APPLICATION OF MICROBIAL RISK ASSESSMENT OF *Escherichia coli* IN IRRIGATION WATER ON LETTUCE CROP

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Abstract

Contaminated water, soil manure, and wildlife are the main sources of contamination of leafy greens. Microbial contamination of lettuce plants, via contact with irrigation water was investigated to aid in the development of irrigation water quality standards for enteric bacteria. Surface irrigation was evaluated with the use of *Escherichia coli* O157:H7 transfer data to the plants. The concentrations of *E. coli* in irrigation water necessary to achieve a 1:10,000 annual risk of infection, the acceptable level of risk used for drinking water by the U.S. Environmental Protection Agency, were calculated with a quantitative microbial risk assessment approach. These calculations were based on the transfer of *E. coli* to fresh produce via irrigation water. The Beta-Poisson model was used to estimate the microbial risk of pathogen annual ingestion. The risk of infection was found to be variable depending on the *E. coli* concentration in water, and the pathogen transfer to the crop days post exposure. The worst-case scenario, in which produce could be harvested and consumed the day after the last irrigation event and maximum exposure is assumed, indicated that concentrations of 10^4 CFU/ml in 30-day-old lettuce plants would result in an annual risk of 1:10,000 if the crop would be consumed. Similarly, concentrations of 10^3 CFU/ml, and 10^4 CFU/ml in 12-day-old lettuce would result in an annual risk of 1:10,000 if the crop would be ingested. It appears that bacterial growth continued for the first ten days after contamination so that the probabilities increased from 0.2 to 0.5 in the 103 CFU/ml concentrations for the lettuce contaminated at 12 days.

Keywords: Risk assessment, *Escherichia coli*, irrigation, lettuce.

Introduction

Foodborne diseases are increasing and becoming widespread; therefore, food safety has been considered as an important global health issue for a long time now (Harris et al., 2003). The number of foodborne outbreaks associated with fresh produce has been observed (Tauxe, 1997). Irrigation with wastewater increases the health risk due to the presence of high concentrations of pathogens such as bacteria, viruses, protozoa and helminths (Toze, 2006). Fresh produce potentially pose an increased food safety risk because they are consumed raw or minimally processed. Produce consumption habits and importation processes, has led to several foodborne outbreaks (Hedberg, 2000; Hedberg et al., 1994; Meng and Doyle, 2002). *Escherichia coli* O157:H7 have been found to be one of the leading causes of the produce-related foodborne outbreaks (Olsen et al., 2000). Outbreaks of food borne diseases caused by *E. coli* are a serious public health concern and a report from CDC indicated that 73,000 cases of infection with *E. coli* and 61 deaths on average occur in the United States annually (Seto et al., 2007). The 2006 outbreak linked to *E. coli*–contaminated spinach that resulted in 205 confirmed cases and three deaths served as a catalyst for research efforts to ensure the safety of leafy greens (CDC, 2006). *E. coli* has been also linked to foodborne outbreaks associated with the consumption of lettuce (Hilborn et al., 1999).

Sources of microbial contamination include irrigation water, soil, inadequately composted manure, and human handling (Beuchat, 1995). Sources of fecal contamination of irrigation water include animal feces (Ackers et al., 1998; Hilborn et al., 1999) wastewater discharge, and runoff from livestock operations (U.S. FDA, 1998). Water from surface sources should not pose a risk of infection from waterborne pathogens greater than 1:10,000 per year according to the U.S. Environmental Protection Agency (U.S. EPA, 1989). This value has been used to evaluate risk associated with the quality of irrigation water (Asano et al., 1992; Takana et al., 1998; Petterson et al 2001). Quantitative microbial risk assessment (QMRA) is a framework and approach that brings information and data together with mathematical models to address the spread of microbial agents through environmental exposures and to characterize the nature of the adverse outcomes. It is a key factor in all decision making for determining the relative urgency of problems and the allocation of resources to reduce risks. (Haas et al., 1999). Since the publication of the WHO guidelines in 1989, the health risks of wastewater use in agriculture have been investigated in greater detail with quantitative microbial risk analysis (QMRA) applied to irrigation (Shuval et al. 1997; Tanaka et al. 1998).

The primary goal was to determine the probability of infection that would result in a 1:10,000 yearly risk of infection from consumption of irrigated lettuce utilizing a QMRA approach. For this objective, the transfer of *E. coli* to the surfaces of lettuce via contaminated irrigation water was reviewed. We use *E. coli* because it is exclusively fecal in origin. Surface irrigation data was assessed. This irrigation method entails the application of water to the soil surface, often resulting in direct contact between the aboveground portion of the plant with the irrigation water. Contamination of 12 and 30 day old lettuce plants, based on sample enrichment after exposure to irrigation water contaminated with different levels of *E. coli* was then used to determine the concentrations of enteric pathogens in irrigation water necessary to achieve the desired annual risk of infection from the consumption of irrigated produce. We also consider the implications of our results for the new WHO guidelines.

Materials and Methods

Quantitative Microbial Risk Assessment (QMRA)

QMRA is useful in quantifying risks of infection. The present study focuses on the risk to consumers of lettuce irrigated with *E. coli*-contaminated water. Risks for farmers and workers are not in the scope of this work. Decay of microorganisms during storage and processing were not considered either, because postharvest activity is outside the control of the irrigation water quality regulation. We used the standard QMRA techniques (Haas et al. 1999) to estimate risks of infection from model pathogen-ingestion scenarios. The risk assessment paradigm involves four steps: hazard identification; exposure assessment; dose response; and risk characterization.

Transfer of *E. coli* O157:H7 from water to Lettuce Plants

The transfer of low numbers of *E. coli* O157:H7 from water to growing lettuce plants was reviewed. Mootian et al. (2009) determined the transfer of low numbers of *Escherichia coli* O157:H7 from soil, manure-amended soil, and water to growing green ice leaf lettuce (*Lactuca sativa* L.). Lettuce plants, young (12 days of age at exposure to contaminated water) or mature (30 days of age at exposure to contaminated water), were irrigated with water containing 10^1, 10^2, 10^3, or 10^4 CFU *E. coli* O157:H7 per ml. Harvested plants were processed to determine whether *E. coli* O157:H7 was associated with the entire plant or within internal locations. Young plants (12 days) were harvested at 1, 10, 20, and 30 days postexposure. Mature plants (30 days) were harvested at 1, and 15 days postexposure. The outer leaves were allowed to touch the soil, to closely model in-field conditions. (Mootian et al., 2009), see Tables 1 and 2.

Table 1. Contamination of 12-day-old lettuce plants, based on sample enrichment after exposure to irrigation water contaminated with *E. coli*, as taken from Mootian et al., (2009).

Treatment	Surface examined	Day(s) after exposure	Concentration of *E. coli* (CFU/ml) in contaminated irrigation water			
			10^1	10^2	10^3	10^4
Water	Total	1	0	0	1/6	1/2
		10	1/2	5/6	2/3	1/2
		20	0	1/6	0	0
		30	1	1/6	0	1/6
	Internal	1	0	0	0	0
		10	1/6	1/6	0	0
		20	0	2/3	0	0
		30	0	0	0	0

*Samples tested positive. Values are number of positive plants/number of plants tested.

Table 2. Contamination of 30-day-old lettuce plants, based on sample enrichment after exposure to irrigation water contaminated with *E. coli*, as taken from Mootian et al., (2009).

Treatment	**Surface examined**	**Day(s) after exposure**	**Concentration of *E. coli* (CFU/ml) in contaminated irrigation water**			
			10^1	**10^2**	**10^3**	**10^4**
Water	Total	1	1/6	1/2	5/6	1/2
		15	5/6	1	1/2	1/6
	Internal	1	0	1/3	0	0
		15	1/6	0	1/6	1/6

*Samples tested positive. Values are number of positive plants/number of plants tested.

Dose–response assessment

The steps taken to quantify the risk of microbial infection and the assumptions used are summarized in Table 3 with surface-irrigated 30-day-old lettuce contaminated by 10^4 CFU/ml of *E. coli* level as an exemplary case.

Table 3. Risk assessment steps and assumptions used to calculate the risk of infection of *E. coli* in lettuce.

Risk Assessment Steps	**Assumptions**
Calculation of the annual risk of infection	1:10,000
Amount of fresh produce consumed per capita	4416.5 g
Contamination rate of 30-day-old lettuce	3/6 samples tested positive
Concentration of *E. coli in* irrigation water to achieve 1:10,00 risk of infection	10^4 CFU/ml

The Beta-Poisson model can be used to quantify the risk of microbial ingestion. The model gives the following equation (Haas et al., 1999):

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

In this equation, P_i is the risk of infection by ingesting pathogens in drinking water, *d* is the dose of microorganisms ingested, N_{50} is the microbial dose resulting in 50% infection, and α is a slope parameter. The best-fit dose-response parameters N_{50}, and α for ingestion of *E. coli* (DuPont et al., 1971) are reported in Table 4.

Table 4. Best fit dose-response parameters (DuPont et al., 1971).

Organism	**Beta-Poisson model**	
	N_{50}	α
Escherichia coli	2.11×10^6	0.155

The annual acceptable risk of infection (P_A) was determined with the following equation (Haas et al., 1999):

$$P_A = 1 - (1 - P_i)^{365} \quad (2)$$

where P_A is the annual risk, which was assumed to be the EPA benchmark annual acceptable risk of infection of 1:10,000 for drinking water.

The dose (*d*) of microorganisms ingested was calculated by multiplying the level of *E. coli* to achieve the levels 10^1, 10^2, 10^3, or 10^4 CFU/ml in irrigation water, by the number of positive plants/number of plants tested, by the amount of produce items consumed which was determined by adjusted annual per capita consumption of lettuce of 4,416.5 g (Alum, A. 2001; Stine et al., 2005). It was assumed that fresh produce was consumed the day it was harvested. Thus, young plants (12 days) were consumed at 1, 10, 20, and 30 days postexposure and mature plants at 1, and 15 days postexposure, according to the study made by Mootian et al., (2009).

Results and Discussion

In this study the risk associated with pathogens in the irrigation water is variable depending on days after the last irrigation event. The worst-case scenario was found when produce is harvested the day after the last irrigation and maximum contamination level is used, for mature lettuce plants, see Table 6. The same worst-case scenario was also found when produce is harvested the day after the last irrigation and maximum contamination level is used, for young lettuce plants, even though they are not “ready” for consumption yet. Both results indicate that the concentrations needed to achieve an annual 1:10,000 risk of infection were as low as 10^4 CFU/100 ml of *E. coli*. If the EPA standard is applied to produce that is consumed raw, then the irrigation water should not contain any *E. coli* above concentrations of 10^3 CFU/ml at least. It should be noted again that these estimates were obtained from data from fresh produce grown with surface irrigation. Also, a concentration of 10^4 CFU/ml was necessary to achieve a 1:10,000 risk of infection in young lettuce after 10 days of exposure, while annual probabilities 0.5 and 0.6 to achieve an annual 1:10,000 risk of infection were found in 10^3 CFU/ml concentrations for young 10 days after irrigation and for mature lettuce 1 day after irrigation respectively. A concentration of 10^3 CFU/ml for mature lettuce after 10 days post exposure has 0.8 probability of infection from consumption.

Table 5. Results of the calculated annual risk of infection from consumption of 12-day-old lettuce, with the four *E. coli* concentrations analyzed.

Treatment	**Surface examined**	**Day(s) after exposure**	**Concentration of *E. coli* (CFU/ml) in contaminated irrigation water**			
			10^1	**10^2**	**10^3**	**10^4**
Water	Total	1	0	0	0.2	1
		10	0	0.1	0.5	1
		20	0	0	0	0
		30	0	0	0	0.8
	Internal	1	0	0	0	0
		10	0	0	0	0
		20	0	0.1	0	0
		30	0	0	0	0

As we can see in Table 5, it appears that bacterial growth continued for the first ten days after contamination so that the probabilities increased from 0.2 to 0.5 in the 10^3 CFU/g concentrations for the lettuce contaminated at 12 days.

Table 6. Results of the calculated annual risk of infection from consumption of 30-day-old lettuce, with the four *E. coli* concentrations analyzed.

Treatment	Surface examined	Day(s) after exposure	Concentration of *E. coli* (CFU/ml) in contaminated irrigation water			
			10^1	10^2	10^3	10^4
Water	Total	1	0	0	0.6	1
		15	0	0.1	0.4	0.8
	Internal	1	0	0	0	0
		15	0	0	0.2	0.8

Fig. 1 shows the dose-response model for consumption of *E. coli* (CFU). Data represent the Beta-Poison dose response model as shown in equations (1) and (2). This model is model is used to describe the probability of infection in human subjects for many enteric microorganisms (Haas, 1983).

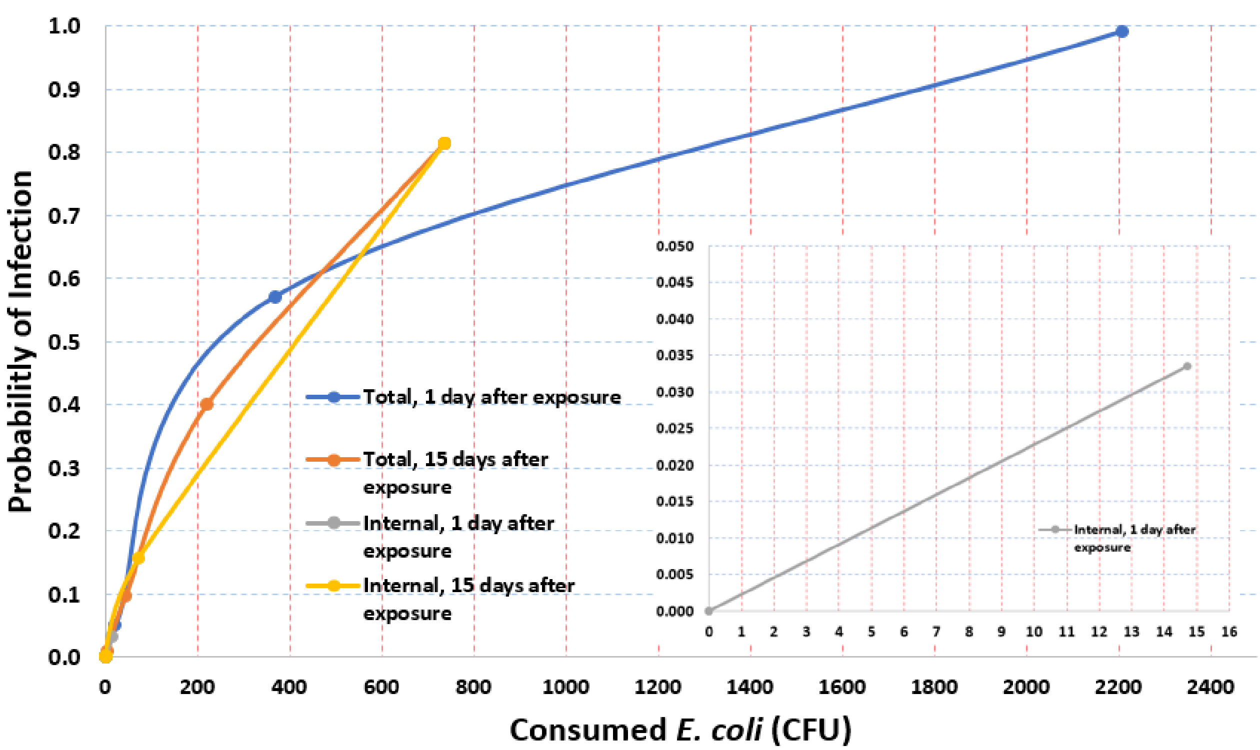

Figure 1. Dose-Response Model for consumption of *E. coli* (CFU).

Conclusions

QMRA is an important tool for assessing the risk involved in irrigation water, both in terms of the formulation of the risk analysis problem and in predicting the probability of infection in different scenarios. Such information could provide guidance for the development of microbial standards for irrigation water. The results suggest that lettuce exposed to and grown in the presence of low numbers of *E. coli* O157:H7 may become contaminated and thus present a human health risk. These results suggest that contamination of lettuce close to harvest may increase the risk of the pathogen being present on the crop. Future efforts must center on avoiding human pathogen contamination of produce.

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