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Quantitative Microbial Risk Assessment of North Carolina Type 2 Reclaimed Water for Agricultural Reuse

Emily S. Bailey [1,2,*] and Mark D. Sobsey [1]

[1] Department of Environmental Sciences and Engineering, Gillings School of Global Public Health, The University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA
[2] Department of Public Health, College of Pharmacy & Health Sciences, Campbell University, Buies Creek, NC 27506, USA
* Correspondence: ebailey@campbell.edu

Abstract: As treated wastewater is increasingly used for agricultural purposes; questions remain about the microbiological quality of produce irrigated by these waters. This study conducted a quantitative microbial risk assessment (QMRA) using microbial data collected from North Carolina Type 2 reclaimed waters, which have been proposed as supplemental irrigation waters. Reclaimed waters were collected from four different water reclamation facilities located in central North Carolina and evaluated for five representative pathogens from the three groups of microorganisms (bacteria, virus, and protozoan parasites). Using these data, produce consumption scenarios were evaluated using a variety of irrigation techniques, including spray irrigation, drip irrigation, and subsurface drip irrigation, and the disability adjusted life years (DALYs) that result from illness by each pathogen as a result of produce consumption were compared to the acceptable level set by the World Health Organization. Based on the types of crop irrigation examined in this study using NC Type 2 reclaimed water, there were irrigation conditions and certain pathogens for which the annual risk of infection was not always reduced below the acceptable DALY risk level of $<1 \times 10^{-6}$ set by the WHO. The risks of viral infection by adenoviruses groups A–F were below the acceptable risk level; however, for *Salmonella* spp., *Cryptosporidium*, and *Giardia*, the annual risk of infection was sometimes greater than would be considered acceptable.

Keywords: reclaimed water; water reuse; water-supply systems; agro-wastewater; risk assessment

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1. Introduction

Treated wastewater used for agricultural purposes has been proposed as a means of enhancing food security while addressing water scarcity issues related to growing water demands [1]. Most reclaimed water usage in the world comes from agricultural uses, with Mexico and Egypt reported to have the highest usage of treated wastewater for irrigation [2]. In North Carolina, high quality reclaimed water, designated Type 2, has been proposed for the irrigation of food crops [3,4]. Despite this level of wastewater treatment, the actual microbial quality and potential health risks from pathogens in this type of reclaimed water are not known and therefore are still of concern.

To evaluate the microbial risks of reclaimed water for agricultural use, the World Health Organization (WHO) has recommended the use of quantitative microbial risk assessment (QMRA) to assess the added disease burden [5]. QMRA is an analytical tool used to estimate the health effects resulting from exposure to microorganisms [5,6]. There are four key steps to the QMRA process: (1) hazard identification, (2) exposure assessment, (3) dose–response assessment, and (4) risk characterization. Previous researchers conducting QMRAs on the agricultural reuse of reclaimed water have focused on the use of reclaimed water on either specific crops [7] or by specific irrigation methods [8]. These studies have also examined the use of secondary effluent, final effluent or reclaimed water

treated by chlorine disinfection only. As the concentrations of pathogens and the associated risks from these pathogens are potentially different in the reclaimed waters that have been previously evaluated, it is necessary and important to evaluate the risks of using NC Type 2 reclaimed water for agricultural proposes.

In this study, five representative pathogens from the three groups of microorganisms (bacteria, virus, and protozoan parasites) included in the NC legislation on reclaimed water were selected. Although the NC reclaimed water legislation is focused on fecal indicator microorganisms to specify water quality and treatment system performance requirements, the goal of this risk assessment was to select representative pathogens from each group to model the risk of exposure posed by reclaimed water via raw fruit and vegetables ingested by consumers under various exposure scenarios. The selected pathogens from each group include *Salmonella* spp. bacteria, Adenovirus groups A–F, Norovirus GII (as representative enteric viruses) and *Cryptosporidium* spp. and *Giardia* spp. as protozoan parasites. The exposure scenarios evaluated in this analysis are focused on three irrigation water application types, specifically, spray, drip, and subsurface drip irrigation.

2. Materials and Methods

2.1. Water Sampling and Microbial Analysis

Reclaimed water samples were collected bi-monthly for 1 year, during and after storm events, as grab samples using approved techniques (Standard Methods for the Examination of Water and Wastewater (SMEWW)) from 4 different water reclamation facilities located in central North Carolina, resulting in 22 reclaimed water samples [9,10]. North Carolina Type 2 reclaimed water (NCT2RW) requires tertiary physical and biological treatment (typically, primary sedimentation, secondary biological treatment and direct granular media filtration) followed by dual disinfection (typically by UV radiation and chlorine disinfection). Reclaimed water samples were collected in 10 L volumes, transported on ice to the laboratory and stored at 4 °C until analysis. A total of 22 samples of reclaimed water were assayed for *Salmonella* spp., *Cryptosporidium* spp., *Giardia* spp., Norovirus GII, and Adenovirus A–F using previously described methods [9,10].

Briefly, *Salmonella* spp. were detected using a quantal assay for selective enrichment in Rappaport–Vassiliadis broth, followed by streak plating for colony isolation on Salmonella-Shigella agar to detect and then confirm presumptive positive colonies. Enteric viruses and protozoan parasites were detected using a hollow fiber ultra-filtration concentration method described by Hill et al., 2007, and Polaczyk et al., 2008 [11,12]. Enteric viruses were further concentrated using polyethylene glycol precipitation methods described by Yamamoto et al., 1970 [13], followed by real-time quantitative PCR for norovirus GII [14] and adenoviruses A–F [15]. *Cryptosporidium* spp. and *Giardia* spp. were detected using the US EPA method 1623 [16].

2.2. Statistical Analysis

Data were entered into a Microsoft Excel (Microsoft Cooperation, Redmond, WA, USA) spreadsheet, and calculations for risk and Monte Carlo simulations were performed using Analytica 4.6 (Lumina Decision Systems, Los Gatos, CA, USA), with random variables sampled 10,000 times for each analysis. Details on the components of the risk assessment model, assumptions, recovery efficiencies, etc., are presented in detail in the sections below. A diagram of the QMRA model designed in Analytica is shown in Figure S1. Uncertainty analyses were conducted for test microorganism in each water type by evaluating the rank order correlation of uncertainty with variables used in the model (Section 3.3).

2.3. Exposure Assessment

The focus of this exposure assessment was the estimation of the likelihood of exposure by an individual to the identified hazard. The key elements and variables included were the concentrations and survival of key pathogens, specifically *Salmonella* bacteria, aden-

oviruses, *Cryptosporidium* and *Giardia* spp., on raw vegetable crops watered by drip, spray or subsurface drip irrigation over a period of 30 days.

To more fully evaluate the exposure of individuals to the ingestion of contaminated foods, the survival (and decay) of those pathogens on food products was considered. Natural processes, such as temperature, dissolved solids, UV/sunlight radiation exposure, relative humidity and moisture content (water activity), may impact the survival of pathogens on food products. As a component of this model, a decay constant was incorporated into the pathogen concentration term (C_C). The decay constants (k) used for this analysis for each class of microorganism are listed in Table 1. Microbial concentrations were calculated using the following equation:

$$C_c = C_{RW}\,[\exp(-kt_d)] \quad (1)$$

where C_c is the concentration of pathogens in organisms/L at elapsed time t_d after irrigation or at consumption, C_{RW} (organisms/L) is the initial pathogen concentration in reclaimed water samples, k is the kinetic decay constant (d^{-1}), and t_d is the elapsed time between final irrigation and consumption in days.

Table 1. Summary of distributions and fit parameters used in models.

Model Parameter and Sample	Symbol	Unit	Distribution and Fit Parameter	Reference
Organism Concentration in NCT2RW	C_{RW}	Log_{10} per L	Normal, fitted to log data	Calculated from 22 reclaimed water samples
Adenovirus A–F			Normal (μ: 3.72,σ: 1.56)	
Salmonella spp.			Normal (μ: 0.13,σ: 0.45)	
Cryptosporidium spp.			Normal μ: 0.22,σ: 0.36)	
Giardia spp.			Normal (μ: 0.22,σ: 0.38)	
Daily fruit and vegetable consumption	M_i	g (kg ca da)$^{-1}$	Point Estimate (PE), 313	US EPA, 2011 [17]
Percentage of fruit and vegetables consumed raw	f_{raw}	-	Triangular (0.25, 0.5, 0.75)	Van Ginneken and Oron, 2010 [18]
Kinetic decay constant				
Viruses	K	Day^{-1}	PE, 0.69	Asano et al., 1992 [19]
Bacteria			PE, 0.147	Reinoso et al., 2008 [20]
Protozoan parasites			PE, 0.0365	
Body mass	M_{body}	kg	Lognormal (μ: 61.429, σ: 13.362)	US EPA, 2011 [17]
Equivalent volume				
Spray irrigation	V_{eq}	g^{-1}	PE, 1.6×10^{-4}	Van Ginneken and Oron, 2010 [18]
Drip irrigation			Triangular (1.6×10^{-7}, 1.6×10^{-6}, 1.6×10^{-5})	
Subsurface drip irrigation			Triangular (1.6×10^{-8}, 1.6×10^{-7}, 1.6×10^{-6})	
Period between irrigation and consumption	t_d	Days	0, 15, 30	-

The exposure to the ingestion of contaminated food can be estimated as the product of the pathogen concentration in the consumed food and the amount of food consumed per day [21], as represented in the equation developed by Hamilton et al., 2006 [7], below:

$$D_i = f_{raw}M_{body}M_iC_cV_{eq}\exp(-kt_d) \quad (2)$$

where D_i is the daily dose of contaminant (organism per capita per day), f_{raw} is the fraction of fruits and vegetables consumed raw, M_{body} is the human body weight (kg), Mi is the daily consumption per capita (ca) per kg of body weight per day (d) or (g/(kg ca d)), Cc is the pathogen concentration (organisms/L) of irrigation water, V_{eq} is the volume of reclaimed water in g^{-1} retained on raw vegetables after irrigation, k is the kinetic decay constant (d^{-1}), and t_d is the elapsed time between final irrigation and consumption in days. This equation evaluates the combined effects of human consumption habits as related to the applied volume of wastewater at a specific microbial quality and application (irrigation) method.

2.4. Exposure Scenarios

For this analysis, data collected on the microbial quality of North Carolina Type 2 reclaimed water were used to model the health risk of pathogens from consuming raw fruits and vegetables irrigated by specific techniques. The irrigation techniques evaluated included spray irrigation (SI), drip irrigation (DI), and subsurface drip irrigation (SDI). Elapsed times of 0, 15, and 30 days between irrigation and harvest were evaluated.

2.5. Irrigation Method and Reclaimed Water Quality

Agricultural crops are typically contaminated in one of two ways: (1) by direct external plant contact with wastewater, and (2) the penetration of microorganisms through the root system or another pathway into a plants' internal parts [22]. Three different types of irrigation methods were considered to describe the risks of both types of agricultural contamination by microbial pathogens. As contact contamination typically depends on the type of irrigation method, it is important to evaluate the three irrigation methods: SI, DI, and SDI. With SI, relatively large amounts of reclaimed water and aerosols are in contact with the crop surface, causing high amounts of contamination. With DI, reclaimed water is provided through on-surface laterals, which only contaminate plants if the laterals are directly attached to the emitters. Oron et al. (1991) estimated that the contamination levels when using DI are at least 2 orders of magnitude lower than when using SI [22]. With SDI, estimated contamination levels are even lower, as reclaimed water will only come into contact with the root of the plant. The distributions and mean values for equivalent volumes of reclaimed water on fruit and vegetable crops are summarized in Table 1.

3. Results

3.1. Dose-Response Modeling

For Adenovirus A–F, *Cryptosporidium* and *Giardia*, the exponential dose–response model was used to determine the probability of infection from ingestion of various numbers of pathogens. The exponential model is

$$P(inf) = 1 - e^{-k * N} \quad (3)$$

where P(*inf*) is the probability of infection resulting from daily ingestion of the number of pathogens (N) and *K* is the average dose, or number of organisms, that must be ingested to initiate an infection. The best-fit *K* values for Adenovirus, *C. parvum*, and *G. lamblia* are 0.67 [23], 0.0042 [24], and 0.0198 [25], respectively.

For *Salmonella* spp., the Beta–Poisson model was used:

$$P(inf) = 1⟦ - [1 + N ((2\hat{}(1/\alpha)-1))/N_{50}]⟧\hat{}(-\alpha) \quad (4)$$

where P(*inf*) is the probability of infection resulting from the daily ingestion of the dose of pathogens (N); α is the pathogen infectivity constant; and N_{50} is LD_{50}, the dose that is lethal to 50% of individuals, divided by ID_{50}, which is the median infective dose. The optimized parameters for non-typhoid *Salmonella* are 2.1×10^{-1} and 4.98×10^{1} for α and N_{50}, respectively [26].

Estimates of daily risk can be extrapolated to the risk of infection over an extended period of time using the equation below [27]. This equation was used to calculate yearly risks of reclaimed water under the exposure scenario of 365 days of exposure to raw vegetables irrigated with Type 2 reclaimed water.

$$P_t = 1 - (1 - P_d)^t \quad (5)$$

Here, P_t is the probability of infection after t days, and P_d is the probability of infection after one day of exposure.

In order to further evaluate the risk of illness from exposure to pathogens in reclaimed waters used for agricultural purposes, it is necessary to calculate the disability adjusted life years (DALYs) as the health effect parameter associated with illness from the pathogens examined. The first step in this calculation is to estimate the risk of diarrheal illness per year (P_{ill}) using the formula

$$P_{ill} = P_t \times P_{ill\,|\,inf} \quad (6)$$

where P_t is the probability of infection after t days (in this case 356 days or 1 year), and $P_{ill\,|\,inf}$ is the probability of illness given infection. This parameter is organism specific; the value for *Salmonella* is 0.3, the values for *Cryptosporidium* and *Giardia* are 0.7, and the value for adenovirus is 0.5 [28]. DALYs per case is also organism specific, and the relevant values are 9.6×10^{-4} for *Salmonella*, 1.5×10^{-3} for *Cryptosporidium* and *Giardia*, and 2×10^{-3} for adenovirus [6]. The health outcome target (*HT*), or DALYs per year, is calculated using the equation below:

$$HT = \text{Pill} \times db \times \text{fs} \div 100 \quad (7)$$

where f_s is the fraction of the population susceptible to a given pathogen; for this analysis, 100% of the population is assumed to be susceptible to each pathogen.

3.2. Risk Characterization

Table 2 displays the DALYs per person per year as well as the upper and lower 95% confidence intervals (CIs) for the risk scenarios based on the irrigation type and period between irrigation and consumption using North Carolina Type 2 reclaimed water. It is important to note that the calculation of risks for *Cryptosporidium* and *Giardia* spp. are based on microscopic protozoan counts (not on infectivity data) and, despite accounting for infectivity in the QMRA model, the risk may be overestimated. Additionally, the US EPA method 1623 does not differentiate between human infectious species and all species of *Cryptosporidium* and *Giardia*, which may also result in an overestimation of human health risks. However, for adenovirus, the fraction of infectious viruses was determined by integrated cell culture-quantitative polymerase chain reaction (ICC-qPCR) and should estimate the infection risk of exposure in these exposure scenarios. In order to obtain risk characterizations, a health-based target was specified as a DALY loss of $<10^{-6}$ per person per year through waterborne exposure by potable reuse water, as recommended by the World Health Organization [5].

SI, which involves the use of sprinklers to distribute reclaimed water onto the land surface, which then either evaporates into the air, deposits on crops or soaks into the soil, causes a large number of airborne particles to come into contact with crop surfaces and the ground, resulting in a large amount of contamination if microorganisms remain in irrigation water and come in contact with the produce. According to this analysis, the DALYs associated with this irrigation method are relatively high compared to DI and SDI. The protozoan parasites had DALYs greater than the acceptable level of 10^{-6} per person per year, with average levels of 8.74×10^{-5} for *Cryptosporidium* and 3.44×10^{-4} for *Giardia*. The DALYs for *Salmonella* were also greater than the acceptable level with an average of 7.49×10^{-6} (95% CI 5.07×10^{-7} to 8.93×10^{-5}). In contrast, the average DALY for adenovirus was 1.15×10^{-9} (95% CI 4.98×10^{-10} to 2.64×10^{-9}), indicating that there is little DALY risk due to adenovirus at this acceptable risk level.

Table 2. Annual risk of infection for irrigation scenarios based on 10,000 Monte Carlo simulations.

Scenario		DALY per Year *		
Irrigation Type	**Organism**	**Average**	**Lower Confidence Limit**	**Upper Confidence Limit**
Spray (SI)	*Salmonella* spp.	$\mathbf{7.49 \times 10^{-6}}$	5.07×10^{-7}	$\mathbf{8.93 \times 10^{-5}}$
	Adenovirus A–F	1.15×10^{-9}	4.98×10^{-10}	2.64×10^{-9}
	Cryptosporidium spp.	$\mathbf{8.74 \times 10^{-5}}$	$\mathbf{1.27 \times 10^{-5}}$	$\mathbf{4.81 \times 10^{-4}}$
	Giardia spp.	$\mathbf{3.44 \times 10^{-4}}$	$\mathbf{5.38 \times 10^{-5}}$	$\mathbf{9.98 \times 10^{-4}}$
Drip (DI)	*Salmonella* spp.	2.25×10^{-7}	1.16×10^{-8}	$\mathbf{4.00 \times 10^{-6}}$
	Adenovirus A–F	3.66×10^{-11}	7.25×10^{-12}	1.28×10^{-10}
	Cryptosporidium spp.	$\mathbf{2.76 \times 10^{-6}}$	$\mathbf{2.61 \times 10^{-7}}$	$\mathbf{2.54 \times 10^{-5}}$
	Giardia spp.	$\mathbf{1.26 \times 10^{-5}}$	$\mathbf{1.14 \times 10^{-6}}$	$\mathbf{1.16 \times 10^{-4}}$
Subsurface drip (SDI)	*Salmonella* spp.	2.28×10^{-8}	1.12×10^{-9}	4.05×10^{-7}
	Adenovirus A–F	3.63×10^{-12}	6.92×10^{-13}	1.28×10^{-11}
	Cryptosporidium spp.	2.73×10^{-7}	2.57×10^{-8}	$\mathbf{2.66 \times 10^{-6}}$
	Giardia spp.	$\mathbf{1.27 \times 10^{-6}}$	1.10×10^{-7}	$\mathbf{1.25 \times 10^{-5}}$

* Time between irrigation and consumption is assumed to be 15 days. Bolded values are greater than the acceptable limit.

In DI, water is delivered directly to the root zone of a plant, where it seeps into the soil. It is expected that less direct contact with plant surfaces will result in less plant contamination and a lower annual risk of infection from microbial contaminants. Based on these results, the annual microbial risks of infection are lower than those estimated for SI. For this type of irrigation, the DALYs for adenovirus (3.66×10^{-11}, 95% CI 7.25×10^{-12} to 1.28×10^{-10}), and *Salmonella* spp. (DALY of 2.25×10^{-7}) were less than the acceptable level, while the DALYs for *Cryptosporidium* and *Giardia* were greater than the acceptable DALY risk levels of 1×10^{-6}, at 2.76×10^{-6} and 1.26×10^{-5}, respectively.

SDI involves the use of embedded pipes or tubing to irrigate crops, typically in rows or fields. As this method involves even less contact with the surface of the plant, Oron et al. (1991) proposed that there may be even less contamination risk associated with this type of irrigation than with DI or SI [22]. In this study and for all pathogens examined, with the exception of *Giardia*, the DALYs were less than the acceptable level. The DALY for adenovirus was 3.63×10^{-12} (95% CI 6.92×10^{-13} to 1.28×10^{-11}), the DALY for Salmonella spp. was 2.28×10^{-8} (95% CI 1.12×10^{-9} to 4.05×10^{-7}), and the DALY for *Cryptosporidium* was 2.73×10^{-7} (95% CI 2.57×10^{-8} to 2.66×10^{-6}). However, for *Giardia* spp., the mean annual risk was 1.27×10^{-6} (the 95% CI 1.10×10^{-7} to 1.25×10^{-5}), and therefore just above the WHO acceptable risk level.

3.3. Uncertainty Analysis

It is important to note that the risk assessments performed here only consider fruits and vegetables consumed in the raw state. Additionally, these results indicate that the irrigation type and exposure of the various crops to reclaimed water (and the resulting microorganisms in reclaimed water) have an impact on the annual risks of infection. Sensitivity analyses were performed for all irrigation scenarios by assessing the rank order correlation of uncertainty for the variables considered in this model, specifically the pathogen concentration, time between irrigation and harvest, human body weight, the equivalent volume of water irrigated onto crops, fraction of fruits and vegetables consumed raw, as well as the time of year that harvesting is conducted. Pathogen concentration contributed the most to uncertainty for all irrigation types; however, the equivalent volume

of exposure also had an important impact on the magnitude of the health outcome for the DI and SDI models.

4. Discussion

Based on the types of produce crop irrigation examined in this study using NC Type 2 reclaimed water, it was found that the annual risk of infection is not always reduced below the acceptable DALY risk level of $<1 \times 10^{-6}$. Based on this analysis of North Carolina Type 2 reclaimed water, for all irrigation types, the risks of viral infection by adenoviruses groups A–F were lower than the acceptable risk level; however, for *Salmonella* spp., *Cryptosporidium*, and *Giardia*, the annual risk of infection was sometimes higher than is considered acceptable. Potential reasons for the higher level of risks for bacteria and protozoan parasites include increased survival on plant surfaces after irrigation, as well as a difference in the volume of water retained on the produce after irrigation. Sensitivity analyses indicated that the pathogen concentration in reclaimed water played the largest role in the pathogen risk differences.

Previous researchers [7,18,29] have evaluated reclaimed water for agricultural purposes and have compared the risks from these exposures to benchmarks set for drinking water risks (10^{-4} infections per year); in this study, the reclaimed water proposed for agricultural reuse in NC was compared to the globally used WHO targets for reuse applications. Van Ginneken and Oron, 2010, found that the estimated average annual risk of infection from SI was 10^{-6}, and the infection risks from DI and SDI were 10^{-8} and 10^{-9}, respectively [18]. Hamilton et al. (2005), using secondary effluent water, also found a similar risk of virus infections modeled using coliphage virus (fecal indicator viruses infecting *E. coli* bacteria) data on different types of vegetables when a 14-day period was considered between irrigation and consumption, with average annual risks ranging from 10^{-5} for lettuce, to 10^{-7} for cucumber and broccoli [7]. Amha et al. (2015) evaluated the average annual risks of infection for *Salmonella* and found that these risks (on average between 10^{-2} to 10^{-3} per year) were higher than those of viral infection [29]. These values for *Salmonella* spp. are within the estimated ranges of average annual risks obtained in this present study.

The risk of *Cryptosporidium* in irrigation waters was evaluated by [30,31]. Mota et al. (2009) found that, assuming 120 days of exposure per year, the annual risks of *Cryptosporidium* infection for tomatoes, bell peppers and cucumbers were all approximately 10^{-5} [30]. The discrepancies between this value and the risks reported by our study could be a result of the shorter period of exposure of the reclaimed to fresh produce. Agulló-Barceló et al. (2012) found average annual risk levels of 4.37×10^{-2} in tertiary treated effluent for total *Cryptosporidium* (not infectious oocysts) [31]. From our research, the DALYs for illness from exposure to agricultural reuse waters were reduced below the WHO proposed DALY level for all irrigation methods, with the exception of SI. As it is not clear if the *Cryptosporidium* oocysts evaluated in this study were infectious, further study is needed to evaluate the infectivity of human infectious *Cryptosporidium* parasites after tertiary wastewater and then UV treatment. If UV treatment reduces the infectivity of the *Cryptosporidium* oocysts, the health risks would be lower than those calculated here. Although infectivity data are possible to obtain for *Cryptosporidium* using a cell culture infectivity assay system, little information is available on *Giardia intestinalis* infectivity due to the lack of a cell culture infectivity system. Therefore, the resulting annual risks of giardiasis are uncertain due to the lack of cyst infectivity data.

In a recent examination of the survival of adenoviruses and norovirus GII, Liu et al. (2021) found that the number of viruses were not significantly reduced by UV and chlorine dual disinfection treatment, which is consistent with our study for adenoviruses [32]. The authors did not specifically evaluate the type of irrigation method but focused on the purpose of irrigation and found that overall, viruses were greater than the WHO acceptable limit in most cases. This has also been reported in analyses of reclaimed water that evaluated antibiotic resistance genes [33], indicating that there are other microbial

health-risk-related questions that require further examination and analyses to determine if reuse water is safe to use for produce irrigation.

The results reported here indicate that the irrigation method plays an important role in the estimation of annual risk from pathogens in reclaimed water used for agricultural purposes. Specifically, the results indicate that SDI reduces the risks of infection to the lowest level. However, in the evaluation of the North Carolina potable reuse for NC Type 2 reclaimed and alternative food crop irrigation schemes for agricultural use, it appears that for some pathogens, there is a significant risk of infection from the application of such reclaimed water to fruits and vegetables. For viruses, however, the annual risk of infection is below the acceptable risk level, despite the high levels of infectious viruses in reclaimed water. Because all of the pathogens studied are usually present in reclaimed water, it is recommended this type of reclaimed water be further compared to other irrigation water sources, such as surface water or ground water, to assess their level of risk and suitability for direct application in produce agriculture.

Limitations of this work include the inherent limitations of microbial detection limits. Such limitations include the use of culture methods that do not address viable but non-culturable bacteria, or the use of analytical methods for microbes that do not distinguish between infectious and non-infectious pathogens, especially for protozoan parasites. Other limitations are our inability to consider and account for phenomena in the plant rhizosphere and general ecological environment that influence the interactions of pathogens with food plants and their soil environments, as well as the influences of other environmental microbes, especially bacteria, that may not be recognized as human pathogens when applied to and present on food crops.

5. Conclusions

In order to identify effective strategies for water conservation in agriculture that address the microbial quality of reclaimed and reused water for food crop irrigation, we investigated alternative irrigation systems used for food crops that would achieve tolerable health risks for bacteria, virus and protozoan parasite pathogens when using NC Type 2 reclaimed water. In this study, we evaluated a variety of irrigation types and key bacterial, viral, and protozoan pathogens using quantitative microbial risk assessment methods to estimate human health risks from them. Our analysis documented that there remain varying risks of infection after irrigation with NC Type 2 reclaimed water. Some irrigation systems, notably spray irrigation, resulted in unacceptable levels of risk for certain pathogens and other irrigation systems resulted in acceptable levels of pathogen risk. As a result, more study is needed on methods to further reduce microbial pathogen concentrations and achieve acceptable risk levels for all irrigation systems, especially for protozoan parasites, when using spray irrigation systems.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/app121910159/s1, Figure S1: Analytica diagram of agricultural reuse model.

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