced
Muffin, cake-style, berry, commercial, uniced
Muffin, cake-style, berry, homemade from basic ingredients, uniced
Muffin, cake-style, raspberry & white chocolate, commercial, uniced
Muffin, cake-style, raspberry & white chocolate, homemade from basic ingredients, uniced
Muffin, cake-style, commercial, not further defined
Cake or cupcake, berry shortcake, undefined fat
Slice, coconut, with jam, commercial
Slice, coconut, with jam, homemade from basic ingredients, fat not further defined
Slice, meringue, with jam, homemade from basic ingredients, fat not further defined
Slice, not further defined
Pie, sweet, mixed berry, homemade
Tart, jam, commercial
Tart, jam, homemade
Crepe or pancake, berry, homemade from basic ingredients
Waffle, berry, fresh, homemade from basic ingredients
Doughnut, jam filled, sugar coated
Doughnut, jam filled, with chocolate icing
Mixed berry, cooked
Raspberry, canned in syrup
Fruit salad, apple, banana & berries, homemade
Fruit salad, apple, banana, berries & other fruit, homemade
Fruit salad, apple, berries & orange, homemade
Fruit salad, banana, berries, melon & other fruit, homemade
Fruit salad, banana, berries & stone fruit, homemade
Fruit salad, banana, berries, mango & other fruit, homemade
Fruit salad, berries & pineapple, homemade
Fruit salad, berries, cherries, watermelon or other red fruit, homemade
Fruit salad, berries, mango, stone fruit & other fruit, homemade
Mixed berry, dried

Crumble, apple & berry, baked, homemade
Souffle, fruit
Yoghurt, natural or Greek, high fat (approx. 6%), added berry pieces
Yoghurt, berry pieces or flavoured, regular fat (approx. 3%)
Yoghurt, berry pieces or flavoured, with added fruit juice, reduced fat (approx. 2%)
Yoghurt, natural or Greek, regular fat (approx. 4%), added berry pieces
Yoghurt, berry flavoured, regular fat (approx. 3%), added cereals
Yoghurt, berry pieces or flavoured, reduced fat (1%)
Yoghurt, tropical fruit or fruit salad pieces or flavoured, with added fruit juice, reduced fat (approx. 2%)
Yoghurt, vanilla flavoured, reduced fat (2%), added berry pieces
Yoghurt, berry pieces or flavoured, low fat (<0.5%)
Yoghurt, berry pieces or flavoured, low fat (<0.5%), intense sweetened
Yoghurt, drinking style, fruit flavoured, reduced fat (1%)
Yoghurt, berry pieces or flavoured, regular fat (approx. 3%), added omega-3 polyunsaturates
Yoghurt, berry pieces or flavoured, reduced fat (1%), added fibre
Yoghurt, berry pieces or flavoured, low fat (<0.5%), intense sweetened, added fibre
Yoghurt, dessert flavoured, low fat (<0.5%), intense sweetened, added fibre
Yoghurt, not further defined
Ice cream, all flavours, homemade from basic ingredients
Yoghurt, frozen, berry flavoured, regular fat
Cake, cheesecake, biscuit base, fruit flavoured cream cheese topping, homemade from basic ingredients
Trifle, homemade from basic ingredients
Smoothie, cows milk, all flavours, added berries
Smoothie, cows milk, all flavours, added mixed fruit
Smoothie, cows milk, all flavours, added mixed fruit & egg
Smoothie, cows milk, all flavours, added mixed fruit, guarana & herbal extracts
Smoothie, cows milk, all flavours, added mixed fruit & nuts or seeds
Smoothie, non-dairy base, all flavours, added mixed fruit
Yoghurt, soy based, berry flavoured, regular fat (approx. 3%)
Jam, mixed berry, regular
Jam, raspberry, regular
Jam, not further defined
Sauce, sweet, mixed berry coulis
Jelly, sugar sweetened, all flavours, prepared, added berries
Bar, muesli or snack, plain or with 10% dried fruit
Bar, muesli or snack, plain or with 10% dried fruit, added vitamins B1, B2, B3, folate & Fe
Bar, muesli or snack, plain or with 10% dried fruit, high fibre, added vitamins B1, B2, B3, B6, E, & folate, Fe & Zn
Bar, muesli or snack, with 10% dried fruit & 5% nuts
Bar, muesli or snack, with 10% dried fruit & 10% nuts
Bar, muesli or snack, with 10% dried fruit & 45% nuts, chocolate-coated
Bar, muesli or snack, with 10% dried fruit & 60% nuts
Bar, muesli or snack, with 10% dried fruit & 60% nuts, yoghurt-coated
Bar, muesli or snack, with 15% dried fruit & 25% nuts, added vitamins B1, B2, B3, C & folate, Fe, & Zn
Bar, muesli or snack, with 20% dried fruit & 5% nuts
Bar, muesli or snack, with 20% dried fruit & 20% nuts, chocolate base

Bar, muesli or snack, with 30% dried fruit & 30% nuts
Bar, muesli or snack, gluten free, with 20% dried fruit & 20% seeds
Bar, muesli or snack, made from breakfast cereal with dried fruit
Bar, muesli or snack, not further defined
Bar, muesli or snack, plain or with 10% dried fruit, chocolate-coated
Bar, muesli or snack, plain or with 10% dried fruit, yoghurt-coated
Bar, muesli or snack, plain or with 10% dried fruit, yoghurt-coated, added vitamins B1, B2, B3, folate & Fe
Bar, muesli or snack, with 15% dried fruit & 15% nuts, yoghurt-coated
Bar, muesli or snack, with 30% dried fruit, yoghurt-coated, added vitamins B1, B2, B3, folate & Fe
Lolly, fruit flavoured, chewy
Wine cooler, wine & fruit juice blend, all flavours
Protein drink, whey based, protein >70%, fortified, prepared with juice
Strawberry
Iced tea, homemade, unsweetened
Juice, apple & forest fruits, commercial
Juice, fruit, commercial, not further defined
Juice, commercial, not further defined
Smoothie, fruit juice based, added berries
Smoothie, fruit juice based, added mixed fruit
Smoothie, fruit juice based, added mixed fruit & nuts or seeds
Juice, apple & berries, home squeezed
Juice, fruit & vegetable blend, apple, berries, beetroot & carrot, commercial
Mineral water, non-citrus flavoured, regular
Bun, sweet, with fruit (other than sultanas), uniced
Bun, sweet, with fruit (other than sultanas), iced
Bun, sweet, with fruit (other than sultanas) & nuts, iced
Bun, sweet, with mock cream & jam, uniced
Bun, sweet, not further defined
Breakfast cereal, whole wheat, small biscuit, with berries, added vitamins B1, B2, B3 & folate, Ca & Fe
Breakfast cereal, whole wheat, small biscuit, with fruit paste, added vitamins B1, B2, B3 & C & Fe
Breakfast cereal, mixed grain (rice & wheat), flakes, berries, added vitamins B1, B2, B3, B6 & folate, Ca, Fe & Zn
Breakfast cereal, mixed grain (wheat & corn), flakes, berries & sultanas, added vitamins A, B1, B2, B3, B6, E & folate, Ca, Fe & Zn
Breakfast cereal, mixed grain (wheat & oat), flakes, berries, added vitamins B1, B2, B3 & folate & Fe
Breakfast cereal, mixed grain (wheat, oat & corn), flakes & clusters, pomegranate & berries, added vitamins A, B1, B2, B3, B6, E & folate, Ca, Fe & Zn
Breakfast cereal, mixed grain (wheat, rice & oat), flakes, dried fruit, added vitamins B1, B2, B3 & E & Fe
Breakfast cereal, mixed grain (wheat, corn, rice & oat), clusters, sultana & berries, added vitamins B1, B2, B3, E & folate, Ca & Fe
Breakfast cereal, mixed grain (wheat, corn, rice & oat), flakes, berries, added vitamins B1, B2, B3, E & folate, Ca & Fe
Breakfast cereal, mixed grain, commercial, not further defined
Breakfast cereal, not further defined
Biscuit, sweet, not further defined
Biscuit, sweet, jam-filled, homemade from basic ingredients, fat not further defined

Biscuit, sweet, sandwich, cream & jam filling, commercial
Biscuit, sweet, cream assorted, commercial, not further defined
Biscuit, sweet, marshmallow filling, chocolate-coated, commercial
Biscuit, sweet, chocolate, commercial, not further defined
Cake or cupcake, sponge, plain, commercial, uniced, filled with jam
Cake or cupcake, sponge, plain, commercial, uniced, filled with jam & cream
Cake or cupcake, sponge, plain, homemade from basic ingredients, uniced, filled with jam &/or cream
Cake or cupcake, sponge, plain, uniced, filled with fruit & cream
Cake or cupcake, sponge, chocolate flavoured, commercial, uniced, filled with jam & cream
Cake, lamington, filled with jam &/or cream
Cake or cupcake, berry, undefined fat, uniced
Cake or cupcake, berry, reduced fat, commercial, uniced
Cake, cupcake or muffin, berry, prepared from dry mix, undefined fat, uniced
Cake, friand, commercial, uniced
Cake, friand, homemade from basic ingredients, uniced
Muffin, cake-style, berry, commercial, uniced
Muffin, cake-style, berry, homemade from basic ingredients, uniced
Muffin, cake-style, commercial, not further defined
Cake or cupcake, berry shortcake, undefined fat
Slice, coconut, with jam, commercial
Slice, coconut, with jam, homemade from basic ingredients, fat not further defined
Slice, meringue, with jam, homemade from basic ingredients, fat not further defined
Slice, not further defined
Pie, sweet, mixed berry, homemade
Tart, jam, commercial
Tart, jam, homemade
Crepe or pancake, berry, homemade from basic ingredients
Waffle, berry, fresh, homemade from basic ingredients
Doughnut, jam filled, sugar coated
Doughnut, jam filled, with chocolate icing
Mixed berry, cooked
Fruit salad, apple, banana & berries, homemade
Fruit salad, apple, banana, berries & other fruit, homemade
Fruit salad, apple, berries & orange, homemade
Fruit salad, banana, berries, melon & other fruit, homemade
Fruit salad, banana, berries & stone fruit, homemade
Fruit salad, banana, berries, mango & other fruit, homemade
Fruit salad, berries & pineapple, homemade
Fruit salad, berries, cherries, watermelon or other red fruit, homemade
Fruit salad, berries, mango, stone fruit & other fruit, homemade
Fruit salad, commercial, fresh
Mixed fruit platter, not further defined
Mixed berry, dried
Crumble, apple & berry, baked, homemade
Souffle, fruit
Yoghurt, natural or Greek, high fat (approx. 6%), added berry pieces

Yoghurt, berry pieces or flavoured, regular fat (approx. 3%)
Yoghurt, berry pieces or flavoured, with added fruit juice, reduced fat (approx. 2%)
Yoghurt, natural or Greek, regular fat (approx. 4%), added berry pieces
Yoghurt, berry flavoured, regular fat (approx. 3%), added cereals
Yoghurt, berry pieces or flavoured, reduced fat (1%)
Yoghurt, tropical fruit or fruit salad pieces or flavoured, with added fruit juice, reduced fat (approx. 2%)
Yoghurt, vanilla flavoured, reduced fat (2%), added berry pieces
Yoghurt, berry pieces or flavoured, low fat (<0.5%)
Yoghurt, berry pieces or flavoured, low fat (<0.5%), intense sweetened
Yoghurt, drinking style, fruit flavoured, reduced fat (1%)
Yoghurt, berry pieces or flavoured, regular fat (approx. 3%), added omega-3 polyunsaturates
Yoghurt, berry pieces or flavoured, reduced fat (1%), added fibre
Yoghurt, berry pieces or flavoured, low fat (<0.5%), intense sweetened, added fibre
Yoghurt, dessert flavoured, low fat (<0.5%), intense sweetened, added fibre
Yoghurt, not further defined
Ice cream, all flavours, homemade from basic ingredients
Yoghurt, frozen, berry flavoured, regular fat
Sundae, vanilla ice cream, strawberry topping, fast food style
Fromais frais, berry pieces or flavour, regular fat (5% fat)
Cake, cheesecake, biscuit base, fruit flavoured cream cheese topping, commercial
Cake, cheesecake, biscuit base, fruit flavoured cream cheese topping, homemade from basic ingredients
Trifle, homemade from basic ingredients
Milk, cow, fluid, flavoured, strawberry, regular fat
Milk, cow, fluid, flavoured, strawberry, reduced fat
Thickshake, strawberry flavour, fast food style
Smoothie, cows milk, all flavours, added berries
Smoothie, cows milk, all flavours, added mixed fruit
Smoothie, cows milk, all flavours, added mixed fruit & egg
Smoothie, cows milk, all flavours, added mixed fruit, guarana & herbal extracts
Smoothie, cows milk, all flavours, added mixed fruit & nuts or seeds
Smoothie, non-dairy base, all flavours, added mixed fruit
Yoghurt, soy based, berry flavoured, regular fat (approx. 3%)
Jam, mixed berry, regular
Jam, strawberry, regular
Jam, not further defined
Jam, all flavours, no added sugar (100% fruit)
Sauce, sweet, mixed berry coulis
Jelly, sugar sweetened, all flavours, prepared, added berries
Pavlova, plain, topped with whipped cream & fresh fruit
Bar, muesli or snack, plain or with 10% dried fruit
Bar, muesli or snack, plain or with 10% dried fruit, added vitamins B1, B2, B3, folate & Fe
Bar, muesli or snack, plain or with 10% dried fruit, high fibre, added vitamins B1, B2, B3, B6, E, & folate, Fe & Zn
Bar, muesli or snack, with 10% dried fruit & 5% nuts
Bar, muesli or snack, with 10% dried fruit & 10% nuts
Bar, muesli or snack, with 10% dried fruit & 45% nuts, chocolate-coated

Bar, muesli or snack, with 10% dried fruit & 60% nuts
Bar, muesli or snack, with 10% dried fruit & 60% nuts, yoghurt-coated
Bar, muesli or snack, with 15% dried fruit & 25% nuts, added vitamins B1, B2, B3, C & folate, Fe, & Zn
Bar, muesli or snack, with 20% dried fruit & 5% nuts
Bar, muesli or snack, with 20% dried fruit & 20% nuts, chocolate base
Bar, muesli or snack, with 30% dried fruit & 30% nuts
Bar, muesli or snack, gluten free, with 20% dried fruit & 20% seeds
Bar, muesli or snack, made from breakfast cereal with dried fruit
Bar, muesli or snack, not further defined
Bar, muesli or snack, plain or with 10% dried fruit, chocolate-coated
Bar, muesli or snack, plain or with 10% dried fruit, yoghurt-coated
Bar, muesli or snack, plain or with 10% dried fruit, yoghurt-coated, added vitamins B1, B2, B3, folate & Fe
Bar, muesli or snack, with 15% dried fruit & 15% nuts, yoghurt-coated
Bar, muesli or snack, with 30% dried fruit, yoghurt-coated, added vitamins B1, B2, B3, folate & Fe
Lolly, fruit flavoured, chewy
Wine cooler, wine & fruit juice blend, all flavours
Protein drink, whey based, protein >70%, fortified, prepared with juice

Appendix 6 – Hepatitis A virus[28]

Hepatitis A virus

Hepatitis A virus (HAV) infects the liver, with disease characterised by liver inflammation and the development of jaundice. HAV infection can be asymptomatic (no clinical symptoms), mild, or lead to severe illness in those with underlying liver disease. Hepatitis A is endemic in many developing countries, while in developed countries sporadic cases occur.

Description of the organism

HAV belongs to the Picornaviridae family of viruses and the genus *Hepatovirus*. The *Picornaviridae* family consists of small (25–28 nm) non-enveloped viruses which are generally more robust and survive better in the environment compared to enveloped viruses, such as herpes simplex virus. HAV particles consist of a single strand of RNA contained within an icosahedral shaped protein shell (Schoub 2003; Cook and Rzezutka 2006; Rozenberg et al. 2011).

HAV has one known serotype and six genotypes (I–VI). Genotypes I–III have been associated with human illness, while genotypes IV–VI are found in Old World monkeys. Genotypes I–III are further divided into A and B. The majority of human strains of HAV belong to genotype I or III (Hollinger and Emerson 2007; FDA 2012). Isolates from a particular HAV outbreak are usually of the same genotype (Normann et al. 2008).

Growth and survival characteristics

HAV requires specific living cells (host cells) in order to replicate. This means that the level of HAV in contaminated food will not increase during processing, transport or storage (Koopmans and Duizer 2004). While not able to replicate outside the host, HAV has been shown to survive in the environment for extended periods of time (Schoub 2003; Cook and Rzezutka 2006). The survival of HAV is influenced by environmental factors such as temperature, pH, chemicals and food composition.

It has been demonstrated that under conditions simulating typical environmental exposure, HAV remains infectious after being dried and stored for 30 days (McCaustland et al. 1982). HAV has also been shown to survive on various non-porous surfaces such as aluminium, china and latex for 60 days, however, it does not survive as well on porous materials (Abad et al. 1994). A study by Mbithi et al. (1992) demonstrated that HAV survives and remains infectious on human hands after 4 hours and can be transferred between hands and inanimate surfaces.

HAV has been shown to survive in fresh river water, seawater, groundwater and untreated tap water (Enriquez et al. 1995; Rzezutka and Cook 2004; Cook and Rzezutka 2006). However, without a standard protocol to determine virus survival it is difficult to compare the survival time of the virus in different environments. An investigation by Arnal et al. (1998) using artificial sterile seawater contaminated with HAV demonstrated that the genetic material of HAV was stable and remained in the water for 232 days, although no infectious HAV particles were detected by 35 days. In general, survival of HAV in water is enhanced at low temperatures (<4°C) (Rzezutka and Cook 2004).

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[28] From FSANZ's *Agents of Foodborne Illness* publication, available at:
https://www.foodstandards.gov.au/publications/Pages/agents-foodborne-illness.aspx

Croci et al. (2002) demonstrated that when fresh produce was stored at 4°C, HAV survived and remained infective on: carrots for 4 days, fennel for 7 days and on lettuce for the study duration of 9 days. The differing survival rates observed on fresh produce may be due to the difference in surface texture of the produce and the presence of anti-viral substances. Shieh et al. (2009) showed that when spinach was stored at 5.4°C a 1 $\log_{10}$ reduction in the level of HAV occurred over a 28.6 day period. These studies imply that HAV can persist under normal domestic storage conditions for extended periods of time.

Chemical and physical factors can affect the heat resistance of HAV. Deboose et al. (2004) investigated the inactivation of HAV in strawberry puree and found that increasing the sucrose concentration resulted in increased heat resistance of HAV. Conversely, lowering the pH was found to decrease the heat resistance of HAV. Changing the calcium concentration had no effect. Bidawid et al. (2000b) demonstrated that increases in fat content also increased the heat resistance of HAV. Dairy products with higher fat content required longer times of exposure to heat than lower fat products to achieve the same level of HAV reduction.

HAV has been found to be resistant to temperatures up to 60°C. The temperature at which 50% of HAV particles disintegrate and release their viral RNA is 61°C (10 minutes). When stabilised by 1 mol/L $MgCl_2$, 50% disintegration of HAV occurs at 81°C (Hollinger and Emerson 2007). In food, complete inactivation of HAV has been observed in shellfish when heated to 85°C for 3 minutes or 95°C for 2 minutes (Millard et al. 1987). These conditions are known to inactivate HAV in shellfish while maintaining a commercially acceptable product (Appleton 2000). For milk and cream, heating to 85°C for 30 seconds is sufficient to cause a 5 $\log_{10}$ reduction in HAV titre (Bidawid et al. 2000b).

Low temperature has little effect on HAV survival. Butot et al. (2008) showed that frozen storage of HAV contaminated berries and herbs had little effect on HAV survival over the study period of 3 months.

HAV is highly resistant to acidic conditions and solvents. Scholz et al. (1989) demonstrated that at pH 1 (24°C) HAV retained high infectivity after 2 hours and was still infectious after 5 hours. Under conditions that simulate the acidity of the human stomach (38°C, pH 1) HAV remained infectious for 90 minutes. Also, being a non-enveloped virus, HAV is resistant to solvents such as 20% ether and chloroform (ether destroys the envelop of some viruses) (Hollinger and Emerson 2007).

Symptoms of disease

HAV infection often causes mild illness in humans, or results in no clinical disease at all. In children this is particularly common, with most children under 6 years of age showing no symptoms (asymptomatic infection) (FDA 2012). For those individuals in which clinical disease occurs, initial symptoms include sudden onset of fever, nausea, anorexia, malaise, vomiting, diarrhoea, abdominal pain, myalgia (muscular pain) and headache. The initial symptoms tend to abate with the onset of jaundice (yellowing of the skin and eyes and a browning of urine due to stimulation of bile pigment production) and pale clay coloured stools. Children with symptomatic infection usually develop flu-like symptoms without jaundice (Brundage and Fitzpatrick 2006; Hollinger and Emerson 2007; FDA 2012).

Most patients show complete recovery from symptoms within 3–6 months of the onset of illness. The fatality rate for HAV is approximately 2.4%, with death more likely to occur in the elderly. Acute liver failure due to severe HAV infection has been reported in children; however, it is more frequent in middle-aged and older people and those with underlying chronic liver disease. Acute liver failure is also a rare complication of HAV infection during pregnancy (Koff 1998; FDA 2012).

The incubation period before onset of disease is 15–50 days (mean time of 30 days) (FDA 2012). HAV is shed in the faeces of infected individuals for up to 2 weeks before the onset of illness. HAV is present in the blood at the same time as viral shedding starts occurring. The virus disappears from the blood shortly after symptoms of disease start, while faecal shedding of the virus continues for another 2 weeks (Cook and Rzezutka 2006; Hollinger and Emerson 2007).

In 3–20% of cases relapses occur, generally with milder symptoms and HAV being shed in the faeces. Multiple relapses can occur (Hollinger and Emerson 2007).

Virulence and infectivity

The target organ of HAV is the liver. HAV is initially ingested, infects the intestinal tract and is then transported to the liver via the bloodstream. In the liver, HAV attaches to receptors on the surface of the hepatocytes, enters these cells and replicates. Replication of HAV within the hepatocytes is not believed to result in immediate cell damage; this is thought to occur subsequent to replication and release of the virus. The host's immune response is responsible for destroying the HAV infected cells. As a consequence of this pathological damage the liver becomes inflamed (WHO 2000; Schoub 2003; Cook and Rzezutka 2006). Released viral particles enter the bile duct and pass into the gastrointestinal tract to be shed in the faeces (Cook and Rzezutka 2006). The resistance of HAV to inactivation by bile and intestinal proteolytic enzymes allows the virus to be shed in the faeces and facilitates faecal-oral transmission (Koff 1998).

Mode of transmission

HAV is transmitted via the faecal-oral route by either person-to-person contact or consumption of contaminated food or water (Guillois-Becel et al. 2009). Person-to-person transmission can involve young children with unrecognised HAV infection (asymptomatic infection) (Brundage and Fitzpatrick 2006).

In contrast to person-to-person transmission, outbreaks of HAV infections usually result from faecal contamination of a single source of food or water. Foods may become contaminated in their growing areas (e.g. shellfish), or during irrigation (e.g. crops), usually by coming into contact with sewage polluted water. Food can also be contaminated by infected food handlers. Infected food handlers may contaminate foods directly or contaminate surfaces on which foods are prepared. A major issue with infected food handlers is that they are often unaware they constitute a hazard, as most of the faecal shedding of HAV occurs prior to the onset of clinical symptoms (Cook and Rzezutka 2006; Hollinger and Emerson 2007). Food establishments with poor sanitary conditions and inadequate treatment and/or disposal of human waste (sewage), along with unsatisfactory manufacturing practices may also contribute to food contamination (Sattar et al. 2000).

Travel to areas in which HAV is endemic from low prevalence areas is known to be a risk factor for HAV infection. The likelihood of becoming infected with HAV depends on local hygienic and sanitary conditions, which vary from country to country (Koff 1998). In 2010, 55.1% of HAV cases reported in Australia were acquired overseas (OzFoodNet 2012).

HAV transmission through blood and blood products is rare. While HAV is present in the blood of infected individuals, this is only for approximately a 2 week period. However, post-transfusion HAV infection has occurred, as have outbreaks of HAV in haemophiliacs who received contaminated blood plasma-derived factor VIII concentrate (Mannucci et al. 1994; Hollinger and Emerson 2007).

Incidence of illness and outbreak data

HAV has a worldwide distribution; however, the prevalence of infection is related to the quality of the water supply, level of sanitation and the age of the individual when infected. In most developing countries, where HAV infection is endemic, the majority of people are infected in early childhood and virtually all adults are immune. In developed countries, HAV infections are less common due to improved sanitation. As a result very few people are infected in early childhood and the majority of adults remain susceptible to infection. Hence in developed countries the risk of epidemics and the occurrence of severe disease may increase as the majority of people infected during an outbreak would be adults (children are often asymptomatic) (Conaty et al. 2000; Issa and Mourad 2001; Koopmans and Duizer 2004).

Hepatitis A is a notifiable disease in all Australian states and territories. The incidence of HAV infection notified in Australia in 2012 was 0.7 cases per 100,000 population (164 cases). This was a decrease from the previous 5 year mean of 1.3 cases per 100,000 population per year (ranging from 0.6–2.6 cases per 100,000 population per year) (NNDSS 2013).

In north Queensland in 1996–1999 the average annual HAV notification rates in Indigenous and non-Indigenous people were 110 and 25 cases per 100,000 population, respectively. In 1999 a HAV vaccination program for Indigenous children in north Queensland was introduced. Consequently, in 2000–2003 the average annual HAV notification rates for Indigenous and non-Indigenous people were 4 and 2.5 cases per 100,000 population, respectively (Hanna et al. 2004). HAV is now included as part of the National Immunisation Program Schedule for Aboriginal and Torres Strait Islander children younger than five years of age living in Queensland, the Northern Territory, Western Australia and South Australia (DOHA 2011). HAV vaccination is also recommended for travellers to endemic areas and those at increased risk because of lifestyle or occupation (DOHA 2008).

The notification rate for HAV in New Zealand in 2011 was 0.6 cases per 100,000 population (26 cases), which was a decrease from the 2010 rate of 1.1 cases per 100,000 population (Lim et al. 2012). The incidence of HAV in the United States (US) has declined from 12 cases per 100,000 population in 1995 to 0.54 cases per 100,000 population in 2010. This reduction has followed the 1999 recommendation for routine vaccination of children in areas of the US with consistently elevated rates of HAV (CDC 2009; CDC 2012). In the European Union there was one strong evidence foodborne HAV outbreak in 2011 and also one in 2010 (EFSA 2012; EFSA 2013).

Foodborne outbreaks of HAV have been recognised for over 40 years, but are infrequently reported. This is because the 2–6 week incubation period for HAV makes it more difficult to associate the source of infection with a particular food (Appleton 2000).

Cold cuts and sandwiches, fruits and fruit juices, milk and milk products, vegetables, salads, shellfish and iced drinks have been implicated in HAV outbreaks (FDA 2012).

Occurrence in food

The types of food most often implicated in HAV outbreaks are those that are either eaten raw or only slightly cooked (e.g. shellfish), or handled extensively prior to consumption (e.g. the picking and packing of raw produce in the field and the preparation of sandwiches and salads) (Koopmans and Duizer 2004; Cook and Rzezutka 2006).

Bivalve molluscs (e.g. oysters, mussels, clams and cockles) live in shallow, coastal and estuarine waters which can be polluted with human sewage. As filter feeders they collect nutrients by filtering particulate matter from the water. If molluscs are grown in water contaminated with human faeces, the molluscs can collect and concentrate HAV from the water (Appleton 2000; Moore 2001; Cook and Rzezutka 2006). HAV has been shown to be concentrated within mussels to 100-fold higher concentrations than the surrounding water and can persist for about 7 days in the mussels (Enriquez et al. 1992). HAV has been detected in oyster samples more than 2 months after the presumed contamination event; this is thought to be due to recontamination of the oysters from sediment in the water (Conaty et al. 2000).

The prevalence of HAV reported in shellfish ranges between 6–27%, depending on the location and analytical technique used. For mussels sampled from markets of major cities in south Italy, 15.6% were found to be contaminated with infectious HAV (n=180) (Croci et al. 2003). For shellfish (clams, mussels, scallops and oysters) collected from the north Adriatic sea located between the Italian and Balkan peninsulas, HAV was detected in 6% of samples (n=235) (Croci et al. 2007). For shellfish collected off the coast of Spain (cultured and wild mussels, wild clams and cockles), HAV was detected in 27.4% of samples (n=164) (Romalde et al. 2002). The methods utilised in these studies detect the genetic material of HAV and some methods are more sensitive than others under different conditions. This suggests that the level of HAV contamination could be higher than reported.

Hernandez et al. (1997) demonstrated that 20% of pooled samples of lettuce wash water collected in Costa Rica were contaminated with HAV (n=10 pools, 5 lettuces per pool), suggesting that lettuces from this region could be a vehicle for HAV transmission.

Host factors that influence disease

People of all ages are susceptible to HAV infection (unless they have had a previous infection or vaccination). The disease is milder in young children under 6 years, with the risk of fatality increasing with age. Thus the risks are higher for unexposed older people (ESR 2001; FDA 2012).

A single HAV infection or administration of the HAV vaccine provides lifelong immunity for the individual against the virus (Leon and Moe 2006). When an outbreak of HAV occurs, if exposure can be recognised before cases begin to occur, treatment with intramuscular immunoglobulin (passive immunisation) within 2 weeks of exposure is >85% effective at preventing HAV infection. However, passive immunisation is only effective for a short time (3–6 months) and people will be susceptible to infection from another exposure (Issa and Mourad 2001; Hollinger and Emerson 2007).

Dose response

The number of HAV particles required to cause infection is not known, however, it is presumed to be 10–100 viral particles (FDA 2012). In fact it has been suggested that a single ingested viral particle may cause infection, however, the probability of this occurring is very low (Cliver 1985). It has been estimated that up to 13,000 infectious HAV particles may be present in 1 mg of faeces (Bidawid et al. 2000a).

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Appendix 7 – *Listeria monocytogenes*[29]

Listeria monocytogenes

Listeria monocytogenes is a bacterium that causes listeriosis, a disease that can have severe consequences for particular groups of the population. It can cause miscarriages in pregnant women and be fatal in immunocompromised individuals and the elderly. In healthy people, listeriosis generally only causes a mild form of illness. *L. monocytogenes* can be found throughout the environment. It has been isolated from domestic and wild animals, birds, soil, vegetation, fodder, water and from floors, drains and wet areas of food processing factories.

Description of the organism

L. monocytogenes is a Gram-positive, non-spore forming rod-shaped bacterium. It belongs to the genus *Listeria* along with *L. ivanovii, L. innocua, L. welshimeri, L. selligeri* and *L. grayi* (Rocourt and Buchrieser 2007). Of these species, only two are considered pathogens: *L. monocytogenes* which infects humans and animals, and *L. ivanovii* which infects ruminants (although there have been rare reports of *L. ivanovii* being isolated from infected humans) (Guillet et al. 2010). There are thirteen known serotypes of *L. monocytogenes*: 1/2a, 1/2b, 1/2c, 3a, 3b, 3c, 4a, 4ab, 4b, 4c, 4d, 4e and 7. The serotypes most often associated with human illness are 1/2a, 1/2b and 4b (FDA 2012).

Growth and survival characteristics

The growth and survival of *L. monocytogenes* is influenced by a variety of factors. In food these include temperature, pH, water activity, salt and the presence of preservatives (refer to Table 1).

The temperature range for growth of *L. monocytogenes* is between -1.5 and 45°C, with the optimal temperature being 30–37°C. Freezing can lead to a reduction in *L. monocytogenes* numbers (Lado and Yousef 2007). As *L. monocytogenes* can grow at temperatures as low as 0°C, it has the potential to grow, albeit slowly, in food during refrigerated storage.

Multiple factors influence the heat resistance of *L. monocytogenes*, including the characteristics of the food, such as salt content, water activiy and acidity. A higher fat content is more protective of *L. monocytogenes.* For example the D-value at 57.2°C for high fat beef (30.5%) and low fat beef (2%) was 5.8 and 2.6 minutes respectively; and for milk the D-value at 60°C in whole milk and skim milk was 1.5-2.1 and 0.95-1.05 minutes respectively. In vegetables, the D-value at 56°C ranged from 0.8 minutes for onions to 5.2 minutes for peas (Doyle et al 2001).

L. monocytogenes will grow in a broad pH range of 4.0–9.6 (Lado and Yousef 2007). Although growth at pH <4.0 has not been documented, *L. monocytogenes* appears to be relatively tolerant to acidic conditions. *L. monocytogenes* becomes more sensitive to acidic conditions at higher temperatures (Lado and Yousef 2007).

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[29] From FSANZ's *Agents of Foodborne Illness* publication, available at:
https://www.foodstandards.gov.au/publications/Pages/agents-foodborne-illness.aspx

Like most bacterial species, *L. monocytogenes* grows optimally at a water activity (a_w) of 0.97. However, *L. monocytogenes* also has the ability to grow at a a_w of 0.90 (Lado and Yousef 2007). Johnson et al. (1988) demonstrated that *L. monocytogenes* can survive for extended periods of time at a a_w value of 0.81. *L. monocytogenes* is reasonably tolerant to salt and has been reported to grow in 13–14% sodium chloride (Farber et al. 1992). Survival in the presence of salt is influenced by the storage temperature. Studies have indicated that in concentrated salt solutions, the survival rate of *L. monocytogenes* is higher when the temperature is lower (Lado and Yousef 2007).

L. monocytogenes can grow under both aerobic and anaerobic conditions, although it grows better in an anaerobic environment (Sutherland et al. 2003; Lado and Yousef 2007).

The effect of preservatives on the growth of *L. monocytogenes* is influenced by the combined effects of temperature, pH, salt content and water activity. For example, sorbates and parabens are more effective at preventing growth of *L. monocytogenes* at lower storage temperatures and pH. Also, adding sodium chloride or lowering the temperature enhances the ability of lactate to prevent *L. monocytogenes* growth. At decreased temperatures (such as refrigeration storage) sodium diacetate, sodium propionate and sodium benzoate are more effective at preventing growth of *L. monocytogenes* (Lado and Yousef 2007).

Table A7-1. Limits for growth of *L. monocytogenes* when other conditions are near optimum (Lado and Yousef 2007)

	Minimum	Optimum	Maximum
Temperature (°C)	-1.5	30–37	45
pH	4.0	6.0–8.0	9.6
Water activity	0.90	0.97	–

Symptoms of disease

There are two main forms of illness associated with *L. monocytogenes* infection. Non-invasive listeriosis is the mild form of disease, while invasive listeriosis is the severe form of disease and can be fatal (FDA 2012). The likelihood that invasive listeriosis will develop depends upon a number of factors, including host susceptibility, the number of organisms consumed and the virulence of the particular strain (WHO/FAO 2004).

Symptoms of non-invasive listeriosis can include fever, diarrhoea, muscle aches, nausea, vomiting, drowsiness and fatigue. The incubation period is usually 1 day (range 6 hours to 10 days) (Painter and Slutsker 2007; FDA 2012). Non-invasive listeriosis is also known as listerial gastroenteritis or febrile listeriosis.

Invasive listeriosis is characterised by the presence of *L. monocytogenes* in the blood, in the fluid of the central nervous system (leading to bacterial meningitis) or infection of the uterus of pregnant women. The latter may result in spontaneous abortion or stillbirth (20% of cases) or neonatal infection (63% of cases). Influenza-like symptoms, fever and gastrointestinal symptoms often occur in pregnant women with invasive listeriosis. In non-pregnant adults, invasive listeriosis presents in the form of bacterial meningitis with a fatality rate of 30%. Symptoms including fever, malaise, ataxia, seizures and altered mental status (Painter and Slutsker 2007). The incubation period before onset of invasive listeriosis ranges from three days to three months (FDA 2012).

Virulence and infectivity

When *L. monocytogenes* is ingested, it may survive the stomach environment and enter the intestine where it penetrates the intestinal epithelial cells. The organism is then taken up by macrophages and non-phagocytic cells. The *L. monocytogenes* surface protein internalin is required for this uptake by non-phagocytic cells, as it binds to the receptors on the host cells to instigate adhesion and internalization. The bacterium is initially located in a vacuole after uptake by a macrophage or non-phagocytic cell. *L. monocytogenes* secrete listeriolysin O protein, which breaks down the vacuole wall and enables the bacteria to escape into the cytoplasm. Any bacteria remaining in the vacuole are destroyed by the host cell. Once located in the cytoplasm of the host cell, *L. monocytogenes* is able to replicate.
L. monocytogenes is transported around the body by the blood, with most *L. monocytogenes* being inactivated when it reaches the spleen or liver. *L. monocytogenes* is able to utilise the actin molecules of the host to propel the bacteria into neighbouring host cells. In the case of invasive listeriosis, this ability to spread between host cells enables *L. monocytogenes* to cross the blood-brain and placental barriers (Montville and Matthews 2005; Kuhn and Goebel 2007; Bonazzi et al. 2009).

Mode of transmission

The most common transmission route of *L. monocytogenes* to humans is via the consumption of contaminated food. However, *L. monocytogenes* can be transmitted directly from mother to child (vertical transmission), from contact with animals and through hospital acquired infections (Bell and Kyriakides 2005).

Healthy individuals can be asymptomatic carriers of *L. monocytogenes*, with 0.6–3.4% of healthy people with unknown exposure to *Listeria* being found to shed *L. monocytogenes* in their faeces. However, outbreak investigations have shown that listeriosis patients do not always shed the organism in their faeces (FDA/USDA/CDC 2003; Painter and Slutsker 2007). Therefore the role of healthy carriers in the transmission of *L. monocytogenes* is unclear.

Incidence of illness and outbreak data

Listeriosis is a notifiable disease in all Australian states and territories. The incidence of listeriosis notified in Australia in 2012 was 0.4 cases per 100,000 population (93 cases). This is a slight increase from the previous 5 year mean of 0.3 cases per 100,000 population per year (ranging from 0.2–0.4 cases per 100,000 population per year) (NNDSS 2013). In Australia the fatality rate in 2010 was 21%, which was an increase from the 14% fatality rate of the previous year (OzFoodNet 2010; OzFoodNet 2012).

The notification rate for listeriosis in New Zealand in 2011 was 0.6 cases per 100,000 population (26 cases). This was an increase from the 2010 rate of 0.5 cases per 100,000 population. The fatality rate in New Zealand in 2011 was 3.8% (Lim et al. 2012).

In the United States (US) the notification rate for listeriosis in 2010 was 0.27 cases per 100,000 population. This was similar to the 2009 rate of 0.28 cases per 100,000 population (CDC 2012). In the European Union (EU) there were 0.32 confirmed cases of listeriosis per 100,000 population in 2011 (ranging from 0.04–0.88 cases per 100,000 population between countries). This was a 7.8% decrease in the number of cases from 2010. The reported fatality rate in the EU in 2011 was 12.7% (EFSA 2013).

Invasive *L. monocytogenes* infections can be life threatening, with average fatality rates being 20–30% among hospitalized patients (WHO/FAO 2004; Swaminathan and Gerner-Smidt 2007)

Most cases of listeriosis are sporadic. Despite this, foodborne outbreaks due to *L. monocytogenes* have been associated with cheese, raw (unpasteurised) milk, deli meats, fruit, salad, fish and smoked fish, ice cream and hotdogs (CDC 2011; Montville and Matthews 2005; Swaminathan and Gerner-Smidt 2007).

Occurrence in food

L. monocytogenes has been isolated from various ready-to-eat products. In a study by Meldrum et al. (2010) the prevalence of *L. monocytogenes* was 4.1% in crustaceans (n=147), 6.7% in smoked fish (n=178), 2% in sushi (n=50) and 0.9% in green salad (n=335) samples in Wales. Wong et al. (2005) isolated *L. monocytogenes* from 1% of ham (n=104) and 1.7% of pate (n=60) samples in New Zealand. *L. monocytogenes* has also been isolated from dairy products. For example, *L. monocytogenes* was detected in 1.3% of fresh cheese samples in Spain (n=78), 0.2% of hard cheese samples in the United Kingdom (n=1242) and 0.3% of ice creams in Italy (n=1734) (Busani et al. 2005; Cabedo et al. 2008; Little et al. 2009). The prevalence of *L. monocytogenes* in bulk milk tank internationally is 1–60% (FSANZ 2009).

The presence of *L. monocytogenes* in ready-to-eat products is probably due to contamination occurring after the product has been processed. This contamination may occur during additional handling steps such as peeling, slicing and repackaging. Also, in the retail and food service environment, contamination may be transferred between ready-to-eat products (Lianou and Sofos 2007). The type of handling that ready-to-eat meat receives may also influence the level of *L. monocytogenes* contamination. In a survey of retail packaged meats there was a significantly higher prevalence of *L. monocytogenes* reported in products cut into cubes (61.5%) (n=13), compared with sliced products (4.6%) (n=196) (Angelidis and Koutsoumanis 2006).

Host factors that influence disease

People at risk of invasive listeriosis include pregnant women and their foetuses, newborn babies, the elderly and immunocompromised individuals (such as cancer, transplant and HIV/AIDS patients). Less frequently reported, but also at a greater risk, are patients with diabetes, asthma, cirrhosis (liver disease) and ulcerative colitis (inflammatory bowel disease) (FDA 2012).

Dose response

Investigations of foodborne outbreaks of non-invasive listeriosis have concluded that consumption of food with high levels of *L. monocytogenes* (1.9×10^5/g to 1.2×10^9/g) is required to cause illness in the general healthy population (Sim et al. 2002).

The number of *L. monocytogenes* required to cause invasive listeriosis depends on a number of factors. These include the virulence of the particular serotype of *L. monocytogenes*, the general health and immune status of the host, and attributes of the food (for example fatty foods can protect bacteria from stomach acid). Some *L. monocytogenes* serovars are more virulent than others; this may be attributed to differences in the expression of virulence factors which could influence the interactions between the bacterium and the host cells and cellular invasion (Severino et al. 2007). The FDA and WHO have developed separate models for both healthy and susceptible populations to predict the probability that an individual will develop listeriosis (FDA/USDA/CDC 2003; WHO/FAO 2004). The probability that a healthy person of intermediate age will become ill from the consumption of a single *L. monocytogenes* cell was estimated to be 2.37×10^{-14}. For more susceptible populations the probability that illness will occur was estimated to be 1.06×10^{-12}. A more recent assessment on invasive listeriosis in

susceptible populations was performed which took into account the different serotypes of *L. monocytogenes* (Chen et al. 2006). This study showed that the probability of a susceptible individual developing invasive listeriosis ranged from 1.31×10^{-8} to 5.01×10^{-11}, suggesting that there are large differences in virulence between *L. monocytogenes* serotypes.

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Appendix 8 – Norovirus[30]

Norovirus

Norovirus (NoV) is the main cause of viral gastroenteritis in humans worldwide. It is highly contagious due to its very low infectious dose, stability in the environment and resistance to many common disinfectants.

Description of the organism

NoV belongs to the *Caliciviridae* family of viruses. NoV is a non-enveloped virus and has a small (27 – 40nm) icosahedral shaped capsid that contains a 7.7kb single stranded RNA genome (Richards et al. 2012; Green 2013).

A standard NoV classification scheme was established by Zheng et al. (2006) which divides NoV sequentially into genogroups, genoclusters and strains. The genogroups and genoclusters are designated numerically, with the genogroup indicated first in roman numerals followed by the genocluster number (Zheng et al. 2006; Donaldson et al. 2008). NoV is classified into five genogroups: GI – GV. Human NoVs belong to genogroups GI, GII and GIV; the former two cause the majority of human infections. The genogroup and genocluster information are combined to generate the genotype, for example GII genocluster 4 is designated as the GII.4 genotype. In humans the GII.4 genotype is most commonly found, causing the majority of outbreaks and approximately 80% of infections (Donaldson et al. 2008; Sharps et al. 2012). Human NoV strains are typically named after the location of the outbreak where they were first isolated, for example Norwalk virus, Hawaii virus and the NoV GII.4 Sydney 2012 strain (Green 2013; van Beek et al. 2013).

NoV has also been isolated from animals, with GIII NoVs detected in cattle and GV NoV detected in mice. GII NoVs have been found in pigs and GIV NoV detected in a dog and lion cub. However, the animal genotypes are different to those found in humans (Martella et al. 2008; Bank-Wolf et al. 2010).

Growth and survival characteristics

NoV requires host specific living cells in order to replicate. As viruses cannot grow in food, the level of NoV contamination cannot increase due to viral replication during processing or storage of food (Zainazor et al. 2010; Green 2013). The environmental spread of NoV is influenced by factors such as pH, temperature and how readily it attaches to the surfaces of fomites (Girard et al. 2010).

NoV is stable in the environment. D'Souza et al. (2006) demonstrated that when NoV was artificially transferred onto formica, stainless steel or ceramic surfaces, the virus could be detected on those surfaces for seven days (study duration). NoV has been shown to persist in groundwater for at least three years and remains infectious in groundwater for at least 61 days (Seitz et al. 2011).

Freezing has little effect on NoV survival. Richards et al. (2012) used NoV positive stool samples to show that freezing at -80°C for 120 days or performing up to 14 freeze/thaw cycles (-80°C/+22°C) did not affect capsid integrity, viral RNA titres or viral infectivity. A study by Butot et al. (2008) demonstrated that the NoV titre in artificially inoculated blueberries was reduced by less than 1 $\log_{10}$ after 2 days and 2.3 $\log_{10}$ after 90 days storage at -20°C.

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[30] From FSANZ's *Agents of Foodborne Illness* publication, available at:
https://www.foodstandards.gov.au/publications/Pages/agents-foodborne-illness.aspx

The effect of heat treatment on NoV is variable and highly dependent on the initial level of contamination, time and temperature of heating, virus strain, and type of food matrix (Codex 2012). Studies performed using artificially inoculated foods showed that consumer practices, such as baking (inoculated) pizza at 200°C for 12 minutes, led to a significant reduction in NoV titres. However, pasteurising spiced tomato sauce at 72°C for 1 minute or heating mussels to 80°C for 15 min did not result in a significant reduction in NoV titre (Mormann et al. 2010; Croci et al. 2012). Heating to an internal temperature of at least 90°C for 90 seconds is considered adequate to destroy viral infectivity in most foods (Codex 2012).

NoV is able to survive acidic conditions. A study by Mormann et al. (2010) showed that there was no significant reduction in NoV titre in tomato ketchup stored at pH 4.5 for 58 days at 6°C. Also, Dolin et al. (1972) demonstrated that NoV stored at pH 2.7 for 3 hours retained the ability to cause illness.

NoV is generally resistant to detergents and ethanol-based reagents used to clean environmental surfaces and fomites, so additional chemical disinfection is required. Disinfectants effective against NoV include hypochlorite, hydrogen peroxide and phenolic-based cleaners (Green 2013). Studies have shown that full inactivation of NoV on stainless steel requires 10 minutes contact time with sodium hypochlorite-based disinfectant, with only a 2-log reduction occurring after 5 minutes of exposure (Girard et al. 2010).

Symptoms of disease

Infection with NoV generally leads to symptoms of gastroenteritis, although asymptomatic infection can also occur. Explosive or projectile vomiting is usually the first sign of illness and is often used to characterise the illness. Other symptoms of NoV infection include diarrhoea, abdominal cramps, nausea, headache, low grade fever, chills, muscle aches and lethargy. The incubation period before onset of disease is usually 24 – 48 hours but may be as short as 12 hours. The illness generally lasts for 12 – 60 hours (Karst 2010; FDA 2012; Sharps et al. 2012). NoV may be shed in the faeces of infected individuals before the onset of any clinical symptom (from 18 hours after infection). Viral shedding continues for a median period of four weeks and can continue for at least eight weeks (Atmar et al. 2008).

In immunocompromised individuals the duration of disease can be prolonged and it can develop into a chronic infection with recurrent episodes (Westhoff et al. 2009). Most patients show complete recovery. However, if severe dehydration occurs due to fluid loss the infection can be fatal (Sharps et al. 2012).

A systematic review of the international literature performed by Desai et al (2012) estimated the NoV hospitalisation rate to be 70 per 10,000 cases and the fatality rate 7 per 10,000 cases when NoV outbreaks were analysed.

Virulence and infectivity

NoV virions are acid stable and survive passage through the stomach. The primary site of viral replication is thought to be the upper intestinal tract. It has been proposed that nausea and vomiting associated with NoV may result from abnormal gastric motor function, and diarrhoea may result from both epithelial barrier and secretory pathway dysfunction (Meeroff et al. 1980; Green 2013).

The infectivity of NoV is linked to the ability of the viral capsid to bind to receptors on host cells and subsequently to enter the host cell. The NoV capsid is predominantly constructed of a major structural protein, VP1. Part of the VP1 protein protrudes from the capsid surface and has a role in binding to the ABO histo-blood group antigen receptor of host cells (Mattison 2011; Green 2013). The "*host factors that influence disease*" section presents

further information on the role of histo-blood group antigens. Mutations in or near the viral receptor binding domain may result in a newly evolved NoV strain that can bind to different host receptors. This could enable the virus to infect a new subset of the population with a different ABO blood type. Mutations may also inhibit the ability of host antibodies to bind to the new NoV variant strain, and so assist the virus to evade the host immune response. This evolution could be associated with the continued prevalence and emergence of new GII.4 strains worldwide. Variant strains of GII.4 NoV emerge every 2 – 10 years and have been responsible for several pandemics (Donaldson et al. 2008; Lindesmith et al. 2008; Sharps et al. 2012; van Beek et al. 2013).

Mode of transmission

NoV can be transmitted via the consumption of contaminated food or water, person-to-person contact, aerosolised vomit particles or contaminated surfaces. NoV is highly contagious and outbreaks frequently occur in semi-closed communities such as nursing homes, military settings, schools, hospitals and cruise ships (Karst 2010; Tuladhar et al. 2013). Asymptomatic individuals can be involved in 'silent' transmission of the virus as both symptomatic and asymptomatic individuals shed similar quantities of NoV in their faeces (Ozawa et al. 2007).

Zoonotic transmission of NoV cannot be completely discounted; however no animal NoV strains have been detected in human samples (Bank-Wolf et al. 2010).

NoV can be transferred between fomites, hands and food. Sharps et al. (2012) showed that 58% of NoV was transferred from artificially inoculated gloved fingertips to food contact surfaces (stainless steel) under wet conditions. Conversely, Tuladhar et al. (2013) demonstrated that when clean finger pads were pressed onto artificially inoculated stainless steel 4.2% of GI.4 NoV strain and 3.5% of GII.4 NoV strain were transferred from the stainless steel to the finger pads.

The level of NoV transfer is reduced when the virus is left to dry, compared to a freshly inoculated surface. For example, D'Souza et al. (2006) showed that when lettuce samples were placed onto freshly inoculated stainless steel, NoV was transferred to 94% of lettuce samples (n=18). However, when NoV was dried onto the stainless steel for at least 30 minutes prior to application, NoV only transferred to 72% of wet lettuce samples (n=18) and no transfer occurred for dry lettuce samples (n=18).

Infected food handlers have been associated with NoV outbreaks. For example, Boxman et al. (2009) described an outbreak where GI.6 NoV was detected on the hands of a food handler working in a restaurant associated with the outbreak. GI.6 NoV is a rare strain and matched the virus isolated from stools of people who became ill and environmental swabs of the restaurant's kitchen and bathroom surfaces.

There have been multiple NoV outbreaks associated with asymptomatic food handlers who shed NoV in their stools yet had no gastrointestinal symptoms (Barrabeig et al. 2010; Nicolay et al. 2011). Food handlers with asymptomatic NoV infection not associated with transmitting disease during an outbreak have been reported at low prevalence: 1.0% in food catering facilities in South Korea (n=6,441) (Jeong et al. 2012), 3.4% in elementary schools in Korea (n=776) (Yu et al. 2011) and 11.9% at a catering facility in Japan (n=159) (Okabayashi et al. 2008).

Therefore, appropriate hand hygiene is very important in order to control transmission and prevent NoV infection. A study by Liu (2010) demonstrated that liquid soap treatment or rinsing with water yielded the greatest reduction in the level of NoV contamination on hands. The alcohol based hand sanitiser was relatively ineffective against the non-enveloped NoV (ethanol based hand sanitisers are more effective against enveloped viruses such as influenza and herpes simplex viruses). As such, the CDC (2011) recommends thorough handwashing with running water and soap as the best method to remove NoV from hands.

Incidence of illness and outbreak data

NoV is not a notifiable disease in Australia. It has been estimated that 18% of NoV infections in Australia are foodborne, with 276,000 cases of foodborne gastroenteritis associated with NoV annually in Australia. This comprises approximately a third of the cases of foodborne illness caused by known pathogens (Kirk et al. 2014). In New Zealand, NoV caused 23% and 16.5% of foodborne outbreaks reported in 2015 and 2014, respectively (Horn et al. 2015; Lopez et al. 2016).

In Europe in 2015, NoV caused 9% of foodborne outbreaks and 26.6% of cases of illness associated with foodborne outbreaks where a link to the implicated food could be established based on strong evidence. This was similar to 2014 when NoV caused 13% of foodborne outbreaks and 28.9% of cases of illness associated with foodborne outbreaks supported by strong evidence (EFSA 2015; EFSA 2016). In the Netherlands it has been estimated that in 2009 there were 662 gastroenteritis cases per 100,000 population and 0.07 fatalities per 100,000 population attributed to foodborne NoV infection (Verhoef et al. 2013).

In the United States (US) it was estimated that NoV accounts for 58% of cases of foodborne illness caused by 31 major pathogens (Scallan et al. 2011). In 2009–2010 NoV was detected in 21% of children under the age of five who sought medical attention for acute gastroenteritis (n=1295), although this included both foodborne and non-foodborne cases (Payne et al. 2013).

NoV infection occurs throughout the year, however, it is most prevalent in the winter season in temperate climates (van Beek et al. 2013). The seasonal occurrence of NoV outbreaks associated with oysters can be attributed to several environmental factors including increased humidity, low temperatures, reduced solar inactivation, low salinity and heavy rains (Wang and Deng 2012). A study by Lowther et al. (2008) demonstrated that in the British Isles during 2004 – 2006 the overall level of NoV was 17-fold higher in oysters harvested in the winter compared to the summer (this increase in NoV level took into account the number of NoV positive samples and the level of NoV contamination).

Outbreaks of NoV have frequently been associated with shellfish, as well as fruit and vegetables and ready-to-eat foods that are typically consumed without additional heat treatment (Zainazor et al. 2010; FDA 2012).

Occurrence in food

Seafood
Seafood such as oysters can be contaminated with NoV in the marine environment. Oysters are filter feeders and filter large volumes of water through their gills. As such, oysters grown in faecally contaminated waters can accumulate NoV within their tissues (Noda et al. 2008; Wang and Deng 2012). Burkhardt and Calci (2000) used a viral indicator and demonstrated that oysters can accumulate up to 99-fold higher levels of virus than the surrounding estuarine waters. NoV binds strongly to oyster tissue and so remains present in the oyster when consumed. Ueki et al. (2007) performed a study which involved artificially contaminating oysters with NoV and then subjecting these oysters to a depuration process. Depuration involves moving oysters to clean water for several days to allow the oyster to purge pathogens. The average NoV concentration did not significantly decrease over the 10 day depuration. NoV is difficult to eliminate from contaminated oysters due to the specific viral attachment to oyster tissues such as gills and digestive glands. As such, depuration is a relatively ineffective method of removing accumulated NoV from oysters (Wang and Deng 2012; Smith et al. 2012).

NoV has been isolated from shellfish in many international studies. The prevalence of NoV in samples collected from harvesting areas was found to be: <2% in oysters across Australia (n=300) (Torok et al. 2018), 37% of shellfish batches in Portugal (n=49) (Mesquita et al. 2011), 49.4% in mussels from Spain (n=81) (Manso and Romalde 2013) and 76.2% in oysters from the United Kingdom (n=844) (Lowther et al. 2012). The prevalence of NoV in samples collected at retail was 3.9% in oysters sampled in the US (n=388) (DePaola et al. 2010), 14.1% of oysters in Korea (n=156) (Moon et al. 2011) and 73.9% in shellfish collected from southern Italy (n=46) (Pepe et al. 2012). The dominant NoV genotypes isolated were GI or GII, with some samples positive for both GI and GII. The difference in geneotype prevalence may be attributed to factors such as season, location, virus extraction method, molecular test employed, and the sensitivity and specificity of the assay (DePaola et al. 2010; Moon et al. 2011). Also, the water quality of the harvesting area in regard to potential effluent contamination and good hygienic practices throughout the supply chain can influence the prevalence of NoV.

The magnitude of NoV accumulation in oysters varies between strains. The GI.1 strain is efficiently concentrated in oysters and accumulates in the midgut and digestive tissues. GII.3 and GII.4 bind to digestive tissues, gills and mantle. GII.3 is moderately accumulated, while GII.4 is poorly accumulated. This matches with outbreak data, as oyster related outbreaks are often associated with GI and GII.3 NoV strains (that accumulate in oysters) but rarely with GII.4 strains. The variation between strains appears to be linked to the binding affinity of particular NoV strains for carbohydrate receptors within different oyster tissues (Maalouf et al. 2011; le Guyader et al. 2012).

Fresh produce
Sewage polluted water can introduce NoV contamination during the growing of fresh produce in the field or during processing, if used. Picking, packing or preparation of fresh produce by hand is another potential NoV contamination source. This is because produce can be contaminated directly by ill or asymptomatic food handlers practicing inadequate hand hygiene or indirectly after a healthy worker touches a contaminated surface (Baert et al. 2011; Sharps et al. 2012).

In surveys of fresh produce collected during 2009 – 2010 in Belgium, Canada and France, NoV was detected on 33% (n=6), 28% (n=641) and 50% (n=6) of individual leafy green samples collected in each country, respectively. NoV was also detected on 34% (n=29) and 7% (n=150) of individual berry samples collected in Belgium and France, respectively. The fresh produce samples were positive for either or both GI NoV and GII NoV strains (Baert et al. 2011).

Host factors that influence disease

People of all ages are susceptible to NoV infection, however infants, the elderly and immunocompromised individuals can have more severe symptoms (Karst 2010). There is a small risk that NoV infection will be fatal in elderly people (Harris et al. 2008).

Hutson et al. (2002) identified a relationship between an individual's ABO histo-blood group antigen and the risk of infection with NoV GI strain (Norwalk virus). Individuals with type O blood had increased susceptibility to infection, while individuals expressing the type B antigen were more resistant to infection. However, this association between ABO histo-blood group antigen and NoV infection appears to be specific to GI NoV strains only. Halperin et al. (2008) studied 176 patients from 2 separate outbreaks caused by GII NoV strains and found there was no association between ABO histo-blood group antigen and the risk of clinical disease.

Expression of most ABO histo-blood group antigens is dependent on the presence of a functional α(1,2)fucosyltransferase (*FUT*2) gene. Individuals with an inactivating *FUT2* gene mutation are called non-secretors (Se⁻); wild-type individuals are called secretors (Se⁺). The Se⁻ phenotype occurs in about 20% of the European and North American populations (Marionneau et al. 2002; Lindesmith et al. 2003; Rockx et al. 2005). Se⁻ individuals are resistant to infection with NoV GI strain (Norwalk virus) as they do not express the appropriate ABO histo-blood group antigens necessary for docking and possibly viral entry (Lindesmith et al. 2003; Lindesmith et al. 2008). However, other NoV strains can infect Se⁻ individuals (Lindesmith et al. 2008; Debbink et al. 2012). GII.4 strains have been shown to infect about 6% of Se⁻ individuals compared to 70–77% of Se⁺ individuals across both outbreak and human challenge settings (Carlsson et al. 2009; Frenck et al. 2012).

Both short-term and long-term immunity to NoV has been demonstrated; however the mechanism mediating the immune response remains unclear (Donaldson et al. 2008). In an early human challenge study Dolin et al. (1972) demonstrated that when volunteers who had previously been susceptible to NoV (Norwalk virus) infection were rechallenged 6–14 weeks later none of the volunteers became ill (n=6). This suggested the development of short-term immunity (for at least 6–14 weeks) against homologous virus infection. In another early study Parrino et al. (1977) showed that when volunteers who had previously become ill were rechallenged with the same NoV strain (Norwalk virus) 27–42 months later they became ill again. However, when challenged a third time, an additional 4–8 weeks later, most volunteers did not become ill. Johnson et al. (1990) performed multiple challenge studies in adult volunteers. Progressively greater resistance to clinical illness occurred with repeated exposure to NoV (Norwalk virus) with 60% of individuals becoming ill after the first exposure (n=42), 18% after the second exposure 6 months later (n=22) and none after the third exposure an additional 6 months later (n=19).

There is currently no licensed vaccine available for NoV infection. Several candidate vaccines are under development, with some progressing to the clinical trial stage.

Dose response

Teunis et al (2008) developed a dose response model for NoV GI strain (Norwalk virus) from human challenge data. The average probability of infection on ingestion of a single viral particle was approximately 0.5. Infected individuals had a dose dependent probability of becoming ill that ranged from 0.1 for a dose of 10^3 viral genomes to a probability of 0.7 for a dose of 10^8 viral genomes. As NoV GI strain (Norwalk virus) does not infect Se^- individuals, this dose response model only utilised data from Se^+ individuals that are susceptible to infection.

The attack rate (proportion of people who become ill) may be influenced by factors such as the amount of infectious virus particles ingested, host susceptibility and virus pathogenicity. A study by Noda et al. (2008) showed that the attack rate in oyster-related outbreaks was higher than in food handler-associated outbreaks. This may be attributed to food handler outbreaks being associated with a single NoV strain. In comparison, various NoV strains (such as GI and GII.3) have been shown to accumulate in oysters in the sea environment and this joint bioaccumulation could result in multiple NoV strains being associated with a single oyster outbreak. Also, the attack rate in outbreaks associated with GII.4 strains was lower than for GII.3 strains. This suggests that GII.4 may cause asymptomatic infection more frequently than other NoV genotypes (Noda et al. 2008; Maalouf et al. 2011).

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Appendix 9 – *Salmonella* spp.[31]

Salmonella (non-typhoidal)

Salmonella spp. are bacteria that cause salmonellosis, a common form of foodborne illness in humans. Outcomes from exposure to *Salmonella* spp. can range from mild symptoms to severe disease and can be fatal. *Salmonella* spp. are carried by a range of domestic and wild animals and birds and have been widely isolated from the environment.

Description of the organism

Salmonella spp. are Gram-negative, non-spore forming rod-shaped bacteria and are members of the family *Enterobacteriaceae* (Jay et al. 2003). The genus *Salmonella* is divided into two species: *S. enterica* (comprising six subspecies) and *S. bongori.* Over 99% of human *Salmonella* spp. infections are caused by *S. enterica* subsp. *enterica* (Bell and Kyriakides 2002; Crum-Cianflone 2008).

Strains of *Salmonella* can be characterised serologically (into serovars) based on the presence and/or absence of O (somatic) and H (flagella) antigens. Phage typing is used to subtype *Salmonella* serovars. The phage type is determined by the sensitivity of the bacterial cells to the lytic activity of selected bacteriophages (Bell and Kyriakides 2002; Jay et al. 2003).

The formal names used to describe types of *Salmonella* are rather cumbersome, for example *S. enterica* subsp. *enterica* serovar Typhimurium. For practical reasons, the abbreviated versions of these names using just the serovar are commonly used, such as *S.* Typhimurium (Crum-Cianflone 2008).

Some *Salmonella* serovars are host-adapted to individual animal species and may differ vastly in the severity of the disease they cause; others such as *S.* Typhimurium have a broad host range, with an ability to infect a wide range of animals, including humans (Jay et al. 2003; Wallis 2006).

S. Typhi and *S.* Paratyphi are specifically associated with infections in humans, leading to severe disease called enteric fever. *S.* Typhi and *S.* Paratyphi produce clinical syndromes referred to as typhoid and paratyphoid fever, respectively. Enteric fever is rare in developed countries, with the majority of cases associated with overseas travel (Darby and Sheorey 2008). In Australia 97.9% of notified cases of typhoid fever were likely to have been acquired overseas in 2010 (OzFoodNet 2012).

Growth and survival characteristics

Salmonella spp. have relatively simple nutritional requirements and can survive for long periods of time in foods and other substrates. The growth and survival of *Salmonella* spp. is influenced by a number of factors such as temperature, pH, water activity and the presence of preservatives (refer to Table A9-1).

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[31] From FSANZ's *Agents of Foodborne Illness* publication, available at:
https://www.foodstandards.gov.au/publications/Pages/agents-foodborne-illness.aspx

The temperature range for growth of *Salmonella* spp. is 5.2–46.2°C, with the optimal temperature being 35–43°C (ICMSF 1996). Although freezing can be detrimental to *Salmonella* spp. survival, it does not guarantee destruction of the organism. There is an initial rapid decrease in the number of viable organisms at temperatures close to the freezing point as a result of the freezing damage. However, at lower temperatures *Salmonella* spp. have the ability to survive long term frozen storage (Jay et al. 2003). Strawn and Dayluk (2010) showed that *Salmonella* was able to survive on frozen mangoes and papayas stored at -20°C for at least 180 days.

Heat resistance of *Salmonella* spp. in food is dependent on the composition, pH and water activity of the food. The heat resistance of *Salmonella* spp. increases as the water activity of the food decreases. Foods which are high in fat and low in moisture, such as chocolate and peanut butter, may have a protective effect against heat. In low pH conditions the heat resistance of *Salmonella* spp. is reduced (Jay et al. 2003; Shachar and Yaron 2006; Podolak et al. 2010).

Salmonella spp. will grow in a broad pH range of 3.8–9.5, with an optimum pH range for growth of 7–7.5 (ICMSF 1996). The minimum pH at which *Salmonella* spp. can grow is dependent on temperature, presence of salt and nitrite and the type of acid present. Volatile fatty acids are more bactericidal than organic acids such as lactic, citric and acetic acid. Outside of the pH range for growth, cells may become inactivated, although this is not immediate and cells have been shown to survive for long periods in acidic products (Bell and Kyriakides 2002; Jay et al. 2003).

Water activity (a_w) has a significant effect on the growth of *Salmonella* spp., with the optimum a_w being 0.99 and the lower limit for growth being 0.93. *Salmonella* spp. can survive for months or even years in foods with a low a_w (such as black pepper, chocolate, peanut butter and gelatine) (ICMSF 1996; Podolak et al. 2010).

Salmonella spp. are similar to other Gram negative bacteria in regard to susceptibility to preservatives commonly used in foods. Growth of *Salmonella* spp. can be inhibited by benzoic acid, sorbic acid or propionic acid. The inhibition of *Salmonella* spp. is enhanced by the use of a combination of several preservative factors, such as the use of a preservative in conjunction with reduction in pH and temperature (ICMSF 1996; Ha et al. 2004; Banerjee and Sarkar 2004).

Salmonella spp. are classed as facultative anaerobic organisms as they do not require oxygen for growth (Jay et al. 2003).

Table A9-1. Limits for growth of *Salmonella* spp. when other conditions are near optimum (ICMSF 1996; Podolak et al. 2010)

	Minimum	Optimum	Maximum
Temperature (°C)	5.2	35–43	46.2
pH	3.8	7–7.5	9.5
Water activity	0.93	0.99	>0.99

Symptoms of disease

Outcomes of exposure to non-typhoidal *Salmonella* spp. can range from having no effect, to colonisation of the gastrointestinal tract without symptoms of illness (asymptomatic infection), or colonisation with the typical symptoms of acute gastroenteritis. Gastroenteritis symptoms are generally mild and may include abdominal cramps, nausea, diarrhoea, mild fever, vomiting, dehydration, headache and/or prostration. The incubation period is 8–72 hours (usually 24–48 hours) and symptoms last for 2–7 days (WHO/FAO 2002; Darby and Sheorey 2008). Severe disease such as septicaemia sometimes develops, predominantly in immunocompromised individuals. This occurs when *Salmonella* spp. enter the bloodstream, leading to symptoms such as high fever, lethargy, abdominal and chest pain, chills and anorexia; and can be fatal. A small number of individuals develop a chronic condition or sequelae such as arthritis, appendicitis, meningitis or pneumonia as a consequence of infection (Hohmann 2001; WHO/FAO 2002; FDA 2012).

Salmonella spp. are shed in large numbers in the faeces of infected individuals at the onset of illness. In the case of non-typhoid disease, bacterial shedding continues for about 4 weeks after illness in adults and 7 weeks in children. It is estimated that 0.5% of individuals with non-typhoid salmonellosis become long-term carriers and continue shedding the bacteria on an ongoing basis (Jay et al. 2003; Crum-Cianflone 2008).

Virulence and infectivity

Once ingested, *Salmonella* spp. must survive the low pH of the stomach, adhere to the small intestine epithelial cells and overcome host defence mechanisms to enable infection (Jay et al. 2003).

Salmonella spp. possess a number of structural and physiological virulence factors, enabling them to cause acute and chronic disease in humans. The virulence of *Salmonella* spp. varies with the length and structure of the O side chains of lipopolysaccharide molecules at the surface of the bacterial cell. Resistance of *Salmonella* spp. to the lytic action of complement (part of the immune response) is directly related to the length of the O side chain (Jay et al. 2003). Other important virulence factors include the presence and type of fimbriae, which is related to the ability of *Salmonella* spp. to attach to host epithelium cells, as well as the expression of genes responsible for invasion into cells (Jones 2005). Some of these virulence genes are encoded on *Salmonella* pathogenicity islands (SPI). SPI-1 is required for bacterial invasion into intestinal epithelial cells, while systemic infections and intracellular accumulation of *Salmonella* spp. are dependent on the function of SPI-2 (Valle and Guiney 2005).

Salmonella spp. produce a heat labile enterotoxin, resulting in the loss of intestinal fluids (causing diarrhoea). This enterotoxin is closely related functionally, immunologically and genetically to the toxin of *Vibrio cholerae* and the heat labile toxin of pathogenic *Escherichia coli* (Jay et al. 2003). Most *Salmonella* strains also produce heat labile cytotoxin which may cause damage to the intestinal mucosal surface and results in general enteric symptoms and inflammation. Infection with non-typhoidal *Salmonella* is generally limited to a localised intestinal event. However, the presence of virulence plasmids has been associated with non-typhoidal *Salmonella* spp. surviving in phagocytes and spreading from the small intestine to the spleen and liver (Jay et al. 2003; Hanes 2003).

Multiple antibiotic resistant strains of *Salmonella* have emerged, an example being *S.* Typhimurium definitive phage type 104 (DT104). Multi-resistant *S.* Typhimurium DT104 infects both humans and animals, such as cattle and sheep. To date, this organism is not endemic in Australia, although it is a significant health problem in European countries, North America, the Middle East, South Africa and South-East Asia (Jay et al. 2003).

Mode of transmission

Salmonella spp. are transmitted by the faecal-oral route by either consumption of contaminated food or water, person-to-person contact, or from direct contact with infected animals (Jay et al. 2003).

Incidence of illness and outbreak data

Salmonellosis is one of the most commonly reported enteric illnesses worldwide, being the second most frequently reported cause of enteric illness in Australia (behind campylobacteriosis). It is a notifiable disease in all Australian states and territories, with a notification rate in 2012 of 49.8 cases per 100,000 population (11,273 cases). This was an increase on the previous 5 year mean of 46.9 cases per 100,000 population per year (ranging from 38.6–54.2 cases per 100,000 population per year) (NNDSS 2013).

The salmonellosis notification rate varied between jurisdictions from 40.5 cases per 100,000 population in New South Wales to 180.1 cases per 100,000 population in the Northern Territory in 2012 (NNDSS 2013). Children between 0–4 years had the highest notification rate, with 218.3 and 160.2 notifications per 100,000 population for males and females, respectively, in 2010 (OzFoodNet 2012) The higher rate of notified cases in this age group may reflect an increased susceptibility upon first exposure, but may also be a result of other factors such as an increased likelihood of exposure and increased likelihood to seek medical care.

The distribution of *Salmonella* serovars in Australia varies geographically, however *S.* Typhimurium was the most commonly reported serovar in 2010, representing 44% of all notified *Salmonella* infections in Australia. Internationally, *S.* Enteritidis is frequently reported as causing human illness; however it is not endemic in Australia. In 2010, 93% of *S.* Enteritidis cases reported in Australia were acquired overseas (Greig and Ravel 2009; OzFoodNet 2012).

The notification rate for salmonellosis in New Zealand in 2011 was 24 cases per 100,000 population (1,056 cases). This was a slight decrease from the 2010 rate of 26.2 cases per 100,000 population (Lim et al. 2012). In the United States (US) 17.73 cases of salmonellosis were notified per 100,000 population in 2010. This was a slight increase from the 2009 rate of 16.18 cases per 100,000 population (CDC 2012). In the European Union the notification rate for salmonellosis was 20.7 cases per 100,000 population in 2011 (ranging from 1.6–80.7 cases per 100,000 population between countries). This was a 5.4% decrease in the number of cases from 2010 (EFSA 2013).

Outbreaks attributed to *Salmonella* spp. have predominantly been associated with animal products such as eggs, poultry, raw meat, milk and dairy products, but also include fresh produce, spices, salad dressing, fruit juice, peanut butter and chocolate (CDC 2010; Jay et al. 2003; Montville and Matthews 2005).

Occurrence in food

The primary reservoir of *Salmonella* spp. is the intestinal tract of warm and cold-blooded vertebrates, with many animals showing no sign of illness. Unlike diseased animals which can be removed from production and/or treated, these asymptomatic (carrier) animals can shed large numbers of *Salmonella* spp. in their faeces and are therefore an important source of contamination. Faecal shedding of *Salmonella* spp. leads to contamination of the surrounding environment including soil, crops, plants, rivers and lakes. A wide range of foods have been implicated in foodborne salmonellosis, particularly those of animal origin and foods that have been subject to sewage pollution (ICMSF 1996; Jay et al. 2003).

At the time of slaughter, *Salmonella* infected animals may have high numbers of organisms in their intestines as well as on the outside of the animal (faecal contamination of hides, fleece, skin or feathers) (Bryan and Doyle 1995; Jay et al. 2003). In Australia, *Salmonella* spp. have been isolated from 3% of chilled cattle carcass samples (n=100) (Fegan et al. 2005). The distribution of *Salmonella* spp. on contaminated meat carcasses is not uniform. For example, a US study by Stopforth et al. (2006) found that the prevalence of *Salmonella* spp. on fresh beef ranged from 0.8% (rib eye roll, n=133) to 9.6% (strip loins, n=52) depending on the cut of meat. Cross-contamination during processing may also lead to increased prevalence of *Salmonella* in finished products (Bryan and Doyle 1995).

Salmonella spp. have been detected in a range of foods. The prevalence of *Salmonella* spp. in bulk tank milk internationally is 0–11.8% (FSANZ 2009a). In shellfish (mussels, clams, oysters and cockles) collected off the coast of Spain, *Salmonella* spp. were detected in 1.8% samples (n=2980) (Martinez-Urtaza et al. 2003). Boughton et al. (2004) isolated *Salmonella* spp. from 2.9% of retail pork sausages samples in Ireland (n=921), and in Spain *Salmonella* spp. were detected in 2% of cooked ham samples (n=53) and 11.1% of cured dried pork sausage samples (n=81) (Cabedo et al. 2008).

An Australian survey found 43.3% of chicken meat at retail (n=859) was positive for *Salmonella* spp. The most prevalent serovar was *S.* Sofia, with 30.5% of chicken meat samples positive for this serovar (Pointon et al. 2008). Although *S.* Sofia accounted for a large proportion of isolates, it appears to be a non-virulent serovar and has been rarely associated with human or animal illness (Gan et al. 2011). The predominance of *S.* Sofia in poultry is unique to Australia as *S.* Sofia is essentially geographically isolated to Australia (Mellor et al. 2010).

S. Enteritidis (in particular phage type 4) is a globally important *Salmonella* serotype that can infect the reproductive tract of poultry and contaminate the internal contents of eggs. However, it is not endemic in Australian egg layer flocks (FSANZ 2009b).

Host factors that influence disease

People of all ages are susceptible to *Salmonella* spp. infection. However, the elderly, infants and immunocompromised individuals are at a greater risk of infection and generally have more severe symptoms (Jay et al. 2003; FDA 2012).

Dose response

Human feeding trials were undertaken during the 1950s to determine the relationship between the dose of *Salmonella* spp. ingested and whether illness occurred. These studies showed that ingestion of $1.3 \times 10^5 – 2.4 \times 10^7$ organisms could cause illness; however, for some strains 1×10^{10} organisms were required for illness to occur (McCullough and Eisele 1951a; McCullough and Eisele 1951b; McCullough and Eisele 1951c; McCullough and Eisele 1951d). However, there are a number of limitations on the use of this feeding trial data. Firstly, the volunteers selected were all healthy adult males, so the results may underestimate the risk to the overall population. Secondly, low doses which are more likely to exist in real food contamination events were not considered (Kothary and Babu 2001; Bollaerts et al. 2008). Investigations of salmonellosis outbreaks have estimated a wide range in the dose of organisms that has caused disease. Ranges reported vary from <10 to 10^9 depending on the food. As such, doses resulting in illness may be much lower than those reported in the feeding trials (Todd et al. 2008). The WHO/FAO (2002) developed a dose-response model based on outbreak data and estimated a 13% probability of illness from consumption of 100 *Salmonella* organisms.

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Appendix 10 – Shiga toxin-producing *Escherichia coli* (STEC)[32]

Shiga toxin-producing *Escherichia coli* (STEC)

Escherichia coli are bacteria that form part of the normal gut flora of humans and other warm-blooded animals. Although most *E. coli* are considered harmless, certain strains can cause severe illness in humans, particularly Shiga toxin-producing *E. coli* (STEC). Infection with STEC is the main cause of haemolytic uraemic syndrome, a condition which can be fatal in humans.

Description of the organism

E. coli are Gram-negative, rod-shaped bacteria and are members of the family *Enterobacteriaceae*. Other species of the genus *Escherichia* include *E. adecarboxylata, E. blattae, E. fergusonii, E. hermanii* and *E. vulneris* (Meng and Schroeder 2007).

Pathogenic *E. coli* are classified into specific groups based on the mechanisms by which they cause disease and clinical symptoms. These categories include enterohaemorrhagic *E. coli* (EHEC), enteroaggregative *E. coli* (EAEC), enteroinvasive *E. coli* (EIEC), enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC) and diffusely adhering *E. coli* (DAEC) (Montville and Matthews 2005). STEC are Shiga toxin-producing *E. coli*, also known as verocytotoxin-producing *E. coli* (VTEC). The STEC strains that cause haemorrhagic colitis (bloody diarrhoea) belong to the EHEC group of pathogenic *E. coli* (Yoon and Hovde 2008). In developed countries EHEC is the most serious of the pathogenic *E. coli*, however, in developing countries EPEC is a major disease causing agent in children (Meng and Schroeder 2007; Ochoa et al. 2008).

Strains of *E. coli* can be characterised serologically based on the detection of specific O (somatic), H (flagella) and K (capsule) antigens. For most *E. coli* strains the O and H antigens are sufficient to identify the strain. For example, *E. coli* O157:H7 is the leading cause of STEC infections internationally (Meng and Schroeder 2007; Gyles 2007).

Growth and survival characteristics

The growth and survival of *E. coli* depends on a number of environmental factors such as temperature, pH, water activity (a_w) and the composition of the food (refer to Table A10-1).

The temperature range for growth of *E. coli* is 7–8°C to 46°C, with an optimum temperature of 35–40°C (ICMSF 1996). Heat resistance of *E. coli* in food is dependent on the composition, pH and a_w of the food. The heat resistance of *E. coli* increases as the a_w decreases. Also, *E. coli* is more resistant to heat when it is in its stationary phase of growth compared to its log phase of growth (Desmarchelier and Fegan 2003). Low temperature has little effect on *E. coli* survival. Strawn and Danyluk (2010) showed that *E. coli* O157:H7 was able to survive on mangoes and papayas stored at -20°C for at least 180 days.

E. coli grow in a broad pH range of 4.4–10.0, with an optimum pH of 6–7 (Desmarchelier and Fegan 2003). A study by Molina et al. (2003) demonstrated that STEC are tolerant to acidic conditions, with many STEC strains able to survive at pH 2.5–3.0 for over 4 hours. *E. coli* O91:H21 was able to survive at pH 3.0 for more than 24 hours. Arnold and Kaspar (1995) found that *E. coli* O157:H7 is more tolerant to acid when it is in stationary growth phase or starved during its log-phase of growth. Therefore STEC may be able to survive and grow in food products previously considered too acidic to support the survival of foodborne pathogens. The effect of pH on *E. coli* survival, however, is dependent on the type of acid present. For example, *E. coli* O157:H7 can survive in a growth medium adjusted to pH 4.5 with hydrochloric acid but not when adjusted to the same pH with lactic acid (ICMSF 1996).

The minimum a_w required for growth of *E. coli* is 0.95. In sub-optimal temperature or pH conditions, a higher a_w value is required for growth of *E. coli* (Desmarchelier and Fegan 2003).

E. coli are facultative anaerobic organisms so do not require oxygen for growth. However, they grow better in aerobic conditions (Meng and Schroeder 2007).

Table A10-1. Limits for growth of *E. coli* when other conditions are near optimum (ICMSF 1996; Desmarchelier and Fegan 2003)

	Minimum	Optimum	Maximum
Temperature (°C)	7–8	35–40	46
pH	4.4	6–7	10.0
Water activity	0.95	0.995	–

Symptoms of disease

Infection with STEC can result in no clinical symptoms (asymptomatic infection) or can cause diarrhoea (may progress to bloody diarrhoea), abdominal cramps, vomiting and fever. The onset of illness is 3–8 days (median of 3–4 days). Most patients recover within 10 days of the initial onset of symptoms (Meng and Schroeder 2007; WHO 2011). In some cases, patients develop haemolytic uraemic syndrome (HUS). HUS is characterised by haemolytic anaemia, thrombocytopenia (decrease in blood platelets) and kidney failure. HUS can also have neurological effects and cause seizures, stroke and coma (WHO 2011). Approximately 6.3% of STEC infected individuals develop HUS, with a fatality rate of 4.6%. Children are more susceptible, with 15.3% of children under five years of age developing HUS following STEC infection (Gould et al. 2009).

STEC are shed in the faeces of infected individuals for several weeks. In children the median shedding time is 13 days (range of 2–62 days) for individuals with diarrhoea. In people who develop HUS, the median shedding time is 21 days (range 5–124 days) (Meng and Schroeder 2007; Pennington 2010).

Virulence and infectivity

STEC strains produce two types of Shiga toxins (Stx1 and Stx2). Stx1 is virtually identical to the toxin produced by *Shigella dysenteriare* serotype 1. The presence of Stx2 is significantly associated with human disease (Spears et al. 2006). Stx are toxic to Vero cells (African green monkey kidney cells) and so are also known as verotoxins (VT). The term STEC is used interchangeably with VTEC. In the laboratory, Vero cells can be used to detect Stx activity, as Stx causes Vero cell death (Desmarchelier and Fegan 2003; Meng and Schroeder 2007).

Due to the acid resistance of STEC, when ingested it is able to survive in the stomach environment and attach to the cells of the intestine. Some STEC strains form a characteristic attaching and effacing lesion on the intestinal cells. The presence of these lesions is a risk factor for the development of HUS (Gyles 2007). Stx produced by STEC is able to bind to specific receptors on susceptible host cells, resulting in the death of these cells. Vascular endothelial cells are a primary target for Stx. Hence production of sufficient Stx results in damage to the blood vessels in the colon and subsequent bloody diarrhoea. If sufficient Stx is taken up by the blood and circulated through the body, this can lead to impaired kidney and neurological function and the development of HUS (Desmarchelier and Fegan 2003; Gyles 2007).

Mode of transmission

STEC are transmitted by the faecal-oral route by either consumption of contaminated food or water, from direct contact with infected animals or via person-to-person contact. It is estimated that 85% of STEC infections are transmitted by food (Meng and Schroeder 2007; Gyles 2007).

Incidence of illness and outbreak data

Infection with STEC is a notifiable disease in all Australian states and territories. The incidence of STEC infections notified in Australia in 2012 was 0.5 cases per 100,000 population (112 cases), which includes both foodborne and non-foodborne cases. This is the same as the previous 5 year mean of 0.5 cases per 100,000 population per year (ranging from 0.4–0.6 cases per 100,000 population per year) (NNDSS 2013). *E. coli* O157 was the most common STEC identified in Australia in 2010 (58.8% of cases), the next most common was *E. coli* O111. There was 1 case of STEC-associated HUS reported in Australia in 2010 (OzFoodNet 2012). Notified cases of STEC infection are influenced by different jurisdictional practices. South Australia routinely tests all bloody stools for STEC via PCR and subsequently they have the highest notification rate in the country (2.8 cases per 100,000 population compared to 0.0–1.4 cases per 100,000 population for the other jurisdictions in 2012) (OzFoodNet 2012; NNDSS 2013).

The notification rate for STEC in New Zealand in 2011 was 3.5 cases per 100,000 population (154 cases). This was a slight increase from the 2010 rate of 3.2 cases per 100,000 population (Lim et al. 2012).

In the United States (US) the notification rate for STEC in 2010 was 1.78 cases per 100,000 population. This was a slight increase from the 2009 rate of 1.53 cases per 100,000 population (CDC 2012). In the European Union there were 1.93 cases of STEC infection per 100,000 population in 2011 (ranging from 0–6.80 cases per 100,000 population between countries). This was a 159.4% increase in the number of cases from 2010 due to the *E. coli* O104:H4 outbreak that affected nearly 4,000 people (EFSA 2013).

The incidence of STEC infections has a seasonal association, with the number of cases increasing during the warmer months. In Australia STEC is most prevalent from November to April (OzFoodNet 2010).

Foods associated with outbreaks of STEC include undercooked ground beef, fresh produce, unpasteurised juices, raw (unpasteurised) milk, uncooked fermented sausage, and raw cookie dough (CDC 2011; Yoon and Hovde 2008; FDA 2012).

Occurrence in food

The major animal reservoir of STEC is ruminants, in particular cattle and sheep (Gyles 2007). Individual animals can carry more than one serotype of STEC (Barlow and Mellor 2010). Meat derived from these animals may become contaminated with STEC organisms if the meat is exposed to faecal material during processing. A study of faecal samples from Australian beef cattle showed 10% of samples (n=300) were STEC positive, with *E. coli* O157 isolated from 1.7% of all samples (Barlow and Mellor 2010). Barlow et al. (2006) isolated STEC from 16% of ground beef (n=285) and 40% of lamb cuts (n=275) sampled in Australia, although the serotypes isolated were not associated with reported human cases in Australia. The detection of STEC at a substantially higher rate in lamb is consistent with the higher concentration and prevalence of *E. coli* on sheep carcasses compared to beef carcasses (Phillips et al. 2001a; Phillips et al. 2001b). The reported prevalence of STEC in bulk tank milk internationally is 0–33.5% (FSANZ 2009).

STEC outbreaks have occurred due to the consumption of fruits and vegetables. Fresh produce may be contaminated due to irrigation with contaminated water or the use of soil treated with farm effluent (Fremaux et al. 2008). The presence of STEC on seafood and poultry at retail may be due to cross-contamination or harvesting seafood from contaminated waters (Desmarchelier and Fegan 2003). STEC has been found to survive for months in soil and manure. It can survive for long periods of time in water and has been isolated from ponds, streams, wells and water troughs. Waterborne transmission of STEC has been reported, both from contaminated drinking water and from recreational water (e.g. swimming) (Fremaux et al. 2008; WHO 2011).

Host factors that influence disease

People of all ages are susceptible to infection with STEC. However, the young and the elderly are more susceptible and are more likely to develop more serious symptoms (FDA 2012).

Dose response

The dose response relationship for STEC is complicated by the number of serotypes and the association of STEC with a variety of foods. The infective dose of *E. coli* O157:H7 is estimated to be very low, in the range of 10–100 cells. The infective dose of other STEC serotypes is suspected to be slightly higher (FDA 2012).

Dose response models have been developed for *E. coli* O157:H7. Teunis et al. (2004) used data from an *E. coli* O157:H7 outbreak at a school in Japan to estimate the dose required to cause disease. In children the estimated ingested dose was 31 organisms, with 25% of exposed children becoming ill. In adults the estimated ingested dose was 35 organisms, with 16% of exposed adults becoming ill.

Haas et al. (2000) used data from a prior animal study undertaken by Pai et al. (1986) and validated their model by comparison with two human outbreaks, one foodborne and the other waterborne, that occurred in the US. This model estimated that the dose required for 50% of the exposed population to become ill was 5.9×10^5 organisms. The corresponding probability of illness for the ingestion of 100 organisms was 2.6×10^{-4}.

Human feeding trial data has been used to generate a dose response model for *E. coli* serotypes other than *E. coli* O157:H7 (*E. coli* O111 and O55) (Haas et al. 2000). The model estimated the dose required for 50% of the exposed population to become ill was 2.55×10^6 and the probability of illness for ingestion of 100 organisms was 3.5×10^{-4}.

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Appendix 11 – Studies reporting the efficacy of commonly used water-based sanitisers for inactivating the in-scope hazards on the surface of in-scope commodities

Produce	Organism	Attachment time	Initial Log$_{10}$CFU numbers	Treatment applied	Contact time (min)	Log$_{10}$CFU reduction	Reference
Chlorine (hypochlorite)							
Rockmelon	*L. monocytogenes*	24h	3.5	1000ppm FC (pH 6.4)	2	>3.2	Ukuku and Fett (2002b)
Rockmelon	*L. monocytogenes*	24h	~6.0	100ppm TC (pH 7.0)	2 - 5	2.0 - >5	Rodgers et al. (2004)
Rockmelon	*L. monocytogenes*	24h	~6.0	200ppm TC (pH 7.0)	2 - 5	2.2 - >5	Rodgers et al. (2004)
Rockmelon	*L. monocytogenes*	18h	4.0	200ppm FC (pH 7.0)	8 - 10	0.8 - >2.7	Webb et al. (2015)
Rockmelon	*L. monocytogenes*	ON	7.6	200ppm FC (pH NR)	5	1.9	Singh et al. (2018b)
Rockmelon	*L. monocytogenes*	2h	5.6	200ppm TC (pH NR)	5	0.9	Upadhyay et al. (2016)
Rockmelon	*Salmonella*	2h	5.6	200ppm TC (pH NR)	5.0	0.5	Upadhyay et al. (2016)
Rockmelon	*S.* Typhimurium	ON	6.6	200ppm FC (pH NR)	5.0	3.8	Singh et al. (2018b)
Rockmelon	*S.* Typhimurium	1.5h	6.0	200ppm TC (pH 7.2) (soaking alone)	1.0	1.8	Parnell et al. (2005)
Rockmelon	*S.* Typhimurium	1.5h	6.0	200ppm TC (pH 7.2) (with scrubbing)	1.0	2.8	(Parnell et al. 2005)
Rockmelon	*S.* Stanley	1h	3.8	1000ppm FC (pH 7.2)	5	3.0	Ukuku and Sapers (2001)
Rockmelon	4 *Salmonella* serovar cocktail	1h	6.9	200ppm FC (pH 6.4)	2	2.7	Ukuku (2006)
Rockmelon	*S.* Poona	12h	4.8	200ppm FC (pH 6.5)	3	0.1	Vadlamudi et al. (2012)
Lettuce	*E. coli*	6h	6.4	100ppm FC (pH NR)	15	2.9	Karaca and Velioglu (2014)
Lettuce	*E. coli*	15 min	~4.0	100 ppm FC (pH NR)	5	1	Francis and O'Beirne (2002)
Lettuce	*E. coli*	6h	6.4	100ppm FC (pH NR)	15	2.9	Karaca and Velioglu (2014)
Lettuce	*E. coli* O157: H7	2 min	~8.0	300ppm and 600ppm (pH 10, free/total chlorine NR)	3	1.5 - 1.8	Niemira and Cooke (2010)
Lettuce	*E. coli* O157: H7	30min	~8.0	300ppm and 600ppm (pH 9.77, 9.99) free/total chlorine NR)	3	<1.0	Niemira (2007)
Lettuce	*E. coli* O157: H7	ON	4.9	100 ppm FC (pH 6.5)	1	0.5	López-Gálvez et al. (2010)
Lettuce	*E. coli* O157:H7	24h	7.1 - 8.7	200ppm FC (pH 6.8)	5	1.2 - 1.5	Lang et al. (2004)
Lettuce	*E. coli* O157:H7	ON	6.9	100ppm FC (pH NR)	5	2.1	Singh et al. (2018b)
Lettuce	*L. innocua*	6h	6.5	100ppm FC (pH NR)	15	2.3	Karaca and Velioglu (2014)
Lettuce	*L. innocua*	15min	~4.0	100 ppm FC (pH NR)	5	1.5	Francis and O'Beirne (2002)
Lettuce	*L. monocytogenes*	NR	3.0 - 5.0	200ppm (pH 7, free/total NR)	10	0.30 - 0.71	Omac et al. (2017)
Lettuce	*L. monocytogenes*	24h	6.9 - 8.9	200ppm FC (pH 6.8)	5	1.2 - 1.5	Lang et al. (2004)
Lettuce	*L. monocytogenes*	ON	7.7	100ppm FC (pH NR)	5	1.7	Singh et al. (2018b)

Produce	Organism	Attachment time	Initial Log$_{10}$CFU numbers	Treatment applied	Contact time (min)	Log$_{10}$CFU reduction	Reference
Lettuce	*S.* Baildon	25min	3.6	120 - 200 ppm FC (pH 6.8)	40s	<1.0	Weissinger et al. (2000)
Lettuce	*Salmonella*	24h	7.4 - 8.9	200ppm FC (pH 6.8)	5	1.4 - 1.9	Lang et al. (2004)
Lettuce	*Salmonella*	24h	~7.0	10 – 20ppm FC (pH NR)	1 - 3	0.7 - 1.4	Lippman et al. (2020)
Lettuce	*Salmonella* spp.	20min - 4h	6.1	200 ppm (pH NR, free/total NR)	3	1.9	Hadjok et al. (2008)
Parsley	*E. coli*	6h	6.6	100ppm FC (pH NR)	15	3	Karaca and Velioglu (2014)
Parsley	*E. coli*	6h	6.5	100ppm (pH NR, free/total NR)	5	3.2	Karaca and Velioglu (2020)
Parsley	*E. coli* O157:H7	24h	6.9 - 8.9	200ppm FC (pH 6.8)	5	2.2 - 3.4	Lang et al. (2004)
Parsley	*L. innocua*	6h	6.6	100ppm FC (pH NR)	15	2.2	Karaca and Velioglu (2014)
Parsley	*L. innocua*	6h	6.5	100ppm (pH NR, free/total NR)	5	1.6	Karaca and Velioglu (2020)
Parsley	*L. monocytogenes*	24h	6.7 - 9.0	200ppm FC (pH 6.8)	5	2.2 - 3.4	Lang et al. (2004)
Parsley	*S.* Typhimurium	1h	7	100ppm (pH NR, free/total NR)	5	1.7	Lapidot et al. (2006)
Parsley	S. Typhimurium	1h	7	200ppm (pH NR, free/total NR)	5	2	Lapidot et al. (2006)
Parsley	S. Typhimurium	1h	7	800ppm (pH NR, free/total NR)	5	2.5	Lapidot et al. (2006)
Parsley	S. Typhimurium	1h	7	1600ppm (pH NR, free/total NR)	5	3	Lapidot et al. (2006)
Parsley	*Salmonella* spp.	24h	6.2 - 8.9	200ppm FC (pH 6.8)	5	1.6 - 4.0	Lang et al. (2004)
Spinach	*E. coli*	6h	6.4	100ppm FC (pH NR)	15	2.7	Karaca and Velioglu (2014)
Spinach	*E. coli*	30min	6.8	100 ppm FC (pH 9.8)	3	2	Rahman et al. (2010)
Spinach	*E. coli* O157: H7			100 ppm	5	1.3	Nei et al. (2009)
Spinach	*E. coli* O157: H7	20min - 4h	6.1	200 ppm (pH NR, free/total NR)	3	0.5	Hadjok et al. (2008)
Spinach	*E. coli* O157: H7	1h	5.4	100 ppm (pH NR, free/total NR)	5	1.1	Lee and Baek (2008)
Spinach	*E. coli* O157: H7	2min	~8.0	300ppm and 600 ppm (pH 10, free/total chlorine NR)	3	1.3 - 1.3	Niemira and Cooke (2010)
Spinach	*E. coli* O157: H7	30min	~8.0	300ppm and 600ppm (pH 9.77, 9.99) free/total chlorine NR)	3	<1.0	Niemira (2007)
Spinach	*E. coli* O157: H7	2h	6.9	200 ppm FC (pH 6.5)	0.5	1	Neal et al. (2012)
Spinach	*L. innocua*	6h	6.4	100ppm FC (pH NR)	15	2.2	Karaca and Velioglu (2014)
Spinach	*L. monocytogenes*	30min	7	100 ppm FC (pH 9.8)	3	2.2	Rahman et al. (2010)
Spinach	*S.* Typhimurium	5h	5	200 ppm (pH 7.0, free/total chlorine NR)	5	1.2	Puerta-Gomez et al. (2013b)

Produce	Organism	Attachment time	Initial Log$_{10}$CFU numbers	Treatment applied	Contact time (min)	Log$_{10}$CFU reduction	Reference
Spinach	*Salmonella spp.*	2h	7	200 ppm FC (pH 6.5)	30s	0.7	Neal et al. (2012)
Spinach	*Salmonella spp.*	20min - 4h	6.4	200ppm (pH NR, free/total NR)	3	0.5	Hadjok et al. (2008)
Blueberry	HAV	1h	~6.0	200ppm FC (pH NR)	30s	2.4	Butot et al. (2008)
Blueberry	Norovirus	1h	~6.0	200ppm FC (pH NR)	30s	3.0 - 3.4	Butot et al. (2008)
Raspberry	HAV	1h	~6.0	200ppm FC (pH NR)	30s	0.6	Butot et al. (2008)
Raspberry	Norovirus	1h	~6.0	200ppm FC (pH NR)	30s	0.9	Butot et al. (2008)
Strawberry	*E. coli* O157:H7	1h	~6.0	50ppm (pH NR)	2	1.6	Zhou et al. (2017)
Strawberry	HAV	1h	~6.0	200ppm FC (pH NR)	30s	1.8	Butot et al. (2008)
Strawberry	HAV	1h	~4.0	50ppm (pH NR)	2	3.4	Zhou et al. (2017)
Strawberry	*L. monocytogenes*	1h	~6.0	50ppm (pH NR)	2	1.6	Zhou et al. (2017)
Strawberry	Norovirus	1h	~6.0	200ppm FC (pH NR)	30s	1.4 - >3.0	Butot et al. (2008)
Strawberry	Norovirus	1h	~7.0	50ppm (pH NR)	2	1.5	Zhou et al. (2017)
Strawberry	*Salmonella* spp.	1h	~6.0	50ppm (pH NR)	2	1.6	Zhou et al. (2017)
Chlorine dioxide							
Rockmelon	*L. monocytogenes*	24h	~6.0	3ppm	2 – 5	2.4 to >5	Rodgers et al. (2004)
Rockmelon	*L. monocytogenes*	24h	~6.0	5ppm	2 – 5	2.9 to >5	Rodgers et al. (2004)
Rockmelon	*L. monocytogenes*	18h	4.4	3ppm	8	-0.8	Webb et al. (2015)
Lettuce	*E. coli* O157: H7	ON	5.4	3ppm	1	0.5	Lopez-Galvez et al. (2010)
Lettuce	*E. coli* O157:H7	18-24h	7.2	200 ppm	2	0.4 - 1.5	Keskinen et al. (2009)
Lettuce	*E. coli* O157:H7	NA	5.5	50 - 200 ppm	1 - 10	2.3	Choi and Lee, 2008
Lettuce	*E. coli* O157:H7	1h	7.9	10 ppm	5	1.2	Singh et al., 2002a
Lettuce	*L. monocytogenes*	NA	4.5	200 ppm	1 - 10	2.0	Choi and Lee, 2008
Lettuce	*S.* Typhimurium	NA	5.1	200 ppm	1 - 10	1.8	Choi and Lee, 2008
Spinach	*E. coli* O157: H7	1h	5.4	100 ppm	5	2.6	Lee and Baek (2008)
Spinach	*S.* Typhimurium	NR	4.9	20ppm	1	1.6	Thi-Van et al. (2019)
Spinach	*S.* Typhimurium	NR	4.9	2ppm	1	0.7	Thi-Van et al. (2019)
Raspberry	Norovirus	1h	~6.0	5 - 10ppm	10	0.6 - 1.0	Butot et al. (2008)
Raspberry	HAV	1h	~6.0	5 - 10ppm	10	0.7 - 0.9	Butot et al. (2008)
PAA							
Rockmelon	*L. monocytogenes*	24h	6.0	80ppm	2 to 5	1.4 to >5	Rodgers et al. (2004)
Rockmelon	*L. innocua*	76h	3.9	30ppm	1 s	1.9	Suslow and Callejas (2015)
Rockmelon	*L. monocytogenes*	ON	7.6	85ppm	5	3.0	Singh et al. (2018b)
Rockmelon	*L. monocytogenes*	ON	7.6	45ppm	5	3.0	Singh et al. (2018b)
Rockmelon	*L. monocytogenes*	ON	7.6	100ppm	5	4.5	Singh et al. (2018b)
Rockmelon	*S.* Typhimurium	ON	6.6	100ppm	5	4.5	Singh et al. (2018b)

Produce	Organism	Attachment time	Initial Log$_{10}$CFU numbers	Treatment applied	Contact time (min)	Log$_{10}$CFU reduction	Reference
Rockmelon	*S. Typhimurium*	ON	6.6	85ppm	5	4.2	Singh et al. (2018b)
Rockmelon	*S. Typhimurium*	ON	6.6	45ppm	5	3.6	Singh et al. (2018b)
Rockmelon	*Salmonella* spp.	76h	3.5	30ppm	1 s	2.1	Suslow and Callejas (2015)
Lettuce	*E. coli* O157:H7	24h	~6.0	50ppm	1.5	1.7	Davidson et al. (2017)
Lettuce	*E. coli* O157:H7	0.5 - 1h	~3.0	40ppm + Lactic Acid (1143 mg/L)	1	< 0.5	van der Linden et al. (2016)
Lettuce	*E. coli* O157:H7	ON	6.9	45 – 100ppm	5	1.8 - 2.2	Singh et al. (2018b)
Lettuce	*L. monocytogenes*	ON	7.7	45 – 100ppm	5	2.0 - 2.4	Singh et al. (2018b)
Lettuce	*S.* Typhimurium	0.5 - 1h	~3.0	40ppm + Lactic Acid (1143 mg/L)	1	< 0.5	van der Linden et al. (2016)
Lettuce	*S.* Typhimurium	0.5	7.5	50ppm	5	2.4	Silveira et al. (2018)
Lettuce	*Salmonella* spp.	24h	~7.0	40 – 80ppm	2	1.3 – 1.5	Lippman et al. (2020)
Spinach	*E. coli* O157:H7	1h	7.1	80 ppm	2	2.2	Zhou et al. (2009)
Spinach	*S.* Typhimurium	NR	4.9	50 - 75 ppm	1	1	Thi-Van et al. (2019)
Aqueous ozone							
Rockmelon	*L. monocytogenes*	18h	6.0	3ppm	2 to 5	3.0 to >5	Rodgers et al. (2004)
Rockmelon	S. Poona	12h	4.8	3ppm	5.0	2.5	Vadlamudi et al. (2012)
Lettuce	*E. coli* O157:H7	1h	7.9	10 ppm	5	1.1	Singh et al. (2002)
Lettuce	*L. innocua*	6h	6.5	12ppm	15min	2.1	Karaca and Velioglu (2014)
Lettuce	*Salmonella* spp.	24h	6.4	0.5 – 1.5 ppm	3 - 10	1.2 – 3.1	Sengun (2013)
Lettuce	*E. coli*	6h	6.4	12ppm	15min	2.1	Karaca and Velioglu (2014)
Parsley	*E. coli*	6h	6.5	12ppm	5	2.2	Karaca and Velioglu (2020)
Parsley	*E. coli*	6h	6.6	12ppm	15min	2.2	Karaca and Velioglu (2014)
Parsley	*L. innocua*	6h	6.5	12ppm	5	1.6	Karaca and Velioglu (2020)
Parsley	*L. innocua*	6h	6.6	12ppm	15min	2.2	Karaca and Velioglu (2014)
Parsley	*Salmonella* spp.	24h	5.4	0.5 – 1.5 ppm	3 - 10	0.3 – 2.6	Sengun (2013)
Spinach	*L. innocua*	6h	6.4	12ppm	15min	2.1	Karaca and Velioglu (2014)
Spinach	*E. coli*	6h	6.4	12ppm	15min	1.7	Karaca and Velioglu (2014)
EOW							
Rockmelon	*L. monocytogenes*	ON	6.6	AEW, pH 2.5, 100ppm chlorine	5	1.7	Singh et al. (2018)
Rockmelon	*L. monocytogenes*	ON	7.6	NNEW, pH 6.2, 100ppm chlorine	5	2.1	Singh et al. (2018)
Rockmelon	*S.* Typhimurium	ON	6.6	AEW, pH 2.5, 100ppm chlorine	5	2.3	Singh et al. (2018)

Produce	Organism	Attachment time	Initial Log_{10}CFU numbers	Treatment applied	Contact time (min)	Log_{10}CFU reduction	Reference
Rockmelon	S. Typhimurium	ON	7.6	NNEW, pH 6.2, 100ppm chlorine	5	3.7	Singh et al. (2018)
Lettuce	*E. coli* O157:H7	1h	7.1	AEW, pH 2.5, 45ppm chlorine	1 - 3	4.2	Park et al. (2001)
Lettuce	*E. coli* O157:H7	ON	6.9	AEW, pH 2.5, 100ppm chlorine	5	2.1	Singh et al. (2018b)
Lettuce	*E. coli* O157:H7	ON	6.9	NNEW, pH 6.2, 100ppm chlorine	5	2.3	Singh et al. (2018b)
Lettuce	*E. coli* O157:H7	18-24h	7.2	AEW, pH 2.6, 50ppm chlorine	2 – 20min	0. – 1.5	Keskinen et al. (2009)
Lettuce	*E. coli* O157:H7	24h	~6.0	AEW and NNEW, pH 4 – 9, 300ppm chlorine	5	1.3 – 2.2	Yang et al. (2003)
Lettuce	*L. monocytogenes*	1h	7.0	AEW, pH 2.5, 45ppm chlorine	1 - 3	3.9 – 4.4	Park et al. (2001)
Lettuce	*L. monocytogenes*	24h	~6.0	AEW and NNEW, pH 4 – 9, 300ppm chlorine	5	1.6 – 2.1	Yang et al. (2003)
Lettuce	*L. monocytogenes*	ON	7.7	AEW, pH 2.5, 100ppm chlorine	5	1.7 – 2.0	Singh et al. (2018b)
Lettuce	*L. monocytogenes*	ON	7.7	NNEW, pH 6.2, 100ppm chlorine	5	1.7 – 2.0	Singh et al. (2018b)
Lettuce	*S.* Typhimurium	24h	~6.0	AEW and NNEW, pH 4 – 9, 300ppm chlorine	5	1.5 – 2.0	Yang et al. (2003)
Spinach	*S.* Typhimurium	NR	4.9	NNEW, pH 6.5 – 6.3, 20 – 60ppm chlorine	1	0.9 – 1.0	Thi-Van et al. (2019)
Spinach	*E. coli* O157:H7	1h	7.1	AEW, pH 2.7, free chlorine 45ppm	2	2.2	Zhou et al., (2009)

TC = Total chlorine
FC = Free chlorine
ON = Overnight
NR = Not reported
NA = Not available
AEW = Acidic electrolysed water
NNEW = Near neutral electrolysed water

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Appendix 12 – Studies reporting the efficacy of hot water for removing in-scope hazards from rockmelons

Produce	Organism	Attachment time	Initial Log$_{10}$CFU numbers	Treatment applied	Contact time (min)	Log$_{10}$CFU reduction	Reference
Rockmelon	*L. innocua*	76h	3.9	65 ˚C Water	0.75	3.4	Suslow and Callejas (2015)
Rockmelon	*L. innocua*	76h	3.9	65˚C Water + 30ppm PAA spray	0.75	2.7	Suslow and Callejas (2015)
Rockmelon	*L. monocytogenes*	76h	3.9	80˚C Water	5.0	> 3.3	Ukuku et al. (2016b)
Rockmelon	*S.* Poona	24h	6.5	82˚C Water	1 and 1.5	2.7 and 5.2	Annous et al. (2013)
Rockmelon	*S.* Poona	24h	6.5	92˚C Water	1 and 1.5	3.0 and 6.1	Annous et al. (2013)
Rockmelon	*S.* Poona	24h	7.0	82˚C Water	1 and 1.5	5.5 and 5.8	Annous et al. (2013)
Rockmelon	*S.* Poona	24h	7.0	92˚C Water	1 and 1.5	5.1 and 5.9	Annous et al. (2013)
Rockmelon	*Salmonella* spp.	24h	5.4	85˚C Water	1 and 1.5	4.7 and 4.5	Solomon et al. (2006)
Rockmelon	*Salmonella* spp.	1h	6.9	96˚C Water	2	4.6	Ukuku and Feet (2006)
Rockmelon	*Salmonella* spp.	76h	3.5	65˚C Water	0.75	3.3	Suslow and Callejas (2015)
Rockmelon	*Salmonella* spp.	76h	3.5	65˚C Water + 30ppm PAA spray	0.75	2.8	Suslow and Callejas (2015)

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