An approach for developing quantitative risk-based microbial standards for fresh produce

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ABSTRACT

A key objective of the Good Agricultural Practices (GAP) program is to reduce the potential for produce to become contaminated with microbial pathogens, such as through irrigation water. Without microbial standards, however, it is impractical to decide whether there is a need to disinfect, a need to institute watershed protection programs, or a need to institute post-harvest disinfection regimes. To develop such standards, quantitative microbial risk assessments can be performed using pathogen monitoring data for produce. This paper presents an approach which can be used towards the application of a risk assessment framework to developing microbial standards for fresh produce. Risks of infection are estimated using typical monitoring data of Salmonella detected on carrots and assuming various scenarios of the likelihood of an individual consuming a contaminated serving of carrots in a given year. Estimated annual risks of infection range from 2.20×10^{-5} to 2.16×10^{-3}, assuming 1% and 100% of an individual's carrot servings are contaminated, respectively. In addition, critical factors are identified which need to be incorporated in such a risk assessment approach as well as their impact on risk estimates to provide growers with benchmarks which may be targeted to reduce health risks.

Key words | carrots, irrigation water, microbial standards, risk assessment, *Salmonella*, vegetable produce

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INTRODUCTION

In the United States, agriculture depends very heavily on surface water sources for irrigation purposes. In many parts of the country, however, municipal wastewater treatment plants also discharge their secondary waste effluent into these same irrigation water sources. Additionally, wildlife, cattle-feeding operations, and septic tanks also contribute to pathogen loadings into these streams (Kuhn *et al.* 2002; McElroy 2002). A number of human illness outbreaks have been associated with the consumption of contaminated produce such as cilantro, tomatoes, cantaloupes, parsley, carrots and green onions (CDC 1996; Beuchat 2002). Although the incidence of illnesses associated with fresh produce is relatively low, the reported number of outbreaks doubled between 1973–1987 and 1988–1992 (Bean *et al.* 1997; Mead *et al.* 1999). This may be due to several factors, including a trend toward increasing consumption of fruits and vegetables as well as a change in geographical patterns of distribution. In addition, outbreaks associated with fruits and vegetables lead to a greater number of reported illness cases than outbreaks associated with other food vehicles (CDC 1996; CDC 2000). Vulnerable populations – such as the elderly, the very young, pregnant women, and the immunocompromised – are at the greatest risk of serious illness and mortality from exposure to foodborne pathogens (Gerba *et al.* 1996).

Fruit and vegetable growers who rely on surface water sources are under increasing pressure to avoid pathogen contamination at the field from irrigation water. However, without *specific* (microbial) irrigation water standards, it is impractical for growers to make decisions regarding the

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suitability of using a particular water source. This is also particularly significant since growers across the United States have to routinely "test" their irrigation water sources without any guidance about what to test for, what the results imply, or furthermore, what actions to take. The agricultural community needs tools which can assist them to decide the suitability of a particular water source. Table 1 shows irrigation water quality data for *Salmonella* from March 2000 – March 2001 from Rio Grande Valley, Texas (unpublished data).

The risk assessment paradigm initially developed in the 1970s by the National Academy of Science/National Research Council to address chemical hazards has been used to evaluate the human health impact (infection, illness and death) associated with exposure to microorganisms in water and food (Regli *et al.* 1991; Haas *et al.* 1993, 1999; Mena *et al.* 2003). Quantitative microbial risk assessment – a four-step methodology of hazard identification, exposure assessment, dose-response assessment (hazard characterization), and risk characterization – has been applied to determine appropriate management strategies regarding water quality and public health (Rose *et al.* 1991; Haas 1995) and can be applied to determine the public health significance of exposure to produce processed with polluted irrigation water (Shuval *et al.* 1997).

This paper describes an approach which utilizes typical monitoring data of the presence/absence of microorganisms (*Salmonella*) on minimally processed produce (carrots) to estimate risks of infection resulting from exposure. Irrigation water quality data for *Salmonella* used by growers in the region is available (Table 1); however, transfer rates of *Salmonella* from these waters to carrots are not part of this present research. Other studies have been conducted which have evaluated the transfer rates of non-pathogenic surrogates to other types of produce and reported transfer rates ranging from 8.8×10^{-7}% to 9.2×10^{-2}% as well as addressed the impact of different types of irrigation methods and timing of harvesting on health risk estimates (Stine *et al.* 2005). Our approach uses pathogen data obtained directly from the produce (carrots) by growers and assumes irrigation water was the source of contamination. This approach evaluates the practical value or usefulness of current routine pathogen monitoring data in conducting risk assessments which could lead to guidelines for growers and determines new directions for data collection from both growers and researchers.

METHODS

Typical monitoring data of the presence/absence of microorganisms on minimally processed produce to estimate risks

Table 1 | Occurrence of *Salmonella* (MPN/100 ml) in irrigation water from Rio Grande Valley, Texas from March 2000 – March 2001

Site	March 2000	April 2000	May 2000	June 2000	July 2000	August 2000	September 2000	November 2000	January 2001	February 2001	March 2001
EP1	ND*	ND	0	ND	ND	ND	ND	ND	ND	ND	ND
EP2	ND	ND	0	0	0	ND	ND	ND	ND	ND	ND
EP3	ND	ND	1.8	0	0	ND	ND	ND	ND	ND	ND
EP4	ND	0	11.2	0	0	0	ND	ND	ND	ND	ND
EP5	ND	0	2	0	0	ND	ND	ND	ND	ND	ND
PR6	12.3	0	ND	8	2	0	0	ND	ND	13.6	6.7
PR7	34.4	0	ND	50.2	4	0	0	ND	ND	4.5	0
WL8	ND	3.7	ND	0	0	ND	0	ND	ND	ND	ND
WL9	ND	9.2	ND	1.8	0	0	0	ND	ND	0	ND
WL10	ND	13.3	ND	6.8	0	0	0	ND	ND	0	ND
WL11	ND	4	ND	4.5	0	0	0	ND	ND	0	1.8

*Not determined due to the unavailability of irrigation water samples from the site in a given month.

of infection resulting from exposure are used in this assessment. Specifically, risks of infection associated with consuming carrots contaminated with *Salmonella* assumed from pathogen-laden irrigation water are estimated. Carrots were chosen as a representative vegetable which is normally consumed raw or with minimal processing. Carrots can become contaminated with pathogens through a variety of ways, including through polluted irrigation water (Endley *et al.* 2003). This assessment involves characterizing two components namely, the concentration of *Salmonella* at the time of exposure (i.e. the number of pathogens per serving of carrot), and the amount of contaminated carrots consumed.

Microbial pathogen monitoring data regarding *Salmonella* obtained from a previous study of carrots are used for the risk estimations (Table 2; Endley *et al.* 2003). As a step toward the construction of a comprehensive risk assessment framework which can be used for criteria development, a dose-response model was developed for *Salmonella*. The beta-Poisson model,

$$P_i = 1 - \left[1 + (d/N_{50})(2^{1/\alpha} - 1)\right]^{-\alpha}$$

is used where P_i = probability (risk) of infection, d = exposure in colony-forming units (CFU), N_{50} = median infectious dose (2.36×10^4), and α = parameter defining the dose-response curve (0.3126) (Haas *et al.* 1999).

Exposure assessment addresses several factors including the amount (concentration) of pathogen in the exposure (such as on a specific produce), the amount of produce consumed, and the frequency and duration of the exposure (such as a one-time event or a repeated exposure over time). Based on *Salmonella* monitoring data for carrots (Table 2), the parameter "d" in the beta-Poisson model was determined by: 1) assuming each *Salmonella*-positive carrot contained 10 CFU (the detection limit of the method); 2) assuming each carrot in the exposure weighs 61 grams, which is the average weight of a medium-sized carrot not including non-edible parts (USDA 2007*a*); and, 3) assuming one serving of carrots weighs 6.5 grams, which is the average serving weight of carrots consumed (adjusted for loss at retail and consumer levels) obtained from the USDA/Economic Research Service database for the years 2000 – 2005 (USDA 2007*b*). Based on these assumptions, the parameter "d" for one serving of carrots is 1.07 CFU of *Salmonella* per serving of carrots.

To estimate annual risks of infection, the following equation was applied:

$$P_{year} = 1 - (1 - P_i)^n$$

where P_{year} = annual probability (risk) of infection, P_i = probability (risk) of infection, and n = the number of contaminated servings consumed during a given year. According to the USDA, an individual consumes 18.6 servings of carrots per year (USDA 2007*a*). In this present assessment, risks are estimated by assuming various frequencies (including those reflecting contamination frequencies observed during routine monitoring) of carrot serving contamination to address the likelihood of an individual consuming a contaminated carrot.

Table 2 | Occurrence of *Salmonella* on carrots from the field, truck and packing shed (modified from Endley *et al.* 2003)

	Occurrence of *Salmonella*-Positive Samples*		
	Field	**Truck**	**Packing Shed**
Salmonella	1/25 (4%)	2/25 (8%)	0/25 (0%)

*Detection limit = 10 organisms per carrot; *n* = 25 carrots.

RESULTS

Table 3 provides yearly risk estimates of infection from exposure to *Salmonella* via carrot consumption using actual bacterial pathogen monitoring data (Table 2). Risks of infection ranged from 2.20×10^{-5} when assuming that only 1% of an individual's yearly number of servings contain *Salmonella* at the detectable level to as high as 2.16×10^{-3} if every carrot serving was contaminated (worst-case). If assuming that the source of *Salmonella* is irrigation water which resulted in the produce monitoring data as shown in Table 2, then inferences may be made which lead to irrigation water quality criteria development for growers. However, interpreting these results subsequently lead to the identification of various data needs regarding factors which

Table 3 | Annual risks of infection associated with consumption of *Salmonella*–contaminated carrots* at different contamination frequencies†

Percentage (%) of yearly servings contaminated (number of servings)	Annual risks of infection
1 (0.19)	2.20×10^{-5}
4 (0.74)	8.60×10^{-5}
8 (1.49)	1.74×10^{-4}
25 (4.65)	5.39×10^{-4}
50 (9.30)	1.08×10^{-3}
75 (13.95)	1.62×10^{-3}
100 (18.6)	2.16×10^{-3}

*beta-Poisson model where $N_{50} = 2.36 \times 10^4$; $\alpha = 0.3126$ (Haas *et al.* 1999); d = 10 colony-forming units (CFU)/carrot; carrot weight = 61 grams at 6.5 grams per serving (USDA 2007*a*, *b*).
†Assuming 18.6 servings of carrots per year at different contamination frequencies (USDA 2007*a*).

impact risk. Table 4 lists these factors to be addressed in quantitative microbial risk assessments of produce impacted by irrigation water.

DISCUSSION

Determining acceptable levels of risk would allow for the identification of appropriate pathogen threshold levels on fresh produce or in irrigation water (as well as in vegetable wash/rinse water) to be used by growers and packers as "action levels" at which management strategies should be implemented by the grower to reduce pathogen contaminant levels. Such an approach has been taken for drinking water by the USEPA with their recommendation that microbial risks of infection should not exceed 1×10^{-4} per year (USEPA 1989). Calculated human health risks associated with exposure to contaminated drinking water can then be compared to the USEPA's recommendation to ensure risks are at negligible levels.

When considering the yearly risks of infection for various scenarios of contamination frequency presented in Table 3, even a contamination frequency as low as 8% (as observed for the truck environment, Table 2) resulted in a risk which does not meet USEPA's annual risk goal. Infection risks calculated in this assessment may be over- or under-estimated depending on the actual yearly carrot consumption value for individuals. In addition, infection risks may be underestimated since the only carrots included as *Salmonella*-positive were presumed to have 10 CFU (the detection limit of the method) yet it is not improbable to assume that other carrots also contained *Salmonella* but below this detection limit. Even if 5 *Salmonella* CFU per carrot were present, after applying the same assumptions as described above, a 10% frequency of consuming a contaminated serving of carrots during a given year just meets USEPA's risk recommendation of 1×10^{-4}.

The Good Agricultural Practices program does not provide specific guidelines or recommendations for growers regarding irrigation water quality. There is no current irrigation water quality criterion in the United States (except for reclaimed water) to alert growers to stop using a particular irrigation water source. The Canadian Environmental Quality Guidelines recommend the limit of 100 *E. coli*/100 ml or 1000/100 ml of total coliforms in irrigation water (Anon 2002). It is generally expected that production agriculture cannot afford irrigation water at drinking water quality. However, the water used in post-harvest processes such as washing and rinsing is critical in terms of potential pathogen loads.

Table 4 lists several assumptions to be considered when addressing the health risks associated with exposure to produce microbially contaminated by irrigation water and

Table 4 | Assumptions for determining exposure (d) in a risk assessment of *Salmonella* on carrots originating from irrigation water and impact on risk estimates

Assumptions	Change	Risk effect
Quality of Irrigation Water Source	↑	↓
Treatment of Irrigation Water	↑	↓
Type of Irrigation (Subsurface Drip rather than Furrow Irrigation)	↑	↓
Transfer Rate of *Salmonella* from Irrigation Water to Carrots	↓	↓
# Days Between Harvest and Last Irrigation	↑	↓
% of Infectious *Salmonella*	↓	↓
Removal of *Salmonella* Due to Washing/Processing	↑	↓
Detection Limit of the Method	↓	↓
# of Contaminated Carrots Consumed	↓	↓

how health risk is impacted when the factor/assumption is changed. Factors which obviously decrease health risks are improved irrigation water quality, an increase in the treatment of irrigation water, a decrease in the transfer rate of *Salmonella* (or any pathogen) from irrigation water to carrots (or any produce), a decrease in the number of infectious microorganisms present, an increase in the removal of *Salmonella* (or any pathogen) during washing, and a decrease in the number of contaminated carrots (or any contaminated produce) consumed. In addition, different types of irrigation applications may impact risk estimates with furrow irrigation potentially increasing the risk of produce contamination as concluded by Stine *et al.* (2005). Furthermore, the timing of harvesting after the last irrigation application impacts risk with longer time periods between events allowing for pathogen die-off (Stine *et al.* 2005). Lastly, growers need detection methodologies with low detection limits which will improve risk estimates, and ideally use methods which address infectivity and generate quantitative data for specific pathogens. Moreover, during pathogen monitoring, the recording of negative samples are just as important as the reporting of positive samples when considering the ultimate goal of identifying risk management strategies.

Components of the exposure assessment step contribute the greatest amount of variability to microbial risk assessments (Haas *et al.* 1999). This variability is due to factors related to source and extent of contamination as well as amount of human exposure. For the assessment presented here, data needs which could be obtained by growers during routine pathogen monitoring include quantitative data for specific pathogens in both agricultural water sources and irrigation water (per sample volume), and quantitative data for specific pathogens on produce (per sample weight). In addition, further research is needed regarding the volume of (microbial-laden) irrigation water which is retained on produce. This has been minimally explored (Shuval *et al.* 1997; Stine *et al.* 2005) and additional investigations are warranted to evaluate the impact of irrigation mode and type of crop on water retainment on produce. Pathogen persistence and survivability from treatment or environmental stressors also need to be investigated for both irrigation water and produce to provide critical information to risk assessments.

As with any risk assessment, uncertainty and variability must be addressed within the assessment to allow for appropriate interpretation of results and to better guide future research directions. Other production practices within the farm environment should also be investigated to identify potential sources of microbial contamination to produce. This would aid in developing (or enhancing existing) Hazard Analysis Critical Control Point (HACCP) programs through the identification of microbial transmission pathways from field to produce to consumer.

Based on estimated risks of infection as shown in Table 3, risk estimates exceeding USEPA's guideline of 1×10^{-4} per year may indicate a problem in the harvesting area, including poor microbial irrigation water quality. Such (high) infection risk estimates may lead a grower to re-evaluate irrigation water source and treatment options, and type of irrigation application. Although it is recognized that different types of irrigation practices pose different risks to produce quality, concerns of microbial irrigation water quality does not influence choice of irrigation practice however; such decisions are driven by other factors like cost, water availability, cropping rotation, and soil type (USFDA 2001). With improved data regarding irrigation water's role in crop contamination, risk assessments of pathogens on produce can provide the information necessary for a grower to make management decisions which are both practical and economical.

CONCLUSIONS

The risk assessment approach described here provides a framework for quantitatively describing the human health significance of various pathogen levels in irrigation and vegetable processing/packing steps. This approach can provide a way for decision-makers to determine whether the quality of the water, for example, used in a particular process needs improvement *via* disinfection or other mechanisms. Developing dose-response models for specific pathogens using current available data and subsequently filling identified data gaps can provide the starting point for identifying acceptable levels regarding different pathogens on produce and in irrigation water. Such an analysis can help identify practices which can have the greatest benefit in reducing health risks in terms of different pathogens and vegetables.

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