Microbial Hazards in Irrigation Water: Standards, Norms, and Testing to Manage Use of Water in Fresh Produce Primary Production

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Short version of title: Microbial hazards in water (less than 40 letters and spaces)

ABSTRACT

Accessibility to abundant sources of high-quality water is integral to the production of safe and wholesome fresh produce. However, access to safe water is becoming increasingly difficult in many parts of the world, and this can lead to the production of fresh produce

contaminated with pathogenic micro-organisms, resulting in increased risk of human disease. Water, an important raw material in the fresh produce chain, is used in considerable amounts in many operations, including irrigation and application of pesticides and fertilizers, but also as a transport medium and for cooling and washing in postharvest practices. In several reported outbreaks related to uncooked fruit and vegetable products, water has been identified as a likely source of the outbreak. The present study, initiated by the ILSI Europe Emerging Microbiological Issues Task Force in collaboration with 8 other ILSI branches and support of WHO/FAO, was undertaken to review the status of, and provide suggestions for, consideration by different stakeholders on water and sanitation and its impact on food safety and public health. A limited number of guidelines and regulations on water quality for agricultural production are available and many of them are still heavily based on microbial standards and (debated) parameters such as fecal coliforms. Data gaps have been identified with regard to base line studies of microbial pathogens in water sources in many regions, the need for agreement on methods and microbial parameters to be used in assessing water quality, the fate of pathogens in water, and their transfer and persistence on irrigated/processed produce.

Key words: water, irrigation, outbreaks, fresh produce, good practices, testing

Introduction

Fresh produce (fresh fruits and vegetables) consumption has been increasing worldwide for several decades (Betts 2014; León and others 2009). The reasons are many, but key is the ‘healthy eating’ advice currently being promoted, where the ‘5 portions per day’ message is being widely advocated. Indeed even 7 portions a day has been recently mentioned. The expansion of the fresh produce market over recent years has resulted in a wide variety of fruit and fresh produce being available throughout the year. There is no doubt that the

consumption of increasing amounts of fresh fruits and vegetables is beneficial to the health of the consumer (Dauchet and others 2005).

Microbiologically, however, there can be some challenges in the production of safe fresh produce (Betts 2014), which has been accompanied by a rise in the number of produce-associated foodborne disease outbreaks. In the US between 1998 and 2007, fresh produce was involved in 684 outbreaks resulting in 26,735 cases of illness. Proportionally, this equates to 14.8% of outbreaks and 22.8% of outbreak-related cases of all foodborne illnesses in the US. Salads, vegetables and fruits were linked to 345, 228 cases and 111 outbreaks, respectively (DeWaal and others 2009; Olaimat and Holley 2012). There has also been an increasing association of food-borne outbreaks with vegetables, juices, and other products in the EU. These products represented 8.7% of reported outbreaks for which a food vehicle was identified in 2010, versus 2.1% in 2009 (EFSA 2012). Based upon EU Zoonoses Monitoring data from 2007 to 2011, Foods of Non-Animal Origin (FoNAO) were associated with 10% of outbreaks, 26% of cases, 35% of hospitalizations, and 46% of deaths (EFSA Panel on Biological Hazards (BIOHAZ) Panel 2012). Trends in outbreak data on FoNAO are however strongly influenced by very large outbreaks of considerable morbidity and mortality, such as the 2011 verocytotoxigenic *Escherichia coli* (VTEC) O104 seed sprout outbreak in Germany. Even excluding this outbreak, FoNAO still caused 10% of outbreaks, 18% of cases, but only 8% of hospitalizations and 5% of deaths in the EU (EFSA Panel on Biological Hazards (BIOHAZ) 2012, 2013). In 2008, the World Health Organization (WHO) categorized leafy green vegetables as the highest priority in terms of fresh produce safety from a global perspective, as the most common produce items associated with outbreaks were greens-based salads and lettuce (World Health Organization 2008b).

Fresh produce can become contaminated with microbiological pathogens during production, at the processing/packing stage, and/or during preparation. Unfortunately, the importance of each of these different phases in the farm-to-fork continuum relative to pathogen

contamination is unknown. However, it is clear that water is an important source of contamination, and over the years, there has been particular interest in the role of irrigation waters in this respect. Natural sources of water for irrigation include lakes and rivers, collected rainwater, desalinated sea water, and deep aquifers or shallow groundwater. The potential for microbial contamination of these water sources varies significantly depending on a variety of factors (Suslow and others 2003).

On an international scale, a particularly important factor when considering the quality of water used in fresh produce primary production is the availability of water resources, which is under increasing pressure. There are many reasons for this. For example, a growing population creates increased demand for water, particularly in urban areas. Climate variability causes greater unpredictability in precipitation, including periods of heavier rainfall as well as drought. Where there is dependence on groundwater, recharge often takes place at specific times of the year, so even relatively brief changes in rainfall patterns can have long-term effects. Even in parts of the world where water is often thought to be more plentiful (such as in India and South East Asia), there are significant pressures on water resources (Shah and others 2014).

In addition to increased water scarcity, human settlements produce a significant amount of wastewater that is rich in organic matter and which may be regarded as a resource if processed and disposed of properly. In fact, more and more regions are considering using this source of water as a valuable addition to the natural water resources available. Already wastewater reuse is practiced in many parts of the world and some countries have extensive experience with this technology (E.P.H.C. 2006). An important use for wastewater, after varying degrees of treatment, has been as a source of water for crop irrigation. This practice also reduces the impact of excess nutrients into surface waters and provides a source of plant nutrients in addition to the water needed for a wide variety of crops. This is practical

particularly in water-stressed regions and where the source of wastewater is reasonably close to where the crops are grown.

Unfortunately, any water source can become contaminated with microbial pathogens. This is well known for drinking water and is the reason why efforts have been made over millennia to separate wastewater containing human and animal fecal matter from sources of drinking water. Consequently, a range of pathogens can also be present in waters used for fresh produce production and processing, and hence enter the food chain. Such hazards include both human-specific pathogens such as *Shigella* spp., norovirus, hepatitis A virus, *Cyclospora cayetanensis*, and zoonotic pathogens including verocytotoxin-producing *Escherichia coli*, *Salmonella* spp., *Yersinia enterocolitica,* and *Cryptosporidium*. In addition, parasites such as tapeworms, that are of little consequence for drinking water (unless present inadvertently), are of major significance for food. While there are some chemical hazards that are of significant concern for drinking water, such hazards are not generally an issue for foods grown using irrigation waters. This document focuses on microbiological issues. Its purpose is to discuss, in detail, the role of water quality in the safety of fresh produce. Accordingly, we describe the following areas:

- The epidemiological evidence supporting the role of water in primary production or at harvest in pathogen contamination and subsequent outbreaks of foodborne disease;
- The sources of water and methods used for irrigation during crop production and their impact on microbiological quality or potential for acting as a contributor to microbial contamination of fresh produce;
- The factors that impact survival of pathogens in water or in irrigated soil and fresh produce;
- Control measures and guidelines for the management of water sources and water treatment, including microbiological criteria, to assure the safety of fresh produce;

- The role of testing and sampling to ensure appropriate water quality.

Fresh Produce and Microbial Food Safety Concerns and Water

Interest in the safety of fresh produce has grown almost exponentially over the last decade. Several comprehensive review articles have been produced, and the interested reader is referred to some selected ones for further details (De Roever 1998; Hanning and others 2009; Johnston and others 2006; Leon and others 2009; Oliveira and others 2012; Sivapalasingam and others 2004; Strawn and others 2011). This section briefly describes produce-hazard pairs that have previously caused recognized outbreaks. The associated epidemiological investigations, with a focus on water serving as the source of contamination, will be described.

Pathogen contamination of fresh produce can occur at multiple locations across the farm-to-fork pathway. While water is an important vehicle for produce contamination, it is not the only one. With the exception of a few documented instances in which seeds have been contaminated with pathogens, the production phase is the earliest point at which fresh produce becomes contaminated with pathogens. This phase includes the steps of planting, growing, irrigating, and other activities and treatments associated with the production of the mature plant. Contamination of produce at the production phase frequently occurs as a consequence of exposure to contaminated water or soil. The former is of great interest to this report and will be discussed in detail throughout the article. Soil can be a source of contamination of crops if the production site was previously used for animal production, industrial dumping, or if biosolids/sludge, manure, or animal waste were applied as fertilizer or for waste disposal. Animal encroachment (birds, mammals, reptiles, and so on) is another important source of produce contamination pre-harvest, as is water run-off from surrounding areas where animal feces contaminates the land.

Outbreak investigations are an important and challenging component of epidemiology and public health and can prevent future problems by identifying contamination sources and mitigation strategies (Reingold 1998). Data availability on outbreaks associated with fresh produce, and in particular detailed information on outbreaks and sources of contamination, are diverse and scattered. Table 1 lists examples of commodities and pathogens that have been reported in various foodborne outbreaks linked to fresh produce consumption. The most common produce items associated with these outbreaks have been leafy vegetables (spinach, lettuce, and lettuce mixes or salads), herbs, sprouted seeds, tomatoes, and berries. Cantaloupes, green onions, peppers, papaya, sugar snap peas, and a number of other commodities have also caused outbreaks. Just as there is a broad range of commodities associated with foodborne illness, the list of foodborne pathogens is also extensive. The biological hazards that dominate most reported produce-associated outbreaks are either zoonotic or human in origin. The most common zoonotic bacteria include *Salmonella enterica* (various serotypes) and verocytotoxin-producing *Escherichia coli* O157, whereas outbreaks associated with *Campylobacter jejuni* and *Listeria monocytogenes* are relatively rare. Recently, however, *L. monocytogenes* has been associated with one of the deadliest produce-associated outbreaks, involving cantaloupe melons (Centers for Disease Control and Prevention 2011). Human-specific bacterial pathogens such as *Shigella* spp., and other pathogenic *E. coli* (such as enterotoxigenic *E. coli*) are included. In addition, some of the human enteric viruses, particularly hepatitis A virus, human noroviruses, and rotavirus, have caused outbreaks, as have the parasitic protozoa *Cryptosporidium* and *Cyclospora*.

A portion of these foodborne illnesses associated with fresh produce originate from poor water quality used in the production or post-harvesting washing. For example, irrigation pond water was responsible for a multistate tomato-associated *Salmonella* Newport outbreak in the US (Greene and others 2008). Iceberg lettuce contaminated with *E. coli* O157 caused a

large outbreak in Sweden, probably due to river water used for irrigation. The organism contained *vt2* genes, which on their own may not be responsible for the outbreak but they are together with other factors involved. However, the strain was only isolated from cattle upstream (Söderström and others 2008). Agricultural water was also the source of contamination in a nationwide 2008 US outbreak of *Salmonella* Saint Paul in peppers (Behravesh and others 2011). A more recent EHEC outbreak in June 2013 in Sweden was caused by fresh salad, components of which could have been contaminated by irrigation water, although this could not be confirmed (Edelstein and others 2013). Most recently (September 2013), a verocytotoxin-producing *E. coli* outbreak associated with the consumption of watercress was attributed to either wildlife entering the farm or contaminated run-off water (Public Health England 2014).

However, identification of the implicated food vehicle and/or the location of the point of food contamination in fresh produce-associated outbreaks are recurrent challenges. In 2012, in the EU, only 6.3% of 5363 outbreaks investigated had the same causative agent identified in the food vehicle or food chain and in human cases (EFSA 2014). Investigations of several *Cyclospora* outbreaks in June-August 2013, associated with the consumption of contaminated iceberg lettuce, were still unable to identify the causative agent (Centers for Disease Control and Prevention 2013). But earlier reports from CDC in 2006 reported that potential water issues may have been related to the fresh spinach outbreak at that time which was attributed to surface runoff from grazing areas onto cultivated fields, construction of irrigation wells, depths to groundwater and groundwater-surface water interaction, and direct use of surface water for irrigation (CFERT and others 2007)

A major limitation of epidemiological investigation is that, in many instances, the true source of contamination is never ascertained and, in the absence of data, investigators can only speculate or assume a source. Such is the case for water; many outbreak investigations assume that the use of non-potable irrigation water just prior to harvest, or contaminated

wash water, is responsible for pathogen contamination of produce. There is substantial danger in such assumptions, not just because they can be incorrect, but also because there is evidence that once a particular transmission pathway is identified, repeated outbreaks and investigations lead to a bias in causation (Lynch and others 2009).

Criteria for reuse of treated wastewater in irrigation are specific to a country or a region. In low-income countries, a range of alternative safety practices like cessation of irrigation prior to harvesting, lowering of watering cans to reduce splashes from the soil, furrow irrigation, and so on, is recommended to safeguard public health as much as possible in the local context (Amoah and others 2011; Keraita and others 2010). It is also important to note that the use of contaminated water in the dilution and subsequent application of fungicides and insecticides can also pose a significant microbial risk in a pre-harvest setting. Special attention to water quality should be taken when using delivery techniques (for example, sprayers) that expose the edible portion of leafy vegetables directly to water, especially close to harvest time (Codex Alimentarius Commission 2003b).

Despite the vast amount of information in Table 1, these reported outbreaks are likely underestimates of the real situation. At national or international levels, an outbreak will receive widespread attention if the event (i) creates serious impacts on public health; (ii) is unusual or unexpected (the agent and/or produce type are unexpected, the circumstances of the outbreak are unique); and/or (iii) poses a significant risk of international spread with consequences for international travel or trade restrictions. The latter criterion is also the one that the World Health Organization's INFOSAN alert system follows to identify potential international food-related events as threats to public health (Rosenkötter and others 2014; World Health Organization 2008a). In point of fact, many of the smaller outbreaks are never investigated. Finally, and perhaps most importantly, the vast majority of foodborne disease illness cases occur sporadically in the population, and these are not at all captured in routine epidemiological surveillance or outbreak investigations (Scallan and others 2011). Hence,

Table 1 is in no way exhaustive, and much more information can be gleaned by consulting other sources of information. Such sources include the scientific literature, annual reports of national public health or food safety agencies (CDC or FDA in the US; ECDC, EFSA, or RASFF in the EU, Food Standards Australia & New Zealand, and so on), and reports of outbreak investigations and epidemiological surveillance systems (such as Eurosurveillance, MMWR, and others). Local news media and dedicated Internet search engines (such as ProMedmail) are also information sources.

Most of the examples of fresh produce-associated outbreaks reported in Table 1 originate from Europe, North America, New Zealand, and Australia, as these locations have well developed epidemiological surveillance systems; such systems are not available in much of the developing world. It is also important to note that, even for these countries, outbreak investigations may be biased or may differ geographically as a function of capacity, organizational structure, differences in trade flow, and so on. Outbreak data are rarely available from developing countries due to the lack of well-functioning surveillance and reporting systems. But it has been shown, in some countries such as Senegal, South Africa, Mexico and India, that the quality of irrigation water and water for washing to maintain produce freshness influences microbial quality (presence of fecal indicator organisms and pathogens) (Castro-Rosas and others 2012; Ibenyassine and others 2007; Minhas and others 2006; Ndiaye and others 2011).

Water Sources and Irrigation Methods Used in Fresh Produce Primary Production

Agricultural practices, including types of crops produced, water sources, and irrigation and harvesting practices vary considerably around the world. Table 2 provides a summary of agricultural practices used in different geographical areas of the world. Clearly, there is considerable variability here. Fecally contaminated irrigation water is certainly a possible,

and sometimes likely, source of pathogen contamination of fresh, ready-to-eat fruits and vegetables. Introduction of enteric pathogens from irrigation water is associated with either the source/type of water or the irrigation method (Brackett 1999; Leifert and others 2008; Steele and Odumeru 2004). Different sources and qualities of water are used for irrigation, with each of these having a different propensity to result in microbiological contamination of the crops. In addition, the method of irrigation plays an important role in the mode of contamination and transfer of bacteria, viruses, or protozoa to produce. In this section, we summarize key issues associated with water sources and irrigation methods applied at the fresh produce production phase of the farm-to-fork continuum.

Irrigation Water Sources

Water used for irrigation may originate from multiple sources: municipal water, rain water, ground water, surface water (open canals, impounded water such as ponds, reservoirs, and lakes), and wastewater (James 2006). The advantages and disadvantages of each are summarized in Table 3. Naturally, municipal water is of the best quality (but only available in some developed regions and quite expensive), followed by groundwater, rainwater, and surface water. The latter may or may not include discharges of treated (or untreated) human or industrial waste water. Because of the acceptable quality and low cost of groundwater, this source of water is increasingly being used. However, the quality and sustainability of natural groundwater reservoirs is threatened in some regions by over-abstraction. This results in the degradation of spring-fed rivers, destruction of wetlands, and chemical and microbiological contamination of the water (Krinner and others 1999; Reid and others 2003). In (semi) arid areas, desalinated seawater or brackish groundwater can also be used for irrigation purposes. Each of these water categories varies in microbiological quality as detailed below.

Rain water or rain-harvested water is generally of relatively good microbial quality, albeit somewhat variable and of a quality less than what is expected of potable water. The quality of rainwater depends in part on the means by which it is collected or transported. This can be

illustrated with roof-harvested rainwater, which can be contaminated with pathogenic bacteria and protozoan parasites because of the presence of bird, insect, and animal droppings on roofs, especially immediately after relatively long periods of drought (Ahmed and others 2002; Burch and Thomas 1998). Water running off fields after heavy rainfall collects in lakes, rivers, or basins and can be heavily contaminated with pathogens from soil or fecal matter.

Ground water (or borehole water) is generally of good microbial quality if infiltration of surface runoff is avoided (Burch and Thomas 1998). There can, however, be large variations between shallow ground water and water from deeper aquifers. Although ground water usually contains less organic matter than surface water, it may contain higher inorganic loads resulting in unpleasant colors and odors. The depletion rates of ground water are accelerating worldwide, as evidenced by the fact that the rate at which humans are pumping dry the vast underground stores of water has more than doubled since the early 1950s (Asano and Cotruva 2004; Foster and Kemper 2014). In general, borehole water shows less variability in terms of microbial load than rainwater (Steele and Odumeru 2004). Nonetheless, the potential for groundwater contamination from surface events, such as flooding or storm-related run-off from areas of concentrated manure accumulation, manure lagoons, or sewage treatment facilities, is well recognized (Ibenyassine and others 2007; Oron and others 2001; Rai and Tripathi 2007). There also are concerns based on well-water surveys and the prevalence of human illness derived from enteric virus contamination in this water (Gerba and Smith 2005; Pillai 1998). Thus, it is equally important to protect groundwater resources from microbial contamination sources.

Surface water includes lakes, rivers, creeks, ponds, and springs that come to the surface. Very often surface waters are contaminated due to discharges of (treated) wastewater, storm water runoff, livestock or wildlife feces, and so on. Also, surface waters show great variation in turbidity (Burch and Thomas 1998). More specifically, lakes tend to have better water quality than rivers, although lakes are also subject to surrounding sources of contamination

from river inflow. Rivers, streams, and creeks have unpredictable water quality since activities upstream can rapidly change the levels of contaminants entering the flowing water. When surface water is used as the irrigation water source, drainage of contaminated water into the surface water reservoir can be avoided by constructing ditches, buffer strips, retention systems, and drainage systems. Potential overflow points should also be eliminated.

Seawater or brackish water, as with other surface waters, is subject to industrial and municipal waste discharges and river or stream runoff, possibly containing a wide range of human enteric pathogens. There are some crops having high salt tolerance, such as wheat and barley, and this property can be enhanced by selecting and breeding, thus providing crop varieties that can be irrigated with diluted seawater (Ghadiri and others 2006). However, in nearly all cases, seawater needs to be properly desalinated (such as seawater from which salt and other minerals are removed to a certain degree) by thermal processes or reverse osmosis before use in agriculture (Guler and others 2010). That process can achieve significant reduction of microorganisms. Although the costs of reverse osmosis membranes are high, the use of desalinated seawater might be economically feasible for high-value crops like greenhouse vegetables and flowers (Yermiyahu and others 2007). Brackish groundwater (i.e. groundwater containing salt, but in lower concentration than seawater) can also be applied for irrigation when desalinated. However, the fact that groundwater is a limited resource, as opposed to seawater, should be taken into consideration (Muñoz and Fernández-Alba 2008).

It is generally believed that the use of untreated wastewater for irrigation presents significant health risks and, hence, is not a recommended practice (Pedrero and others 2010). Wastewater is usually of very poor physicochemical and microbiological quality and, consequently, requires intensive treatment prior to use in irrigation, unless other safety measures are in place when treatment is not feasible. Unfortunately, wastewater used for

irrigation is often untreated or treated inadequately, particularly in developing countries (World Health Organization and others 2000). For example, it has been estimated that the percentage of effectively treated wastewater was 14% in Latin America and the Caribbean, 35% in Asia, 66% in Europe, and 90% in North America (Carr and Blumenthal 2004). Homsi (2000) estimated that only 10% of wastewater is treated in developing countries, resulting in about 20 million hectares of irrigation with insufficiently treated or diluted wastewater (Raschid-Sally and Jayakody 2009; Scott and others 2010). Municipal wastewaters generally contain pathogenic enteric bacteria, viruses, and intestinal parasites. Primary and secondary water treatment processes can eliminate 1 to 3 $\log_{10}$ units of enteric microorganisms with an additional of 1 to 3 $\log_{10}$ units reduction achieved using tertiary treatments like filtration, all also depending upon the exact treatments used and the type of microorganism (bacteria, viruses or protozoan (oo)cysts (World Health Organization 2006). However, high microbial numbers might still be present in these purified wastewaters and additional disinfection practices should be applied if further elimination is required, as is frequently the case (Dell'Erba and others 2004; Falsanisi and others 2006; Koivunen and Heinonen-Tanski 2005; Liberti and others 2000, 2001). Treated wastewater is an increasingly relevant water source for irrigation, as it offers a year-round water supply and reduces the exploitation of natural sources, in particular the slow-recharging water layer (Lopez and others 2006). Minimum requirements of good practice to protect the health of the people using wastewater or excreta, or consuming products grown with wastewater or excreta, are provided by WHO (World Health Organization 2006) and its more recent Guidance Notes (World Health Organization 2010).

Worldwide, however, most irrigation water derives from 2 main sources: surface water or ground water reserves such as aquifers (Gleick 2000). In general, irrigation with surface water is expected to pose greater risk to human health than irrigation with water from deep aquifers drawn from properly constructed and protected wells, largely because of the ability

to prevent animal fecal contamination and run-off water from adjacent fields using the latter method (Suslow and others 2003). Most of these water sources are naturally replenished by precipitation. The exception to this is wastewater whose volume depends more on the population size contributing to the pool of wastewater. It is also important to note that different sources of water are very often mixed to obtain sufficient volume needed for certain water-intensive crop production settings and climatic conditions. Surely in times of water shortage, sources must be mixed, but the quality of the final water produced can vary and be unknown by the user. The identification of source water, combined with the definition of appropriate water quality, are vital to assure the safety of irrigated products (Stine and others 2005).

Irrigation Methods

Irrigation methods vary (usually by region) and each method may have its own potential to introduce human pathogens or, on occasion, even promote human pathogen growth on the product (Stine and others 2005). Irrigation methods range from very simple manual practices in the developing world to more sophisticated mechanical practices in the developed world. Commonly used irrigation methods include watering cans and buckets, motorized pumps with hosepipe (Obuobie and others 2010) (the latter are usually used in Africa and other developing countries), while sprinkler irrigation systems, irrigation by canals (furrows), drip irrigation, hydroponic cultivation, and son on, tend to be used in the developed world. Each irrigation method is discussed below in some detail.

Watering cans and buckets: small-scale farmers may use watering cans and buckets to fetch and manually carry water, from a water source, mostly shallow dug wells, streams, or dugouts, to the fields, followed by watering of crops through the spout or shower head of the can. This is, therefore, an overhead irrigation method. When men use this method, they usually carry 2 watering cans at a time, while the bucket system is mostly practiced by women and children (IPTRID 2001; Keraita and others 2002). Farmers using buckets and

watering cans come in direct contact with water mainly by stepping in it while fetching it, or from water splashing on them while carrying it and during watering; highly contaminated water can present a health risk to the farmers themselves. If the water is contaminated with microbial pathogens, the likelihood for subsequent crop contamination is very high because of the combination of overhead application and large surface area.

Other *surface irrigation methods* include *flood irrigation* (water applied over the entire field to infiltrate the soil); *canal or furrow irrigation* (water applied between ridges, for example, level and graded furrows, contour furrows, corrugations, and son on); and *sprinkler irrigation* (in which water is applied in the form of a spray and reaches the soil more or less like rain, from travel sprinklers, spray guns, and portable and solid-set sprinklers, and so on). The flood irrigation system results in complete coverage of the soil surface with water and is normally not an efficient irrigation method. This system can also result in contamination of root crops or vegetable crops growing near the ground. Because it results in direct farm worker exposure, more so than any other method, flood irrigation poses the greatest health risks to both farmers and consumers when contaminated irrigation water is used. Similarly, sprinkler irrigation facilitates the contamination of ground crops (exposing the edible portion of the produce directly to water, a particular problem if applied close to harvest time), fruit trees, and farm workers. Splashing of sprayers can create recontamination of the crop surface from the soil (Marites and others 2010). In addition, pathogens contained in aerosolized effluent may be transported downwind and create a health risk to nearby residents (Fattal and others 1987). Risk associated with spray-irrigation may increase if the irrigation event occurs immediately after a high wind lapse (Barker-Reid and others 2009).

In *subsurface irrigation*, water is supplied through deep surface canals or buried pipes beneath the root zone in such a way that it wets the root zone by capillary action, whereas in *drip irrigation* water is applied around each plant or a group of plants so as to wet only the root zone and to limit the moisture to a relatively local application. Relatively speaking, these

methods certainly provide the greatest degree of health protection for farm workers and consumers, especially if the methods are automated. Drip irrigation and well-maintained furrow irrigation also limit contamination of leaf surfaces (Qadir 2008). Plants grown without soil, such as in hydroponic systems, absorb nutrients and water at varying rates, constantly changing the composition of the re-circulated nutrient solution. Hence, water used in hydroponic culture should be changed frequently or, if recycled, a water treatment method should be applied.

The method of irrigation plays an important role in the transfer of contamination to crops. It is important to note that irrigation distribution networks are designed to meet peak demands, which might create, in some parts of the network, low-flow conditions that can contribute to the deterioration of microbial quality of water. Also, maintenance of the water delivery systems is important as biofilms can increase the contamination between the source and the tap (Hallam and others 2001; Szewzyk and others 2000).

The most often applied systems of irrigation in professional crop production and the advantages and disadvantages associated with these different irrigation methods are summarized in Table 4. It is clear that contamination and transfer of pathogens depends on the irrigation method and on the nature of the produce (SCF 2002). Subsurface or drip irrigation lowers the risk of transfer to growing plants compared to furrow and sprinkler irrigation, by minimizing the exposure of the irrigated water to the produce (Enriquez and others 2003; Hamilton and others 2006; Oron and others 2001; Song and others 2006). Furthermore, subsurface or drip irrigation lowers the risk of splashing of contaminated soil on vegetables (Cevallos-Cevallos and others 2012; Franz and others 2008; Girardin and others 2005; Ntahimpera and others 1999; Pietravalle and others 2001).

Pathogen contamination by irrigation water is of greatest concern when irrigation Is done right before harvest. For example, water containing 2.5 log CFU *Salmonella* spp. was sufficient for contamination and persistence of the pathogen on plants for at least 48 h after

spray-irrigation (Kisluk and Yaron 2012). Other studies have reported *E. coli* persistence after spray-irrigation for up to 27 days (Erickson and others 2010). Hence, unless the water quality is well-controlled and of potable quality, spray-irrigation is best applied in the early stages of plant growth, thus maximizing the opportunity for pathogen die-off.

Based on the above reflections, Table 5 demonstrates the risk ranking of lower to higher risk of combinations of source of water with different irrigation methods and type of crop. Highest risk of contamination can be attributed to the combination of raw/poorly treated wastewater to be used for surface-irrigation with watering cans as applied to low-foliar plants such as lettuce or root crops such as onions or carrots. Ultimately, however, the type of irrigation method chosen by a grower depends on several issues, including the ground water depth, types of water sources available, local cost of these water sources, cost of irrigation equipment/infrastructure, soil type and slope, and crop type or applicability of crop rotation(s) (Mena 2006).

The Behavior of Microbial Hazards in the Production Environment

In considering microbial pathogen contamination of fresh produce, it is important to understand that, once the microbes are introduced into water and via water into soil or plants, the factors impacting their ability to survive, and perhaps even grow, under given climatic and environmental conditions or stages of crop production is important. The ability of pathogenic organisms to attach, survive, and grow on the surface of various fruit and vegetables is dependent upon (i) the metabolic capabilities of the pathogens themselves; (ii) the unique set of intrinsic factors possessed by a particular produce item; and (iii) the extrinsic ecological factors that naturally occur in or on the produce at various stages of production, processing, distribution, and /or preparation (Beuchat 2002). The survival of pathogens is important as it can impact the likelihood of an outbreak (Fonseca and others 2011). In general, the survival of pathogens in pristine water decreases with increasing temperatures (González and Hänninen 2012; Rhodes and Kator 1988). However, increasing

nutrients and high organic load can also increase survival. For example, the viable counts of *Campylobacter* spp. decreased below detection limits within 5 days at 25 °C and within a maximum of 70 days at 4 °C (González and Hänninen 2012). Thomas and others (2002) found 18 times higher decay rates for *Campylobacter* spp. at 20 °C. The survival of *E. coli* O157 in surface water strongly decreased with increasing temperatures; it survived 8 weeks at 25 °C compared to 13 weeks at 8 °C (Wang and Doyle 1998). *Salmonella* spp. survived 24 weeks in freshwater microcosms at ambient temperature (30 °C) compared to 58 weeks at temperatures of 5 °C (Sugumar and Mariappan 2003).

After irrigation, the ability of enteric bacteria to survive in the hostile environment of the phyllosphere is debatable. Stress conditions on plant surfaces can restrict pathogenic bacterial surviva (Brandl 2006; Warriner and Namvar 2010). Enteropathogens can adapt to the phyllosphere environment but may fail to compete with indigenous epiphytes (Brandl and Mandrell 2002; Cooley and others 2006; Janisiewicz and others 1999; Warriner and Namvar 2010). Between 30 and 80% of the total bacterial population on a leaf surface is located in biofilms having an increased survival rate (Morris and Monier 2003). Even if human pathogens cannot produce homogeneous biofilms, they may become entrapped in heterogeneous biofilms produced by nonpathogenic bacteria, making them much more restive to stress conditions (Fett 2000). However, it has been reported that *E. coli* O157:H7 may not preferentially colonize biofilms produced by natural microbiota on lettuce leaves (Seo and Frank 1999).

A number of key factors are likely to influence bacterial death on the phylloplane, the most important being low humidity, high temperatures, exposure to UV, and wind-mediated drying of the leaf surface (Gras and others 1994; Hutchison and others 2008; Moyne and others 2011; Oliveira and others 2012). The survival and growth of certain enteric pathogens on plants depends on the relative humidity (RH). Low RH has been proposed as one of the main factors limiting survival of bacteria on plant surfaces (Medina and others 2012; Oliveira and

others 2012). For instance, *Salmonella* spp. populations declined rapidly under low RH on cilantro, whereas the organisms were able to grow on cilantro leaves under humid conditions. Phylloplane bacteria are also efficient in UV-induced DNA damage repair (Heaton and Jones 2008). Enteropathogens encounter osmotic stress when passing through the host gut, which may induce cross-resistance to stresses encountered on the leaf (Brandl 2006). Protection from environmental stresses may by facilitated by movement into the internal tissue of the plant. Enteropathogens in irrigation water can be taken up by the root system, or via wounds or other structures such as stomata, and enter the edible portion of the plant (Janisiewicz and others 1999; Seo and Frank 1999; Solomon and others 2002; Zhang and others 2009). However, despite a lower survival of pathogens in the field in the warmer seasons, there are higher chances of pathogen introduction at these times (Fonseca and others 2011).

Several studies have examined the persistence and survival of pathogens on lettuce through the application of irrigation water, manure, or direct inoculation of lettuce with soil and manure. Nevertheless, the comparison of data from individual studies is difficult due to variability in experimental design and conditions, plant species, cultivars, maturity at inoculation, bacterial strains and their cultivation, and analytical methods (Delaquis and others 2007). A summary of individual studies carried out with leafy vegetables is provided in Table 6 and Table 7. The tables emphasize the big differences in experimental design between studies. Sometimes artificially high inoculation levels (5-9 $\log_{10}$ CFU/g or mL) were used, such as in experiments to investigate the use of contaminated compost and irrigation water on the ability for cross-contamination, survival, and internalization of human pathogens in soil or lettuce. Still, the survival of pathogens after application of irrigation water or manure ranged from 1 day up to 2 months on lettuce and more than 7 months in soil depending on inoculation level and season (Hutchison and others 2005; Liu and others 2013). Survival of foodborne pathogens on produce is significantly enhanced once the protective epidermal

barrier has been broken either by physical damage, such as punctures or bruising, or by degradation by plant pathogens or spoilage organisms (bacteria or fungi). These conditions can also promote the multiplication of human pathogens, especially at ambient temperatures. Injured cells and released cell fluids provide a nourishing environment for microbial growth. Certain crop management practices and/or extreme weather conditions (such as heavy rain, hail, or strong winds) might influence tissue susceptibility for contamination and internalization with foodborne pathogens by affecting plant physiology, tissue structure, and microbial ecology.

Prevention and Control Measures for Irrigation-Water Quality

To assure the safety of fresh produce, and simultaneously safeguard the health of crop producers and their staff, a set of guidelines, namely, Good Agricultural Practices (GAP), have been released. These good practices are defined at the international level in the 'Codex General Principles on Food Hygiene' (Codex Alimentarius Commission 2003b), with guidance specific to fresh produce production further developed in 'CAC/RCP 53-2003 Code of practice for fresh fruits and vegetables'. This particular Codex document provides explanations of good practices to minimize the contamination of fresh produce during cultivation and (post)- harvest practices. There are many suggested practices, but, because of the importance of water as a potential source of contamination, significant parts of GAP documents focus on water. The Codex Committee on Food Hygiene guidelines for control of virus contamination of food (Codex Alimentarius Commission 2012) also recommends that efforts should be made to use only potable or clean water (this is water quality that does not affect the wholesomeness of the food) during production. In parallel to the Codex Alimentarius documents, several guidelines and quality assurance standards were developed for the primary production of fresh produce on the initiative of national competent

authorities, fresh produce industry associations, or as voluntary private standards and marketing agreements in the fresh produce supply chain. For example, in the US there are general and specific guidance documents provided by the U.S. FDA (FDA 1998, 2009a, 2009b, 2009c, 2009d). Guidance is also provided by specific commodity groups (United Fresh Produce Association 2002, 2005, 2008 2010). GlobalGap is the European retailers private collective standard set and is also acting as an organization for benchmarking other voluntary standards in agricultural production (including fresh produce production) around the globe enabling certification of GAPs (www.globalgap.org). An alternative organization, SQF (Safe Quality Foods), initially developed in Australia in the early 1990's and currently owned and managed by the Food Marketing Institute in the USA, has elaborated the SQF 1000 Code for primary production as quality assurance standard for certification of GAPs (www.sqfi.com). Another quality assurance standard developed on a national level is *Integrated Chain Quality Management* (ICQM) in Belgium (www.vegaplan.be). The ICQM Standard applies specifically to agricultural crop production and horticulture and describes the minimum requirements for producers and workers on good practices to gain access to the high-value fresh produce market. In Norway, KSL Matmerk is a private initiative providing guidance and a quality system for agriculture. McDonald's Corporation has issued its own rigorous food safety standard for fresh produce production, and there are many other private collective or individual company- based standards and quality assurance programs available. Some of the guidelines on use of water sources and prerequisites on water quality and sampling and testing are shown in Table 8.

With regard to the legal framework demanding implementation of GAPs, in Europe the EC Regulation No 852/2004 on the hygiene of foodstuffs lays down general hygiene requirements to be respected by food businesses at all stages of the food chain, including at primary production. There is thus a legal obligation to comply with requirements for good hygiene practice and thus to prevent the contamination of food of plant origin also at primary

production. Some European countries also have explicit legislation referring to the quality of water to be used in primary production (for example, Spain) (Table 8). In association with the US-FDA Food Safety Modernization Act (FSMA), regulations that result in mandatory GAPs adherence will inevitably be instituted, although these remain in developmental stages at the time of this writing.

Overall, several preventive control measures can be practiced on the farm in an effort to avoid microbial contamination of irrigation water. Although it is impossible to completely prevent and, if occurring, eliminate such contamination, careful attention to controls can minimize risk. For a further review of the most effective preventive measures and interventions, one is referred to the above-mentioned Codex Alimentarius Commission documents, the opinions issued in 2014 by EFSA on the risk posed by pathogens in food of nonanimal origin (EFSA 2014) or the review by Gil and others (2015).

As mentioned in many Codes of Practice for primary production of fresh produce, the importance of selecting a high-quality irrigation water source cannot be overemphasized. It is essential that, on a regular basis, sanitary surveys of water reservoirs and distribution systems are executed. These should focus on the integrity of surrounding protective structures, identifying potential point source and nonpoint source confluences (such as drainage into these systems). If the evaluation concludes that (human or animal) fecal contamination of the water in a specific area is at levels that may compromise the quality of the water and thus the safety of crops, appropriate interventions should be taken. The most important intervention used to address pathogen risks in irrigation water, if judged to be of insufficient quality, is water treatment. Treatment methods correspond approximately to what is used for sewage water treatment and include coagulation, flocculation, filtration, and disinfection. Solar irradiation is also suggested to reduce the levels of pathogenic microorganisms in irrigation water. Other options that have been considered to improve the microbial quality of surface waters include sand filtration or storage in catchments or

reservoirs to achieve partial biological treatment before use (Carr and Blumenthal 2004). Overall, it is recommended to use a disinfection treatment if using water from open reservoirs that are prone to human or animal fecal contamination (and thus likely pathogen contamination). Disinfectant treatments of surface or well water include chlorination, use ofperoxyacetic acid, and UV treatment. Ozonation has also been described as a possible disinfection treatment for irrigation water (Suslow and others 2003). It can be difficult to choose the technology that is the best fit for a specific situation, as the performance of the water treatment process will depend upon physicochemical and microbial parameters associated with the water to be treated. Selection of technology will also relate to aspects including capital and operational costs, complexity of the technology, required monitoring, and safety issues (Van Haute and others 2013).

The Role of Testing and Monitoring in ensuring safe water in fresh produce production

Some Codes of Practice demand growers to have the water they use periodically tested for microbial contaminants. Depending on the type of water source and method of irrigation, microbial sampling may be recommended at different frequencies to verify the functionality of good agricultural practices. Testing is costly; if testing is applied, it is important that an agreement is made on the frequency and location of sampling; the sampling method and volume; the microbial parameters to be analyzed; the method of detection or enumeration of these microbial parameters; the interpretation of test results, including specifications and/or microbiological criteria; and the types of actions to be taken upon noncompliance. At present, there is no widespread agreement regarding the microbiological guidelines for irrigation water. In most cases, actual pathogen contamination of waters and fresh produce is probably quite rare, particularly in the developed world. Furthermore, direct pathogen screening is expensive, time-consuming, and difficult to interpret (Savichtcheva and Okabe 2006).

Pathogens tend to be nonuniformly distributed in water, which complicates the interpretation of negative test results. In most cases, generic *E. coli* are used as indicator organisms, as their presence relates to fecal (animal or human) pollution. Alternatively, fecal coliforms may be used for this purpose. Total coliforms can be analyzed to indicate failures in control measures. As operational indicators, total coliforms may provide information on the adequacy of water treatment and on the microbial condition of the water distribution system at the point of application. It should be noted, however, that the presence of total coliforms in the (tank) water or the distribution system, without further discrimination, is of no immediate public health significance. Nonetheless, the presence of coliforms is still an indicator of inattention to "best practices" and should prompt further actions, such as sanitary survey of the construction of the water network, the input to the tank water, control of the water treatment, storage conditions, potential for regrowth of micro-organisms, and so on.

As the pathogens associated with fresh produce outbreaks are almost always of fecal origin, a good indicator should correlate with the presence of fecal contamination. Historically, a subset of the coliforms, the fecal coliforms (those coliforms which ferment lactose with the production of acid and gas within 48 h at 44.5-45.5 ^{o}C in EC broth) have been the most widely used in sampling and testing of water quality for this purpose. The major genera represented in the fecal coliform group are *Enterobacter*, *Citrobacter*, and *Klebsiella*, which are not always of fecal origin, although the majority of the fecal coliforms are strains of *E. coli*. A WHO world survey indicated that most European rivers contain mean fecal coliform counts of 1,000 to 10,000 per 100 mL (World Health Organization 1989). There are, in some countries or states, guidelines on appropriate microbial quality specifications for surface water or (treated) wastewater to be used for irrigation based on testing for fecal coliforms (Table 9). Guidelines for microbial water quality of surface water for irrigation are usually less stringent than those of wastewater for unrestricted irrigation due to the assumption that enteric viruses and other human pathogens are present in lower concentrations in surface

water (Gerba and Choi 2006); or in the context of microbiological criteria, fecal coliforms in surface water may originate from sources other than sewage or waste effluents, which is certainly the case in hot climates.

Actually, with better detection methods available, *E. coli* is now the indicator of choice for fecal contamination originating from warm-blooded animals (including humans) (Mossel 1978, 1983). The presence of generic *E. coli* provides evidence of an increased likelihood of potential contamination of food or water by ecologically closely related pathogens. Holvoet and others (2014) showed that the use of water with *E. coli* levels higher than 2 $\log_{10}$/100 mL needs to be avoided, as 42% of the water samples with values exceeding this contained a pathogen (*Salmonella* or *Campylobacter* isolates) or the presence of verocytotoxin genes (indicative of the presence of pathogenic *E. coli*). This is contrasted to less than 10% if the value was below 2 $\log_{10}$ *E. coli*/100 mL. It has been suggested that monitoring of water quality in primary production for fecal indicator organisms such as *E. coli* can help inform farmers on deviations in good practices and situations that need corrective measures, thereby contributing to the assurance of a microbiologically safe product. This is especially the case since detection of pathogens in water (or fresh produce) is not always reliable for reasons described above, including statistical limitations, leading to a false sense of security.

The limit of an *E. coli* criterion in water (or fresh produce) is set according to what is generally obtainable when applying good practices and is not a direct indicator of risk. However, an increased number of *E. coli* cells (above the level normally observed) indicates a higher degree of exposure to fecal contamination from pathogen reservoirs and/or cross-contamination or growth (EFSA 2014). But indeed the utility of *E. coli* screening is debatable with regards to public health. For example, Ahmed and others (2010) found that 12% of roof harvest rain water samples had <1 CFU *E. coli*/100 mL but were positive for one or more pathogens.

It has been suggested that the enterococci perform better than *E. coli* in terms of indicating fecal contamination and pathogen presence in environmental waters (perhaps because they are more environmentally persistent), although data are mixed (Harwood and others 2005; Hörman and others 2004; Kinzelman and others 2003; Lemarchand and Lebaron 2003;). Alternative indicators such as *Bacteroides* spp., *Bifidobacterium* spp., and *Clostridium perfringens*, as well as bacteriophages (for example, coliphage MS2 or φX174 or *Bacteroides fragilis* phage B40-8) and adenoviruses have also been proposed. Again, there are no compelling data about their utility to date.

The frequency of testing and the maximum allowed indicator level are still points of debate. Sampling once or twice a year provides some information on water quality, but eventually a high variability in the water quality, in particular for surface waters or during the growing season, may occur. Overall, the frequency of testing should depend upon the exact farm management and operation practices and climatic conditions. For instance, borehole water is less vulnerable to contamination and will demand less frequent testing than open reservoirs (of course, depending upon the construction of the reservoir). Furthermore, climate incidents such as flood, runoff of storm water, and son on, would by necessity increase the frequency of testing. However, the presence of an effective and well-operated water treatment system implies a need for less frequent testing, which would be done merely for verification purposes.

Future perspectives: the role of risk assessment in managing the use of water in fresh produce primary production

Guidelines and regulations dealing with microbial standards are often empirically designed, based upon prior experience on what is achievable under good practices, and has been shown to function by prior history and epidemiological evidence as appropriate in protecting consumers' health. But other strategies for managing health risks may also be effective (Carr and Blumenthal 2004). For example, the latest guidelines by the World Health Organization

(2006) for use of wastewater in agriculture have been revised substantially by replacing the fecal coliform guideline with health-based targets defined through attributable risk and disability-adjusted life years (DALYs). As such, governments in developing countries have been given greater flexibility in achieving these targets (World Health Organization 2006). These guidelines are intended to be used as the basis for the development of national and international approaches to managing the health risk from hazards associated with treated wastewater use in agriculture. An example of implementation of this approach is illustrated in the AGWR report (O'Toole and others 2010). This study showed how to translate a health outcome target to performance targets for water treatment, and irrigation and farming practices. It shows how microbial risk assessment can be used in a regulatory framework to guide food producers to the appropriate risk management interventions (based on a combination of barriers) in the chain from irrigated fresh produce to consumer. Another example is a quantitative microbial risk assessment (QMRA) study elaborated in Sweden by Ottoson and others (2011). The QMRA indicated that reducing the maximum contamination level of irrigation water from 4 $\log_{10}$ CFU to 2 $\log_{10}$ CFU *E. coli*/100 mL would lead to a 5-fold reduction in verocytotoxin- producing *E. coli* illnesses due to consumption of iceberg lettuce. Besides controlling the microbiology of the irrigation water source, other recommendations could be made using this model, such as increasing the time between irrigation and harvest. Specifically, cessation of irrigation for, respectively, 1, 2, 4, and 7 days, which reduced the risk 3, 8.8, and 18 times. However, depending on the weather conditions, cessation of irrigation may not be possible in all cases.

Stine and others (2005) computed the maximum concentration of *Salmonella* and hepatitis A virus (HAV) in irrigation water that would result in a 10^{-4} annual risk of infection for individuals consuming different types of fresh produce that were irrigated under different conditions. Their findings indicated *Salmonella* concentrations could range from a low of 1.5×10^{2} CFU/mL to a high of 7.2×10^{6} CFU/100mL for furrow-irrigated lettuce, depending upon when

the last irrigation event occurred (1 or 14 days before harvest, respectively). Hamilton and others (2006) developed a microbial risk assessment (MRA) model to estimate the risk of enteric virus illness when secondary effluent was used to irrigate horticultural crops (broccoli, cucumber, cabbage, and lettuce). The model computed the daily exposure based on the human body mass, daily consumption, virus concentration in water, volume of irrigation water deposited on the product, virus die-off, and time between last irrigation and harvest. A dose-response model for rotavirus was used as a proxy. Across the various produce crops, the annual risk of infection ranged from a low of 10^{-9} to 10^{-3} when irrigation using reclaimed water was ceased 2 weeks before harvest, to a high of 10^{-3} to 10^{-1} when irrigation use was ceased 1 day before harvest.

Few site-specific data points were available for most of these MRAs, meaning that many assumptions were necessary. Specific parameters lacking hard data included the rates of pathogen transfer from irrigation water to crops, pathogen penetration in food crops, and pathogen survival on or in food crops. Data on these factors have been accumulating over the last decade, and this should improve the reliability of future MRA estimates. However, the sheer number of different fresh produce commodities and pathogens, combined with water sources and irrigation practices in different locations, means that developing risk models that can span the breadth of fresh produce safety will be a considerable challenge.

Overall conclusions

Outbreaks of foodborne disease associated with fresh produce are not uncommon. The true disease burden is unknown due to under-reporting, the impact of sporadic disease, and poor epidemiological surveillance. There have been several outbreaks linked to contaminated irrigation water. Many different sources of water and methods are used for irrigation of fresh produce around the world. There are 2 main sources of irrigation water: i) surface water or treated wastewater (more prone to contamination and variable in water quality); and ii) ground water reserves or collected rainfall water (less prone to contamination or more

controlled water quality if stored properly). Drip or subsurface irrigation limits direct contact between edible plant tissue and irrigation water (splashes) and thus is less likely to introduce pathogens than furrow or sprinkler irrigation. Codes of practice stress the importance of the quality of the irrigation water source for ensuring safety of fresh produce. A few general principles of preventive measures are i) regular execution of and response to sanitary surveys; ii) maintenance of irrigation water reservoirs and distribution systems; iii) adequate water treatments to gain better water quality; and iv) fecal indicator tests to monitor water quality. These measures are particularly helpful after climatic incidents. If working under conditions of good agricultural practices, in most cases, pathogen contamination of waters and fresh produce is expected to be an infrequent and temporary event, so direct pathogen screening of water (or produce) is likely to be ineffective. Nevertheless, this might be different in regions or under conditions in which contaminated surface water or insufficiently treated wastewater are used due to lack of access to clean water. Still, sanitary surveys and observational audits might also be more useful in these situations, as testing alone should never be relied upon as a food safety management tool, but rather should complement existing strategies (GAPs). An emerging alternative is the use of MRA to guide risk management directions for effective pathogen control and to select the most appropriate control measures to manage the use of water in fresh produce production.

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Table 1: Foodborne outbreaks linked to consumption of fresh produce

Causative agent	Year	Country	Human cases	Implicated food	Country of origin	(Likely) source of contamination	Reference
VTEC 0157	August – September 2013	UK	19 cases	Watercress	Domestic production	Wildlife entering the watercress farm or run-off water	(Public Health England 2014)
EHEC	June 2013	Sweden	19 cases	Fresh salad	Domestic	Irrigation water could be the likely source but not confirmed.	(Edelstein and others 2013)
Salmonella Saintpaul	April – July 2008	US	1500 cases	Jalapeno and Serrano peppers	Import from Mexico	The outbreak strain was isolated from two environmental samples, agricultural water, and Serrano peppers on a farm in Mexico which grew the peppers	(Behravesh and others 2011)
Salmonella Newport	2002 & 2005	US	510 cases & 72 cases	Sliced tomatoes	Domestic production	the outbreak strain was traced back to farms on the Eastern Shore of Virginia, specifically to on-site ponds used for irrigation water	(Greene and others 2008)
Salmonella Thompson	October-December 2004	Norway (and probably larger EU outbreak)	21 cases	Rucola lettuce	Import from Italy	It is speculated that using water of non-potable quality for irrigation of vegetables close up to harvest may lead to contamination of the products with a variety of pathogens.	(Nygård and others 2008)
Salmonella Litchfield	2006/2007	Australia	26 cases	Papaya	Domestic	*Salmonella* Litchfield was not detected in papaya samples, of the inspected farms however at one farm other serotypes of *Salmonella* were detected in untreated river water that was used for washing papaya	(Gibbs and others 2009)
E. coli 0157	September – December 2006	US	205 cases	Pre-packaged spinach	domestic	The outbreak strain was isolated from one of the fields and in addition from river water, cattle faeces on a farm nearby and wild pig faeces. A potential cause was that the river functioned as a vector between the contaminated faeces and the irrigation wells used.	(CFERT and others 2007)
E. coli 0157	July - September 2005	Sweden	135 cases	Iceberg lettuce	domestic	The lettuce was irrigated by water from a small stream, and water samples were positive for Stx 2 by PCR. The identical VTEC O157 Stx 2 positive strain was isolated from the cases and in cattle at a farm upstream from the irrigation point	(Söderström and others 2008)
Norovirus and enterotoxigenic E. coli	January 2010	Denmark and Norway	260 cases	Lettuce (lollo bionda) used in sandwiches	Import from France	Unknown; mentioned that since neither norovirus nor ETEC are zoonotic agents, human fecal matter may have been the source of the contamination, possibly via contaminated water.	(Ethelberg and others 2010)
Norovirus	June - September 2005	Denmark	More than 1000 cases	Frozen raspberries	Import from Poland	Unknown; mentioned that contamination with norovirus may have occurred at farm level by fcally-contaminated irrigation water, during harvesting by infected farm workers and/or during processing and freezing by infected workers at company level.	(Falkenhorst and others 2005)
Cyclospora cayetanensis	May 2011	Canada	17 cases	basil	Import from US	Unknown (usually contamination of the produce with sporulated oocysts through irrigation with contaminated water,	(Hoang and others 2005)

Causative agent	Year	Country	Human cases	Implicated food	Country of origin	(Likely) source of contamination	Reference
Cyclospora cayetanensis	May - June 2009	Sweden	18 cases	Sugar snaps	Import from Guatemala	or by spraying with pesticides or fungicides prepared using contaminated water. Other possibilities includes handling of the produce by workers who were infected	(Insulander and others 2010)
Cyclospora cayetanensis	May - June 1996	Canada and US	More than 1400 cases	Raspberries	Import from Guatemala		(Herwaldt and Ackers 1997)
Cyclospora cayetanensis	2000	Germany	34 cases	fresh green leafy herbs	Southern Europe	Probably fertilization with human waste or fecal contaminated water used to irrigate crops, prepare pesticides, or freshen or clean produce at their origin	(Döller and others 2000)
Salmonella enterica	November 1999 - January 2000	Brazil	26 cases	Mango	Domestic	Mangoes imported from Peru which were due to exposure to untreated water (inadequately chlorinated water) in the final step of the fruit fly control programme	(Beatty and others 2004; Sivapalasingam and others 2004)
Cryptosporidium parvum	October 1995	US	31 cases	apple cider	domestic	The cider mill did not use drop apples, and apples were washed and brushed before pressing; however, cattle were present near the farm, and the apples were washed with water from a source later determined to contain *E. coli*	(Center for Disease Control and Prevention 1997)

Table 2 Overview of agricultural practices in fresh produce production in different geographical areas

Geographical area	Irrigation practice	Irrigation water	Cultivated fresh produce	Harvesting practices	Washing processes	Storage of produce	Reference
Sub-Saharan Africa	• Watering cans and buckets • Motorized Pumps and hose • Sprinkler • Furrow • Border or flood • Drip irrigation • Basin	• Rivers/streams • Underground water • Untreated wastewater (grey water) • Underground water • Rainwater stored in reservoirs	• Green leafy vegetables: e.g. lettuce, cabbage, spring onions • Some fruit crops	• Mainly manual harvesting using knifes, hand picking, cutlasses etc • Mechanical harvesting (minimal) • Special harvesting containers not used • Harvesting implements mostly not cleaned	• Sometimes washed on farm with polluted irrigation water • Clean water used for washing in markets	• Non refrigerated transport • Storage at room temperature • No storage facilities	• (Amoah and others 2007; Barno and others 2009; Ibenyassine and others 2007; Keraita and others 2017, 2010; Khalil and others 2014; Mdluli and others 2013; Yengoh and others 2010)
Middle East	• Mostly surface irrigation • Sprinkler • Furrow • Border or flood • Drip (micro irrigation) • Basin	• Rivers/streams • Underground water	• Green leafy vegetables • Fruits • Some tree crops	• Manual harvesting • Mechanical harvesting	• Ground water • Potable water used for fresh-cut produce	• Refrigerated transport • Storage at appropriate temperatures in cold storage room and refrigerator • For non-RTE storage at ambient temperature	• (Bashour and Nimah 2004; Feenstra and others 2000; Hussain and others 2002; Ongley 1996; Qadir 2008)
Central and South East Asia	• Sprinkler • Furrow • Border or flood • Drip (micro irrigation) • Basin • Motor pumps	• Rivers/streams • Underground water • Untreated wastewater (grey water) • Underground water	• Salads & green vegetable • tomatoes • fruits • some tree crops	• Manual harvesting • Mechanical harvesting	• Collected rainfall • Tap water • Ground water • Potable water used for fresh-cut produce • Surface water (for first washing) • Recycled surface water for transportation	• Refrigerated transport • Storage at appropriate temperatures in cold storage room and refrigerator • For non-RTE storage at ambient temperatures • Ambient temperature for rural areas	• (Ahmad and Chua 2013; Basu and Scholten 2012; Gorton and others 2011; Huong and others 2013)
Latin America	• Sprinkler • Furrow • Border or flood	• Rivers/streams • Underground water	• Fruits and leafy vegetables	• Manual harvesting • Mechanical	• Collected rainfall • Tap water • Ground water	• Refrigerated transport • Storage at appropriate temperatures in cold	• (Cardenas and others 2013; de Quadros Rodrigues and others

	• Drip (micro irrigation) • Basin	• Untreated wastewater • Underground water	• Other tree crops	harvesting (minimal)	• Potable water used for fresh-cut produce • Surface water (for first washing)	storage room and refrigerator • For non-RTE storage at ambient temperatures • Ambient temperature for rural areas	2014; Pereira and others 2002; Scott and others 2010)
OECD[#] countries	• sprinklers • drip systems • sheet irrigation • furrow • border strip • hydroponic mechanism	• surface waters (rivers, reservoirs) • ground water (wells, boreholes) • rainwater source for irrigation • Tap water (to a lesser extent)	• Salads • green vegetable • fruit crops many others • some tree crops	• Hand picking of fruits • Mainly mechanical/ machine harvesting	• Collected rainfall • Tap water • Ground water • Potable water used for fresh-cut produce • Surface water (for first washing)	• Refrigerated transport • Storage at appropriate temperatures in cold storage room and refrigerators	• (Steele and Odumeru 2004; Tyrrel and others 2006)

[#] Organization for Economic Co-operation and Development

Table 3: Comparison between the different types of water sources used for irrigation

Aspect	Municipal Water	Groundwater	Collected Rainfall water	Surface water
Definition (Codex Alimentarius Commission 2003c; Jacxsens 2010)	Water of potable quality offered by water companies	Water, seeped through from the surface and present in porous rocks below the surface, shallow wells or deep aquifers	Collected water from precipitation (rain, snow, …)	Water from a source that is exposed to the environment like rivers/canals/lakes/open wells
Cost (example from one European country i.e. Belgium)	Capacity compensation + approximately 3.3 euro/m³ (www.pidpa.be)	First 499 m³ are free of charge, between 500 and 30 000 m³, one m³ cost around 0.08 € (VMM, 2013)	Free	Charging depending on the surface water if > 500 m³/year
Contamination sources	Pipelines, biofilm	Failing of septic systems, leaking sewer lines and from land discharge by passage through soils and fissures or interaction with surface water (Fong and others 2007; Hunt and others 2005; Lucena and others 2006; Steele and Odumeru 2004).	Dust, organic matter, leaves, bird and animal excreta on the catchment areas (Evans and others 2006; Sazakli and others 2007).	Treated wastewater, discharge of raw sewage, municipal wastewater, storm-water runoff, runoff from urban and agricultural areas. Animals like birds, farm animals, and even humans are both indirect and direct contributors to the contamination (Geldreich 1991; Savichtcheva and Okabe 2006; Sliva and Dudley Williams 2001).
Weather impact	/	Heavy rainfall may lead to changes in the direction of water flow systems and flow through channels that would not normally occur which could lead to contamination (Hunter 2003).	Microbial profile found in rainwater systems was dependent on local environmental conditions and wind speeds/directions (Evans and others 2006). Rainfall after longer dry periods results in an increased presence of bacteria in the reservoirs (Schets and others 2005; Yaziz and others 1989). The first flush of rainwater carries most contaminants into storages (Yaziz and others 1989).	Storms, tides, or strong winds cause sediment resuspension, bacteria will also resuspend, resulting high bacteria levels in the water column (Ahn and others 2005; Bai and Lung 2005; Parker and others 2010; Stumpf and others 2010). An additional increase in the numbers of organisms in the surface water is obtained due to heavy rainfall or storm flow through sewage overflow and surface runoff (Ahn and others 2005; Astrom and others 2009; Goyal and others 1977; Parker and others 2010; Rechenburg and others 2006).

Table 4: Comparison of the (dis)advantages of the different irrigation methods

Aspect	Canal/furrow Irrigation	Sprinkler/overhead Irrigation	Drip /subsurface Irrigation
Definition (Eurostat, 2003)	Leading of water along the ground, either by flooding the whole area or leading the water along small furrows between the crop rows, using gravity as a force	Irrigating the plants by propelling water under high pressure as rain over the parcels	Irrigating the plants by placing water low by the plants drop by drop or with micro-sprinklers or by forming fog-like conditions
Advantages (Ghassemi and others 1995; Verbeten 1998)	Low capital costs	Suited for a wide range of slopes, soils and crops Avoidance of uneven penetration of water and its subsequent waste	Increased uniformity, soil structure is preserved, water is saved because of reduced evaporation and a Correct control of water quantities and nutrients reaching plants is possible
Disadvantages (Ghassemi and others 1995; Verbeten 1998)	Uneven penetration of the water Water application onto the field may be uncontrolled Not suited for all slopes and soils	High initial cost of equipment The higher operation costs compared with surface irrigation The need of a pumping plant and the requirement of energy	High capital costs Obstruction of small drippers because of water impurities Creation of an area of permanently saturated or near-saturated soil favoring the development of plant or animal pests

Table 5: Levels of risk associated with different source waters, irrigation methods and crop types

Level of risk	Source Water[a]	Irrigation method	Crop type
Lower  Higher	Municipal potable water	• Subsurface	• Root crops (e.g. onions)
	• Groundwater collected from deep wells/bores • Rainwater (collected in closed systems)	• Drip	• Low foliar (e.g. lettuce)
	• Groundwater from shallow wells/bores	• Furrow	• Off ground (e.g. tomatoes)
	Adequately treated wastewater Rainwater (collected in closed systems) Surface waters in proximity to animals/human habitation Raw/poorly treated wastewater	• Spray • Surface irrigation with watering cans	• Fruit trees (e.g. apple, mango) • Low foliar (e.g. lettuce)/root crops (e.g. onions)

[a] from FAO/WHO Microbiological hazards in fresh leafy vegetables and herbs 2008

Table 6: Irrigation with contaminated water on leafy vegetables and the subsequent survival of enteric bacteria in lettuce and soil

Setting	Produce	Bacteria	Inoculum (log CFU/ml)	Irrigation method	Survival in soil after inoculation	Survival on produce after inoculation	Reference
Field	Leafy green lettuce	*E. coli* O157	8	Spray	ND	27 days	(Erickson and others 2010)
	Iceberg lettuce	*E. coli*	8-9	Spray	7 day	1 day	(Fonseca and others 2011)
	Iceberg lettuce	*E. coli*	8-9	Drip	7 day	< 1 day	
	Iceberg lettuce	*E. coli*	8-9	Furrow	15 days	< 1 day	
	Lactuca sativa L.	*E. coli* O157:H7	5	Spray	140 days	56 days	(Islam and others 2004)
	Parsley	Salmonella enterica	8.5	Spray	ND	4 weeks	(Kisluk and Yaron 2012)
	Lactuca sativa L.	E. coli O157:H7	4	Surface	ND	15 days	(Mootian and others 2009)
	Lettuce, parsley, tomato and pimento	Untreated wastewater	ND		ND	3 days	(Melloul and others 2001)
Lab	*Lactuca sativa* var. *longifolia*	*L. innocua*	7	Spray	ND	4 weeks	(Oliveira and others 2011)
	Lactuca sativa var. *Longifolia*	*E. coli* O157:H7	7	Spray	ND	4 weeks	(Oliveira and others 2012)
	Green ice lettuce	*E. coli* O157:H7	7	Surface	ND	20 days (6/32 samples positive)	(Solomon and others 2002)
	Green ice lettuce	*E. coli* O157:H7	7	Spray irrigation	ND	20 days (29/32 samples positive)	
	Butterhead lettuce	*E. coli* O157:H7	4	Spray	ND	30 days	(Solomon and others 2003)
	Butterhead lettuce	*E. coli* O157:H7	2	spray	ND	15 days	
	Spinach	*E. coli* O157:H7	5	Spray	ND	6 days	(Wood and others 2010)

Table 7: Inoculation of soil or leafy greens and the subsequent survival of enteric bacteria

Setting	Produce	Bacteria	Inoculation (log CFU/g)	Survival in soil after inoculation	Survival on produce after inoculation	Reference
Field	Lactuca sativa	E. coli O157:H7	Composts: 7	154 days	77 days	(Islam and others 2004)
	Lactuca sativa L.	E. coli O157:H7	Soil and manure-amended soil: 1, 2, 3 and 4	ND	15 days	(Mootian and others 2009)
	Romain lettuce	E. coli O157:H7	Plants: 5.4-6.4 soil 4.7	Soil Up to 15 days	Lettuce, up to 35 days	(Moyne and others 2011)
Lab	Spinach	Salmonella Enterica	Soil: 6	ND	All samples after 7 days 40 % after 14 days 20 % after 21 days	(Arthurson and others 2011)
	Romain lettuce	E. coli O157:H7	Lettuce leafs 6.5 log CFU/leaf 24h, 23°C, <50% humidity	ND	Reductions of 1.8 to 3.3 log CFU/leaf	(Theofel and Harris 2009)
	Romain lettuce	E. coli O157:H7	Fresh-cut lettuce 3.5 5 days, 5°C and 20°C, < 50% humidity	ND	Increase at 20°C after 24h decrease at 5°C after 1, 2 and 5 days	(Theofel and Harris 2009)
	Romain lettuce	E. coli O157:H7 and S. enterica	Plants: 4 3 days, 28°C, 100% relative humidity	ND	100 fold increase E. coli O157:H7 155 fold increase S. enterica	(Brandl and Amundson 2008)
	Romain lettuce	E. coli O157:H7 and S. enterica	Harvested leaves: 4 3 days, 28°C, 100% relative humidity	ND	500 fold increase E. coli O157:H7 and 740 fold increase S. enterica	(Brandl and Amundson 2008)
	Romain lettuce	E. coli O157:H7	Soil: 5 20°C , 70% relative humidity	ND	36 days	(Ibekwe and others 2006)
	Lactuca sativa L cv. Dublin	E. coli O157:H7, Salmonella serovar Typhimurium	Manure: approximately 7	*E. coli* O157:H7 up to 56 days *Salmonella* Typhimurium > 56 days	One lettuce root was positive lettuce E. coli O157	(Franz and others 2005)
	NA	*E. coli* O157:H7	Inoculated manure mixed with unautoclaved or autoclaved soil: 6-7	Autoclaved soil: 231 days 21°C	ND	(Jiang and others 2011)
Green-house	Crisphead Lettuce	*E. coli* O157:H7	Bovine manure: 4	8 weeks	Not detected	(Johannessen and others 2005)

Table 8: Water sources and quality guidelines proposed in different manuals on good practices in fresh produce primary production

Criteria	Codex Fresh Produce, (Codex Alimentarius Commission 2003a)	GlobalGap (Global Gap 2013)	SQF 1000 (SQF Institute 2010)	McDonalds (McDonald's Worldwide Quality Systems 2011)	FSMA (New England Farmers Union 2013)	ICQM (Primary Production 2013)	Spain (BOE 2007)	Norway (Matmerk KSL 2010)
Sources of irrigation water	identify the sources of water, assess its microbial quality, and its suitability for intended use	no untreated sewage water	a known clean source or treated to make it suitable for use	Adequate distances between water sources and potential sources of contamination must be maintained. Water sourced from a well or bore hole must not be closer than 200 feet (60 m) to areas of untreated manure accumulation. Water sourced from open surface water must not be closer than 100 feet (30 m) to areas of untreated manure accumulation for sandy soils and no closer than 200 feet (60 m) for loamy or clay soils. Reclaimed recycled water is not permitted unless it meets (treated or	adequate for its intended use	creek, open well, drilled well, rain, potable or vegetable wash water, or water used in the processing of vegetables such as blanching, sterilizing	microbial criteria have only been established for the use of treated wastewater for the irrigation of crops that are likely to be eaten uncooked	the irrigation water source should be protected against contamination

				untreated) certain criteria				
Microbio-logical standards	/	If treated sewage water is used, water quality complies with WHO guidelines. If water might be polluted must comply with local or WHO guidelines	Based on the hazard analysis, best practices within country of production and any application legislation, if applicable.	The geometric mean of generic *E. coli* of the five most recent samples must be lower than 126 MPN/100 ml, with no single sample > 235 MPN per 100 ml.	Water that may come in contact with the harvestable portion of produce must meet a standard of no more than 235 CFUs of generic *E. coli* per 100 ml throughout the growing season.	/	*E. coli:* ≤100 CFU/100 ml, sampling plan 3 classes: n = 10; m = 100 CFU/100 ml; M = 1000 CFU/100 ml; c=3 Intestinal nematode: ≤1/10 liters	Water quality has to be “close to drinking water quality” (not specified) and the last day of irrigation before harvesting should be documented
Frequency of testing	depend on the water source and the risks of environmental contamination	a frequency according to the results of the risk assessment every year	decided by the hazard analysis, best practices within country of production and any application legislation	A set of five samples must be collected prior to harvest Samples taken must be at least 18 hours apart and not longer than 30 days since the last sample was taken.	River or Natural lake water: every 7 days during growing season, Water reservoir from groundwater: once a month, groundwater: at the beginning of the season and every 3 months thereafter	/	/	At least one water sample should be analyzed each year,

Table 9 Irrigation Water Quality Guidelines and Regulations

Country/Region	Water Type	Regulation/Guideline	Criterion a, b	Reference
Australia New Zealand [c]	Irrigation water for non- food crops (trees/flowers): Secondary treatment or primary treatment with lagoon detention	Guideline	< 1000 *E. coli* per 100 mL	
Australia New Zealand	Irrigation water for commercial crops raw or unprocessed (salads crops and spray irrigation): Advanced treatment to achieve total pathogen removal required (e.g., secondary, filtration and disinfection)	Guideline	< 1 *E. coli* per 100 mL	
Australia New Zealand [e,f]	Irrigation water for commercial food crops: Secondary treatment with >25 days lagoon detention and disinfection	Guideline	< 100 *E. coli* per100 mL	
Canada	All	Guideline	< 1000 total coliforms per 100 mL < 100 fecal coliforms per 100 mL	(Steele and Odumeru 2004)
Canada (Alberta)	Surface water	Guideline	< 1000 total coliforms per 100 mL < 100 fecal coliforms per 100 mL	(Steele and Odumeru 2004)
Canada (British Columbia)	All	Guideline	< 200 fecal coliforms per 100 mL < 77 *E. coli* per 100 mL: < 20 fecal streptococci per 100 mL	(Steele and Odumeru 2004)
Canada (Saskatchewan)	Surface water	Guideline	< 1000 total coliforms per 100 mL < 100 fecal coliforms per 100 mL	(Steele and Odumeru 2004)
Italy	(Treated) Wastewater	Regulation	< 10 *E. coli* per 100 mL *Salmonellae* absent in 100 mL	(Cirelli and others 2008)
Spain [c]	(Treated) Wastewater	Regulation	< 100 *E. coli* per 100 mL < 1 nematode egg in10 L	(BOE 2007)
USA	Surface water	Guideline	<126 *E. coli* per 100 mL	(LGMA 2012; US Environmental Protection Agency 2003)
USA	(Treated) Wastewater	Guideline	Fecal coliforms absent per 100 mL	(US Environmental Protection Agency 2004)
California (USA)	?	Regulation	< 2.2 totoal coliforms per 100 mL Fecal coliforms absent	(Steele and Odumeru 2004)
WHO	Wastewater	Guideline	< 1000 fecal coliforms per 100 mL < 1 nematode egg per L	(Blumenthal and others 2000; World Health Organization 2006)

[a] All values per 100 ml, unless otherwise stated, TC = total coliforms, FC = fecal coliforms, EC = *E. coli*. [b] Specifics of sample value calculation, such as geometric mean, minimal number of samples, period of sampling, percentage of samples that may deviated from the target value etc. are not mentioned here. [c] Direct contact of irrigation water with edible parts. [d] No direct contact of irrigation water with edible parts. e Crops with limited or no ground contact and eaten raw (e.g. tomatoes, capsicums) —drip irrigation and no harvest of wet or dropped produce, [f] Crops with ground contact with skins removed before consumption (e.g. watermelons) — if spray irrigation, minimum 2 days between final irrigation and harvest