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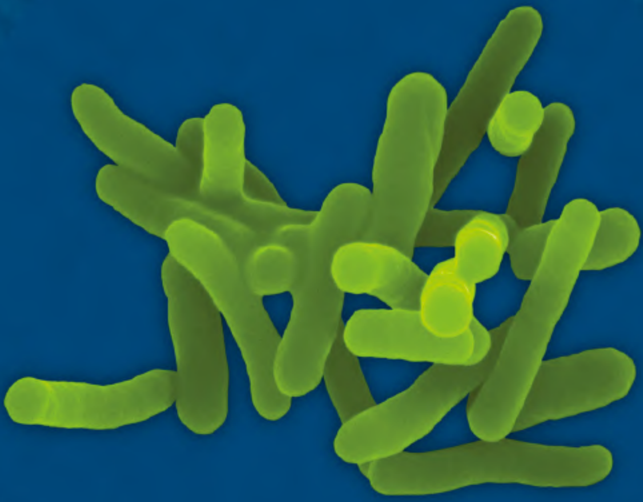
Prevention and control of microbiological hazards in fresh fruits and vegetables Parts 1 & 2: General principles

Meeting report



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MICROBIOLOGICAL RISK
ASSESSMENT SERIES

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Food and Agriculture Organization of the United Nations
World Health Organization

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Abbreviations

CAC	Codex Alimentarius Commission
CCFH	Codex Committee on Food Hygiene
CEA	controlled environment agriculture
FAO	Food and Agriculture Organization of the United Nations
FCS	food contact surfaces
FFV	fresh fruit and vegetables
GAPs	good agricultural practices
GHPs	good hygiene practices
HACCP	hazard analysis and critical control points
JEMRA	Joint FAO/WHO Expert Meetings on Microbiological Risk Assessment
RTE	ready-to-eat
QMEA	quantitative vmicrobial exposure assessment
QMRA	quantitative microbial risk assessment
STEC	shiga toxin-producing *Escherichia coli*
WHO	World Health Organization

Declarations of interests

All participants completed a Declaration of Interests form in advance of their involvement in this work. The interests declared by the participants were not considered by FAO and WHO to present any conflict in light of the objectives of the meeting.

All of the declarations, together with any updates, were made known and available to all the participants at the beginning of the meeting.

All the experts participated in their individual capacities and not as representatives of their countries, governments or organizations.

Ir. Liesbeth Jacxsens, Xiaojun Liao and Julio Isabel Lopez Montes were invited as resource people due to scheduling conflicts. They were not available to participate in the entire meeting, including on the final day for the adoption of the conclusions and recommendations.

Executive summary

Introduction

The purpose of the meetings was to collect, review and discuss relevant measures for control of microbiological hazards from primary production to point of sale in fresh, ready-to-eat (RTE), and minimally processed fruits and vegetables, including leafy vegetables.

The scope of the meetings included aspects of primary production in open fields or in protected facilities (such as high and low tunnels, production under cover, greenhouses and net houses, and address hydroponic and aquaponic systems, and other systems as required) and post-harvest activities, including activities performed prior to packing, minimal processing, distribution, maintenance of the cold chain where applicable, transportation, and handling at point of sale. Emphasis was placed on the identification and evaluation of solutions to reduce microbiological risks that result in foodborne illnesses associated with fresh fruits and vegetables produced in various regions of the world, taking into consideration their effectiveness and suitability.

Fresh, ready-to-eat, and minimally processed fruits and vegetables

Foodborne illness

The experts noted that surveillance and outbreak data from many countries, including developing nations, are generally sparse, if not missing all together. Such data are needed to more accurately assess the burden of illnesses associated with fresh fruits and vegetables and to identify salient causes of contamination for food. All data are useful, including illness, outbreak, and recall data from research or other sources, and effort was placed on gathering this information. The experts sought to update and include any recent trends in commodity and pathogen pairing, and pathogen occurrence and presence.

Microbiological hazards

The experts developed tables to capture information about microbiological hazards, implicated commodities, dates, geographic location, number of illness cases, level of contamination, region and references. It was decided that pathogens would not be ranked by the most prevalent due to the lack of illness and outbreak data for many countries and regions. Effort was focused on collecting information from as many countries as possible to expand the understanding of new, emerging, re-emerging, or neglected pathogens and commodity-pathogen pairings, as well as practices that may be contributing to increased illnesses.

Commodities of concern

The experts concluded that some commodities such as leafy vegetables, herbs, sprouts and cantaloupes (rock melons) remain predominant in produce-associated illnesses but noted that any commodity can become contaminated, as demonstrated by an outbreak involving bulb onions.[1] It was also noted that bias may be introduced by under-reporting from some countries, and that some commodities may not yet be recognized as vehicles for the transmission of foodborne pathogens given limitations to the current foodborne illness surveillance programmes.

Overview of production systems

It is recognized that a range of production systems (e.g. conventional, organic, urban, peri-urban agriculture, less-defined systems) exist in highly diverse geographic regions with varying environmental conditions, fauna, and climate subjected to extreme events and changing patterns due to changing climate. Variable market channels, distribution networks, cultural practices, consumption patterns, and regulatory frameworks influence specific risks that may be associated with each production system. This report provides examples, but it is understood that each grower should employ a food safety management system, including microbiological risk assessments, and plan for extreme weather events relevant to their location. There are universal practices and resources required for food safety, including sanitary facilities, good personal hygiene practices, training and sanitation that are critical to risk reduction, which should be adopted in all production systems. In the field environment, good agricultural practices (GAPs) and good hygiene practices (GHPs) are recommended. As production moves into partially or fully enclosed facilities, good manufacturing practices (GMPs) become practical and as minimal processing occurs, operations should consider application of the hazard analysis and critical control points (HACCP) system.

International production and trade

International trade requirements should be based on validated practices that reduce microbiological risks. Many audit schemes, certifications, private standards

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[1] USFDA. 2021. https://www.fda.gov/food/outbreaks-foodborne-illness/outbreak-investigation-salmonella-oranienburg-whole-fresh-onions-october-2021

and other trade requirements have moved beyond food safety and are less relevant to microbiological risk reduction. It is also important that food grown for domestic markets be produced with food safety practices to reduce risks to local consumers. It is a concern that food safety practices which are prioritized for commodities that enter international trade because of their economic value are abandoned for commodities destined for local or domestic markets. Public health requires that all fresh fruits and vegetables be produced in a safe manner.

Primary production in open fields

Location, adjacent land use, topography, and climate

Climate and weather (including local variability and extreme weather events related to the changing climate), topography, geographic location and adjacent land use can influence the magnitude and frequency of transfer of microbiological hazards from environmental sources to growing crops. Some geographical locations are clearly more at risk from climate-related events. Measures can be applied to mitigate such risks, for example, intercropping, crop rotation, water management through suitable drainage, and the establishment of buffers and barriers. However, the experts recognized that there are significant data gaps, which preclude accurate assessment of both the magnitude of the risks and the efficacy of strategies for their mitigation. The full report will include details about the scope and impact of these factors on the potential transmission of microbiological hazards to fruit and vegetables, including leafy vegetables.

Prior land use and assessment

Use of land for human settlement, animal rearing, industrial activities, open defecation, and sewage and waste disposal introduces microbiological hazards that may persist in the growing environment. The experts noted that it is critical to perform a risk assessment to determine the most appropriate mitigation steps. Mitigation steps could include crop rotation, fallowing, improvements in infrastructure including drainage, and longer-term considerations such as landscape planning. However, there is a lack of data concerning long-term survival of microbiological hazards in land used for purposes other than growing fruit and vegetable crops.

Unintentional contact of crops with contaminated water

Unintentional contact of crops with contaminated water due to numerous causes, such as extreme weather events, which cause flooding from streams, rivers and canals, can introduce microbiological hazards to the production environment.

Not all water that is involved in unintentional contact has the same hazards. Irrigation practices that result in only in-field flooding are different than water from overflowing rivers that, in addition to microbiological hazards, may also contain physical and chemical hazards. This is why there is always a need to assess risks resulting from unintentional contact. Experts also recognized that sometimes water can unintentionally contact crops through equipment malfunction (e.g. broken pipes, sprinkler heads) or overpumping during irrigation. There is insufficient data in this area, but some mitigation strategies can be applied to minimize the occurrence. These include land management practices such as sloping to lessen water intrusion, pumping into deep wells or other underground storage, barrier construction along water bodies and creating drainage channels, trenches or drain tiles.

Wildlife, livestock, and human intrusion

Wildlife, livestock and humans are a part of the environment, but they can also introduce microbiological hazards through faeces as well as distribute microbial hazards via field intrusion. Risk mitigation steps can include practical applications such as harvest buffers and preharvest inspections, and physical interventions such as fences. The experts noted that there are a wide variety of species, cultural practices including integrated livestock and produce production systems, and various human activities that impact risks. Risk assessment must include identifying most likely risks from geographically relevant wildlife, livestock, cultural practices, and human intrusion as well.

Water quality

Water applied to crops can impact the microbiological safety of fruits and vegetables. The experts recognized that water of highly variable microbiological quality is used in crop production, for numerous agricultural activities, during production, handling and/or processing, and it may adversely affect produce safety. Water testing is recommended to assess water quality and to determine if it is fit to use for certain purposes. Once the quality of the water available is known, the most appropriate treatment options and their efficacy and/or the application of multiple barrier processes can be implemented, such as irrigation methods that limit contact with the harvestable portion of the crop.

Soil amendments: animal manures, biosolids and other natural fertilizers

Application of untreated animal and human manures to soil used to grow fruits and vegetables results in significant microbiological risks. Extending the time between application and harvest reduces risks, but best practice is to avoid contact of all soil amendments with the harvestable portion of the crop, minimize runoff into waterways, and avoid crop contamination via dust. Treatment of manures through controlled and validated composting or other processes (e.g. heat treatment) prior to field application will reduce risks.

Harvest, field packing, and packing house packing

This section encompasses an extremely diverse set of practices that could introduce microbiological hazards from humans, food contact surfaces, equipment and water as well as through cross-contamination. Extended time between harvest and consumption, and improper cold storage can increase the opportunity for pathogens that may be present to multiply, thereby increasing the likelihood of illness. The experts acknowledged the global diversity of farms and farming practices. Hazards should be assessed for each farm; however, some control measures should be applied in all settings. Worker education and training, as well as ensuring resources necessary for workers to practice proper personal hygiene are provided and are critical to the proper implementation of food safety practices. All farms should have a sanitation programme to ensure food contact surfaces and equipment do not introduce microbiological hazards. Water that comes into contact with fruits and vegetables at harvest and during post-harvest activities should be fit-for-purpose, with microbiological quality being extremely important for minimizing risks.

Primary production in protected facilities

Fresh fruits and vegetables are grown in a range of protected facilities. The full report provides examples of facilities that are considered protected facilities and summarizes relevant risks and mitigation practices that are unique to each. Protected production systems are not inherently safer than open systems. Protected facility structures should be located, designed and constructed to avoid contamination and harbourage of pests such as insects, rodents and birds. Worker training and sanitation practices are necessary in all operations. Proper water management and soil amendment use are critical to controlling and reducing risks. Use of GAPs and GHPs are recommended. Each operation should assess specific hazards and implement mitigation practices to reduce risks associated with the identified hazards.

Minimally processed

Available research indicates that several practices can be implemented to identify and reduce microbiological risks. Grading/culling and pre-washing showers prior to processing, the use of biocides to maintain the microbiological quality of processing water, and ensuring raw products are separated from final products will reduce cross-contamination during processing. Many data gaps were noted, mostly linked to the use of different biocides and their effectiveness to maintain the microbial quality of water. Concerns have also been raised on the role played by the natural microbiota and microbial attachment and infiltration during minimal processing in the potential colonization of pathogenic microorganisms in fresh produce. Maintaining the cold chain will reduce multiplication of bacterial pathogens that may persist during processing. Good hygiene practices, good manufacturing practices and the HACCP system support hazard identification and implementation of effective control practices. As mentioned previously, sanitation and worker training programmes are critical steps for reducing microbiological risks.

Transport, distribution and point of sale

This section covers all steps from field packing, as well as packing in a building, to the point of sale. The microbiological risks encountered along this chain include the potential for bacterial growth during transport, distribution and at point of sale. Contamination and/or cross-contamination can also occur as a result of improper handling during loading and unloading, comingling and displaying with raw commodities and animals and animal products, and exposure to unsanitary surfaces and water at point of sale. Mitigation strategies include training of operators, produce handlers, and point of sale retailers, as well as the use of clean, enclosed, refrigerated transport vehicles, a clean and sanitary point of sale environment, and fit-for-purpose water for cleaning, sanitizing and cooling. Maintaining cool temperatures will limit growth of bacterial pathogens. Storing produce in cool locations and moving fruits and vegetables quickly to the point of sale when refrigerated storage is not available can reduce risk. Cold storage is not suitable for some fruits and vegetables, as it may cause product deterioration that may result in bacterial growth. There is a need for additional research to further assess the risks and identify practical mitigation strategies that cover the diversity of transport, cold chain, and distribution channels for fresh produce globally.

Significant gaps in mitigation and intervention measures

Fruit and vegetable production includes many different commodities grown in diverse geographic regions that are often distributed globally. It is unlikely there will be sufficient research data to clearly identify all hazards or define practices to reduce all associated risks. Acknowledging these challenges, the experts identified research areas that would be most valuable for study in the full report, including both hazard identification and mitigation interventions.

1

Introduction

1.1. BACKGROUND

Fruits and vegetables are an important part of a healthy diet and are protective against many chronic health conditions. Yet, fresh fruits and vegetables have been consistently implicated in food safety incidents involving microbiological hazards around the globe for decades. Fresh produce contaminated with foodborne pathogens (e.g. bacteria, viruses, protozoa, helminths) have resulted in numerous outbreaks of foodborne disease and trade disruptions (Carstens, Salazar and Darkoh, 2019; Lynch, Tauxe, and Hedberg, 2009; de Oliveira Elias, Decol and Tondo, 2018; Raymond *et al.,* 2022).

The Codex Alimentarius Commission (CAC) initially developed the "Code of Hygienic Practice for Fresh Fruits and Vegetables" in 2003 then later revised it following a JEMRA meeting, held in 2008, to address microbiological hazards associated with leafy vegetables and herbs (FAO and WHO, 2008). Several commodity specific annexes were added to the code of practice in 2012, 2013 and 2017 (FAO and WHO, 2017).

Subsequently, in 2018, FAO and WHO published the report "Shiga toxin-producing *Escherichia coli* (STEC) and food: attribution, characterization and monitoring (MRA31)" (FAO and WHO, 2018a) wherein fresh fruits and vegetables were identified as important sources of STEC infections. In 2019, following a request from the Codex Committee on Food Hygiene (CCFH), the CAC approved new work at its 42nd Session on the development of guidelines for the control of STEC in leafy greens and in sprouts (FAO and WHO, 2018b). Also in 2019, the JEMRA meeting on the Safety and Quality of Water Used in Food Production and Processing highlighted that any water used through the production chain of fruits and vegetables may potentially contain human pathogens, albeit at low concentrations (FAO and WHO, 2021). More recently, in October 2020, a JEMRA meeting on *Listeria monocytogenes* in Ready-to-Eat (RTE) Foods noted increased reports of listeriosis acquired from fresh and minimally processed fruits and vegetables (FAO and WHO, 2022).

To meet the requests of the CCFH and to update and expand the information available in MRA14, FAO and WHO convened a series of expert meetings on preventing and controlling microbiological hazards in fresh fruits and vegetables. The goal of these meetings was to gather recent data and evidence, and to provide scientific opinions on the topic.

1.2. OBJECTIVES

The purpose of the meetings was to collect, review and discuss relevant measures for control of microbiological hazards from primary production to point of sale in fresh, ready-to-eat, and minimally processed fruits and vegetables, including leafy vegetables.

The scope of the meetings included aspects of primary production in open fields, protected facilities (such as high and low tunnels, production under cover, greenhouses and net houses, and address hydroponic and aquaponic systems and other systems as required) and post-harvest activities, including activities performed prior to packing, minimal processing, distribution, maintenance of the cold chain where applicable, transportation, and handling at point of sale. Emphasis was placed on the identification and evaluation of solutions to reduce microbiological risks that result in foodborne illnesses associated with fresh fruits and vegetables produced in various regions of the world, taking into consideration their effectiveness and suitability.

Regulatory expectations and limitations of individual countries were not the focus of the meeting. It is understood that individual country regulations may not align with the definitions provided in this report, but it is expected that the information presented will still be useful and can advance the understanding of hazards and risk mitigation.

The objectives of the meetings included:

- identifying and characterizing fresh fruits and vegetables and microbiological hazard combinations of concern to public health;
- reviewing publicly available literature and guidelines from competent authorities and industry associations (e.g. compliance guidelines, code of

practices) to assess the current state of knowledge regarding the control of microbiological hazards in fresh fruits and vegetables. Effort was made to increase understanding of hazards and their control in areas where reporting is not common. To this end, unpublished data from reputable researchers were included in this assessment, especially from countries that lack surveillance systems; and

- reviewing mitigation and intervention measures being used at different points along the food continuum (e.g. preharvest, packing, transportation, market, point of sale) and assessing their effectiveness at reducing microbiological hazards.

1.3. DEFINITION AND TERMS

Fruits and vegetables: Fruits and vegetables, including leafy vegetables and herbs, are considered as edible parts of plants (e.g. seed-bearing structures, flowers, buds, leaves, stems, shoots, roots), either cultivated or harvested wild, including fungi (modified from FAO, 2020).

Exclusions from this definition and this document include, but are not limited to:

- starchy root and stem tubers such as cassavas, potatoes, sweet potatoes and yams (although leaves of these plants consumed as vegetables are included);
- dry grain legumes (pulses) unless harvested when immature;
- cereals including corn, unless harvested when immature;
- nuts, seeds, and oilseeds such as coconuts, walnuts and sunflower seeds;
- medicinal plants, unless used as vegetables;
- macroalgae;
- spices;
- stimulants such as tea, cacao and coffee; and
- processed and ultra-processed products made from fruits and vegetables such as alcoholic beverages (e.g. wine, spirits), plant-based meat substitutes, or fruit and vegetable products with added ingredients (e.g. packed fruit beverages, ketchup).

Fresh (fruits and vegetables): Fruits and vegetables are those that are not processed in a manner that changes their physical properties. Cooked, canned, juiced, frozen, candied, dried, pickled, fermented, or otherwise preserved foods derived from fruits and vegetables are excluded from this definition and this report.

Ready-to-eat (fruits and vegetables, including minimally processed): These are fruits and vegetables intended for direct human consumption without any additional steps or action taken to reduce or eliminate microbial contamination (modified from FAO and WHO, 2017).

Minimally processed (fruits and vegetables): Fruits and vegetables that have undergone processes that do not affect their fresh-like quality, such as washing, trimming and cutting (modified from FAO, 2020). Fruits and vegetables that are peeled, cut into pieces, chopped, frozen, or dried, with the exception of leafy vegetables, are not included in this report.

Microbiological hazard: Foodborne microbiological hazards include, but are not limited to, pathogenic bacteria, viruses, algae, protozoa, fungi, parasites, prions, toxins and other harmful metabolites of microbiological origin (FAO and WHO, 2013).

1.4. MODELLING OF MICROBIOLOGICAL HAZARDS AND RISKS IN FRESH FRUITS AND VEGETABLES

The experts noted that mathematical models and tools for the prediction of microbiological growth and inactivation as well as assessment of risks posed by some foodborne microbiological hazards in fresh fruits and vegetables have been described in recent years. The ultimate goal of the use of mathematical models is to supply science-based evidence to facilitate decision-making in the prevention and mitigation of food safety issues (Allende *et al.*, 2022). Quantitative microbial risk assessment (QMRA) needs to incorporate predictive microbiological models to quantify microbial growth, inactivation or decay for each of the steps and risk factors included in the production, processing, distribution and storage of fresh fruits and vegetables. Several examples are presented in **Table 1** and **Table 2**.

Predictive mathematical models have great value in risk assessment and risk management to predict future outcomes by analysing historical and current data. Thus, predictive modelling involves collecting data, formulating a statistical model, predicting, and validating the model. The use of predictive microbiology allows the characterization of the behaviour of microorganisms, such as growth, inactivation, transfer, toxin production and so forth in different food matrices, including fresh fruits and vegetables. Different factors can be evaluated within these models to determine the effect of different conditions (scenarios) in the behaviour of microorganisms. The impact of intrinsic and extrinsic factors of the food matrices as well as the microbial interactions are some examples already found in the literature.

The usefulness and value of any mathematical model will be directly linked to the quality and representativeness of the data used to build and apply it. Therefore, one of the most important limitations when developing and implementing predictive models is the lack of accurate data and knowledge about many relevant factors that influence the output of the models, including knowledge about foodborne hazards, food matrices as well as production, processing, distribution, and storage conditions. Another potential limitation of the mathematical models already available in the literature is that comparison among the different models is difficult, mostly because of a lack of harmonization in the applied mathematical tools and compatible data. It is acknowledged that predictive models should only be reliably used when they have been validated in the specific hazard/food combination. Despite all these limitations, there are many attempts already available in the literature. The predictive models described in **Table 1** can be successfully applied by scientists and stakeholders of the agrifood sector to make the right decisions when selecting corrective actions.

Quantitative microbial risk assessment (QMRA) has been defined as a risk assessment that provides numerical expressions of risk and indication of the attendant uncertainties (FAO and WHO, 2014). Quantitative microbial risk assessment, based on predictive microbiology, represents a valuable tool to assess different scenarios and to establish safety limits of production, processing, and distribution chains. Quantitative microbial risk assessment models provide a comprehensive modelling framework that can be adapted as future research elucidates contamination routes and levels, as well as microbial ecology processes along the supply chain of fruits and vegetables (Pang *et al.*, 2017). Quantitative microbial risk assessment also allows producers to predict outcomes before actual implementation of specific corrective actions, adding value to the development of targeted intervention measures.

In the last decade, several QMRA models have been developed to estimate the exposure and/or risk of enteric foodborne pathogens in fresh fruits and vegetables delivered through specific distribution systems (**Table 1**). In these studies, the behaviour of the pathogens has been simulated at different steps of the fresh produce continuum from production to consumption, showing the estimated risks for each specific scenario. Some of the already available QMRA models incorporate the whole farm-to-fork production and value chain of different fruits and vegetables, including field production. However, the low prevalence and concentration of foodborne pathogens in field- or greenhouse-grown crops and the limited availability of microbiological models mimicking the behaviour of these pathogens in agricultural settings have been identified as a major data gap when assessing exposure to pathogenic microorganisms associated with fresh produce (Weller *et al.,* 2017). In fact, data gaps are the main limitation of most of the QMRAs. A solution is to establish faecal indicator conversion ratios between indicator bacteria and specific enteric bacterial pathogens. The implementation of these conversion ratios would allow the use of currently available data sets of the concentration of indicator microorganisms for potential risk factors (e.g. manure, irrigation water, process water, equipment, workers) as well as for fresh fruits and vegetables in different stages of the production, processing, distribution and storage. However, in most of the cases, the established ratios are based on scarce data, which might lead to an underestimation of health risks when compared with using data on an actual pathogen (Owusu-Ansah *et al*., 2017). Some authors have suggested the use of quantitative microbial exposure assessment (QMEA) modelling of faecal hygiene indicator microorganisms (e.g. *E. coli*) instead of QMRA models of enteric pathogenic microorganisms in the fresh produce chain (Allende *et al*., 2018). This approach might be a good strategy to reduce uncertainty associated with the lack of data, helping stakeholders to address the need for a risk-based approach in their good agricultural and hygiene practices.

Table 1 Examples of predictive models for foodborne pathogens in fresh fruits and vegetables

Model classification	Microbial behaviour	Commodity	Foodborne pathogen	Factor(s) studied and range	Predicted parameter(s)	Stage	Reference
Primary	Transfer	Fresh cucumbers	*Salmonella*	Time (0–7 days), temperature (7 and 21 °C), unwaxed or waxed (mineral oil, vegetable oil, or petroleum wax)	Transfer rate (%)	Waxing and storage	Jung, J. & Schaffner, D.W., 2021
Secondary	Inactivation	Lettuce	*Salmonella*	Cut size (3×3, 3×4, 3×5, 3×6, 3×7 cm^2), contact time (30, 50, 70, 90 and 110 s), benzyl-isothiocyanate (0, 20, 40, 60 and 80 mg/L) and free chlorine (0, 50, 100, 150 and 200 mg/L) concentrations	Number of log reductions (log CFU/g)	Washing	Cuggino *et al.*, 2020
Primary and secondary	Growth	Ready-to-eat lettuce	*Salmonella enterica* and *L. monocytogenes*	Storage temperature (7–30 °C), packaging under modified atmosphere (5% O_2, 15% CO_2 and 80% N_2)	Growth rate (μ) and lag time (λ)	Consumption	Sant'Ana, Franco and Schaffner, 2012
Primary and secondary	Growth	Ready-to-eat lettuce	*E. coli* O157:H7	Dynamic temperature conditions during distribution from processing to retail	Growth rate (μ) and die-off	Storage and transportation in the different stages (processor, distribution centre and retail)	McKellar *et al.*, 2012
Secondary	Growth/growth-death	Packaged fresh-cut romaine mix	*E. coli* O157:H7 and *Listeria monocytogenes*	Dynamic modelling for *E. coli* O157:H7 and *Listeria monocytogenes* using real time-temperature profiles during transport, retail cold storage and display. Dynamic modelling using growth-death data for *E. coli* O157:H7	Anticipated CFU/g	Transportation, retail storage and display	Zeng *et al.*, 2014

Primary and secondary	Growth	Spinach	*E. coli* O157:H7, *Salmonella,* and *Listeria monocytogenes*	Temperature ranging from 10 to 40 °C for up to 10 h	Anticipated CFU/g	Transportation from retail to home	Mishra *et al.*, 2017
Primary and secondary	Growth	Baby spinach	*E. coli* spp. and *Salmonella* Typhimurium LT2	Temperature ranging from 10 to 37 °C	Growth rate (μ), lag time (λ) and maximum population (y_{max}) and CFU/g	Processing and distribution, under slow temperature changes	Puerta-Gomez *et al.*, 2013
Primary and secondary	Growth	Cabbage	*E. coli* O157:H7	Temperature (15, 25 and 35 °C) and relative humidity (60%, 70%, and 80%)	Growth rate (μ), maximum population (y_{max})	Distribution from farm to table	Ding *et al.*, 2012
Primary and secondary	Growth	Lettuce	*L. monocytogenes*	Temperature (4–35 °C)	Growth rate (μ) and lag time (λ)	Storage as affected by washing with or without alkaline electrolyzed water	Ding, Jin and Oh, 2010
Primary and secondary	Growth	Iceberg lettuce	*E. coli* O157:H7, *Salmonella* spp., *L. monocytogenes*	Temperature (5–25 °C), variable temperature during distribution	Growth rate (μ), lag time (λ) and maximum population (y_{max}) and CFU/g	Distribution from farm to retail	Koseki and Isobe, 2005
Secondary	Inactivation	Fresh-cut celery	*E. coli* O157:H7 and *Salmonella* Typhimurium	Thermo-ultrasound (temperature: 50, 55, 60 °C; time: 10, 15, 20 min, 40 KHz frequency), calcium propionate concentration (1, 2, 3%, w/v)	Number of log reductions (log CFU/g)	Processing	Kwak, Kim and Rhee, 2011

Source: Authors' own elaboration.

Table 2 Examples of risk assessment models for foodborne pathogens in fresh fruits and vegetables

Foodborne pathogen	Commodity	Type of risk assessment	Stage(s)	Scenarios	Highlights	Output(s)	Reference
E. coli O157:H7	Fresh-cut lettuce	Quantitative	Farm-to-fork	Baseline model (no intervention) and different interventions (chlorine, ultrasound and organic acid, irradiation, bacteriophage, and consumer washing)	Soil and irrigation water as sources of contamination. Retail and home storage temperature were the most important factors affecting the number of illnesses cases.	Estimated mean number of illness cases	Pang *et al.*, 2017
Salmonella	Lettuce, cabbage, broccoli	Quantitative	Exposure	Baseline model only	Consumption of raw and unwashed vegetables irrigated with water from Bogotá river	Daily probability of illness	Henao-Herreño, 2017
Salmonella	Lettuce/ leafy greens	Quantitative	Farm-to-fork	Baseline model and scenarios with different contamination levels, fraction of contaminated produce and chlorine concentrations	Most predicted illness arises from cross-contamination when chlorine is < 5 mg/L. Chlorine concentration should be kept at above 10 mg/L to minimize the risk of illness.	Risk of infection, estimated number of illnesses and estimated percent of illnesses arising from cross-contamination	Maffei *et al.*, 2017
Salmonella	Lettuce, cabbage and cucumber	Quantitative	Exposure	Scenarios: end users that wash or that do not wash vegetables prior to consumption, variable withhold period (time between the last irrigation and harvest)	Vegetables irrigated with treated wastewater. Lettuce led to the highest risk of infection, cucumber the lowest. Washing of vegetables before consumption and for longer withhold periods reduced risk.	Daily probability of infection. Annual risk of infection. Disease burden (disability-adjusted life years per person per year)	Amha, Kumaraswamy and Ahmad, 2017

Salmonella and *Listeria monocytogenes*	Ready-to-eat vegetables	Quantitative	Processed product to consumption	Scenarios evaluated different occurrence (%) and levels of pathogens and the impact of stricter temperature controls during transportation, retail and home storage (< 5 ^{0}C).	Reduction of both occurrence and levels led to reduced risk. Temperature control during transportation, retail and home storage (< 5 ^{0}C) was more effective against *Salmonella* than against *L. monocytogenes*. Lower occurrence and levels of pathogens in the field or during processing lessen risk more than temperature control during transportation, retail and home storage.	Risk of infection per month per serving. Number of cases per month in exposed populations	Sant'Ana, Franco and Schaffner, 2014
Salmonella, Campylobacter spp., and *E. coli* O157	Minimally processed mixed salads	Quantitative	Post-harvest to consumption	Scenarios considered production aspects (e.g. amounts and composition of the mixed salads), portion size, salad-specific contamination (prevalence, estimated level), and processing steps (e.g. washing and mixing of raw produce).	Distinction between infection and illness for dose-response modelling of *Salmonella* and *Campylobacter*	Probability of becoming ill	Pielaat, Van Leusden, Wijnands, 2014
E. coli O157:H7, *Salmonella enterica,* and *Listeria monocytogenes*	Leafy greens	Quantitative	Processing plant to arrival in food service	Baseline and various scenarios (time and temperature in supply chain, fixed temperature of 18 ^{0}C in a salad bar). Model allows the assessment of the impact of various logistic scenarios on risk.	Pathogen growth modelled using time-temperature profiles in the overall supply chain. Temperature was generally maintained < 5 ^{0}C in the chain, resulting in minimal growth of *E.* O157:H7 and *Salmonella enterica*, greater growth of *Listeria monocytogenes*. First- and second-order Monte Carlo modelling	Probability of infection. Estimated number of infections per year	Franz *et al.*, 2010

E. coli O157:H7, *Salmonella enterica,* and *Listeria monocytogenes*	Leafy greens	Quantitative	Processing plant to arrival in food service	Three scenarios: i) baseline based on supply chain logistics and temperature trajectories, ii) increase in delivery frequency from 2 days/week to 5 days/week, and iii) fixed delay times	Modelling logistics (storage time distributions) instead of assuming fixed times and fitting to statistical distributions provides better estimates of risk for psychrotrophic pathogens such as *Listeria monocytogenes*. The risk of listeriosis due to the consumption of fresh-cut leafy greens at salad bars can be amplified in the supply chain. First- and second-order Monte Carlo modelling	Probability of infection Estimated number of cases	Tromp, Rijgersberg and Franz, 2010
Cryptosporidium and *Giardia*	Tomatoes, bell peppers, cucumbers and lettuce	Quantitative	Exposure	Scenario assumed irrigation with water contaminated with *Cryptosporidium* and *Giardia* oocysts and cysts, respectively. The models considered (i) (oo)cyst levels, (ii) the amount of (raw) produce consumed, and (iii) consumer exposure frequency and duration.	Findings underlined the importance of farm practices (irrigation water source, crop type, and type of irrigation application) and measures to reduce sources of contamination at pre- and post-harvest stages for the mitigation of infections caused by *Cryptosporidium* and *Giardia*.	Annual risk of infection	Mota *et al.*, 2009

E. coli O157:H7	Fresh cut-cos lettuce	Quantitative	Farm-to-fork (pre- and post-harvest)	Baseline scenario (summer), and different scenarios with variable growing time, rainy days, sun hours per day, solar decay rate, and prevalence of the pathogen entering the processing plant in winter, fall and autumn. Different irrigation water sources and mode of application, manure quality and the use of chlorine or peroxyacetic acid (PAA) in the washing water also considered. QMRA combined two previously published QMRA models: i) QMRA model focused on the preharvest stage (considering the effect of growing time, rainfall, solar radiation, and microbial quality of irrigation water and manure-amended soil), and ii) QMRA model focused on post-harvest stage considering washing (chlorine, peroxyacetic acid [PAA], and their combination), occurrence of cross-contamination and time and temperature during storage and transportation.	Exclusion of manure and drip irrigation reduced *E. coli* O157:H7 levels in cos lettuce at harvest; the exclusion of solar radiation led to the highest levels. Results indicated that the use of local data that take into consideration unique environmental conditions and agricultural practices are key for risk outcomes. The use of a sequential washing treatment could reduce the public health risk associated with *E. coli* O157:H7 compared to water washing only. Control of temperature was the most important post-harvest practice to mitigate the predicted number of illness cases per year and probability of illness per serving due to the consumption of Cos lettuce.	Number of illnesses per year Probability of illness per year	Bozkurt *et al.*, 2021

Human norovirus, hepatitis A virus and human adenovirus	Lettuce (Romaine and butterhead), raspberries, strawberries	Quantitative	Farm-to-fork	Scenarios assessed the impact of various contamination routes on risk: irrigation, harvesters' hands, conveyor belts, food handlers' hands, rinsing and consumption. Contamination occurring in the home, contamination during production via faeces or pesticides prepared with surface water, and virus uptake via roots or leaves were not considered in the model due to lack of data.	Contamination of produce was affected by hand contact more than other potential routes such as irrigation water, surfaces (conveyor belts) or water used for produce rinsing. Hand hygiene is key in mitigating health risks from viruses.	Estimated risk per serving	Bouwknegt, M. *et al.*, 2015
E. coli O157:H7	Leafy greens	Quantitative	Farm-to-fork	Model assessed the impact of *E. coli* O157:H7 prevalence and levels in the produce, the impact of chlorination and cross-contamination during washing as well as the influence of times and temperature during home storage on the risk of illness.	The largest number of cases resulted from cross-contamination during washing. Levels in the field of -1 log CFU/g and 0.1% prevalence could have resulted in an outbreak approximately the size of the 2006 *E. coli* O157:H7 spinach outbreak that occurred in the United States of America.	Number of illnesses from directly contaminated pieces. Number of illnesses derived from cross-contaminated produce during washing	Danyluk and Schaffner, 2011

Source: Authors' own elaboration.

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2

Fresh, ready-to-eat, and minimally processed fruits and vegetables

2.1 FOODBORNE ILLNESS

The experts noted that surveillance and outbreak data from many countries including developing nations are generally sparse, if not missing all together, and are needed to more accurately assess the burden of illness associated with fresh fruits and vegetables or to identify causes of contamination. All data regarding illnesses, outbreaks, recalls, research and other sources were considered useful to this end. The experts made an effort to update and include any recent trends in commodity and pathogen pairing or pathogen occurrence and presence with a focus on emerging and neglected pathogens.

2.2 MICROBIOLOGICAL HAZARDS

The experts developed tables to capture information about microbiological hazards, implicated commodities, dates, geographic locations, cases, level of contamination, and associated references. Hazards were not prioritized due to the lack of monitoring and occurrence data, and illness and outbreak data from many countries and regions. Information was collected on outbreak data as well as occurrence and prevalence data from as many sources as possible to expand the understanding of new, emerging, re-emerging, or neglected pathogens and commodity-pathogen pairings, as well as practices that may be contributing to increased illnesses, with a focus on countries and regions where data are typically sparce.

In December 2022, the European Union One Health 2021 Zoonoses report was published. Based on the report, in 2021, foodborne outbreaks associated with the consumption of foods of non-animal origin were reported by 14 Member States (Austria, Belgium, Denmark, Finland, France, Germany, Hungary, Italy, Lithuania, Luxembourg, the Kingdom of the Netherlands, Poland, Spain and Sweden) and two non-MSs (Switzerland and Norway) (EFSA and ECDC, 2022). Based on the data, the number of outbreaks of foodborne disease associated with foods of non-animal origin was more than double in the year 2020 (45 versus 23). Foodborne outbreaks were mostly linked to *Salmonella* (11 outbreaks), followed by STEC (four outbreaks). Other microorganisms responsible for outbreaks of foodborne disease were *Yersinia enterocolitica, Clostridium botulinum, Staphylococcus aureus,* and bacterial toxins. Viruses and parasites were also responsible for outbreaks associated with food of non-animal origin. In 2021, nine large (≥ 50 cases) or very large (≥ 100 cases) outbreaks associated with "vegetables and juices and other products thereof" were reported in the European Union. Ready-to-eat vegetables have also been linked to five foodborne outbreaks, mostly reported in Finland, and one of them classified as very large (728 cases) was associated with *S.* Typhimurium. Particularly relevant were the outbreaks associated with "Alfalfa sprouts" caused by *Salmonella* Coeln in Sweden, with "Galia melons" imported from Honduras caused by *Salmonella* Braenderup in several countries, and with berries and small fruits caused by norovirus in Switzerland.

Based on the United States Centers for Disease Control and Prevention (CDC) database, foodborne outbreaks linked to produce and fresh produce in the United States of America in a similar time frame (2017–2020) have been mostly associated with *Salmonella* (43 outbreaks), Shiga toxin-producing *E. coli* (1 outbreak), *Yersinia enterocolitica* (7 outbreaks), *Clostridium* (6 outbreaks), *Campylobacter* (9 outbreaks), *Streptococcus* (1 outbreak), *Legionella* (18 outbreaks), and *Shigella* (22 outbreaks). Foodborne outbreaks have also been linked to viruses (856 outbreaks) and parasites (*Cryptosporidium parvum* [14 outbreaks] and *Giardia* [6 outbreaks]). From 2017 through 2020, there were 134 multistate outbreaks with a confirmed source in the United States of America. Among these foodborne outbreaks, 43 resulted in product recalls or withdrawing the contaminated product from the market (CDC, 2023). Fruits were identified as the source of the most solved foodborne outbreaks (22), while root/underground vegetables (e.g. dry bulb onions) were identified as the source of the most outbreak-associated illnesses (1 400) of any food category (CDC, 2023).

In general, there is a trend of increasing foodborne outbreaks where viruses and parasites are implicated, particularly in the United States of America. On the other hand, *Campylobacter* also seems to be a pathogen of concern in food of non-animal origin, although surveillance is still very limited.

From the literature search, information from 39 outbreaks was gathered, resulting in a total number of 4 436 cases. **Table 3** summarizes examples of foodborne outbreaks caused by food of non-animal origin. Some of these foodborne outbreaks have already been included in the information above from international organizations such as the CDC or European Centre for Disease Prevention and Control (ECDC) and European Food Safety Authority (EFSA), however, sometimes with updated figures on the number of cases after additional investigations including more extensive interviews and typing of patient samples. The foodborne outbreaks were linked mostly to bacteria (26) followed by viruses (7) and parasites (6).

The occurrence data were collected from peer-reviewed review articles and publications, publicly available monitoring results from competent authorities, and unpublished sources provided by the experts, ranging from 2001 to 2022 (**Table 4**). In total, data were collected from 81 sources, representing at least 43 countries, possibly more as some sources reported results for imported fruits and vegetables with country of origins not specified. The data was divided into the six WHO regions: European region (EUR), America region (AMR), Western Pacific region (WPR), African region (AFR), Eastern Mediterranean (EMR), and South-East Asia region (SEAR), and by microbiological hazard type: bacteria, parasite and virus. The summary in **Table 4** is merely an example of the types of data available and is not intended to be an exhaustive list.

Globally fresh fruits and vegetables are most often sampled and tested for bacteria (73 percent). *Salmonella* spp. (34 percent), STEC (29 percent) and *Shigella* spp. (18 percent) are the most commonly tested bacterial pathogens. Despite the large amount of samples collected, the positive rate for these common pathogens are low; *Salmonella* spp., 1.2 percent; STEC, 0.8 percent and *Listeria,* 1.2 percent. Bacterial pathogens with high positive rates include *Cronobacter* spp. (43 percent) in AMR and *Enterococcus* spp. (42 percent). Of the data reviewed, the America region collected a vast majority (90 percent) of the samples with the other five WHO regions each collecting 1–3 percent each of the remaining samples.

Sampling and testing for parasites made up 21 percent of the data. Fresh fruits and vegetables were the most commonly tested for *Cyclospora* spp. (22 percent) and *Cryptosporidum* spp. (22 percent), while parasites such as *Balatidium* spp. (20 percent), *Taenia* spp. (20 percent), and *Strongyloides stercoralis* (19 percent) had the hightest positive rates in samples collected from AFR and SEAR. Samples for parasite testing were more evenly distributed throughout the WHO regions with only 43 percent of samples coming from AMR, and substantially more coming from AFR (18 percent), WPR (13 percent) and EMR (11 percent) than for bacteria.

Sampling and testing of fresh fruits and vegetables for viruses made up 6 percent of the data, with a bulk of the samples being tested for norovirus (59 percent). Norovirus had a positive rate of 10 percent, while rotavirus (12 percent of the samples) had a positive rate of 9 percent, and hepatitis A (16 percent of samples) had a positive rate of 5 percent. For the samples included as part of these data, 50 percent were collected in WPR and 22 percent in AMR. The Eastern Mediterranean region and the European region both collected 14 percent, and none of the data on viruses was representative of the AFR.

Table 3 Examples of foodborne illness outbreaks caused by fresh fruits and vegetables contaminated with bacterial, parasitic and viral microbiological hazards

Hazard	Commodity	Year	Country	Number of cases	References
BACTERIA					
Aeromonas hydrophila	Cucumbers	2012	China	349	Zhang *et al.*, 2012
Aeromonas hydrophila	Leaf salad mix	2017	Iceland	128	EFSA foodborne outbreak data
Bacillus cereus	Pineapple	2013	France	5	Glasset *et al.*, 2016
Bacillus cereus	Salad	2010	France	44	Glasset *et al.*, 2016
Bacillus cereus	Fruits – unspecified	2016	United States of America	39	NORS Data
Campylobacter jejuni	Unknown	2007	Finland	7	EFSA foodborne outbreak data
Campylobacter spp.	Fruits – unspecified	2012	United States of America	51	NORS Data
Campylobacter spp.	Sweetcorn, peas	2014	United Kingdom	39	EFSA foodborne outbreak data
Cryptosporidium parvum	Kale	2019	Sweden	49	EFSA foodborne outbreak data Food Safety News, 2020a
Cryptosporidium parvum	Loose leaf salad	2012	United Kingdom	305	EFSA foodborne outbreak data
Listeria monocytogenes	Cantaloupe (whole)	2018	Australia	19	NSW Government, 2018

Listeria monocytogenes	Sprouts	2008	United States of America	20	Cartwright *et al.*, 2013
Listeria monocytogenes	Mushrooms, Enoki (whole)	2019	United States of America, Australia	42	Food Safety News, 2020b
Listeria monocytogenes	Prepackaged salads	2021	United States of America	18	USFDA, 2022c
Other pathogenic *E. coli*	Spring onions	2021	Denmark	85	Food Safety News, 2022
Salmonella enterica	Onions	2021	United States of America	1040	CDC, 2022
Salmonella spp.	Fresh spinach leaves (baby spinach)	2007	Denmark	19	Denny *et al.*, 2007
Salmonella spp.	Cucumber used in ready-to-eat food products	2018	United Kingdom	76	EFSA foodborne outbreak data
Shigella sonnei	Baby corn	2007	Denmark	200	EFSA foodborne outbreak data
Shigella sonnei	Snow peas	2019	Norway	28	EFSA foodborne outbreak data
Shigella spp.	Basil	2011	Norway	46	Guzman-Herrador *et al.*, 2011
STEC	Leafy greens	2018	United States of America	64	NORS Data
STEC	Salad leaves	2016	United Kingdom	170	EFSA foodborne outbreak data
Yersinia enterocolitica	RTE Fruits and vegetables	2011	Norway	21	MacDonald *et al.*, 2011
Yersinia enterocolitica	Leafy greens	2019	Sweden and Denmark	57	Espenhain *et al.*, 2019

Yersinia pseudotuberculosis	Raw grated carrot	2008	Finland	50	EFSA foodborne outbreak data
PARASITE					
Angiostrongylus cantonensis, costarrisensis	Salad	2000	Jamaica	12	Lindo *et al.*, 2002
Cryptosporidium spp.	Spinach drink	2019	Sweden	122	Food Safety News, 2020b
Cyclospora cayatenensis	Sugar peas	2009	Sweden	12	Insulander *et al.*, 2010
Giardia duodenalis	Leafy greens	2007	United States of America	15	NORS Data
Toxoplasma gondii	Fruits – unspecified	2013	Brazil	73	Pinto-Ferreira, 2019
Trypanosoma	Berries	2007	Brazil	25	De Barros Moreira Beltrão *et al.*, 2009
VIRUSES					
Calicivirus	Mixed salad	2016	Sweden	400	EFSA foodborne outbreak data
Hepatitis virus	Frozen strawberries	2018	Austria	16	Ruscher *et al.*, 2020
Hepatitis virus	Frozen strawberries	2020	Germany	65	Ruscher *et al.*, 2020
Hepatitis virus	Frozen strawberries	2018	Sweden	20	Ruscher *et al.*, 2020
Norovirus	Fruits - unspecified	2012	United States of America	125	NORS Data
Norovirus	Lollo bionda lettuce	2010	Denmark	566	Ethelberg *et al.*, 2010
Sapovirus	Fruits – unspecified	2014	United States of America	14	NORS Data

Table 4 Examples of bacterial, parasitic and viral contamination rates and prevalence in fresh fruit and vegetable commodities from the various World Health Organization regions

Hazard	AFR		AMR		EMR		EUR		SEAR		WPR		References
	Prev.	Pos. rate	Prev.	Pos. rate	Prev.	Pos. rate	Prev.	Pos. rate	Prev.	Pos. rate	Prev.	Pos. rate	
BACTERIA													
Campylobacter spp.	--	--	0.0%	3/ 8 946*	--	--	5.1%	17/ 335	--	--	--		Buyukunal *et al.*, 2015; de Carvalho *et al.*, 2013; Denis *et al.*, 2016; McMahon and Wilson, 2001
Cronobacter spp.	--	--	43.3%	13/30	--	--	--	--	--	--	--	--	Vasconcellos *et al.*, 2018
Enterobacter spp.	12.9%	27/ 210	26.0%	26/ 100	9.5%	10/105	--	--	--	--	--	--	Akoachere, Tatsinkou and Nkengfack, 2018; Al-holy *et al.*, 2013; Oluboyo *et al.*, 2020; Santos *et al.*, 2020; Shin *et al.*, 2019
Klebsiella spp.	15.0%	9/60	--	--	10.5%	11/105	--	--	--	--	--	--	Al-holy *et al.*, 2013; Oluboyo *et al.*, 2020
Listeria spp.	0.1%	1/989	0.8%	127/ 8 480*	2.5%	3/120	0.3%	3/932	--	--	3.5%	21/ 608	Abadias *et al.*, 2008; Calonico *et al.*, 2019; Cardamone *et al.*, 2015; CFIA, 2022; Denis *et al.*, 2016; Duvenage and Korsten, 2017; USFDA, 2017, 2018a, 2022a; Jang *et al.*, 2021; Korir *et al.*, 2016; Kuan *et al.*, 2017; Kyere *et al.*, 2020; Li *et al.*, 2017; Richter *et al.*, 2021; Roth *et al.*, 2018; Sant'Ana *et al.*, 2012; Buyukunal *et al.*, 2015; Soltan Dallal *et al.*, 2015

Prev.: Prevalence; Pos. : Positive

Salmonella spp.	9.3%	189/ 2023	0.6%	240/ 38761*	0.7%	2/ 299	1.4%	14/ 1006	--	--	9.7%	78/ 808	Abadias *et al.*, 2008; Al-holy *et al.*, 2013; Calonico *et al.*, 2019; Cardamone *et al.*, 2015; CFIA, 2022; Denis *et al.*, 2016; Duvenage and Korsten, 2017; USFDA, 2017; 2018b; 2022a, 2022b; Hammad *et al.*, 2008; Jang *et al.*, 2021; Korir *et al.*, 2016; Kuan *et al.*, 2017; Li *et al.*, 2017; Maffei *et al.*, 2013; McMahon and Wilson, 2001; Najafi and Bahreini, 2012; Oluboyo *et al.*, 2020; Richter *et al.*, 2021; Roth *et al.*, 2018; Sant'Ana *et al.*, 2011; Buyukunal *et al.*, 2015; Vital *et al.*, 2014
Shigella spp.	16.1%	29/ 180	0.0%	0/ 23286*	--	--	--	--	--	--	--	--	Akoachere, Tatsinkou and Nkengfack, 2018; Denis *et al.*, 2016
Vibrio spp.	9.4%	34/ 360	--	--	0.0%	0/ 105	--	--	--	--	20.7%	57/ 276	Al-holy *et al.*, 2013; Igbinosa *et al.*, 2021; Oluboyo *et al.*, 2020; Tunung *et al.*, 2010
STEC	15.8%	106/ 670	0.2%	88/ 34809*	6.3%	22/ 348	5.6%	35/ 620	0.0%	0/6	4.8%	39/ 807	Abadias *et al.*, 2008; Al-holy *et al.*, 2013; Al-Kharousi *et al.*, 2016; Ceuppens *et al.*, 2014; CFIA, 2022; Denis *et al.*, 2016; Duvenage and Korsten, 2017; USFDA, 2022b, 2022a, 2017, 2018b, 2019; Jang *et al.*, 2021; Korir *et al.*, 2016; Kuan *et al.*, 2017; McMahon and Wilson, 2001; Najafi and Bahreini, 2012; Oliveira *et al.*, 2011; Richter *et al.*, 2021; Roth *et al.*, 2018; Buyukunal *et al.*, 2015; Silva *et al.*, 2003; Vital *et al.*, 2014

Staphylococcus spp.	12.1%	29/ 240	37.1%	13/35	66.0%	169/ 256	0.0%	0/276	0.0%	0/6	3.6%	4/112	Akoachere, Tatsinkou and Nkengfack, 2018; Al-Kharousi *et al.*, 2016; Calonico *et al.*, 2019; da CRUZ *et al.*, 2019; Duvenage and Korsten, 2017; Hammad *et al.*, 2008; Jang *et al.*, 2021; Najafi and Bahreini, 2012
Enterococcus spp.	--	--	0.0%	0/3	46.9%	46/ 98	50.0%	3/6	0.0%	0/6	25.0%	3/12	Al-Kharousi *et al.*, 2016; Hammad *et al.*, 2008
Aeromonas spp.	16.7%	5/30	--	--	23.2%	29/125	17.0%	17/100	--	--	--	--	Hammad *et al.*, 2008; Jiménez *et al.*, 2009; McMahon and Wilson, 2001; Xanthopoulos, Tzanetakis and Litopoulou-Tzanetaki, 2010
Citrobacter spp.	--	--	--	--	9.5%	10/105	--	--	--	--	--	--	Al-holy *et al.*, 2013
Escherichia coli	--	--	--	--	1.9%	2/105	--	--	--	--	--	--	Al-holy *et al.*, 2013
Bacillus spp.	--	--	--	--	--	--	--	--	--	--	5.0%	5/100	Jang *et al.*, 2021
Yersinia spp.	--	--	--	--	--	--	0.8%	1/ 123	--	--	--	--	Cardamone *et al.*, 2015
Clostridium spp.	--	--	--	--	--	--	0.0%	0/ 270	--	--	--	--	Calonico *et al.*, 2019
PARASITES													
Blastocystis spp.	--	--	15.0%	15/100	--	--	--	--	--	--	--	--	Li *et al.*, 2017
Cryptosporidium spp.	11.5%	112/ 976	0.8%	28/ 3 624*	6.6%	33/ 496	2.4%	23/ 971	7.7%	14/ 183	2.0%	32/ 1600	Alemu, Nega and Alemu, 2020; CFIA, 2022; Ferreira *et al.*, 2018; Lalonde and Gajadhar, 2016; Silva, Andrade and Stamford, 2005; Utaaker *et al.*, 2017

Cyclospora spp.	6.2%	57/ 922	0.9%	46/ 5 300*	21.4%	6/28	0.0%	0/48	--	--	3.1%	53/ 1696	Akoachere, Tatsinkou and Nkengfack, 2018; Alhabbal, 2015; Barlaam *et al.*, 2021; CFIA, 2022; USFDA, 2022a; Ahmed *et al.*, 2018; Li *et al.*, 2019, 2020; Ortiz Pineda, Temesgen and Robertson, 2020
Entamoeba spp.	9.3%	126/ 1 353	10.9%	77/ 712	7.1%	132/ 1 870	--	--	7.0%	14/ 200	--	--	Abdi *et al.*, 2014; Akoachere, Tatsinkou and Nkengfack, 2018; Alhabbal, 2015; Gomes Neto *et al.*, 2012; Ahmed *et al.*, 2018; Li *et al.*, 2020; do Nascimento Ramos *et al.*, 2019
Hook worm	--	--	--	--	--	--	--	--	11.5%	23/ 200	--	--	Ahmed *et al.*, 2018
Rhabditoid Larvae	--	--	--	--	--	--	--	--	--	--	7.0%	14/ 200	Li *et al.*, 2020
Strongyloides stercoralis	18.5%	33/ 180	--	--	--	--	--	--	18.7%	87/ 465	--	--	Akoachere, Tatsinkou and Nkengfack, 2018; Ahmed *et al.*, 2018; Punsawad *et al.*, 2019
Taenia spp.	20.0%	30/ 150	--	--	--	--	--	--	--	--	--	--	Akoachere, Tatsinkou and Nkengfack, 2018
Toxocara spp.	16.7%	30- 180	--	--	--	--	--	--	5.6%	15/ 270	--	--	Akoachere, Tatsinkou and Nkengfack, 2018; Punsawad *et al.*, 2019
Toxoplasma gondii	--	--	0.5%	18/ 3 686*	--	--	9.9%	49/ 496	40.0%	6/15	3.6%	10/ 279	Barlaam *et al.*, 2021; CFIA, 2022; Danaya Amina Bethea, 2014; Lalonde and Gajadhar, 2016; Lass *et al.*, 2012; Li *et al.*, 2020; Marchioro *et al.*, 2016

Trichuris trichiura	0.0%	0/ 150	--	--	--	--	--	--	2.8%	13/ 465	--	--	Akoachere, Tatsinkou and Nkengfack, 2018; Ahmed *et al.*, 2018; Punsawad *et al.*, 2019
Trypanosoma cruvi	27.8%	8/ 30	9.3%	13/ 140	--	--	--	--	--	--	--	--	Akoachere, Tatsinkou and Nkengfack, 2018; Ferreira, 2016
Ascaris spp.	20.0%	30/ 150	6.3%	1/16	5.6%	5/85	--	--	15.9%	74/ 465	--	--	Akoachere, Tatsinkou and Nkengfack, 2018; Alhabbal, 2015; Ahmed *et al.*, 2018; Punsawad *et al.*, 2019; Da Silva *et al.*, 2014
Balantidium spp.	13.6%	76/ 555	62.0%	106/ 171	--	--	--	--	4.0%	8/ 200	--	--	Akoachere, Tatsinkou and Nkengfack, 2018; Ahmed *et al.*, 2018; Li *et al.*, 2020; de Lima *et al.*, 2018
Enterobius spp.	50.0%	15/ 30	--	--	7.1%	6/84	--	--	4.9%	10/ 200	--	--	Akoachere, Tatsinkou and Nkengfack, 2018; Alhabbal, 2015; Ahmed *et al.*, 2018
Giardia spp.	7.1%	72/ 1 012	4.5%	50/ 1 118*	5.6%	80/ 1 431	5.2%	17/ 329	2.9%	19/ 651	--	--	Alhabbal, 2015; CFIA, 2022; Colli *et al.*, 2015; Khawaja *et al.*, 2018; Li *et al.*, 2020; Ortiz Pineda, Temesgen and Robertson, 2020
Ancylostoma spp.	14.6%	26/ 180	10.0%	3/30	--	--	--	--	15.1%	40/ 265	--	--	Akoachere, Tatsinkou and Nkengfack, 2018; Gomes Neto *et al.*, 2012; Punsawad *et al.*, 2019
Cystoisospora spp.	1.4%	8/580	19.0%	19/ 100	--	--	--	--	--	--	--	--	Akoachere, Tatsinkou and Nkengfack, 2018; Li *et al.*, 2020

Fasciola spp.	12.0%	18/150	1.0%	1/100	7.1%	2/28	--	--	--	--	--	--	Akoachere, Tatsinkou and Nkengfack, 2018; Alhabbal, 2015; Ferro, Costa-Cruz and Barcelos, 2012
Echinococcus spp.	0.0%	0/30	0.0%	0/24	--	--	2.0%	1/48	--	--	--	--	Akoachere, Tatsinkou and Nkengfack, 2018; Barlaam *et al.*, 2021
Enterocytozoon spp.	40.0%	12/30	7.6%	19/250	--	--	--	--	--	--	14.4%	125/868	Akoachere, Tatsinkou and Nkengfack, 2018; Li *et al.*, 2020
VIRUSES													
Adenovirus	--	--	--	--	--	--	--	--	--	--	0.0%	0/541	Shin *et al.*, 2019
Astrovirus	--	--	--	--	--	--	--	--	--	--	0.0%	0/541	Shin *et al.*, 2019
Enterobacter spp.	--	--	--	--	--	--	--	--	--	--	0.0%	0/244	Akoachere, Tatsinkou and Nkengfack, 2018; Al-holy *et al.*, 2013; Oluboyo *et al.*, 2020; Santos *et al.*, 2020; Shin *et al.*, 2019
Hepatitis A	--	--	0.0%	0/799	27.9%	86/308	--	--	--	--	0.2%	1/541	CFIA, 2022; Shaheen, Elmahdy and Chawla-Sarkar, 2019; Shin *et al.*, 2019; Sofy *et al.*, 2018
Norovirus	--	--	12.5%	181/1 440	26.5%	282/1 066	5.3%	71/1 355	0.0%	0/60	4.2%	84/1 982	CFIA, 2022; Cook, Williams and D'Agostino, 2019; El-Senousy *et al.*, 2013; Gao *et al.*, 2019; Shaheen, Elmahdy and Chawla-Sarkar, 2019; Shin *et al.*, 2019; Sofy *et al.*, 2018
Rotavirus	--	--	--	--	--	--	33.0%	2/6	--	--	9.1%	109/1 197	Baert *et al.*, 2011; Gao *et al.*, 2019; Shin *et al.*, 2019

* Sampling data represents both domestic and imported fresh fruits and vegetables of unknown origin in Canada and the United States of America.

Table 5 Monitoring results by pathogen and hygiene indicator obtained by Food Compass

	Years 2013–2018		**Years 2019 - 2021**	
Microorgansims	Total number of samples	Positive samples	Total number of samples	Positive samples
L. monocytogenes	6400	97 (1,52%)	4053	55 (1,36%)
Salmonella	6750	3 (0,04%)	4120	0
E. coli	3732	62 (1,66%)	4133	79 (1,91%)
STEC	5675	3 (0,05%)	4048	4 (0,10%)
Staphyloccus aureus	3016	21 (0,70%)	744	10 (1,34%)
Bacillus cereus	2414	413 (17,11%)	1559	344 (22,07%)
Campylobacter jejuni	163	1 (0.61%)	114	2 (1,75%)

Note: The boundaries apply for pathogens: *Listeria monocytogenes* not detected in 25 g (*Listeria monocytogenes* < 100 cfu/g); *Salmonella* not detected in 25 g; presumptive STEC not detected in 25 g; *Staphylococcus aureus* < 100.000 cfu/g; presumptive *Bacillus cereus* < 100.000 cfu/g; *Campylobacter* not detected in 25 g; *E coli* is seen as a hygiene indicator, not as a pathogen. **Detection limit:** *Listeria monocytogenes* detection in 25 g (*Listeria monocytogenes* < 1 cfu/g); *Salmonella* detection in 25 g; presumptive STEC detection 25 g; *Staphylococcus aureus* < 10 cfu/g; presumptive *Bacillus cereus* < 100 cfu/g; *Campylobacter* detection in 25 g.

The group of experts also had access to different databases, including databases generated by food business operators and other stakeholders (e.g. producer associations). Of particular interest was the database provided by Food Compass. Food Compass is an independent, Dutch national non-profit organization which brings together trading companies, importers and exporters as well as growers among others. Since 2013, Food Compass has been taking microbiological samples of fresh, unprocessed fruits, vegetables and mushrooms from its associates (Food Compass, 2023). The first report was published in June 2019 and includes information covering 5 years (2013–2018) (Food Compass, 2019). In this report, Food Compass showed the results obtained for pathogens and hygiene indicators of approximately 1 400 samples per year, divided over various product groups (**Table 5**). After 5 years of research, it could be concluded that generally only a few pathogens were found on fresh and unprocessed fruits and vegetables. The highest prevalence was found for presumptive *B. cereus* (17.11 percent) followed by *E. coli* (1.66 percent) and *L. monocytogenes* (1.52 percent). Other pathogenic microorganisms found in the monitoring of fruits, vegetables and mushrooms were *Salmonella*, *STEC*, *Staphyloccous aureus* and *Campylobacter jejuni*, all showing prevalence lower than 1 percent. An additional dataset from 2019–2021 was also available from Food Compass (**Table 5**). A total of 18 909 data points were received corresponding to microbiological data from berries and small fruits, bulb vegetables, citrus fruits, flowering brassica, fruiting vegetables, head brassica, herbs and leaf crops, leafy brassica, legumes vegetables, melons, mushrooms, pome fruits, root and tuber vegetables, sprouts, stem vegetables, stone fruits, tropical fruits and witloof.

The two datasets (2013–2018 and 2019–2021) demonstrate a similar prevalence for *L. monocytogenes*; however, slightly higher prevalences were observed in the first database (2013–2018) compared to the second one (2019–2021) for *Salmonella*, while the opposite was observed for presumptive STEC, *Staphylococcus aureus*, presumptive *Bacillus cereus* and *Campylobacter jejuni*. When a positive sample was detected, in most of the cases, values were very low.

2.3 COMMODITIES OF CONCERN

The experts concluded that some commodities such as leafy greens, herbs, sprouts and cantaloupes (rock melons) remain leading causes of produce-associated outbreaks but noted that any commodity can become contaminated, as demonstrated by a recent outbreak involving bulb onions. It was also noted that bias may be introduced by variation in the rate of reporting between countries and because some commodities of concern remain unknown.

There are intrinsic and extrinsic factors that might have an impact on the final microbiological risks of different commodities (EFSA Panel on Biological Hazards, 2013). Some of them are summarized as follows:

- potential for growth of the bacterial pathogens (e.g. pH of the produce) or no growth (e.g. low water content);
- processing that may inactivate some pathogens (e.g. blanching), or alter the physicochemical composition to prevent pathogen growth (e.g. fermentation, addition of salt, lowering pH); and
- production volume, pre- and post-harvest practices, and consumption practices leadingto isolating a single commodity from the broader category (e.g. "strawberries" versus "other berries").

2.4 OVERVIEW OF PRODUCTION SYSTEMS

It was recognized that a range of production systems (e.g. conventional, organic, urban, peri-urban agriculture, less-defined systems) exist in highly diverse geographic regions with varying environmental conditions, subjected to extreme events and changing patterns due to the climate crisis. Fruits and vegetables are regularly colonized by diverse microbiota and can become contaminated with human pathogens and parasites during production, harvesting, post-harvest handling, processing and distribution. This is why preharvest and post-harvest handling and processing operations will influence the microbiological contamination and safety of fresh produce, and multiple risk factors (e.g. contaminated agricultural water, manure, food contact surfaces) have been identified. In addition, variable market channels, distribution networks, cultural practices, consumption patterns, and regulatory frameworks will also impact the specific risks. This report provides examples, but each grower should employ a food safety management system, including microbiological risk assessments, and plan for extreme weather events relevant to their region and farm. There are recommended general good hygiene practices required for food safety applicable to sanitary facilities, personal hygiene, training and sanitation that are critical for the production of safe food and should be adopted in all production systems. There are different types of intervention strategies that represent the strongest strategic approach to deal with problems originating from the complexity and variation of food products and processes, including:

- managerial interventions, which refer to fostering an operational culture of food safety and committing to excellence in implementing the preventive control strategies;

- equipment interventions, which refer to tools and utensils used for the intervention; and
- method interventions, which refer to chemical and physical interventions.

Knowing the source mode of action of the hazard, and the measures for controlling microbiological hazards, can help focus the practical use of technologies and strategies (Gil *et al.,* 2015).

2.5 INTERNATIONAL PRODUCTION AND TRADE

International food trade requirements should be based on food safety practices proven to reduce risks. Many audit schemes, certifications, private standards, and other trade requirements have moved beyond food safety and are not necessarily relevant to controlling microbiological risks. It is also important that food grown for domestic markets also be produced with food safety practices to reduce risks to local consumers. It is a concern that sometimes food safety practices are prioritized for commodities that enter international trade and are abandoned for commodities destined for local or domestic markets. For public safety, all fresh fruits and vegetables must be safely produced.

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3

Primary production in open fields

3.1 LOCATION, ADJACENT LAND USE, TOPOGRAPHY, AND CLIMATE

3.1.1 Problem scope

The safety of fruits and vegetables that are grown in open fields is influenced by different factors, many of which are difficult to control. Microbiological hazards and sources of contamination vary significantly from one particular setting/context to another, even for the same crop. Climate and weather (including local variability and extreme weather events related to the climate crisis), topography, geographic location, and adjacent land use can influence the extent and frequency of transfer of microbiological contamination from environmental sources to growing produce. It is important to take into account that some geographical areas are more at risk than others. Food safety hazards must be managed for each field where produce is located as moving field location is not an option in most instances.

3.1.2 Potential impact

The impact of the climate crisis on agriculture is due to variations in the seasons, changes in areas suitable for growing crops, fluctuations in crop yields, and changes in soil quality, such as modifications of soil minerals, variation in their bioavailability, and alteration in soil microbial ecosystems (FAO, 2008; Gil *et al.*, 2015). The climate and weather changes, such as changes in temperatures, extreme weather events, changes in rainfall, drought, and wind may all have impact on the transfer, survival and persistence of pathogenic microorganisms in the open field primary production system. Current trends in climate change are related to changing disaster risk patterns mainly by the increase in frequency and intensity of extreme events. For example, an increase in frequency and severity of extreme precipitation events may lead to contamination of soil, agricultural lands, ground or surface water, and food with pathogens originating from sewage, agriculture, urban, and industrial settings as well as flooding events and tailing ponds (IPCC, 2007). These changes may also have an impact on the physiological properties of the produce and products as well as their susceptibility to plant and human pathogens. The location of production areas and fields, including the geographical region, and the topography may also have an impact on the transfer of pathogenic microorganisms. Fields can be situated in areas prone to flooding, downstream of facilities that may impact irrigation water movement and quality, adjacent to land that supports animal presence and movement, and downhill from grazing lands that lead to faecal runoff.

As briefly mentioned above, adjacent land use, which includes all kinds of operations that may influence the occurrence of pathogens in the environment and consequently the potential for transfer of pathogens to the crops, is an important factor to take into consideration. The adjacent land use includes operations such as mixed farms (i.e. animals with fruit and vegetable production), game farming, informal settlements, landfills, sewage treatment plants, proximity to abattoirs, concentrated animal feeding operations (CAFOs), and any other processing industries with potential for runoff. Mixed farms and integrated livestock in produce fields with animals such as chickens, dogs, cats and horses can contaminate crops with faeces if they enter the growing areas. Working animals are needed for cultivation and crop protection in some geographic regions, but pets in fields, such as walking with dogs, also represent a risk. Peri-urban or urban areas with poor sanitary facilities are also at risk for contaminating the production environment of fresh produce with human faeces. Operations, like the ones mentioned here, may attract other pests such as congregating birds, insects, rodents and other pests that may carry and transfer pathogens. The factors that are mentioned here each has impact individually; however, their cumulative effect and how these factors interact are more challenging to foresee and control.

3.1.3 Mitigation and intervention measures

There are mitigation measures that can be applied in order to reduce the risk of transfer of pathogenic microorganisms from the primary production environment to the product, and several will be discussed in the sections below. Preventive measures to avoid contamination coming from growing field and adjacent land include the development of risk assessment to identify potential point (from a single identified source) and non-point (from possibly many sources that may be difficult to identify) sources (FAO and WHO, 2008). For instance, buffers and barriers may be established to protect fields from unwanted excess water or the water could be managed by suitable drainage, e.g. creation of channels to divert runoff and potential floods. Selecting produce less susceptible to flooding, such as those off the ground, and agricultural management practices, including site management that fits produce needs such as drainage furrows. Intercropping (growing two or more crops in proximity) or crop rotation can be used to deter wildlife intrusion to reduce the potential for transfer of pathogenic microorganisms from wildlife. However, due to the many factors that have an impact on risks, such as water and wildlife, and the complex interplay between them, many mitigation measures may be needed to effectively reduce risks.

Karp *et al.* (2015) demonstrated that pathogen prevalence in fresh produce is rapidly increasing on farms closer to land suitable for livestock grazing, and that vegetation clearing is associated with increased pathogen prevalence over time. The findings from these authors contradict widespread food safety reforms that champion vegetation clearing as a pathogen mitigation strategy. Berry *et al.* (2015) demonstrated that there is a decrease in contamination as distance from the feedlot is increased. These authors indicated that there is a great risk for planting fresh produce 180 m or less from a feedlot. However, the minimal distance needed to significantly reduce the risk of produce contamination near cattle feedlots will depend on various site-specific factors such as ground cover, humidity and wind.

Based on the literature, the main mitigation and intervention measures to reduce microbial contamination associated to location, adjacent land use, topography and climate include:

- intervention strategies focused on the construction of ditches and establishment of buffer areas to reduce water intrusion and limit impacts from water, wind, wildlife, and other risks;
- use of selected crops that are suitable for growth in areas most affected by droughts, floods, saline soils, or other challenges;
- crop rotation: the establishment of a crop, typically a small grain or legume, in between cultivations of a cash crop might reduce pathogen survival in soil;
- avoiding proximity of the fields to surface water and available water storage;
- avoiding proximity of produce fields to grazable lands, rangelands, pastures, or poultry farms; and
- avoiding proximity of produce fields to urban areas.

3.1.4 Available data

Due to the complex nature of these issues, there is only limited information focused on the overall impact of location, adjacent land use, topography and climate. Most of the available literature is focused on the impact of climate and weather in combination with management variables or agricultural factors present in the United States of America and Europe. Little is published on other countries. Although there are several publications investigating pathogenic bacteria, there are also many looking into generic *E. coli.* For example, results from Litt *et al.* (2021) indicated that rainfall affected *E. coli* survival in soils and transfer to cucumbers. Liu, Hofstra and Franz (2016) reported that climate variables and agricultural management practices had a systematic influence on *E. coli* presence and concentration in leafy greens. Data from Sharma *et al.* (2019) indicated that spatio-temporal factors such as site, year and season influenced survival of generic *E. coli* and attenuated *E. coli* O157 to a greater extent than weather effects such as average daily temperature and rainfall. Park *et al.* (2015) conducted a repeated cross-sectional study to identify farm management, environment, weather, and landscape factors that predict the numbers of *E. coli* on spinach at preharvest. The results from this study indicated that farm management, environment, and weather factors determine the odds of a contamination event taking place. However, when a contamination event had taken place, the numbers of *E. coli* on the spinach was determined by weather only (Park *et al.*, 2015). There are other studies indicating that temperature has an impact (Castro-Ibáñez *et al.*, 2015a), while seasonality, solar radiation and rainfall were also predicted to have an important impact on *E. coli* contamination (Allende *et al.*, 2017). A study by Strawn *et al.* (2013a) indicated that landscape and meteorological factors affecting the prevalence of *Listeria monocytogenes, Salmonella* and STEC differed between the pathogens. While temperature was one of the factors affecting the likelihood of detecting *L. monocytogenes*, precipitation was one of the important factors in *Salmonella* detection. It has been shown that vegetated filter strips can reduce numbers of *E. coli* and load in overland flows (Devarajan *et al.,* 2023). In a study from Kenya, the grass species and the root system of the different plant species influenced the filtering of *E. coli* (Olilo *et al*., 2016). Vegetated filter strips are also effective in reducing *Cryptosporidium parvum* oocysts during overland transport (McLaughlin, Kalita and Kuhlenschmidt, 2013). Although there is not sufficient knowledge on the fate and removal of faecal indicator bacteria and pathogens in constructed wetlands, there are indications that these may have potential to remove a significant portion of faecal bacteria present as reviewed by Wu *et al.* (2016) and Shingare *et al.* (2019). Results published by Castro-Ibañez *et al.* (2015b) confirmed previous knowledge which defined flooding as a main risk factor for the microbial contamination of leafy greens. This study evaluated the impact of a flooding event that occurred in an open field in the southeast of Spain based on the resulting microbial contamination of leafy greens, including indicator microorganisms (coliforms, *Escherichia coli* and Enterococcus) and pathogenic microorganisms (*Salmonella* spp., STEC, and *Listeria monocytogenes*).

The impact of adjacent land use, such as proximity to different animal operations or fields where animal manures and/or biosolids are being spread, has not been extensively studied, but some relevant information is available. Strawn *et al.* (2013b) evaluated risk factors associated with *Salmonella* and *L. monocytogenes* contamination of produce fields and found a higher prevalence of these pathogens in fields with no grassy buffer zones. In line with these findings, it has been demonstrated that removing non-crop vegetation does not improve produce safety (Devarajan *et al.*, 2023). In the last years, growers have been pressured to remove surrounding non-crop vegetation to reduce the intrusion of wildlife onto their farm fields. However, information reported by Karp *et al.* (2015) demonstrated that enterohemorrhagic *E. coli* (EHEC) prevalence in fresh produce was not reduced, but rather it increased by more than an order of magnitude in 5 years, despite extensive vegetation clearing at farm field margins. Similar results have been provided by Benjamin *et al.* (2013) who found that generic *E. coli* was more likely to be detected in surface water on produce farms located near as opposed to far from rangelands. Berry *et al.* (2015) carried out an experiment on the impact of proximity to a beef cattle feedlot. *E. coli* O157 with the same pulsed-field gel electrophoresis (PFGE) subtypes were isolated from both the feedlot surface manure and leafy greens suggesting a link between the feedlot and the leafy greens. The results indicated that the risk of airborne transport increased when the cattle pen surfaces were very dry combined with cattle activity (Berry *et al.*, 2015). Another study from the United States of America showed that microorganisms moved from a poultry operation into an almond orchard and that the greatest impact was in the rows closest to the poultry operation (Theofel *et al.*, 2020). Jahne *et al.* (2016) carried out a quantitative risk assessment for bioaerosol deposition to food crops near manure application sites. They recommended a setback distance between the crop production site and the manure application. An experimental study by Kumar *et al.* (2017) investigated airborne soil particulates as vehicles for contamination with *Salmonella* and found that dust contaminated with *S.* Newport could contribute to contamination. Runoff from different operations into water will be covered in other sections, but it is clear that this is a risk. The establishment of a crop, typically a small grain or legume, in between cultivations of a cash crop has been proven to improve crop productivity and soil health and maintain the sustainability of agroecosystems. Reed-Jones *et al.* (2016) evaluated the impact of the use of cover cropping on enteric bacterial survival and found that the impacts of cover crops and green manures on bacterial population dynamics in soil varied, being influenced by bacterial species, time from inoculation, soil temperature, rainfall and tillage, revealing the need for longterm studies. However, during a 7-week study, Rothrock, Franz and Burnett (2012) demonstrated that *E. coli* O157:H7 survival was significantly lower in soils maintained at either near water-holding capacity (45 percent soil volumetric water content) or under clover growth. These findings support the use of crop rotation as a potential mitigation measure. Weller *et al.* (2016) confirmed previous findings showing that samples of produce collected near water and pasture had a significantly increased likelihood of *L. monocytogenes* isolation compared to that for samples collected far from water and pasture. This study also identified additional land cover factors associated with an increased likelihood of *L. monocytogenes* isolation, such as proximity to wetlands.

3.1.5 Uncertainty and data gaps

Data gaps in this area are significant. The experts recognize that all these factors will have an impact, but there is not sufficient data and information available as to the extent. There will be differences in the impact of these factors in the different fruit and vegetable producing areas around the world, and it will be difficult to obtain data from all practices and all geographic regions. When evaluating how different factors affect survival and growth of pathogens in fresh produce, several studies concluded that results were context dependent, which means that they are influenced by bacterial species, time from inoculation, soil temperature, rainfall and tillage. These observations support the need for long-term studies.

3.2 PRIOR LAND USE AND ASSESSMENT

3.2.1 Problem scope

In order to increase food production, land formerly used for other purposes needs to be used for fruit and vegetable cultivation. Land previously used for urban or informal human settlements, waste disposal, industrial activities, Concentrated Animal Feeding Operations (CAFOs), dairy farms, game farms, and other activities may have contaminants such as pathogenic microorganisms that can be transferred to the produce. Raw manure and other untreated soil amendments of animal origin are the most commonly applied in land used for animal grain and forage, meaning that prior land use might represent a risk of the presence of faecal contamination. It is well known that pathogenic microorganisms such as bacteria and parasites may persist in the environment such as in soils, sediments and water for a prolonged period of time.

3.2.2 Mitigation and intervention measures

In order to prevent contamination of fresh fruits and vegetables grown in such areas, it is important to have an understanding of the former land use (i.e. the history of previous land uses that may introduce food safety risks). Primary production should not be carried out in areas where the known or presumptive presence of pathogens would lead to an unacceptable likelihood of transfer to horticultural crops intended for human consumption without a validated process kill step (FAO and WHO, 2022, 2017). It is not always possible to control the land history of the field because information about the level of pathogens in the soil or required time to reduce these to acceptable levels is not known by growers (Suslow, 2003; Gil *et al.,* 2015). The main mitigation measure would be the evaluation of potential hazards. If the evaluation concludes that contamination in a specific area is at levels that may compromise the safety of crops, such as contamination with human sewage and untreated biosolids, intervention strategies should be managed to limit the use of this land for primary production of fresh produce. In other instances, practices could be put into place to reduce risks. For example, the grower might not be aware that the growing land was recently affected by an event of heavy rainfall and flooding, but the evaluation of potential hazards reveals high levels of faecal indicator bacteria. In this case, growers could plant crops less likely to be impacted by splash or they could plant a cover crop to minimize splash. It would also be important to minimize future flooding by establishing efficient gullies and drain systems that can be used to prevent the additional dissemination of contaminated water (FAO and WHO, 2008). As another example, if previous land use includes grazing activities, a minimum 120-day waiting period between grazing and harvest should be established.

In those cases where there is evidence of contamination in a specific area, crops can be selected to reduce microbiological risks, e.g. growing crops that will undergo a validated treatment/processing step, not in close proximity to the ground. In some cases, the use of plastic mulch can be considered a barrier to avoid the contact between the soil and the crop. On the other hand, if contamination only affects a specific area of the field, rapid detection methods of pathogens and microbiological contaminants (mycotoxins) should be used to delineate the contaminated zone (Tirado *et al.*, 2010). Buffer zones can also be used to increase the distance from the contaminated area to the edible crop, while fallowing, i.e. leaving arable land without planting/sowing for one or more growth cycles, which can also be a strategy to reduce risks.

3.2.3 Available data

While there is little data available in the scientific literature on biological hazards related to former land use, there are more available data related to chemical hazards such as heavy metals, potential toxic elements, pesticide residues, persistent organic pollutants and others. Buscaroli *et al.* (2021) published a review in relation to risks in urban agriculture. However, the chemical risks are outside the scope of this document.

Several studies have evaluated potential safety risks associated with grazing practices. Patterson *et al.* (2018) showed persistence of generic *E. coli* in the soil up to 140 days after post-sheep grazing, indicating that the establishment of research-based waiting periods between grazing and harvest is important to inform best practices for farmers and food safety regulators. Based on scientific evidence, a 120-day waiting period between grazing and harvest was proven to reduce the mean generic *E. coli* concentrations. Studies already mentioned in the previous section (3.1. Location, adjacent land use, topography and climate) also provide scientific evidence for this section, particularly on the impact of flooding in the soil and crop contamination (Castro-Ibañez *et al.,* 2015a, b).

3.2.4 Uncertainty and data gaps

There are significant data gaps in this area. It is apparent that former land use can have an impact on the products grown on such land, but as to which extent will differ between the fruit and vegetable areas around the world. It is acknowledged that it is difficult to obtain information from all practices and geographic regions.

3.3 UNINTENTIONAL CONTACT OF CROP WITH CONTAMINATED WATER

3.3.1 Problem scope

Sometimes fresh fruits and vegetables are unintentionally contacted by contaminated water such as during extreme weather events that lead to flooding. Flooding may have multiple food safety consequences, particularly if the agricultural land is adjacent to livestock farms, industrial sites, or residential areas (Miraglia *et al.,* 2009). Fresh produce grown in contaminated land after flooding has been recognized as a potential vehicle for transmission of pathogenic microorganisms

(FAO and WHO, 2017; EFSA Panel on Biological Hazards, 2013) as well as chemical and physical contaminants.

Unfortunately, when such an event occurs, it is often associated with other food supply restrictions and limitations because whatever event caused the flooding often interferes with transportation as well, such as hurricanes, earthquakes, typhoons, tropical storms, and other events. This increases the value of the crop as both a food source and an income-generating crop.

3.3.2 Potential impact

Contamination of the crop may be caused by microbiological hazards from water as well as by chemical and physical hazards that may also be in the water. Flooding can also be a source of contamination of the soil. If the soil is contaminated, it may compromise the safety of crops.

3.3.3 Mitigation and intervention measures

It is recommended that in the immediate time after crops have been unintentionally contacted with water that may be contaminated, an assessment of the crop should be conducted. In this assessment, it is important to determine if the edible part of the crop has been affected by the flooding or not. For instance, crops that are staked or tree crops, might not be contaminated by direct contact with the flood waters. A risk assessment should also be performed to determine the safe use of previously flooded outdoor areas (e.g. growing fields used for the next planting).

To minimize risks, it is best not to harvest crops that have been contacted by flood waters since it is likely not possible to identify or control all the potential hazards. In some countries, such as the United States of America, produce contacted by flood waters from overflowing rivers is considered adulterated and not allowed for human or animal consumption (USFDA, 2011). One way to mitigate risks is to prevent flooding before it happens. Muhadi and Abdullah (2015) suggested the use of river modelling to determine the potential impact of flooding as a strategy to mitigate the floods. They developed a model with the aim of creating a flood damage map as well as evaluating the effect of mitigation to flood damage. When the model was applied to one specific area, the output of the model suggested the development of a retention pond as a flood mitigation structure.

Other unintentional water contact, such as forgetting to turn off irrigation water or water pooling in the field due to heavy rains is different and would require assessment of other risks that may be relevant such as the presence of animal or human faecal material that may have been spread by the event.

The main mitigation measures to minimize microbial risks due to unintentional contact of crop with contaminated water can be summarized as follows:

- Assess flood potential: There are available models that can be applied to evaluate the probability of a growing area to be affected by flooding (e.g. modelling of flood damage maps).
- Crop rotation: If it is established that a specific area can be affected by flooding, the best mitigation measure would be the rotation of crops of less risk to the most affected areas. If so, lands subject to flooding should be planted with crops that are not consumed raw. Crops destined for the fresh market should be grown in fields less likely to flood and less likely to receive unintentional contact with water. However, in the case of small plot farmers, this may not be an option.
- Creation of buffer zones: Creating buffer zones around water sources likely to flood or lead to unintentional water contact of fresh produce can also reduce risks, but it also decreases land available for cultivation. Buffer zones may be planted with crops that are not consumed raw or with plants that deter animals from consuming the main crop.
- Physical barriers along rivers or water bodies: Constructing barriers or berms can prevent water sources from flooding into production fields, but it also prevents water from draining off the field such as during heavy rain events. The practicality and effectiveness of these mitigation measures should be assessed by the specific farm.
- Land management: Crop fields can be adapted to reduce the effects of flooding. For example, land can be sloped to lessen flooding. Some attempts have been made using channels to move surplus surface water from flood-prone rivers or their distributary canals during the wet season when there is a high flood risk. In some areas, water is moved to ponds which allow the water to flow swiftly down below ground, where they infiltrate the local aquifer. This water can then be pumped back up again during the dry season so that farmers can maintain or intensify their crop production (Nair, 2015).

3.3.4 Available data

Orozco *et al.* (2008) demonstrated the presence of *E. coli* and *Salmonella* Newport in tomato during and after a flooding event. Similarly, Castro-Ibañez *et al.* (2015b) evaluated the impact of a flooding event on the contamination of leafy greens and soil. The aim was to establish a safe amount of time between the flood and the harvest to avoid microbial risks. The results of this study confirmed previous research which established flooding as a primary risk factor for the microbial contamination of leafy greens. However, based on the results, the climatological factors during and after the flooding event considerably affect microbial survival in leafy greens. Casteel *et al.* (2006) reported high levels of faecal contamination in agricultural soils after an extensive flooding in North Carolina. Jacxsens *et al.* (2017) reported that flooding of growing fields increased the risk considerably, with an odds ratio (OR) 10.9 for *Salmonella* and 7.0 for STEC. A systematic review demonstrated that more than 50 percent of the outbreaks of human illness following extreme water-related weather events reported heavy rainfall and flooding as the most common combination of events leading to contamination and illness (Cann *et al.,* 2013).

A review article focused on urban flooding mitigation techniques briefly summarizes multiple mainstream techniques for flooding mitigation (Qin, 2020). Although the study is focused on urban flooding, many of the mitigation measures included in this review can be applied to avoid flooding of growing fields including infiltration trenches, vegetated filter strips, soakaways and other underground water storage units.

3.3.5 Uncertainty and data gaps

Data gaps in this area are significant. The evaluation of the microbial contamination risk after flooding is a challenge mostly due to the difficulties in developing an adequate experimental design in advance. The sporadic nature of these events makes it difficult to repeat the sampling in a specific setting. Thus, attempting to establish a safe interval between the flood and the harvest to avoid microbial risks is challenging (Castro-Ibañez *et al.,* 2015b). Climatic conditions also have an impact of the die-off of microorganisms, which might affect the duration of the safe interval between the flood and the harvest. The applicability of the different flooding mitigation measures should be validated for each specific location, making extrapolation of the available studies very difficult.

3.4 WILDLIFE, ANIMAL AND HUMAN INTRUSION

3.4.1 Problem scope

Wildlife, domestic animals and humans are a part of the environment, but they can also introduce microbial hazards through faeces as well as distribute microbial hazards as they move through fields. While domestic animals may be easily separated from growing operations, it is difficult to control wild animals (e.g. frogs, lizards, snakes, rodents, foxes, boars) and birds (Harris *et al.,* 2003; Gil *et al.*, 2015). Fruit and vegetable growers must address the risks in the area where their land is located. Most farmers are already trying to control hazards associated with wildlife and human intrusion mostly due to economic losses as they can lead to crop destruction or loss. With the growing popularity of urban agriculture, feral animal intrusion and rodents may also represent a risk for transfer of pathogens to fresh produce. In addition, dumping of human waste or direct contamination through human defecation may also directly or indirectly contaminate crops. Human intrusion is more likely in urban situations while animal intrusion are more common in agricultural settings.

3.4.2 Potential impact

Humans and animals, wild and domestic, can shed human pathogens. When they enter a growing field, they represent a vector of contamination of crops directly through faecal deposition or indirectly via faecal contamination of agriculture water or soil in contact with the crop (Jay-Russell, 2013). Bird presence in growing fields is an important risk to food safety since they carry foodborne pathogens, including *Salmonella* and *E. coli*, from sources to destinations that may include agricultural areas (Rivadeneira, 2019). In addition to the potential contamination from faecal pathogens, wild animals are also carriers of other human diseases that are not directly related to food safety (i.e. transferring diseases like leishmaniosis to humans). Controlling hazards associated with humans and animals can have an impact in the control of other human diseases.

3.4.3 Mitigation and intervention measures

Mitigation measures related to wild and domestic animals are in general less specific and more difficult to implement than interventions focused on the reduction of other risk factors such as agricultural water, mostly because they cannot be quantified and audited using microbiological testing criteria (Jay-Russell, 2013). Despite these difficulties, there are specific preventive measures which include the development of an environmental risk assessment of the field and the installation of physical barriers to avoid access of farm and wild animals to the growing field and water sources (CCFRA, 2002; FAO and WHO, 2017, 2022). The installation of physical barriers such as ditches, small hills, mounds, berms and even vegetative buffers has been recommended to re-direct animals and also to reduce runoff from animal production or waste management (James, 2006; Gil *et al.,* 2015). Gil *et al.* (2015) reported that removing animal attractants and harbourage in the production environment could reduce the animal activity in the growing fields. Regarding human intrusion, fencing is the surest way to protect an area. Maintenance of a good riparian area width contributes to long-term protection from any outside factor such as human or animal intrusion (Palone and Todd, 1997).

Airborne transmission has been shown to play a role in dissemination of foodborne pathogens from cattle operations to growing fields. In these cases, windbreaks and hedgerows may reduce aerosol drift (Gil *et al.,* 2015). Distress machines and substances, such as those emitting noise or calls (i.e. predator calls such as sonic fences and ultrasonic rodent repellents) can reduce animal activity close to the growing field (Caro, 2005; Gil *et al.,* 2015). Growers can also use scarecrows and reflective strips to scare off birds and pests from crops. The use of mechanical traps has been suggested as an effective mechanism to trap field mice in lettuce-growing operations. This simple but effective measure has been demonstrated to be very efficient while preserving the product integrity (James, 2005). Poison bait stations to control rodent and bird populations are practices that have been applied by growers, but they have also been cited as detrimental to environmental stewardship goals (Jay-Russell, 2013).

The co-management concept, which has been developed to align food safety and conservation goals, is being promoted among growers to minimize conflicts caused when food safety goals negatively impact conservation goals, with the final aim of achieving a sustainable outcome (CLGMA, 2021). Rivadeneira (2019) suggested that trained birds and native wild owls could be used as protective tools to reduce animal nuisance over significantly larger areas than traditional methods. Based on the results obtained, the use of falconry seems to be an option as a successful way to deter birds and control rodents in agricultural settings. However, its feasibility will depend on many factors and in some cases, this might not be a measure easy to implement. In addition to these preventive measures, there are also mitigation measures that balance food safety and conservation including monitoring for animal intrusion and faecal contamination of the production environment during growth and harvest and establishing no-harvest zones when contamination is found to prevent contaminated produce from being harvested. The creation of buffer zones of unharvested product near the edges of fields, increased surveillance or stage harvesting and processing so that higher-risk material (i.e. produce grown near field edges) is harvested and processed last have also been identified as good mitigation measures (Weller *et al*., 2019). These mitigation measures require that farm workers are trained to recognize, report and mitigate these risks (Jay-Russell, 2013). New technology such as real-time object detection algorithms combined with the use of drones may be able to detect animal intrusion in the growing fields in the future and provide additional tools for risk mitigation.

3.4.4 Available data

Patterson *et al.* (2018) reported the contamination of soil with *E. coli* after allowing grazing activities in the growing field. They confirmed that contamination of the soil with *E. coli* was observed even after 149 days. These studies are necessary to develop science-based waiting periods between grazing and harvest. Larson *et al.* (2016) evaluated the impact of riparian fencing in controlling animal intrusion. They confirmed that riparian fences effectively excluded cattle. It was also reported that fences deterred grazers from entering riparian areas, which may reduce stream contamination (Devarajan *et al*., 2023). Similarly, Sunohara *et al.* (2012) demonstrated that riparian fencing was effective at restricting pastured livestock to within 3 to 5 m of intermittent streams that improved water quality. On the other hand, Park *et al.* (2014) looked at the association between produce contamination and the combination of farm management and environmental and weather factors, finding no significant association between the odds of detecting generic *E. coli* in a spinach sample and a farmer using wildlife control fences versus not using fences. Strawn *et al.* (2013b) reported that wildlife observed in the field within 3 days of sample collection increased the likelihood of an *L. monocytogenes*-positive produce sample. However, in the same study, the authors observed that farms with management practices that attempt to repel wildlife did not reduce the occurrence of generic *E. coli*.

Karp *et al.* (2015) found that extensive vegetation clearing at farm field margins increased EHEC prevalence in fresh produce from 2007 to 2013. The authors concluded that pathogen prevalence seems to increase the most on farms where non-crop vegetation was removed, highlighting the risk of promoting vegetation removal to improve food safety (Devarajan *et al.,* 2023). On the other hand, Weller *et al.* (2016) reported that proximity of the growing fields to forest and scrubland were significantly associated with *Listeria* positive samples, indicating that with a 100-m increase in the distance of a sampling site from forests, the likelihood of *Listeria* spp. isolation decreased by 16 percent and the likelihood of *L. monocytogenes* isolation decreased by 14 percent. Importantly, the authors concluded that the effect of proximity to natural cover may not be a function of the presence of natural cover per se but instead may be driven by the fact that the natural-agricultural border represents an ecotone (i.e. the transitional area where two ecological communities meet).

Berry *et al.* (2015) demonstrated that the risk for airborne transport of *E. coli* O157:H7 from cattle production or wild animals is increased when cattle pen surfaces are very dry and when this situation is combined with cattle management or cattle behaviours that generate airborne dust.

Researchers from the United States Department of Agriculture (USDA) are now involved in a project to develop automated drone-based sensing technologies that can aid producers in rapidly identifying and marking problem sites and their surrounding areas for exclusion from harvesting (USDA, 2022). The objective of the project is to develop a new multimodal imaging-based drone system with real-time image processing and analyses capabilities to provide detection and classification of animal intrusion and faecal contamination in farm fields and to monitor microbial quality of irrigation water.

3.4.5 Uncertainty and data gaps

Most outbreak investigations lead to correlation and not causation when it comes to determining the origin of the contamination. In some outbreaks, animal intrusion events with faecal contamination are indicated as contributing to widely distributed contamination with significant illnesses and health impacts. In these cases, the investigations often do not reveal how and how often limited intrusion and faecal contamination resulted in widespread contamination. Lack of clarity in investigations as well as in research into how contamination is distributed and amplified makes reducing risks difficult. It seems likely there are other mechanisms impacting amplification and distribution of the contamination, including spread through packing and processing facilities, ineffective sanitation practices, and complex distribution networks.

3.5 WATER QUALITY DURING PREHARVEST

3.5.1 Problem scope

Water is critical to plant health and survival but can also impact the microbiological safety of fruits and vegetables. There are many factors that impact the source of water used on the farm. The first is water availability. In many regions of the world, water is a scarce and valuable resource. Though using municipal or ground water that is free from microbiological contamination reduces risks, sometimes this is not an option because water scarcity limits this water to drinking only, or the cost of using this water is prohibitive. Human pathogens can contaminate water, be distributed in water, and be transferred to growing crops through irrigation, application of protective sprays, frost protection, heat mitigation, and splash. In order to avoid contamination of fresh produce via water, many different factors should be considered, including the selection of water source, potential water treatment, and "fit-for-purpose" water quality for use throughout the fresh produce supply chain, which might include water use on a wide variety of crops, production practices, and consumption patterns (De Keuckelaere *et al.*, 2015).

The previous Joint FAO/WHO Expert Meetings on Microbiological Risk Assessment

(JEMRA) focused on the safety and quality of water used with fresh fruits and vegetables (FAO and WHO, 2021) and summarized clear and practical guidance on the microbiological criteria and parameters that can be used to determine if water is fit-for-purpos when used in the preharvest production of fresh fruits and vegetables (FFV). One of the main concerns to determine if water is fit-for-purpose is how to establish the quality of the water. There are many options for assessing water quality to make sure it is fit-for-purpose. Basic microbiological assessment usually focuses on faecal coliforms or generic *E. coli* to assess the presence of faecal contamination in water sources. However, it should be noted that no single water quality indicator is appropriate or useful for all water types, and the indicator should be selected based on the purpose and information needed (FAO and WHO, 2021). Research indicating the correlation of faecal contamination to presence of pathogens is highly variable, but microbiological tests for faecal coliforms and generic *E. coli* tend to be the most widely available and affordable. There is value in knowing if water is contaminated by faeces since it provides an opportunity to identify the source of the contamination and provides important information for water management decisions. In some areas, producers may test water for specific pathogens of concern, but this is not a wide-spread or recommended practice except in very specific situations where a very targeted assessment and mitigation is required. More information regarding the water quality required for a specific purpose at a particular point in FFV production is provided in the above-mentioned report (FAO and WHO, 2021).

3.5.2 Potential impact

The use of water of poor quality in fresh produce production may cause foodborne illnesses. Water is used throughout the FFV production chain at different points and for different purposes. Each point of water use can present specific microbial risks associated with the multiple risk factors that can be present. In the primary production of fruits and vegetables, the likelihood of faecal contamination of different water sources differs. Generally, the risk of microbial contamination of different water sources increases according to the following ranking from low to high risk: 1) protected rainwater, 2) groundwater collected from deep wells, 3) groundwater collected from shallow wells, 4) surface waters, and 5) raw or inadequately treated wastewater (FAO and WHO, 2021). Properly constructed and protected wells should generate water that is free of microorganisms, but all wells are not created equally. Many shallow wells are subject to contamination by surface water and in many areas, wells are hand dug or constructed in a way that does not ensure protection of source water from contamination. During post-harvest handling and processing operations, water that comes in direct contact with edible portions of fruits and vegetables should have potable water quality (FAO and WHO, 2017). However, the quality of water used depends on the stage of the operation. For example, during initial washing stages, clean water could be used in contrast to final rinses where water should be of potable quality (FAO and WHO, 2017). The JEMRA report on safety and quality of water used with fresh fruits and vegetables summarizes all the relevant information for application of a fit-for-purpose concept to be successful in producing safe fruits and vegetables (FAO and WHO, 2021). It is important to note that, based on the expert opinion, water quality criteria for use in fruits and vegetables supply chains should be established within the framework of national food and water regulations and guidelines and take into consideration local resources, infrastructure and capability.

3.5.3 Mitigation and intervention measures

A risk assessment, appropriate to the national or local production context, should be conducted to assess the potential risks associated with a specific water source or supply in order to devise the appropriate risk mitigation strategies (FAO and WHO, 2021). During the 2018 JEMRA meeting on water quality in food production (FAO and WHO, 2019), experts considered how a risk-based approach could be practically implemented at even the simplest farm or processing operation. The experts discussed the use of decision support processes and prepared examples. These decision support tools were visualized as decision trees and risk mitigation selection tables and can be accessed in the Microbiological Risk Assessment Series No. 33 (FAO and WHO, 2019). Many factors were taken into consideration including the water source and its microbial quality; the stage in the supply chain and how water is used; whether the water comes into contact with or infiltrates edible FFV parts; the end-use of the crop (e.g. eaten raw, eaten cooked), and the efficacy of risk mitigation measures, if applied. There are several fit-for-purpose risk mitigation measures including: 1) alternative water sources, 2) crop rotation (e.g. change from vegetables eaten raw to those eaten cooked), 3) reducing the contact of the water with the edible part of the crop (e.g. changing from overhead irrigation to drip irrigation), 4) water treatment, 5) irrigation cessation for 3 days prior to harvest, 6) peeling fresh produce (e.g. root crops, fruits, removal of outer leaves), and 7) washing. If national guidelines or regulations are available and include methodologies for assessing vulnerability and risk, selecting appropriate risk mitigation measures and monitoring the process, such guidelines should be followed.

3.5.4 Available data

Most of the available scientific evidence relating to the impact of poor water quality on the safety of fresh produce has been summarized in previous JEMRA reports (FAO and WHO, 2019; 2021). New scientific articles have been published since these reports became available. Most of these studies focus on the potential factors affecting quality of water used during preharvest activities and post-harvest handling and processing of fruits and vegetables, as well as suitable water treatments to be applied at different stages of the production and processing (Alegbeleye and Sant'Ana, 2023; López-Gálvez, Allende and Gil, 2021). The evaluation of alternative water sources (e.g. secondary-treated wastewater, reclaimed water, chlorine-treated wastewater, green wall-treated greywater) as fit-for-purpose water sources for specific commodities and uses needs to be considered on a case-by-case basis to determine their suitability in pre- and post-harvest activities (Fernandes *et al.,* 2023; Truchado, Gil and Allende, 2021).

3.5.5 Uncertainty and data gaps

The previous JEMRA report (FAO and WHO, 2019) has already acknowledged that current data gaps are mostly linked to the lack of understanding regarding the behaviour and persistence of microbial hazards introduced via water, the interaction of water with the diverse range of products and in different environments at different steps along the supply chain, the effectiveness of risk reduction measures at these steps to improve water quality, and of unforeseen contamination events in water reuse. These data gaps limit the application of risk-based approaches and introduce uncertainty. The main problems are the limitations in quality and lack of quantitative data for use in risk assessments. In most cases, there are only data available for specific conditions, and from only a very limited number of countries.

3.6 SOIL AMENDMENTS (ANIMAL MANURES, BIOSOLIDS, OR OTHER NATURAL FERTILIZERS)

3.6.1 Problem scope

Soil amendments, particularly biological soil amendments of animal origin, are widely used in agriculture as they provide soil and crops with essential nutrients and have a reduced cost. Among biological soil amendments, manure, including raw and aged manure and incompletely composted manure, are the most commonly used for crop production and as part of soil health management (Ramos *et al.,* 2021). However, the use of raw manure or incompletely composted manure represents a high risk, as they can contain pathogenic microorganisms (e.g. *Esherichia coli* O157, *Salmonella*, *Listeria*, *Campylobacter*, *Cryptosporidiu*, *Giardia*). There is not only the risk of direct contamination of the crop with raw or incompletely composted manure containing foodborne pathogens, but also the possibility of indirect contamination through runoff, as previously mentioned in other sections (Sections 3.1 and 3.2). In mixed farms (i.e. both animal and fruit/vegetable production), growers use on-farm sources of manure to manage their own wastes. However, many growers purchase off-farm manure and use raw manure because it is widely available, locally produced, and low cost (Ramos *et al.,* 2021). The risks linked to the use of raw manure or incompletely composted manure on produce crops are very high, and these types of manure should only be used in organic fresh produce systems when science-based data on application to harvest time intervals, based on minimizing the risks for the survival of foodborne pathogens in soil amended with these amendments, are followed. Manure management practices, such as manure source and type, storage, and treatment, will influence the occurrence, survival and transfer potential of pathogens (Alegbeleye and Sant'Ana, 2020).

Sewage sludge, also referred to as human biosolids, is a by-product of sewage treatment processes. Biosolids represent a good source of organic and inorganic plant nutrients, which are used to improve the quality of the land dedicated to farming. In some countries, the reuse of biosolids as soil amendments for horticultural crops is a common practice. However, the presence of potentially pathogenic microorganisms represents a risk if biosolids are not correctly treated. There can also be chemical risks associated with biosolids including heavy metals.

Litter or solid manure may be stockpiled in the open environment or accumulated in large volumes (stacked piles) under cover and protected from weathering (Harris *et al.,* 2013). There is a risk of waste spills or seeps from the manure piles to adjacent land and water sources, which will depend on many different factors including storage conditions, weather, location, and management practices (Alegbeleye and Sant'Ana, 2020).

3.6.2 Potential impact

Horticultural producers, due to their social and educational background, do not always consider food safety risks during the production of fruit and vegetable crops. Often their practices are based on tradition, experience, culture, available materials and supplies, and not the most up to date science because producers have neither the time nor the opportunity to learn about the importance of food safety. This behaviour leads to situations in which pathogenic organisms that are not easily detected or considered a priority in the daily work environment could exist and persist, resulting in diseases in humans who consume these fruits and vegetables.

Untreated amendments of animal origin, such as raw manure, are added to soil. These soil amendments can lead to contamination of fresh produce with human pathogens that survive long enough to cause human illness when the fruits and vegetables are consumed (Shah *et al.*, 2019). Of utmost relevance is the application of untreated manure or incompletely composted manure on fruits and vegetables eaten raw and grown on the ground. Less critical is the use of raw manure for non-soil contact crops, such as orchards (Ramos *et al.*, 2021). On the other hand, composted soil amendments represent a very good alternative for organic agriculture, where the use of synthetic fertilizer and pesticides is prohibited, and farms rely on natural soil amendments (Ramos *et al.,* 2021). Therefore, management of soil amendments of animal origin should be based on scientific data (i.e. time-intervals between application and planting/harvesting, validated compost treatments) to reduce food safety risks due to the presence of foodborne pathogens.

3.6.3 Mitigation and intervention measures

Based on the literature, one of the main mitigation measures to prevent microbial contamination of crops due to the use of raw or incompletely composted manure is based on time-interval criteria between the application of animal-based soil amendments and time of crop harvest (Ramos *et al.,* 2021). There are numerous guidelines that suggest different time-interval criteria (European Union, 2017; Pires *et al.,* 2018). For instance, USDA-National Organic Program (NOP) standards and the European Commission stipulate between 3 to 12 months waiting period between incorporating raw manure into the soil and crop harvest, depending on whether the edible portions of the crops come into indirect or direct contact with the soil (Ramos *et al.,* 2021; European Union, 2017). The general rule of longer time intervals between application of the manure and harvest of the crop lessening risks should be considered.

Another alternative to mitigate risks associated with the use of manure is to treat the manure to inactivate potential pathogens that might be present (Devarajan *et al.*, 2023). There are many different treatments that have been validated as efficacious treatments to inhibit microbial growth. For example, composting, fermentation, or biological purification would reduce microbiological risks present in raw manure. High temperature, anaerobic digestion and pressure treatments are technologies useful for treating organic materials leading to their stabilization (Alegbeleye and Sant'Ana, 2020; González *et al.,* 2021). Treatment processes (including composting) are highly variable. Ramos *et al.* (2021) reported that, although it is relatively easy to compost raw manure, many growers indicate that they do not have the capacity or dedicated space for full composting on their own operation, which might facilitate the emergence of composting facilities close to the production areas as well as large-scale, centralized composting facilities.

In the case of human biosolids, such as sewage sludge, different stabilization treatments have proven effective in removing foodborne pathogens. These include composting, thermal drying, thermophilic anaerobic digestion, and liming. However, the efficacy of these treatments needs to be validated for each type of sewage sludge. Ensuring proper treatment of animal and human wastes will decrease risks.

Finally, it is important to mention that soil rotation and avoiding use of non-composted amendments for fruits and vegetables intended for fresh consumption are effective at reducing risks. Tilling operations have been also recommended as a potential mitigation measure, but current scientific data provide conflicting results. It should be considered that for specific markets, such as export markets, where private standards are well established, the use of non-composted animal manure in the soil, where fruits and vegetables intended for consumption without heat treatment are produced, is not allowed. However, this is not the same for domestic markets, and growers have more flexibility for the use of soil amendments.

Covering the planting beds with mulch, such as plastic mulch, helps keep fruits and vegetables protected from direct contact with the soil surface. This reduces cross-contamination from foodborne pathogens that might be present in the soil to produce. Properly installed plastic helps keep soil from splashing onto the plants during rainfall, which can also be a risk of contamination. The use of low-pressure drip irrigation systems to avoid water splashing and distribution of potential pathogens that might be present in the soil has also been recommended. Litter or solid manure may be stockpiled in the open environment or accumulated in large volumes (stacked piles) under cover and protected from weathering. To avoid the risk associated with runoff of wastes to the field or water sources, manure storage and treatment sites should be placed as far away as practical from fresh produce growing and handling areas. Special attention should be given to cover the manure piles to prevent exposure to wind or rain. An effective way to reduce and contain runoff is to cover the stacked piles or to protect them in closed facilities. The use of vegetative buffers might be useful to reduce manure runoff.

3.6.4 Available data

Many research studies have demonstrated that the use of raw or incompletely composted manure for fresh produce cultivation increased the prevalence of foodborne pathogens such as *E. coli* and *Salmonella* spp. (Harris *et al.,* 2013; Sharma *et al.,* 2016, 2019; Strawn *et al.,* 2013b). Although there are studies evaluating the impact of specific factors on the prevalence of pathogens in the soil and the fresh produce, such as type of amendments of animal origin applied (i.e. cattle manure, chicken manure), the type of crop, and agricultural practices (i.e. organically managed soils, conventionally managed soils), there are still knowledge gaps that require further research (Ma *et al.,* 2012, 2014; Sharma *et al*., 2016; Alegbeleye and Sant'Ana, 2020). For instance, some authors reported that soils amended with chicken manure were better substrates for survival of foodborne pathogens (e.g. generic *E. coli*, *Salmonella*, *E. coli* O157:H7) than other animal-based manures (Sharma *et al.,* 2016; Gu *et al.,* 2018). However, no data are available regarding cross and drift contamination, especially on chicken manure when used as an amendment.

Science-based data are already available to help growers establish an appropriately protective time interval between untreated manure application and crop harvest to reduce the risk of surviving foodborne pathogens contaminating organic fresh produce. However, there are many factors affecting the time interval required for each specific situation including type of manure, type of soil, and weather. For instance, Sheng *et al.* (2019) found the use of non-composted fresh dairy manure to amend soil did not have an impact on food safety when applied at least 120 days before harvest. However, other studies reported survival of *E. coli* and *Salmonella* in soil even after 100 days when non-composted fresh dairy manure was applied to the soil (Ingham *et al.,* 2004; You *et al.,* 2006). Litt *et al.* (2021) identified several physical and chemical factors that affect the survival of *E. coli* in amended soil. In particular, specific soil properties that increase soil moisture content due to the rain affected *E. coli* levels over short periods of time, but they have a long-lasting effect regarding the transfer of *E. coli* to the produce.

Mukherjee and Diez-Gonzalez (2007) found that the use of aged manure (> 6 months) was associated with a decreased likelihood of *E. coli* detection. On the other hand, Mukherjee *et al.* (2004) found no significant differences in the levels of *E. coli* in produce between farms using aged or composted animal manure as fertilizer (> 1 year). However, organic samples from farms that used manure or compost aged less than 12 months had a prevalence of *E. coli* 19 times greater than that of farms that used older materials.

Implications of manure applications on mean annual runoff concentrations and loadings of *E. coli* associated with runoff have been described by different authors (Alegbeleye and Sant'Ana, 2020). Rees *et al.* (2011) demonstrated that after three successive annual supplementary additions of poultry manure, annual flow-weighted concentration of *E. coli* was 48 percent greater than the untreated plot, concluding that plots amended with fresh poultry manure increased the *E. coli* concentrations in runoff water by 20 to 230 percent. In general, research studies evaluating the impact of biosolids in the prevalence and concentration of enteric pathogens found no significant differences between soils amended with biosolids and plots amended with conventional fertilizers (Rahube *et al*., 2014). However, Major *et al.* (2020) found that the use of composted sewage sludge substantially altered the prokaryotic community composition and increased the persistence of *Salmonella enterica* serovar Typhimurium and *S. enterica* serovar Senftenberg in soil. The authors conclude that the use of sewage sludge compost is an interesting option, but safety measures should be applied in order to avoid contamination of crop plants by human pathogens.

There is limited data available on the food safety impact of implement beds covers such as plastic mulch. Devarajan *et al.* (2023) summarized several studies indicating that in some cases, *E. coli* prevalence in produce was not associated with mulch application, while another study found that *E. coli* sometimes survived longer in soils with mulch applied. Regarding the differences between till versus no-till soil, Bezanson *et al.* (2012) reported that the soil treatment had no significant overall effect on the survival of *E. coli* O157:H7 on romaine lettuce. On the other hand, Reed-Jones *et al.* (2016) reported that in organic fields tillage negatively impacted *L. innocua* populations but found no significant differences in the persistence of *E. coli* based on samples collected before or after tillage.

3.6.5 Uncertainty and data gaps

Many of the products used as amendments do not have certification processes, and this generates a limitation in terms of the components that they provide as well as the degree of contamination that they can provoke in fresh fruits and vegetables. More research is required to develop protocols and recommendations that allow their use in fresh consumption crops.

3.7 HARVEST, FIELD PACKING, AND PACKING HOUSES

3.7.1 Problem scope

Foodborne pathogens can be introduced from the environment either directly or indirectly during harvesting, packing (field or packing house), and transportation. Various unit operations should be considered within the context of harvest and packing of fresh fruits and vegetables, all of which can significantly change the likelihood of microbiological hazards being introduced. Crops can be harvested by hand or mechanically as a mono- or polyculture. The packing of produce can occur directly in the field or in a facility, commonly referred to as a packing house, which can have varying levels of sophistication. Transportation of the crop from the field to the packing operation can occur in enclosed, temperature-controlled vehicles or vehicles open to the environment. Packing operations are very diverse and may include curing, ripening, culling, fungicide application, hydrocooling, washing, waxing, sorting, bundling, cooling, and placing in a package or other container for transport to the next point in distribution.

Given the wide-ranging practices used for the harvest and packing of produce, routes of contamination will vary across countries and regions, commodities, and scale of production. However, there are some common routes of contamination which should be considered in order to mitigate food safety risks. It is well understood that fruits and vegetables may become contaminated at various stages during production, but given the large volume of crop handled during harvesting and post-harvest activities (e.g. storing, cooling, packing), these steps can act as a point where contamination is distributed more widely or introduced from new sources.

3.7.2 Potential impact

Cross-contamination can be a significant issue during handling at harvest and packing, with environmental factors (e.g. temperature, humidity) also influencing survival of pathogens during these activities. Anything the commodity touches has the potential to cross-contaminate crops during harvesting and packing, further disseminating localized contamination from the field. A focus on sanitary control of contact surfaces (e.g. equipment, harvest bins) and water as well as proper training of employees is paramount given the potential for spread of pathogens. Lack of proper controls and training, and limited options for the inactivation of microorganisms in fresh produce, increase the likelihood of illness due to cross-contamination during harvest and post-harvest handling as well as transportation.

3.7.3 Food contact surfaces (FCS)

Contaminated food contact surfaces as well as adjacent surfaces on equipment and in the packing facility (e.g. drains, floors, coolers) are a documented route of contamination for produce during harvest and post-harvest handling. In 2011, a listeriosis outbreak that was linked to cantaloupe resulted in 147 cases and 33 deaths in the United States of America (McCollum *et al.* 2013). Of 39 environmental samples that were collected in the packing facility, one third tested positive for *L. monocytogenes*. Brush and felt rollers downstream from a retrofitted cantaloupe washer were amongst the food contact surfaces (FCS) which were positive for the outbreak strains (McCollum *et al.* 2013). In 2014, a multistate caramel apple listeriosis outbreak was the first outbreak related to whole fresh apples. This outbreak resulted in 35 cases of listeriosis and the death of seven individuals in the United States of America (Angelo *et al.* 2017). Once more, environmental samples were positive for *L. monocytogenes* that matched outbreak strains from packing equipment FCS such as brushes, conveyor belts, and a wood harvest bin, underscoring the likely role cross-contamination of FCS played in subsequent produce contamination.

The prevalence of *Listeria monocytogenes* and *Listeria* spp. has been recently evaluated in produce packing houses. The prevalence of *Listeria* spp. on FCS in apple packing houses was 4.6 percent (136/2 988), and the bacteria were most frequently isolated from the wax coating unit operation (17.3 percent; n=110) (Ruiz-Llacsahuanga *et al.*, 2021). The FCS that showed the greatest prevalence of *Listeria* spp. were polishing brushes (19.6 percent; n=92), dividers under fans/blowers (17.4 percent; n=46), dryer rollers (10.5 percent; n=143), and brushes under fans/blowers (9.7 percent; n=206) (Ruiz-Llacsahuanga *et al.,* 2021). When evaluating 1 588 non-food contact surfaces in a cohort of fresh, whole produce packing houses, 6.4 percent tested positive for *L. monocytogenes* or *Listeria* spp., with *L. monocytogenes* isolated most frequently amongst the *sensu stricto* clade (3.8 percent) (Estrada *et al.,* 2020). This study also underscored the importance of drain and moisture management, as *Listeria* were most prevalent in samples collected from drains, cold storage, and sites which were kept wet during production.

While most typically think of built packing environments when considering sanitation, it is also important to consider hand tools, picking bags, harvest bins, and mechanical harvester sanitation programmes as equally important, given the role these surfaces can play as a potential source of pathogen contamination (McEvoy *et al.*, 2009; Taormina *et al.*, 2009).

3.7.3.1 Mitigation and intervention measures

It is imperative that growers and packers prioritize cleaning and sanitizing any surface that produce encounter as a mitigation strategy. Sanitation schedules should be established and strictly adhered to for FCS, and equipment adjacent to FCS, as well as packing rooms, coolers, and transport vehicles (FAO and WHO, 2017).

Harvest tools, equipment and bins should also be placed on the master sanitation schedule with sufficient frequency of cleaning and sanitizing events to prevent biofilm formation and inactivate pathogens which are introduced during harvesting and packing. Field-based sanitation poses many challenges, but operators should be cognizant to provide barriers so that soil splash does not re-contaminate equipment, bins, and tools during sanitation, such as the use of concrete or gravel pads which drain well. Additionally, harvest tools and equipment should be cleaned immediately after harvest rather than letting the equipment sit fouled which can support the growth of biofilms that are naturally harder to remove and inactivate with typical sanitation approaches.

Wet cleaning and sanitizing are most frequently thought of when considering sanitation approaches, but it is important to recognize that equipment and surfaces which are not designed to be wet cleaned, where the commodity is dry packed or in areas where a source of uncontaminated water is lacking, a dry cleaning and sanitizing approach is preferred. The primary driver in this approach is the fact that dry packing environments do not support the growth of bacterial foodborne pathogens, and once water is applied, niches within the equipment and packing areas will remain wet and support their growth. Dry cleaning has the same tenant as wet cleaning, but soil is physically removed by sweeping, vacuuming, or abrasive blasting with dry ice (Burnett and Hagberg, 2014). In many instances, an alcohol-based solution which evaporates quickly may be used for wiping down food contact surfaces since soil removal is often difficult. Once soil is removed, sanitization can be achieved through application of an isopropyl alcohol and quaternary ammonium compound sanitizer which evaporates quickly and effectively reduces foodborne pathogen populations, such as *Salmonella* (Du *et al.,* 2010; Kane *et al.*, 2016).

When wet cleaning and sanitizing approaches can be utilized, they are preferred given the increased efficacy in cleaning with water and detergent compared to dry cleaning. It is important to remove soil with water or detergents prior to application of sanitizers. Chlorine (sodium or calcium hypochlorite), peroxyacetic acid (PAA), quaternary ammonium compounds (QAC), or iodophors have shown good efficacy at reducing bacterial contamination when used on non-porous surfaces such as stainless steel, plastic polymers (polyester, polyurethane), and cement as well as porous surfaces such as rubber, even when present as a biofilm (Fouladkhah, Geornaras and Sofos, 2013; Hua, Korany, El-Shinawy and Zhu, 2019; Joseph *et al.*, 2001; Krysinski, Brown and Marchisello, 1992).

As resources allow, FCS which are not easily cleaned and sanitized, such as wood, carpet and foam should be exchanged for materials which are more easily cleaned (e.g. plastic polymers, stainless steel) in order to remove potential harbourage sites for foodborne pathogens.

Once sanitation programmes are well established and are being routinely implemented, growers can look towards other mitigation strategies such as environmental monitoring programmes and incorporation of hygienic design principles for new equipment so that equipment is easier to access for cleaning and has fewer niches capable of harbouring foodborne pathogens.

Environmental monitoring programmes (EMPs) have been widely implemented in processed food facilities and minimally processed fruit and vegetable facilities that produce ready-to-eat products, especially where risk of cross-contamination with *Listeria monocytogenes* or *Salmonella* has been documented (Spanu and Jordan, 2020). Environmental monitoring programmes can also be used to assess the performance of a cleaning and sanitation programme. Utilizing this approach, the facility will sample surfaces for foodborne pathogens (e.g. *L. monocytogenes, Salmonella* sp.) and/or indicator organisms (e.g. *Listeria* spp., *Enterobacteriaceae*, coliforms, *E. coli,* aerobic plate count). Several guidance documents have been developed to assist with development of EMPs for fresh produce operations (USFDA, 2017; United Fresh Food Safety and Technology Council, 2018; United Fresh Produce Association, 2021) as well as other non-produce raw agricultural commodities (Almond Board of California, 2010). It is imperative that growers prioritize sanitation programme implementation first and foremost before any EMP is implemented.

3.7.3.2 Uncertainty and data gaps

Many FCS such as wood, foam rollers, brush rollers, rubber and burlap pose challenges to cleaning and sanitizing due to their porous nature making them difficult to clean. It has yet to be established whether sanitation programmes can overcome less-than-ideal hygienic design, but the evaluation of sanitation approaches for these surfaces must be established given their widespread use within the industry. Science-based recommendations for frequency of cleaning of both food contact and non-food contact surfaces are also needed in order to manage introduction of foodborne pathogens more effectively with limited resources.

3.7.4 Personnel health, hygiene, sanitary facilities and training

In production, harvesting, sorting and packaging for markets, personal hygiene of farmers and workers as well as sanitary infrastructure to adhere to hygienic practices is important (FAO and WHO, 2017). The responsibility for adoption of good agricultural practices will fall on a number of key people along the production line and should be clearly identified at the outset. Provision of fit-for-purpose water is key to adhering to good sanitary practices; therefore, an understanding of the landscape of production sites, the supply chain, and ultimate reach of the market should be envisioned to ensure these good practices. One of the important factors is raising awareness and providing knowledge to farmers and workers about the biological hazards that could be transmitted by failure to adhere to good farming and production practices.

3.7.4.1 Mitigation and intervention measures

Farmers and other workers could be carriers of disease. Workers can carry microbial pathogens on their skin, in their hair, on their hands, and in their digestive systems or respiratory tracts. Unless workers understand and follow basic good agricultural practices, they may unintentionally contaminate fresh produce, fresh-cut produce, food contact surfaces, water supplies, or other workers, and thereby create the opportunity to transmit foodborne illness. Basic protection practices related to worker health and hygiene fall into two categories; disease control and cleanliness. For every geographic region, it may be useful to have a list of likely hazards, so that any illness among workers can be detected quickly and thereby ensure that workers are healthy. People known or suspected to be suffering from, or to be carriers of, a disease or illness likely to be transmitted through fresh fruits and vegetables should not be allowed to enter any food handling areas. Proper quarantine periods should be enforced to prevent microbiological agents from entering the supply chain through workers. It is important to ensure that personnel are following proper personal cleanliness.

The same risks posed by workers may also be introduced from visitors. For this reason, it is important to control visitor access to areas where produce is grown, packed, or held. If visitors are allowed to enter these areas, they should be trained as to the policies and expectations related to personal health and hygiene and be supervised.

It is important to provide hygienic and sanitary facilities for workers in the field, packing houses, and any other place where harvest and packing is taking place. Drainage from these facilities should not enter the soils where agriculture is being done. Such facilities should be located in close proximity to the fields and indoor premises, and in sufficient numbers to accommodate personnel. The facilities should be designed in a way that ensures sanitary waste removal and avoids contamination of growing sites, fresh fruits and vegetables, and agricultural inputs. An adequate means of hygienically washing and drying hands should be provided. Sanitary conditions and good repair standards have to be maintained at all times.

It is important to provide regular training on sanitary practices for all personnel working in fields and in packing houses. It is also important to conduct up-to-date training on a regular basis as labour may change every season or during the season. Within a country, it is also important to build capacity in order to conduct risk assessments, and implementation of mitigation and interventions, as well as develop resources such as personal hygiene and cleanliness manuals for farms and packing houses to utilize.

3.7.4.2 Uncertainty and data gaps

Although there are more available studies evaluating how improvements in personal hygiene of farmers and workers reduce food safety risks, it is still an area where growers and packers struggle to effectively implement practices. This knowledge is still needed to develop food safety education programmes for produce growers and packers.

3.7.5 Water applied during harvest and post-harvest handling operations

Water is used extensively for the harvest and packing of FFV, including washing, cooling, conveyance, hydration and waxing. It is also used for employee hygiene (e.g. handwashing) and cleaning of equipment, surfaces, and the production environment. Measures and practices need to be in place to prevent water being the source of product contamination or being the carrier to spread and cross-contaminate produce or food contact surfaces.

The FAO/WHO report "Safety and quality of water used with fresh fruits and vegetables" (FAO and WHO, 2021) summarizes the use of water in the post-harvest production of fresh fruits and vegetables and provides guidance on the microbiological criteria and parameters that can be used in determining whether water is fit-for-purpose.

The report concludes that post-harvest water in contact with edible portions of FFV or food contact surfaces should be of potable quality, and the quality should be monitored and maintained during processing. The drinking water standard has also been recommended in many industry guidelines and required by certain government regulations. For example, in the United States of America, the water used during or after harvest that will directly contact produce or food contact surfaces or is used for washing hands must meet the microbial drinking water quality standard (i.e. no detectable generic *E. coli* in 100 millilitres [mL] of water).

3.7.5.1 Mitigation and intervention measures for source water

Practical interventions that could be applied post-harvest to mitigate food safety risks when water does not meet the requirement of fit-for-purpose were considered. The report provides an overview of different categories of water source, potential microbial hazards associated with each category of water, treatment systems, and the ability of treatment to inactive pathogens (FAO and WHO, 2021). The most relevant interventions are summarized as follows:

- The water sources, water storage, holding systems, water distribution systems, facilities, and equipment must be adequately maintained and regularly inspected to prevent introduction of hazards to produce or food contact surfaces. Care should be taken to keep the source free of debris, trash, animals, and other possible sources of contamination.
- Wells that supply untreated ground water should be properly located and constructed to avoid contamination. The well, its casing, sanitary seals, and piping tanks should be properly constructed to protect against environmental contamination and must be adequately maintained.
- Consideration should also be given to water delivery systems from municipalities and off-farm wells. All water lines must be designed or equipped with suitable devices to prevent backflow or cross-connections between piping systems that discharge wastewater or sewage and piping systems that deliver water that will be in contact with FFV or food contact surfaces.
- If untreated groundwater is used for post-harvest activities, the microbial quality of the water source needs to be tested to determine whether the water quality is fit-for-purpose.
- If the source water does not meet the microbial quality criterion for the intended use, treatment of the water may be needed. Chemical and physical methods are available for treatment of surface water (e.g. chemical disinfection, filtration, coagulation, flocculation, ultraviolet [UV] light).
- The effectiveness of water disinfection treatments should be monitored at a frequency adequate to ensure that treated water consistently meets the relevant microbial quality criteria for its intended use.

3.7.5.2 Mitigation and intervention measures for water used during harvest and post-harvest handling operations

3.7.5.2.1 Application of water disinfection treatments

During post-harvest washing (in dump tanks, flumes, or wash tanks) or cooling (in hydrocoolers) of FFV, pathogenic microorganisms present in individual crops can spread via water to other crops. As the same wash water is often used for washing large volumes and multiple batches of product, bacteria present in water could further spread to subsequent batches and result in cross-contamination. The potential for transfer of pathogenic microorganisms from contaminated produce to wash water within and between production batches have been demonstrated (Allende *et al.*, 2008; Luo *et al.,* 2012; Buchholz *et al.*, 2014; Davidson, Kaminski and Ryser, 2014).

Application of disinfection treatments (or antimicrobials) in water used in harvest and post-harvest handling operations is needed to prevent water being a vehicle to contaminate produce. Commonly used antimicrobials include sodium hypochlorite (common names include Bleach, Eau-de-Javel), calcium hypochlorite, acidified sodium chlorite, peracetic acid, hydrogen peroxide, chlorine dioxide, and ozone (Gombas *et al.*, 2017). It is important that the use of antimicrobials does not introduce chemical hazards. Label instructions should be closely followed to ensure safe application. Several alternative water disinfection treatments have been tested with the aim to maintain the microbiological quality of process water (Gil *et al.,* 2015). It should be noted that most of these alternative methods have only been tested under laboratory-based experiments and are not yet commercially available or their efficacy is not yet fully validated in an industrial setting.

The effectiveness of antimicrobials in preventing cross-contamination can be greatly influenced by the characteristics of wash water. Chlorine-based sanitizers form various compounds in water that make up free chlorine, including the most effective disinfectant, hypochlorous acid (HOCl). The percentage of free chlorine present as HOCl in water is pH and temperature dependent (Deborde and von Gunten, 2008; Suslow, 2001; White, 2010). Higher temperatures also favour chlorine disinfection (CDC, 2012; Erkmen, 2010). The organic matter, dirt, and plant debris that accumulate over time in recirculated wash water will impact the antimicrobial efficacy of certain sanitizers. An increase in organic matter results in a greater depletion of free chlorine and the presence of solids, and enables the pathogen to better survive chlorination; both led to a lower antimicrobial efficacy (Fu *et al.*, 2018). The antimicrobial efficacy of peracetic acid, on the other hand, is not pH dependent and is less influenced by the presence of organic matter (Gombas *et al.,* 2017).

Industry and government guidelines have recommended that the level of antimicrobials in wash water be maintained and monitored. The level of antimicrobials needed to prevent cross-contamination depends on a number of factors such as type of pathogen, pathogen population, effectiveness and concentration of antimicrobials, and environmental and operating conditions such as organic load and solid in the water, pH and temperature of the water, product feed rate, contact time, and water agitation (USFDA, 2018b). It has been recommended that the minimum concentration of antimicrobials used in wash water be scientifically established, either based on published scientific literature or by conducting washing experiments (USFDA, 2018b). Washing experiments should be conducted under the worst-case conditions (e.g. at a product feed rate where the organic load in the water accumulates most rapidly and to the highest level) with either a surrogate organism or the target pathogen, using laboratory-scale, pilot-scale, or production-scale washing equipment (USFDA, 2018b).

Validation of the effectiveness of control process in preventing occurrence of hazards is recommended (FAO and WHO, 2021) and required by certain government agencies (USFDA, 2018b). Guidelines to validate control of cross-contamination during the washing of fresh-cut leafy vegetables have been developed (Gombas *et al.*, 2017). An example of how to validate sodium hypochlorite added to wash water as a process preventive control measure during the washing of fresh-cut leafy greens is available (USFDA, 2018b).

Certain segments of the produce industry have set specific performance criteria for antimicrobial levels in water used in contact with large quantities of produce (i.e. the same water in a tank is used to wash hundreds of kilos of produce). For example, food safety guidelines issued by the Arizona and California Leafy Greens Market Agreement specify that wash water be maintained at a free chlorine level of ≥ 1 ppm (pH 5.5–7.5) for multipass water (Arizona LGMA, 2021; California LGMA, 2021). Gombas *et al.* (2017) summarized all the available studies and concluded that the published literature points to a concentration of 10 ppm of free available chlorine at the optimum pH range (6.5 to 7) as an approximate target for minimizing cross-contamination during the washing of fresh-cut leafy vegetables. Studies performed mimicking industrial conditions also demonstrated that free chlorine levels around 25 ppm are effective to avoid accumulation of microorganisms in water used in harvest and post-harvest handling operations of leafy greens (Tudela *et al.*, 2019a, b).

3.7.5.2.2 Monitoring and maintaining the microbiological quality of water used during harvest and post-harvest handling operations

Development of an effective monitoring programme is essential to ensure consistent microbiological quality of the water used during harvest and post-harvest handling operations. Rapid and accurate measurements of residual antimicrobial levels in wash water are required. Measuring devices must have sufficient precision to ensure levels are within established limits, and accuracy should be verified periodically to ensure that measurements, particularly those close to the established threshold, are reliable. Since the quality of the water in contact with fresh produce accumulates high loads of organic matter, effective monitoring, and maintenance of the process water should involve supplementing chlorine measurement with assessment of water quality. This can be achieved by either visually monitoring the water for build-up of organic material (such as soil and plant debris) or by using sensors or analytical instruments to measure specific water characteristics such as organic load, turbidity, and pH, which need to be well managed to ensure the microbiological quality of the water.

Water use in harvest and post-harvest activities should be changed when there is excessive build-up of dirt and organic material and/or when consistent antimicrobial levels can no longer be maintained. Industry guidelines recommend that dump tanks be cleaned and sanitized, and the water changed as often as needed to maintain its microbiological quality (USFDA, 2018a; Texas International Produce Association and United Fresh Produce Association, 2020). The Food Safety Best Practices Guide for the Growing and Handling of Mexican Papaya (Texas International Produce Association and United Fresh Produce Association, 2020) set a threshold limit of turbidity at 300 NTU above which fresh water should be added to dump tanks.

Microbial testing of water used in harvest and post-harvest activities is not an efficient way to monitor its microbiological quality. It has been reported that pathogens suspended in water were easier to eliminate than pathogens attached to leaves (Davidson, Kaminski and Ryser, 2014; Shen, 2014, Fu *et al*., 2018). Absence of pathogens in water may not accurately indicate the absence of pathogens on leaves or the contamination status of the production batch (Fu *et al.,* 2018).

3.7.5.2.3 Maintaining appropriate water temperature

Certain industry guidelines require that water temperature in dump tanks be maintained at a temperature that is warmer (at least 10 °F or 5 °C) than the pulp temperature of the commodity (USFDA, 2013, 2018; Texas International Produce Association and United Fresh Produce Association, 2020) to limit infiltration of water into fresh produce. If the temperature of the water in the dump tank or flume is cold and the internal temperature of the commodity (e.g. tomato, papaya, cantaloupe) is hot from field heat, the water temperature differential may promote infiltration of water and microbial pathogens (if present) into the internal tissue of fresh fruits and vegetables. It has been reported that internalization of *Salmonella* in mangoes (Branquinho Bordini *et al*., 2007) and of *E. coli* O157:H7 in apples (Burnett, Chen and Beuchat, 2000) was affected by a temperature differential between the contaminated wash water and the fruits. However, the impact of temperature differential is not clear for the washing of leafy greens. Internalization of *Salmonella* during the washing of parsley or baby spinach was shown to be independent of a negative temperature differential between the wash water and the produce (Duffy *et al*., 2005; Gómez-López *et al.,* 2013).

3.7.5.3 Uncertainty and data gaps

Validated antimicrobial concentrations required to prevent cross-contamination under different uses, water qualities, and commodities are still lacking. The performance standards recommended in industry guidelines need to be validated to build confidence that the minimal antimicrobial concentrations recommended are scientifically sound.

A grower-focused document summarizing treatment methods (both physical and chemical) for treatment of post-harvest water at the source and during use is lacking. Although characteristics, advantages and disadvantages of some physical and chemical technologies for treatment of irrigation water have been reviewed (Allende and Monaghan, 2015), a summary of how these methods may be used in actual applications treating water from different sources (e.g. groundwater, surface water, wastewater) and under what operating conditions and parameters will be useful.

3.7.6 Time and temperature control during cooling, packing, and storage

From the point of harvest, produce should be kept at optimal temperature conditions for the specific crop and production system. Stepwise cooling to remove field heat is a well-known process to prevent physiological deterioration and spoilage (Yahaya and Mardiyya, 2019) as well as foodborne pathogen growth (Duvenage *et al.,* 2017; Duvenage and Korsten, 2017). This may require rapid timely movement of produce into a cold chain which should be according to crop-specific ideal conditions. Previous studies showed that cold chain management systems are time-temperature dependent and relate to slowing down biological processes to retain quality. These best practices also benefit food systems assurance to prevent possible foodborne pathogen growth (Mercier *et al.,* 2017). Cold chain management includes forced air cooling, retaining cooling conditions, and cold storage, including modified atmosphere packaging, that will be discussed in Section 5 (Minimal processing) and Section 6 (Transport, distribution, and point of sale).

Crops should be stored at optimal temperature and humidity conditions and stabilized as soon as practical once harvested. While typically done for quality, there are added benefits from cooling products which deter bacterial growth. Cooling is typically carried out with the use of forced cold air, cold water (hydrocooling), contact with ice (top icing), or through evaporation of water when the product is placed in a vacuum (vacuum cooling). Water used for ice or hydrocooling should be fit-for-purpose as to not contaminate product when cooling (FAO and WHO, 2021). During cooling, condensate can readily form and has been shown to result in cross-contamination if allowed to drip onto product below. Therefore, condensate should be removed or barriers put in place to prevent cross-contamination to produce.

There are several resources to aid in determining optimal storage conditions from organizations such as the UC Davis Postharvest Center. Growers should keep in mind that some crops are prone to chilling injury if stored at low temperatures which may result in tissue breakdown, potentially promoting survival and growth of bacterial contaminants. More details on the importance of temperature controls during distribution are covered in Section 6 of this report.

It is important that growers remove damaged or decayed product when conducting post-harvest activities. Some crops are stored for extended periods of time in refrigerated or controlled atmospheres and can experience decay during this period. It is imperative that damaged or decayed product be removed during intermediate post-harvest handling steps prior to distribution because these conditions could enhance survival of contaminants by allowing for increased access to nutrients and neutralization of acidic pH during mould growth arising from decay.

3.7.7 Transport in the field, from the field to the packing house or processing facility

Transport of produce from the field to a storage site or packing facility can be a point of contamination. When staging product for transport, it is important to take steps to prevent contamination from unsanitary surfaces, bioaerosols, and animal activity. Transport vehicles are commonly used to move product from the field to packing houses or processing facilities and are commonly used for multiple purposes within an operation that can lead to increased risk of contamination with foodborne pathogens. Risks and appropriate mitigation steps should be considered when moving produce.

Crops should be protected during transport from contamination by animals, bioaerosols, or transport vehicles through sanitation and barriers (e.g. containers, coverings, packaging). Additionally, during staging at the field, storage site, or packing house, produce can act as an attractant for birds, rodents and other animals. Deterrents can be utilized to help prevent their contact with crops, and crops should be destroyed if they have any signs of faecal contamination or damage (e.g. bird pecks, rodent faeces).

Transport vehicles should be cleaned and sanitized on a set schedule to prevent cross-contamination to produce with direct contact with the vehicle as well as those in open containers. Prior to loading, they should be inspected for signs of contamination such as rodent droppings, off odours, or other visible potential sources of contamination. If found, the vehicle should be recleaned and sanitized prior to use.

When possible, use dedicated vehicles for transport of produce. When this is not practical, it is important to clean and sanitize the vehicles before they are used for transport of FFV to reduce risks that may be introduced during other activities.

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4

Primary production in protected facilities

4.1 PROBLEM SCOPE

Many commodities are produced in protected facilities to extend the growing season by protecting them from frost, protecting the crop from rain, reducing plant pathogens, and having year-round production by supplementing light and heat. Protected agriculture can include high tunnels, low tunnels, greenhouses, and controlled environment agriculture. Though these facilities protect the crop from the environment, they do not protect from all food safety hazards.

Controlled environment agriculture (CEA) takes a technology-based approach to produce optimal growing conditions inside controlled environments such as greenhouses and indoor vertical farms. Plants are typically grown year-round using hydroponic, aeroponic, or aquaponic methods. The crops are less affected by climate or weather conditions and less exposed to animal and bird intrusion. Controlled environment agriculture production has evolved from simple shade structures and hoop houses to full indoor vertical, highly sophisticated greenhouse facilities with controlled lighting, water, and ventilation.

The following production systems were considered in this report:

» High and low tunnels (semi-closed systems, soil based, two sides open)

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Figure 1. Examples of net houses (A) and greenhouses (B) using soil as substrate

» Net houses that offer physical protection against some animal pests and light.

- Examples of net houses (Figure 1A and B)

» Greenhouses (closed systems constructed of glass or plastic, regulation of temperature and humidity), with either low or high technological inputs. Greenhouses can use soil or many different substrates (e.g. hydroponics, aquaponics) to grow crops.

- Examples of greenhouses using hydroponics (Figure 2A) and substrate (Figure 2B)

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Figures 2. Examples of greenhouses using hydroponics (A) and substrates (B)

- Examples of greenhouse using soil (Figure 3).

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Figure 3. Examples of greenhouses where crops are grown in soil

- Examples of greenhouses using different hydroponic systems (Figure 4).

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Figure 4. Examples of greenhouses using different hydroponic systems

» Aquaponic systems wherein plant production is coupled with aquaculture. Fish may be raised in the same stream as the plants or separately. The most common foodborne pathogens are usually not found, but little is known about other pathogens.

» Novel and emerging crop production systems are in shipping containers, warehouses, urban roofs, or vertical farms, which may be done on a commercial scale and on a small scale via in-house or in-home systems.

These protected systems have as a main objective the efficient production of high-value crops at maximum productivity in an environmentally friendly way (e.g. reduce water use, reduce land use), but they are not inherently safer than open systems as contamination can occur through production practices and procedures that introduce hazards into the environment. The main risk in primary production in protected systems is the spread of microbiological hazards from the source of contamination to potential niches. Protected systems are generally assumed to be less prone to contamination because they provide primary barriers against sources of hazardous microorganisms, such as animal pests. However, other contamination routes (e.g. water, organic amendments, workers, infrastructure) persist, and hazards can spread quickly across large crop volumes (Holvoet *et al.*, 2015; Delbeke *et al.,* 2015). This was the case in a *Salmonella* outbreak linked to the consumption of greenhouse-grown salad greens in the United States of America in the summer of 2021 (CDC, 2021). The most important contamination sources in the greenhouse are irrigation water and the introduction of manure. During the summer of 2021, a recall of greenhouse leafy green products was initiated following a positive test of *L. monocytogenes* from a routine facility test of rainwater holding tanks in the greenhouse facility (USFDA, 2021).

4.2 POTENTIAL IMPACT

Some environmental factors in protected production systems (e.g. constant temperature, humidity) may create conditions that facilitate the survival and expansion of bacterial hazards, leading to enhanced risk of crop contamination. Environmental monitoring best practices may also be needed if the popularity of protected production systems continues to increase (Misra and Gibson, 2021). This concern is enhanced in regions where large volumes of widely consumed fresh fruits and vegetables are primarily produced in protected systems, which enhances the risk of population exposure.

The main microbiological hazards associated with protected production systems include enteric pathogens (*Salmonella* spp., pathogenic *E. coli*) linked to the use of fertilizers, animals, or caused by cross-contamination*,* as well as *Listeria monocytogenes* derived from the production environment (soil, water). *L. monocytogenes* can also provoke a biofilm or niche from which produce can be (systematically) contaminated. Very persistent strains have been identified. In addition, workers can spread human pathogens, especially viruses such as norovirus and hepatitis A.

While many new or alternative types of protected production systems have emerged in recent years, few have benefitted from microbiological assessments. In addition, fruit and vegetable production guidelines for food safety and existing legislation may be difficult to apply in the context of entirely novel production systems.

The food safety implications of integrated animal and food plant production in aquaponic systems need to be examined in more detail as some bacterial species associated with fish (*Vibrio* spp., *Aeromonas* spp. Enterobacteriaceae, etc.) are potentially pathogenic to humans.

4.3 MITIGATION AND INTERVENTION MEASURES

The most important mitigation measures for protected fresh fruit and vegetable production systems include:

- adherence to GAPs and a food safety management system; and
- establishing environmental monitoring programmes for *Listeria* (*monocytogenes*).

There is an underlying assumption that fruit and vegetable crops produced in protected environments have a lower risk of contamination than crops grown outdoors. In general terms this is true for several risk factors, but preventive measures have to be taken by growers. It should be considered that environmental conditions that are allowing optimal yields in protected crops are also adequate for promoting growth of human pathogens.

When leafy vegetables are grown indoors (e.g. tunnels, semi-tunnels, greenhouses), structures should be located, designed and constructed to avoid contamination and harbouring pests such as insects, rodents and birds (FAO and WHO, 2017). Each food business operation should be evaluated individually to identify specific sanitation requirements for each product (European Union, 2004). Food safety priorities to prevent contamination of protected crops include water quality, handwashing, environmental controls, enforceable worker health policy, cleaning and sanitation of reusable plastic containers, reused retail-ready containers, and irrigation water testing for human pathogens to prevent contamination (Illic *et al.*, 2017).

The USFDA has recently published a list of requirements and recommendations applicable to growers engaged in CEA (USFDA, 2022), which includes:

1) developing a keen understanding of potential sources and routes of contamination including the raw materials and inputs used, as well as possible sources of contamination throughout the operation;

2) implementing effective sanitation procedures and sampling plans while also paying close attention to hygienic operations and equipment design, ensuring that cleaning procedures do not contribute to the dispersion of microbial contaminants that may be present;

3) assessing growing operations to ensure implementation of appropriate science- and risk-based preventive measures, including applicable required provisions of the current legislation and good agricultural practices (GAPs);

4) verifying the effectiveness of routine monitoring of processing and storage environments to prevent pathogen growth in harvested crops;

5) ensuring that all growing pond water is safe and of adequate sanitary quality for its intended use, which includes implementing measures (such as water treatment) necessary to reduce the potential for contamination by known or reasonably foreseeable hazards;

6) performing a root cause analysis when a pathogen is identified in the growing environment, in raw agricultural inputs such as water, or in the agricultural commodity to determine how the contamination likely occurred, and implementing appropriate prevention and verification measures; and

7) assessing and mitigating risks associated with adjacent and nearby land uses that may impact CEA operations, in both rural and more urbanized settings.

Intervention strategies aimed at inactivating or eliminating pathogens to reduce them to acceptable levels include using integrated pest management (IPM) systems and biocontrol measures for pest and disease control. These approaches take measures to exclude domestic animals and wildlife from crops, adequately treating manure to destroy pathogens and stabilizing nutrients, and testing and remediating (if necessary) irrigation water quality at regular intervals (Suslow *et al.*, 2003; Islam *et al.*, 2004; Pachepsky *et al.*, 2011; Gil *et al.*, 2015).

A research study based on an iterative systematic Delphi expert elicitation approach concluded that contaminated greenhouse surfaces that are in contact (direct or indirect) with the vegetables and fruits are important sources of pathogen transfer to crops, highlighting the need for environmental controls in greenhouses. The importance of adequate worker management practices including handwashing and health policy highlights the role of worker education in reducing the risks of foodborne outbreaks linked to greenhouse grown produce (Illic *et al.*, 2017).

Other relevant documents include the CanadaGAP Food Safety Manual for Greenhouse Product (CanadaGAP, 2021).

4.4 AVAILABLE DATA

There are already specific guidelines describing good agricultural practices (GAPs) and good hygiene practices (GHPs) for CEA operations (FAO and WHO, 2017), which include general recommendations such as: i) protected facility structures should be located, designed and constructed to avoid contamination and harbourage of pests; ii) worker training and sanitation practices are necessary in all operations; and iii) proper water management and soil amendment use are critical to controlling and reducing risks. However, they are often not tailored to a defined production situation (e.g. greenhouse, high tunnel). Large and well-established greenhouses have effective food safety management systems (FSMS) in place which reduce the risks (Kireziva *et al.,* 2015a, b). However, many small producers are less prepared because they do not have enough people, knowledge and resources to implement GAPs and GHPs in their facilities (Nanyunja *et al.,* 2015). Based on a systematic Delphi expert elicitation study, food safety priorities in greenhouses include handwashing, environmental controls, enforceable worker health policy, cleaning and sanitation of reusable plastic containers and reused retail-ready containers, and management of irrigation water (Ilic *et al.,* 2017).

Growers and workers routinely access protected facilities where crops (e.g. tomatoes, peppers, berries) are often picked by hand. Moreover, production in protected systems may take place where other farming activities, such as animal production, take place. Here, growers and workers can become a source of cross-contamination between humans, animals, the production environment, water reservoirs and crops (Delbeke *et al.,* 2015).

Nutritional solutions used in hydroponic systems have been shown to allow the growth of bacterial foodborne pathogens (López-Galvez *et al.,* 2016). Consequently, the use or storage of such solutions for extended periods of time may introduce significant risk. The same applies to aquaponic systems, particularly where filtration is relied upon to remove particulates from waters derived from aquaculture. There is significant public interest in aquaponic systems, and tours are popular. However, public access to the growing area represents a potential risk.

Some protected systems incorporate very sophisticated technology including humidity control and water treatment systems. The impact of these technologies on the safety of the crops is not well known, and new challenges might be introduced. This is the case of urban farming and new production systems that try to incorporate aspects of circular economy mostly by the reuse of raw materials, such as the use of compost and organic fertilizers and the reuse of water, but little is known about the safety. If not practiced properly, urban agriculture can indeed be both unsanitary and polluting (Smit, Nasr and Ratta, 2001). Novel and alternative food production systems are often under-regulated, and little information is known regarding their potential to support to a greater or lesser extent the growth and survival of foodborne pathogens. Intensified inclusion of aspects of circular economy, such as water reuse and use of organic fertilizers puts pressure on the contamination status of raw food sources and challenges the preharvest food safety management systems.

The quality of the air in protected facilities is a potential risk factor, particularly in closed systems. Unfortunately, little is known about the presence of airborne pathogens and the formation of aerosols in these environments.

4.5 UNCERTAINTY AND DATA GAPS

Most of the data gaps relate to the impact of the production environment on the contamination of fresh produce. Growers lack a good understanding about how efficacious the cleaning and sanitation activities are against foodborne microorganisms. In general, more training is needed regarding cleaning and sanitation of the environment and the harvesting equipment. There are a variety of technologies that can be applied for cleaning and sanitation, but they are also linked to the size of the facilities. The use of sanitation needs a good monitoring programme and knowledge for the management of the sanitizers. Apart from the data gaps related to cleaning and sanitation, little is known about the air recirculation, water reuse, impact of specific production systems in microbial growth, and so forth. More research is needed to fulfil these data gaps.

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5

Minimal processing

5.1 PROBLEM SCOPE

Minimally processed fruits and vegetables are subjected to a variety of unit operations which provide opportunities for contamination with foodborne pathogens, including grading, removing outer or damaged leaves, coring, cutting, washing, drying, and packaging, among others. One of the main problems is cross-contamination with surfaces and equipment, but also between different batches of produce and water. Personnel hygiene is also critical to avoid contamination during processing. It is important to consider that, after packaging and during storage and distribution, some pathogens can survive or even grow.

Raw material should be inspected upon receipt to minimize risks. Inspection is part of quality control and the first barrier to avoid low quality products entering the processing plant. The production environment and equipment are of special concern because if one contaminated batch of produce is introduced in the processing facilities, cross-contamination of subsequent batches of produce may occur (Gil *et al.,* 2015). Food contact surfaces such as containers, conveyors, equipment and utensils represent a potential source of contamination, especially if they are cleaned infrequently and biofilms form. Biofilms are challenging to remove once they have become established. Minimally processed fruits and vegetables often are mechanically sliced using high-speed machines. Fruit and vegetable tissue may be damaged when knives are not well maintained (Barry-Ryan and O'Beirne, 1998).

Water is used in large volumes during minimal processing. The previous JEMRA meeting on safety and quality of water used with fresh fruits and vegetables (FAO and WHO, 2021) summarizes clear and practical guidance on the microbiological criteria and parameters that can be used to determine if water is fit-for-purpose when used in the pre- and post-harvest production of fresh fruits and vegetables (FFV). Additionally, Section 3.7.5 summarized the most relevant risks associated with the use of water during harvest and post-harvest handling operations. It is important to highlight that microorganisms present in the water and on the produce may be internalized via stomata and natural openings and cannot be completely removed during washing operations.

Drying and dewatering systems, aiming to reduce the surface water of the product after washing, are critical steps. The time and speed of centrifugation, or alternative dewatering systems, need to be adjusted for each product to reduce tissue damage and subsequent microbial deterioration. In the case of baby leafy greens, which are too delicate to withstand centrifugation, intervention strategies such as the use of forced air or air-bed conveyors may be used. However, if forced air is used, it must be filtered to avoid the contamination of the product (Gil *et al.*, 2015).

After cutting, washing and drying, the final operation is packing. In most cases, the assembly and packaging room are separated from the reception and washing sections, but this is not always possible. The environment of the packing rooms can be a source of contamination (Gil *et al.*, 2015). On the other hand, Lehto *et al.* (2011) found that air inside a processing plant can be a vehicle for contamination of food by pathogens if not properly controlled.

Breaking the cold chain during distribution and storage of minimally processed fruits and vegetables is the main problem which contributes to the amplification of foodborne pathogens during minimal processing, and particularly during distribution and storage.

5.2 POTENTIAL IMPACT

It is accepted that contamination of produce with foodborne pathogens can occur during primary production and harvest, but produce can also be contaminated during minimal processing or be amplified during minimal processing, and subsequent storage and distribution activities. If a low proportion of contaminated produce is introduced in the processing plant, this initial contamination can contaminate a large proportion of the product during processing operations. This amplification of the contamination contributes to the occurrence of large foodborne outbreaks. Cross-contamination of produce via contact with contaminated surfaces and equipment, water and workers as well as breaking the cold chain are critical factors that contribute to both initial contamination and amplification of the microbial contamination during processing.

5.3 MITIGATION AND INTERVENTION MEASURES

Processors should ensure that their suppliers (e.g. growers, harvesters, packers, distributors) have adopted the principles outlined in the Code of Hygienic Practice for Fresh Fruits and Vegetables (FAO and WHO, 2017, 2022; Suslow, 2003). Preventive sanitation programmes such as GHPs, GMPs, and Sanitation Standard Operating Procedures (SSOPs) should be implemented to reduce the risk of contamination by pathogenic bacteria, viruses and parasites (CLGMA, 2021). Prerequisites for HACCP-based food safety programmes are required at all stages of minimal processing.

The first barrier to prevent contamination from entering the processing plant is the inspection of the raw material. Product of visibly inferior quality or compromised in some manner relative to food safety risks, such as insect damage or bruising, should be rejected. Grading and selection are very important activities (i.e. discarding or trimming damaged or decayed material) to reduce the level of contamination, since the external parts of the crop which are eliminated during trimming are usually the most contaminated with dust and soil.

To avoid cross-contamination between final product and raw materials, product flow and segregation from incoming raw product to outgoing washed product is particularly important, as is exposure to chilling environments (e.g. forced air cooling, cold storage) to maintain quality and reduce microbial growth potential. To avoid damage to the product while cutting or peeling, which might favour microbial growth, intervention strategies aiming to maintain equipment such as replacing and/or sharpening knives on a regular basis are necessary (FAO and WHO, 2017, 2022).

The periodic maintenance and cleaning during shifts, and daily cleaning and sanitizing of equipment surfaces with careful attention to the cutting equipment is essential to reduce microbial hazards (Sapers, 2003). The processing environment and equipment should be periodically cleaned and maintained during each working shift. In most cases, processing facilities are cleaned and sanitized at least once a day and special attention is given to the cutting equipment to reduce potential persistence of pathogens in the equipment. Packaging under hygienically controlled conditions immediately after cleaning and sanitizing provides microbiological protection of fresh-cut produce (FAO and WHO, 2008; Turatti, 2011; Gil *et al.,* 2015).

Washing (primary and secondary) removes soil, other gross debris, and plant tissue exudates that occur during cutting and contributes to overall microbial reduction. To minimize cross-contamination in those operations where water is used in contact with the produce (e.g. cooling, washing, rinsing, transporting), water disinfection treatments, and particularly antimicrobials, are routinely added to the water. It has been reported that the mechanical force of the water during washing allows the reduction of the overall microflora of fruits and vegetables. In most cases, the use of antimicrobials to maintain the microbiological quality of the water only has a limited impact on the reduction of the microorganisms present on the surface of the produce. Most of the available studies report a limited lethality (~2 log cycles) on the epiphytic microorganisms and no effect on internalized microorganisms. Therefore, minimizing the potential for contamination in the field from the seed onward is key to reduce food safety risk in fresh produce.

Maintenance of the cold chain is responsible for the preservation of the organoleptic and microbiological quality of fresh fruits and vegetables. Studies show that the efficiency of the cold chain is often less than ideal, as temperature abuses above or below the optimal product-specific temperature range occur frequently, a situation that significantly endangers food safety (Mercier *et al.,* 2017). Therefore, temperature control and maintenance of adequate refrigeration at all stages is a key mitigation measure for produce safety, particularly during processing, distribution and storage.

In addition to the above-mentioned preventive measures, there are also specific intervention strategies that can be applied as additional barriers to inhibit the growth of foodborne pathogens if present in fresh produce. It is important to highlight that decontamination practices can be a useful tool in further reducing the number of pathogenic microorganisms, but the use of substances intended to remove microbial surface contamination should only be permitted if a fully integrated control programme is applied throughout the entire food chain (EFSA, 2016). Several commercial treatments based on bacteriophages and bacteriocin-producing cultures have been proposed as treatments to control various foodborne pathogens, which can be used to complement GHPs and GMPs (Truchado *et al.,* 2020).

5.4 AVAILABLE DATA

Gil *et al.* (2015) described the mitigation measures to be implemented during minimal processing of fruits and vegetables, from the point of the reception to distribution and storage. Relevant information about the impact of conventional and emerging techniques used for the minimal processing industry to guarantee the safety of fruits and vegetables has been summarized by Artés and Allende (2014).

Buchholz *et al.* (2012) generated baseline data for *E. coli* O157:H7 transfer from inoculated equipment surfaces to uninoculated lettuce during pilot-scale processing without the use of antimicrobials. They observed that the greatest *E. coli* O157:H7 transfer was observed from inoculated lettuce to the shredder and conveyor belt. On the other hand, Gu *et al.* (2019) reported that cleaning and sanitizing performed in the processing environment of fresh-cut processing plants resulted in both quantitative and qualitative reductions of microorganisms. However, the potential formation of biofilm in the equipment surface is a great concern. Liu *et al. (*2015) demonstrated the ability of *E. coli* O157:H7 to form dual-species biofilms in equipment surfaces, suggesting an effective protection against external stresses.

Multiple studies have assessed the efficacy of different treatments to reduce accumulation of microorganisms in the water. Section 3.7.5 of this report included a description of the intervention strategies needed to monitor and maintain the microbiological quality of water used during harvest and post-harvest handling operations. This is also valid for minimal processing operations where water is used. Additionally, the previous JEMRA meeting on safety and quality of water used with fresh fruits and vegetables (FAO and WHO, 2021) summarizes clear and practical guidance on the microbiological criteria and parameters that can be used to determine if water is fit-for-purpose when used in the pre- and post-harvest production of fresh fruits and vegetables (FFV).

Truchado *et al.* (2020) evaluated the efficacy of bacteriophages and bacteriocin-producing cultures to inhibit growth of *L. monocytogenes* in minimally processed leafy greens. They concluded that bacteriophages seem to be a promising decontamination treatment for leafy greens aiming to reduce growth of bacteria.

5.5 UNCERTAINTY AND DATA GAPS

Minimally processed fruits and vegetables include many different handling and processing operations, and each of them might have an impact on the microbiological safety of the final product. As minimal processing happens during primary production, it is unlikely that there is sufficient research data to clearly identify all hazards or define practices to reduce all associated risks. One should keep in mind that one-size-fits-all approaches do not serve the minimal processing industry mostly because no two processing or packing facilities are exactly the same. Knowing the limitations of the available science-based evidence, the experts have summarized the most acknowledged risk factors, impacts, and available mitigation measures. The following data gaps have been identified by the experts:

- Washing effectiveness: Research methods should more closely reflect real conditions encountered in commercial processing and should include impact on risk assessment outputs.
- Sanitizers: Research on new products and technologies for produce disinfection is lacking, and it should include impact on risk assessment outputs.
- Data gaps exist on the extent of microbial attachment and infiltration after harvest and during processing under real commercial conditions.
- Data gaps exist on the use of novel technologies for post-packaging decontamination of produce that does not affect their physical properties.

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6

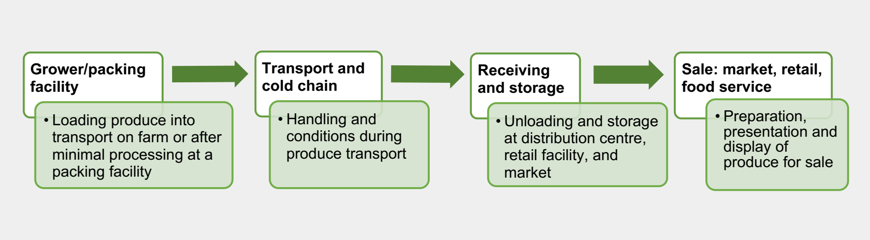
Transport, distribution, and point of sale

6.1 PROBLEM SCOPE

This section covers all stages from both the field packing of fresh produce and the produce packing house through transport and distribution, to the point of sale which includes retail, fresh markets, food service, and restaurants (**Figure 5**). It includes all the infrastructure and logistics which preserve the safety and integrity of fresh produce from the production environment to the consumer and is often referred to as the cold chain.

Activities and operations along this continuum include the loading and unloading of produce into transport vehicles, storage at the end facility (e.g. refrigerated, chilled, temperature controlled, modified atmosphere), and display and presentation of produce at the point of sale.

Figure 5 Major stages in the supply chain post farmgate



Source: Authors' own elaboration.

The microbiological status of fresh produce is influenced by conditions encountered along this supply chain including the possibility of contamination and the growth of pathogens during transport, distribution, storage, and at the point of sale.

Contamination is a result of improper handling during loading and unloading; contamination can also occur through comingling with other raw produce or during transport with other commodities, animal products, and animals, and can occur through exposure to unsanitary conditions during storage and at the point of sale.

Environmental conditions during transport will vary depending upon the nature of the produce and access to equipment designed to ensure freshness. Some vegetables and tropical fruits are vulnerable to elevated temperatures and temperature fluctuations. However, in the majority of cases, temperature control is a vital component in fresh supply chains, with the goal of maintaining the freshness and quality of fresh produce and preventing the growth of pathogens.

Importantly, access to cold chain facilities (during transport or storage) may not be available, and this is especially prevalent in emerging economies and remote settings.

6.2 POTENTIAL IMPACT

The microbiological issues encountered along this part of the supply chain include contamination that occurs through handling during loading, unloading, storage, and display; contamination through comingling with other types of fresh produce; and potential contamination during transport. All are impacted by the equipment, facilities, and the practical realities on the ground. Access to enclosed, hygienically designed, refrigerated transport vehicles is a key requirement. This is usually not an issue in developed economies but is problematic in emerging economies where transport is used for a variety of purposes and may not meet requirements of being sanitary and temperature controlled.

The operation of transport vehicles is impacted by the following:

- temperature monitoring of vehicles and smart sensors in/on individual packs – temperature recording devices, sensors, and indicators of microbial growth potential are useful if available;
- packing strategies and use of fans in the cold rooms to facilitate uniform airflow around the produce;
- cleaning of vehicles, bins, and pallets to reduce cross-contamination risks; and
- scheduling transport to reduce risks – where there is no refrigeration, there is

a need to improve transportation and avoid the heat of the day, traffic delays, or periods where there may be high insect/vermin activity.

Basic education and training of drivers and operators in aspects of food hygiene is a fundamental requirement. Importantly, this needs to address the ever-present risk of cross-contamination. Training and guidance materials need to be provided in the appropriate language and educational level.

The sources of contamination that may be encountered along the supply chain include:

- unclean bins, baskets, and packing boxes used to transport produce;
- transport vehicles – If vehicles are not enclosed, open-air conditions may expose produce to contamination or ingress from insects, birds, vermin, dust, and fumes;
- transport vehicles – Contamination may also arise because of prior carriage of non-food grade substances, animals, and incompatible food (e.g. meat from abattoir, fertilizer, chemicals);
- comingling with live animals being transported to a market; and
- handlers not practicing proper hygiene, which may contaminate produce.

A further concern is the failure to adequately control temperatures during transport as this may provide opportunities for growth of bacterial pathogens on fresh produce. This is a particular concern where there are prolonged periods of transport without adequate temperature control. The risks increase where minimally processed produce has been cut (e.g. fresh cut leafy greens) as the release of nutrients from cut surfaces will promote bacterial growth. The way transport trucks are loaded is also important. Microclimatic zones may result depending on how the vehicles are stacked and the extent to which air can circulate amongst the cargo.

Maturation and deteriorative processes may also predispose fresh produce to deterioration and may result in bacterial growth. Comingling different types and lots of produce can cause issues where produce has different respiration patterns during maturation and ripening (i.e. exposure of unripe climacteric fruit to ethylene can cause earlier than desirable ripening). Products that generate ethylene include apples, apricots, avocados, cantaloupe, feijoa, kiwifruit (ripe), nectarines, papaya, peaches, pears and plums. Therefore, mixed transport of different fruits and vegetables may advance ripening, predisposing the produce to spoilage and the potential emergence of pathogens or conditions which favour pathogen growth.

Control of sanitation and temperatures are also essential during storage of produce at a distribution centre or in a retail setting. Produce may be placed in a cold storage or go directly into a retail storage facility, and the issues of the comingling of produce, the production of ethylene, and multiple use chillers may be encountered. An important issue is exposure to warm humid air during unloading or failure to operate cold chain facilities correctly as this can result in temperature fluctuations which lead to condensation on overhead fittings, lights, walls and ceilings – condensation containing pathogens (*Listeria monocytogenes*) may drip onto and contaminate exposed produce.

Cross-contamination is also a potential risk in retail settings, especially fresh food markets. This includes proximity to raw foods such as meat or fish; consumer handling of produce and access by domestic animals, insects, and vermin. During the COVID-19 pandemic, there was a reintroduction of packaging fresh produce to avoid consumers contacting food for sale, which can either reduce or enhance microbiological risks depending on how it is done.

Access to hygienic facilities and services is important in markets and retail settings. Water quality can impact produce safety – water is used for washing fresh produce, ice-making operations, and for cleaning programmes. Poor quality water or ice can contaminate produce.

Incidents usually arise when multiple factors combine. For example, contamination in the field, followed by failure of a washing or a sanitation step, can be exacerbated by problems along the cold transport chain or by inadequate handling at retail or in the consumer's home. Some of these may be amplified in developing country situations – due to absence of effective controls or failure along the cold chain.

6.3 MITIGATION AND INTERVENTION STRATEGIES

Interventions that address food safety along the transport chain include access to appropriate transport vehicles and education and training of operators. Both aspects are impacted by practicality, feasibility and realities on the ground.

The provision of hygienic facilities and services during produce storage and at point of sale are also a key to risk reduction. Well-maintained and fully functional cold storage and hygiene display surfaces are essential. Where appropriate, vulnerable points should be identified, and comprehensive food safety plans to address the vulnerabilities should be developed. All operations should develop an effective traceability system.

In developing suitable training programs, it is important to:

- identify priority groups for training and education;
- develop training materials and teaching strategies that are culturally appropriate;

- include basic hygiene and provide training which develops competencies in personal hygiene as well as cleaning and sanitizing surfaces; and
- be adapted to the audience level of education and experiences – wet markets and farmers markets may require tailored information.

6.4 UNCERTAINTIES AND DATA GAPS

There is a paucity of data covering this aspect of the supply chain in emerging economies, where challenges include the inability to access suitable transport and the absence of effective temperature control at all stages. These are persistent challenges.

The absence of good monitoring data impedes the ability to determine where contamination occurs along the transport, storage, and sale continuum, or the extent to which storage conditions impact pathogen growth.

Where produce is destined for high value markets such as export markets, there are financial incentives to improving this part of the supply chain. The Australian Cold Chain Guidelines for Food 2017 provide best practice recommendations for transport, logistic, and safety requirements to ensure the safety and quality of chilled and frozen foods including fresh produce (AFGC, 2017).

6.5 REFERENCES IN SECTION 6

AFGC (Australian Food and Grocery Council). 2017. *Australian cold chain guidelines.* Griffith, ACT. [Cited 01 May 2023]. https://www.afgc.org.au/wp-content/uploads/2019/07/Australian-Cold-Chain-Guidelines-2017.pdf

7

Significant gaps in mitigation and interventions measures

Fruit and vegetable production includes many different commodities grown in diverse geographic regions around the world. It is unlikely there will ever be sufficient research data to clearly define all risks or to define practices to reduce all risks. Acknowledging these challenges, the experts note elsewhere in this report areas of research that would be most valuable for both risk identification and the development of improved mitigation and intervention measures:

- Primary production in open fields: There are significant data gaps mostly because there is a lack of scientific evidence about the impact of different agricultural practices, contexts on the survival and growth of pathogens, and regionally specific data. Fruits and vegetables are produced all around the world, but most of the data have been obtained in a limited number of countries, while data is still missing for many countries. Available research on the survival and growth of pathogens in fresh produce indicates that results are usually context dependent. Therefore, the information obtained in one specific area cannot be directly extrapolated to another area. This affects most of the relevant risk factors in primary production including: 1) location, adjacent land use, topography and climate; 2) prior land use; 3) unintentional contact of crop with contaminated water, such as flooding; 4) wildlife, livestock, and human intrusion; 5) water quality and availability; 6) soil amendment types and uses; and 7) harvest, field packing, and packing house practices and infrastructure.
- Primary production in protected facilities (e.g. high and low tunnels, greenhouses, net houses, hydroponic and aquaponic systems): The use

of protected facilities to grow fruits and vegetables is gaining popularity. However, growers still lack knowledge about the main risk factors in each type of production. The closed production environment can be seen as a more controlled environment with fewer risks, but this is not always true. If not well managed (i.e. pest control, proper sanitation, worker training programmes), protected facilities can have just as many risks and can represent a source of cross-contamination. More knowledge is needed to have a good understanding about microbiological risks and how efficacious the cleaning and sanitation activities against foodborne microorganisms in these types of environments are.

- Processing: As for primary production, it is unlikely that there are sufficient research data to clearly identify all hazards or define practices that help to reduce microbiological risks in all the processing environments and under all manufacturing practices. Relevant data are still needed to understand the efficacy of water disinfection treatments to maintain the microbiological quality of process water. There are also knowledge gaps linked to the capacity of microorganisms to be internalized in the produce during the different operations. Additionally, there is a need to search for advanced post-packaging decontamination intervention that could reduce or eliminate the risk of contamination.
- Storage, distribution, and point of sale: The global complexity and diversity of how fresh produce is stored, distributed and marketed at point of sale creates extreme data gaps, particularly in developing economies. For this reason, the experts decided to focus on the impacts of cold chains both the lack of and improperly managed cold chains. Although the amount of available data about the time and temperature conditions needed for the survival and growth of pathogenic microorganisms in produce under real conditions could be representative of the situation in developed economies, there are still many challenges in emerging economies mostly due to the difficulties for suitable transport and the absence of effective temperature control. The lack of reliable monitoring data represents an impediment to determine which situations should be avoided. Therefore, more data in different settings is necessary to identify the riskiest practices.
- Retail, food service, consumer education and training: This is the forgotten part of the supply chain because there are very limited studies highlighting the significance of education and training on the safety of fresh produce. However, it has been demonstrated that this is really relevant. More research is needed to understand what the most suitable mechanisms are to communicate potential risks linked to this last link of the chain.

Annex

A1.

Response to Codex Committee on Food Hygiene (CCFH) regarding specific interventions for leafy greens

The following questions that were presented by the CCFH electronic working group (eWG) on the development of the "Guidelines for the control of STEC in raw beef, fresh leafy vegetables, raw milk and raw milk cheeses, and sprouts" on 27 July 2021 were addressed separately by the experts convened as part of the Joint FAO/WHO Expert Meeting on Microbiological Risk Assessment (JEMRA) on the Prevention and Control of Microbiological Hazards in Fresh Fruits and Vegetables.

Q1. Most control measures in Annex 2 "Fresh leafy vegetables" of the draft "Guidelines for the control of STEC in raw beef, fresh leafy vegetables, raw milk and raw milk cheeses, and sprouts" are not specific for STEC (and thus information in the *Code of Hygienic Practice for Fresh Fruits and Vegetables* would suffice). JEMRA – Please provide input on control measures that have been studied scientifically with respect to control of STEC and thus warrant inclusion. (These measures may also control other pathogens, but we need to know if there is sufficient scientific information related to control of STEC to warrant including them in this annex.)

A1. Many potential measures have been scientifically studied with respect to control of microbiological hazards in fresh fruits and vegetables, including leafy greens. However, based on the experts' opinions, while much of this research was not carried out with STEC, the conclusions are valid for STEC control as well. Specific experiments using different STEC are not necessary; there is no evidence to indicate that STEC behaves differently in response to these control measures. The most significant control measures include:

- maintaining the cold chain at every stage along the farm-to-fork continuum;
- adding sanitizers to wash water to prevent cross-contamination. It is noted that sanitizer use can reduce microbiological load on product, but the data are not sufficient to provide consistent outcomes; however, inclusion is prudent to prevent cross-contamination;
- avoiding direct application of untreated animal manures (e.g. ruminant species, pigs, poultry) to leafy vegetable fields as it may increase the likelihood of STEC contamination. Composting reduces risk of contamination, but the quality and effectiveness of composting can be variable, so the primary recommendation is to avoid the application of untreated raw manures to leafy vegetable fields in the year of production; and

- ensuring water that contacts the crop directly is fit-for-purpose. If growers do not have the resources to monitor or determine water quality, adopting practices that prevent direct water contact with the edible part of the crop are recommended.

Q2. It has been suggested that the guidelines address HACCP system principles. Please provide input on whether good hygiene practices (GHPs) or good agricultural practices (GAPs) at a step provides adequate control of STEC or whether there are applicable critical control points (CCPs).

A2. GHPs or GAPs provide an effective means of establishing farming practices, which minimize potential contamination by microbiological hazards, including STEC. Providing guidance to producers on minimizing contamination should be encouraged. For example, the introduction of HACCP system prerequisite programmes in fruit and vegetable production will reduce contamination as they include practices captured in GHPs and GAPs. It is appropriate to use the HACCP system during minimal processing activities; however, there are no CCPs that eliminate microbiological hazards.

Q3. It has been proposed that we add here that growers should be looking at distances between fields and nearby animal operations, and should be considering a minimal distance, if possible, based on recent scientific studies and publications. Is there scientific evidence to support recommendations for distance between fields growing leafy vegetables and animal operations? If not, is there specific guidance you can provide on what to consider in evaluating and controlling the risk from animal operations close to leafy vegetable growing fields?

A3. There are insufficient data to determine a minimum distance between fields and nearby animal operations, though it is noted that risks should decrease as distance increases. It is important for each operation to make an assessment based on its situation. Factors that should be considered include wildlife (e.g. type, abundance, movement), air movement and prevailing winds, hydrologic system and likely runoff, topography, human factors including intrusion and movement, and other related conditions.

Evidence indicates that the risk of airborne transport of *E. coli* O157:H7 from cattle production increases when cattle pens are very dry and when this situation is combined with cattle management or cattle behaviours that generate airborne dust (Berry *et al.,* 2015). Based on these results, distances between fields and nearby animal operations higher than 180 m would be recommended because *E. coli* O157:H7 positive leafy greens were found at that distance. However, additional research is needed to determine safe set-back distances between cattle feedlots and crop production.

Berry, E.D., Wells, J.E., Bono, J.L., Woodbury, B.L., Kalchayanand, N., Norman, K.N., Suslow, T.V., Lopez-Valesco, G. & Millner, P.D. 2015. Effect of proximity to a cattle feedlot on *Escherichia coli* O157:H7 contamination of leafy greens and evaluation of the potential for airborne transmission. *Applied and Environmental Microbiology*, 81(3): 1101.

Q4. Should we indicate that fresh leafy vegetables should not be harvested in areas where animal faeces are found and evaluate the risk when other evidence of animal intrusion is found? If so, what is the size of the area (e.g. around/right next to where faeces were observed? Or larger areas/field?). Is it practical to delineate an area that should not be harvested? What is the scope of vegetables which should not be harvested (e.g. Would this be limited to vegetables which are damaged by wild animals and/or contaminated by wild animal faeces?)?

A4. Fresh leafy vegetables that have direct faecal contamination (visible) on the edible portion of the crop must not be harvested. There is insufficient data to provide a standard no-harvest buffer zone recommendation, but there are several considerations that should be taken into account when considering a no-harvest buffer zone. Where there is animal intrusion and evidence of localized faecal contamination, an assessment of the extent of contamination should be conducted. Factors that should be considered in the size of the no-harvest zones should include the extent (e.g. volume/mass/area of contamination), the distribution of contamination (e.g. localized, widespread), type of harvest (e.g. hand, mechanical), impact of irrigation or rain influencing splash or spread, and the perceived timing of the contamination (e.g. recent, past). The purpose of establishing a buffer zone is to minimize risks of direct faecal contamination as well as prevent cross-contamination with equipment, hands, and harvest tools.

Q5. Can JEMRA provide advice on the role of testing of water to control STEC in fresh leafy vegetables? Is testing for STEC warranted and under what circumstances? What results would indicate a concern? Are there appropriate indicator organisms that could be used in lieu of or in addition to testing for STEC? What would acceptable levels (or levels of concern) be? What should the frequency of water testing be?

A5. JEMRA does not recommend the routine testing of irrigation water for the presence of STEC. Information on testing and indicator organisms were addressed during a JEMRA meeting on the use and reuse of water in vegetable production. More detail can be found in FAO and WHO (2021).

FAO & WHO. 2021. *Safety and quality of water used with fresh fruits and vegetables.* Microbiological Risk Assessment Series No. 37. Rome. https://www.fao.org/publications/card/en/c/CB7678EN

Q6. It has been suggested that we include a recommendation for storage under 7 °C here. JEMRA, does the science support this as an appropriate temperature for preventing the growth of STEC in fresh leafy vegetables? Are there other temperatures combined with time that could apply?

A6. There is no convincing scientific evidence that *E. coli* O157:H7 can grow on leafy vegetables at temperatures lower than 7 °C. Moreover, there is little data available concerning the growth of non-O157 STEC in leafy vegetables. The following references are offered in support of this assessment:

Luo, Y., He, Q., McEvoy, J.L. & Conway, W.S. 2009. Fate of *Escherichia coli* O157:H7 in the presence of indigenous microorganisms on commercially packaged baby spinach, as impacted by storage temperature and time. *Journal of Food Protection*, 72: 2038–2045.

McKellar, R.C & Delaquis, P. 2011. Development of a dynamic growth-death model for *Escherichia coli* O157:H7 in minimally processed leafy green vegetables. *International Journal of Food Microbiology,* 151: 7–14.

Posada-Izquierdo, G.D., Perez-Rodriguez, F., Lopez-Galvez, F., Allende, A., Selma, M.V., Gil, M.I. & Zurera, G. 2013. Modelling growth of *Escherichia coli* O157:H7 in fresh-cut lettuce submitted to commercial process conditions: chlorine washing and modified atmosphere packaging. *Food Microbiology,* 33: 131–8.

Kim, J., Chung, H., Cho, J. & Yoon, K. 2013. Evaluation of models describing the growth of nalidixic acid-resistant *E. coli* O157:H7 in blanched spinach and iceberg lettuce as a function of temperature. *International Journal of Environmental Research and Public Health* 10: 2857–2870.

Song Y.S., Stewart, D., Reineke, K., Wang, L., Ma, C., Lu, Y., Shazer, A., Deng, K. & Tortorello, M. 2019. Effects of package atmosphere and storage conditions on minimizing risk of *Escherichia coli* O157:H7 in packaged fresh baby spinach. *Journal of Food Protection,* 82: 844–853.

Q7. The eWG is considering these alternative sentences. What is the role of testing fresh leafy vegetables for STEC and/or indicator organism (including acceptable levels of organisms or levels of concern and frequency of testing)? (See Q5 where we asked about testing water for questions that also apply to product testing.)

The working group is also considering these alternative sentences:

> Microbiological testing of fresh leafy vegetables and of water for primary production for STEC is currently of limited use due to difficulty in detecting STEC resulting from low prevalence and low numbers of STEC in fresh leafy vegetables and in water.

> STEC, if present, is usually only present in low numbers in fresh leafy vegetables, and this makes direct testing for these pathogens technically challenging.
>
> Question: What is the role of testing fresh leafy vegetables for STEC and/or indicator organisms (including acceptable levels of organisms or levels of concern and frequency of testing)?

A7. Routine STEC testing at any stage is not recommended by the experts because the information derived from testing does not provide an accurate estimate of risk. It is strongly suspected that most contamination is sporadic and is non-homogeneously distributed within a lot and with low or very low number of contaminating microorganisms. This combination results in statistical challenges in most lots testing negative regardless of the contamination status of the lot. There are situations where targeted testing for STEC may be valuable, for example, to test system or product integrity where gross contamination is suspected. Product testing for indicators is also not recommended, but like STEC testing, can be useful in limited and specific situations where there is a need to verify or test a system.

FAO/WHO Microbiological Risk Assessment Series

1 FAO and WHO. 2002. Risk assessments of *Salmonella* in eggs and broiler chickens: interpretative summary.

2 FAO and WHO. 2022. Risk assessments of *Salmonella* in eggs and broiler chickens.

3 FAO and WHO. 2003. Hazard characterization for pathogens in food and water: guidelines.

4 FAO and WHO. 2004. Risk assessment of *Listeria monocytogenes* in ready-to-eat foods: interpretative summary.

5 FAO and WHO. 2004. Risk assessment of *Listeria monocytogenes* in ready-to-eat foods: technical report.

6 FAO and WHO. 2004. *Enterobacter sakazakii* and other microorganisms in powdered infant formula: meeting report.

7 FAO and WHO. 2008. Exposure assessment of microbiological hazards in food: guidelines.

8 FAO and WHO. 2005. Risk assessment of *Vibrio vulnificus* in raw oysters: interpretative summary and technical report.

9 FAO and WHO. 2005. Risk assessment of choleragenic *Vibrio cholerae* O1 and O139 in warm-water shrimp in international trade: interpretative summary and technical report.

10 FAO and WHO. 2006. *Enterobacter sakazakii* and *Salmonella* in powdered infant formula: meeting report.

11 FAO and WHO. 2008. Risk assessment of *Campylobacter* spp. in broiler chickens: interpretative summary.

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13 FAO and WHO. 2008. Viruses in food: scientific advice to support risk management activities: meeting report.

14 FAO and WHO. 2008. Microbiological hazards in fresh leafy vegetables and herbs: meeting report.

15 FAO and WHO. 2008. *Enterobacter sakazakii* (*Cronobacter* spp.) in powdered follow-up formula: meeting report.

16 FAO and WHO. 2011. Risk assessment of *Vibrio parahaemolyticus* in seafood: interpretative summary and technical report.

17 FAO and WHO. 2009. Risk characterization of microbiological hazards in food: guidelines.

18 FAO and WHO. 2010. Enterohaemorragic *Escherichia coli* in raw beef and beef products: approaches for the provision of scientific advice, meeting report.

19 FAO and WHO. 2009. *Salmonella* and *Campylobacter* in chicken meat: meeting report.

20 FAO and WHO. 2020. Risk assessment tools for *Vibrio parahaemolyticus* and *Vibrio vulnificus* associated with seafood: meeting report.

21 FAO and WHO. *Salmonella* spp. In bivalve molluscs: risk assessment and meeting report, in progress

22 FAO and WHO. 2016. Selection and application of methods for the detection and enumeration of human-pathogenic halophilic *Vibrio* spp. in seafood: guidance.

23 FAO and WHO. 2014. Multicriteria-based ranking for risk management of foodborne parasites.

24 FAO and WHO. 2016. Statistical aspects of microbiological criteria related to foods: a risk managers guide.

25 FAO and WHO. 2020. Risk-based examples and approach for control of *Trichinella* spp. and *Taenia saginata* in meat: revised edition.

26 FAO and WHO. 2022. Ranking of low moisture foods in support of microbiological risk management: meeting report and systematic review.

27 FAO and WHO. 2022. Microbiological hazards in spices and dried aromatic herbs: meeting report.

28 FAO and WHO.2016. Microbial safety of lipid based ready-to-use foods for the management of moderate acute and severe acute malnutrition: first report.

29 FAO and WHO. 2021. Microbial safety of lipid based ready-to-use foods for the management of moderate acute and severe acute malnutrition: second report.

30 FAO and WHO. 2016. Interventions for the control of non-typhoidal *Salmonella* spp. in beef and pork: meeting report and systematic review.

31 FAO and WHO. 2018. Shiga toxin-producing *Escherichia coli* (STEC) and food: attribution, characterization, and monitoring.

32 FAO and WHO. 2019. Attributing illness caused by Shiga toxin-producing *Escherichia coli* (STEC) to specific foods: report.

33 FAO and WHO. 2019. Safety and quality of water used in food production and processing: meeting report.

34 FAO and WHO. 2019. Foodborne antimicrobial resistance: role of the environment, crops and biocides: meeting report.

35 FAO and WHO. 2021. Advances in science and risk assessment tools for *Vibrio parahaemolyticus* and *V. vulnificus* associated with seafood: meeting report.

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37 FAO and WHO. 2021. Safety and quality of water used with fresh fruits and vegetables.

38 FAO and WHO. 2022. *Listeria monocytogenes* in ready-to-eat (RTE) foods: attribution, characterization and monitoring, meeting report.

39 FAO and WHO. 2022. Control measures for Shiga toxin-producing *Escherichia coli* (STEC) associated with meat and dairy products, meeting report.

40 FAO and WHO. 2023. Safety and quality of water use and reuse in the production and processing of dairy products, meeting report.

41 FAO and WHO. 2023. Safety and quality of water used in the production and processing of fish and fishery products, meeting report.

42 FAO and WHO. 2023. Prevention and control of microbiological hazards in fresh fruits and vegetables. Part 1 and 2: General principles, meeting report.

43 FAO and WHO. 2023. Prevention and control of microbiological hazards in fresh fruits and vegetables. Part 3: Sprouts, meeting report.

44 FAO and WHO. 2023. Prevention and control of microbiological hazards in fresh fruits and vegetables. Part 4: Specific commodities, meeting report.

Microbiological Risk
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Fruits and vegetables are an important part of a healthy diet and are protective against many chronic health conditions. Yet, fresh fruits and vegetables have been consistently implicated in food safety incidents involving microbiological hazards around the globe for decades.

In response to requests of the Codex Committee on Food Hygiene concerning microbiological hazards in fresh fruits and vegetables and to update and expand the information available in microbiological hazards in fresh leafy vegetables and herbs (MRA14), which was published in 2008, FAO and WHO convened a series of expert meetings in 2021 to 2022. The purpose of the meetings was to collect, review and discuss relevant measures to control microbiological hazards from primary production to point of sale in fresh, ready-to-eat (RTE) and minimally processed fruits and vegetables, including leafy vegetables.

The experts made an effort to update and include any recent trends in commodity and pathogen pairing or pathogen occurrence and presence with a focus on emerging and neglected pathogens. The primary production in open fields was investigated by considering the location, adjacent land use, topography, and climate; prior land use; water; wildlife, animal and human intrusion; soil amendments; and harvest and packing. The experts also worked on: primary production in protected facilities; minimal processing; transport, distribution, and point of sale; and also the gaps in mitigation and interventions measures. The advice herein is useful for both risk assessors and risk managers, at national and international levels and those in the food industry working to control the relevant hazards in the fresh fruits and vegetables.

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