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Abstract: The human health risks associated with multimedia exposure pathways of *Ascaris lumbricoides (A. lumbricoides)* are poorly understood including use of surface water contaminated with untreated wastewater in agriculture, consumption of uncooked raw vegetables irrigated with polluted stream-water, and incidental ingestion of fecally contaminated water by recreators. We analyzed a total of 65 water samples for the presence of helminthes collected from 5 monitoring locations in Salta City over a 13-month period on the Arias-Arenales river in the northwestern region of Salta province in Argentina. The average density of *A. lumbricoides* in the surface water was 5 eggs/liter. *A. lumbricoides* was detected in 35 of the 65 samples with a maximum concentration of 28 eggs/L. The risk of infection from accidental recreational ingestion of water was highest in children (1.31×10^{-4}) compared to the adults (6.47×10^{-5}) and secondary recreators (6.50×10^{-6}) whereas the annual probability of mean infection risk from incidental consumption of surface water in children was 2.62% followed by 1.33% in adults, and least in secondary recreators i.e. 0.13%. Consumers of wild water-cress (assumed mean single exposure raw consumption of 100 grams/person) collected from Arenales river had the highest single exposure mean infection risk compared to consumers of other raw vegetables in the community due to the higher grams of consumption and higher contamination per gram. We observed that the annual mean *Ascaris* infection risk (4×10^{-4} in β-Poisson and 2×10^{-2} in β-binomial D-R) from accidental ingestion of irrigation water by growers, assuming 75 days of active irrigation exposure, was higher using β-binomial D-R than the recommended annual tolerable risk level of 1.2×10^{-3} per person per year for wastewater use in agriculture. This research also showed the impact of using different D-R models in human health risk assessment. Application of conditional D-R models led to higher risk estimates resulting in higher estimates of extreme risks. The concentration of the infectious nematode in the surface water and the rates of accidental ingestion of contaminated water were the key drivers in the risk estimates and had the greatest effect on the total uncertainty of the estimated risks in all of the scenarios..

***Keywords**:* intestinal parasitic nematodes; microbial risk assessment; dose-response (D-R) models; intestinal helminth infections; censored data

1. INTRODUCTION

The World Health Organization (WHO)/United Nations Children's Fund (UNICEF) Joint Monitoring Program (JMP) estimated that 1.2 billion people lacked improved sanitation facilities and 783 million (11%) used unimproved water sources (WHO, 2010), respectively. WHO recommend a criterion of ≤ 1 viable intestinal nematode egg/L for wastewater used for irrigation based on epidemiological studies (World Health Organization, 2006). Ascariasis is a common geohelminthic infection of the small intestine by the human roundworm, *Ascaris lumbricoides*. The geohelminth or soil-transmitted nematode parasite is so called because it has a direct life cycle that involves no intermediate hosts or vectors and is transmitted by faecal contamination of soil, food and water supplies (Holland and Kennedy, 2002). Ascarisis eggs are passed in the feces of infected persons and if the person openly defecates outside the house then eggs can be deposited into the soils. Ascariasis can either come from direct contact with soils via hands or indirectly through a) consumption of raw foods that are contaminated with either feces , b) irrigated with contaminated water, or c) exposed to use of biosolids in agriculture that are polluted with helminth eggs. They are normally not considered waterborne and eggs in wastewater are not usually infective (Jimenez, 2007). *Ascaris* was chosen as the specific hazard in the risk assessment model for the following reasons: (1) *Ascaris* is very prevalent in most parts of the world, especially in low and middle income countries, (2) *Ascaris* eggs are the most hardy and resistant of all excreted pathogens, (3) the female worms produces vast quantities of eggs (~200,000 eggs per day), and (4) they can survive in the environment for periods of months or even years before becoming infective (Feachem et al., 1983). Quantitative microbial risk assessment (QMRA) is a modeling approach to predict the human health risks from exposure to pathogens. It estimates the risk of infection or illness based on the concentration of infectious pathogens in surface or drinking water or another ingested substance (e.g., food, aerosols, air), the estimated ingestion rates and established dose-response models for a given population. QMRA has been applied to assess risks through exposure to helminths in fecally polluted irrigation water, contaminated soils and contaminated carrots (Cutolo et al., 2012; Mara and Sleigh, 2010, b). *Ascaris* ova were selected in the present study because they are considered an indicator of the presence of other helminth ova due to their high environmental resistance especially in untreated and treated wastewater and sludge as previously described by Jimenez (2007). This is the first study to assess helminth risks through exposure to fecally contaminated water, different vegetables consumption, and use of water for irrigation purposes and to develop a risk-based management plan in a site-specific context. The main objectives were (i) to assess risk related to primary or secondary incidental ingestion of water in the Arenales river in three scenarios: primary contact by adults, primary contact by children and secondary contact recreation; (ii) to assess risk related to consumption of several raw vegetables (lettuce, two serovars of cabbage (Savoy King/Grand Slam and Winter Head), broccoli, cucumber and water-cress) irrigated with/grown in contaminated water; and (iii) to assess risk associated with accidental ingestion of irrigated water by individuals working in fields.

2. METHODS AND MATERIALS

2.1 Area description and laboratory methodology

The Arias-Arenales river is in the north-western region of Salta province in Argentina (Figure 1). The river experiences seasonal fluctuations in flow; it has very high flows during the summer (November to April) and very low flows during the winter season (May to October). The eleven points were identified as critical as the river crosses the city (7.8 miles). The monthly monitoring was done for 13 months (from Feb 2009 to Feb 2010). There were a total of 11 monitoring points – five points were directly located on the river and six points were selected on the channels that discharge directly into the river. Five points on the river were chosen for use in three risk assessment scenarios to estimate the human health risk from incidental ingestion of *A. lumbricoides* in Arenales River water. These points were P1, P2, P6, P10 and P11, where P1 and P2 were selected as low pollution controls on the Arias and Arenales river, P6 was a recreational area called Parque los Sauces with picnic tables, grills and a place for children play area, and P10 and P11 were located upstream and downstream of the wastewater treatment plant (WWTP) and municipal landfill (Figure 1).

The hollow fiber ultrafiltration (HFF) methodology was used as previously described by Rajal et al. (2007) and Poma et al. (2012). Briefly, 20 L of water was collected and filtered through two sieves (74 and 37 μm) to remove solids and concentrated to 30 mL using a ultrafiltration unit with a 50,000 MW cutoff membrane (Microza AHP 1010, Pall Life Sciences, East Hills, NY). The samples were eluted with 0.5 M glycine/NaOH (pH 7.0) and 0.1% Tween 80 solutions to recover materials sorbed to the tubing. The final concentrated sample of 50~70 mL consisted of a mixture of the eluate from HFF unit and the final retentate. 20 mL of the concentrated sample was again filtered through three layers of gauze to eliminate microscopic particles according Poma et al. (2012). The identification of *Ascaris* eggs was performed by direct microscopic examination (1000X) of the preserved samples. Further, wet-mount preparations with Lugol's solution were used to identify the eggs. Intestinal nematode egg count was performed by the Stoll quantification method (Stoll and Hausheer, 1926). The overall concentration factor is composed of the factors from the concentration by UF: 400 X and sedimentation stage (or flocculation) estimated at 10 X. Sample limit of detection for the intestinal parasite is 2.5 eggs / L, this arises from the following relationship Equation (1):

$$SLOD_{parasites} = \frac{Detection - \lim it - of - the - microscope}{Concentration - factor} = \frac{10000\,p/mL}{4000} \quad (1)$$

where detection limit of the microscope is 10X (particles/mL).The method used here did not allow the distinction between human versus animal *A.eggs*, fertile versus infertile eggs, viable versus non-viable eggs and infective (embryonated) versus non-infective eggs (unembroynated).

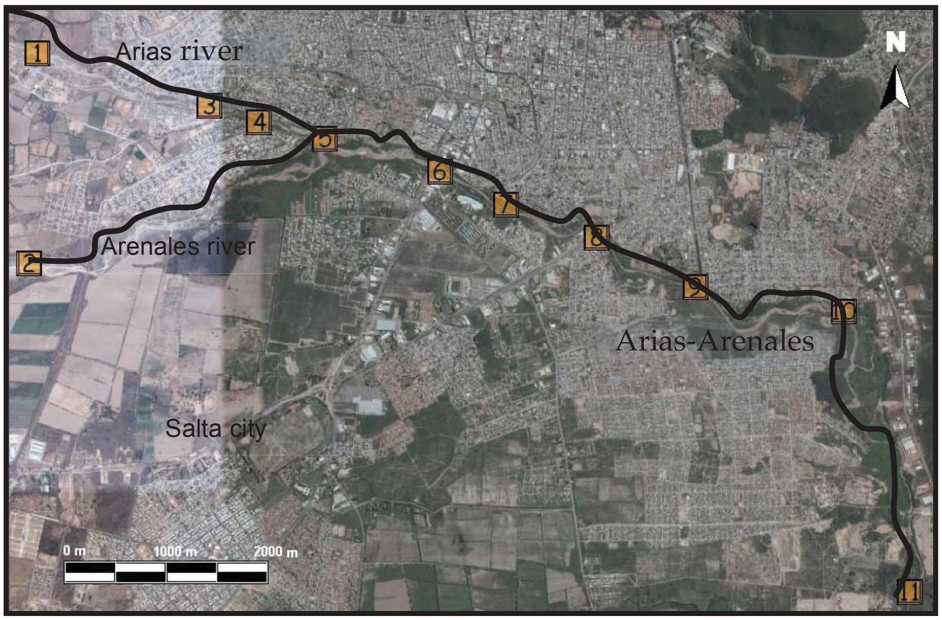

Figure 1. Geographical representation of the monitoring stations (P1-P11) along Aria-Arenales river

2.2 QMRA methodology

The probabilistic model was based on the concentration of nematode eggs in the surface water, exposure duration, ingestion rates (children, adults & secondary recreators, produce growers, consumers), and dose-response models for exposure risks, at Arenales River in Salta. The assumptions and parameters used in the research study were described in Table 1.

Table 1. Distributions and fit parameters used in the Monte Carlo Simulations Model (MCSM)

Model parameter	Unit	Distributions and fit parameters	References
Volume of water caught by produce	mL/g	Lettuce: Normal, μ = 0.108, σ = 0.019 (truncated at 0) Broccoli: Log logistic, α = 4.246, β = 0.01583, λ = 0.001085 (truncated at 0) Savoy King/Grand Slam cabbage: Empirical CDF (μ = 0.0352) (truncated at 0) Winter Head cabbage: Empirical CDF (μ= 0.0889) (truncated at 0)	Hamilton et al., (2006)
		Cucumber: Normal, μ = 0.0036, σ = 0.0012 (truncated at 0) Water-cress: 10 – 15 mL/100 g (uniform	Assumed in this study

		distribution)	
Consumption rate	g/kg day	Empirical CDFs for total populations as well as consumers only Consumers only: Mean consumption rate of lettuce (g/day) = 33.11* Mean consumption rate of broccoli (g/day) =65.79 Mean consumption rate of cabbage (g/day) = 49.70 Mean consumption rate of cucumber (g/day) = 28.07 Mean consumption rate of water-cress (g/day) = 100	U.S Department of Agriculture Continuing Survey of Food Intakes by Individuals (CFSII).
Body mass	kg	Log normal, μ = 61.429, σ = 13.362	Hamilton et al., (2006)
Recreational exposure duration	h/day	PERT distribution (0.25, 0.50, 2)	McBride et al., (2013)
Recreational ingestion rate	mL/h	PERT distribution – Primary contact, children and secondary contact recreators Primary contact - (10,50,100), Children - (20,100,200), Secondary contact -(1,5,10)	McBride et al., (2013)
Irrigation water ingestion rate	mL/event	Uniform distribution (1, 5)	Seidu et al., (2008)
Dose-response model	P_{infday}	β-Poisson, β = PERT (0.822,1.096,1.37), α = PERT (0.078,0.104,0.13) and, N_{50} = PERT (644.25,859,1073.73)	Navarro et al., (2009)

Three scenarios were considered in the research study as described below:

Scenario 1
The risks of *Ascaris* infection from incidental ingestion of contaminated surface water by three groups of recreators were estimated (primary contact by adults, secondary contact regardless of age and primary contact by children) in Arias-Arenales river in Salta, Argentina.

Scenario 2
The hypothetical risk of *Ascaris* infection from ingesting raw vegetables grown in fields irrigated with polluted Arenales river surface water was estimated. In addition, we also estimated the risks associated with the consumption of raw aquatic plant, water-cress in Arenales river.

Scenario 3
A hypothetical scenario where we estimated the risk of *Ascaris* infection in vegetable growers using contaminated Arenales surface water for spray irrigation of fields. We did not monitor Ascaris eggs in the soil and even though it is one of the primary routes of transmission of Ascariasis, it was not considered as one of the transmission pathways in this research study. Three dose-response relations were compared and evaluated for risk assessment in different scenarios as described in equations (2), (3) and (4):

(a) β-Poisson D-R model

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

(b) β-Binomial D-R model

$$P_I^o = 1 - \frac{\binom{\alpha+\beta-1}{\alpha}}{\binom{\alpha+\beta+I-1}{\alpha}} \quad (3)$$

(c) Exponential D-R model

$$P_{infday} = 1 - \exp(-r.d) \quad (4)$$

Previously, various researchers had used a value of *r* of one as a worst case scenario (Cutolo et al., 2012; Seidu et al., 2008; Westrell et al., 2004). We wanted to compare our risk estimates with the previous work done by various researchers using different D-R models. Therefore, we assumed two different values of "*r*" from data i.e. *r* = 1 as exact single hit model and *r* = 0.039 (Navarro et al., 2009a) to indicate how the D-R model changes with different parameter values.

Monte Carlo (MC) simulations for 10,000 trials using Latin hypercube sampling (LHS) were used, to estimate the human health risk for each exposure scenario associated with *A. lumbricoides* in the surface water using @RISK version 6.0.0, professional edition (Palisade Corporation, Newfield, New York).

3. RESULTS

A total of 65 samples were collected during a 13-month study from 5 monitoring stations on the Arias-Arenales river and tested for Ascaris lumbricoides (Poma et al., 2012). Ascaris was detected in 54% of the samples and the concentrations ranged from 0 to 27.5 eggs/L (Table 2). Most of the positives samples were in the range of 2.5-5 eggs/L, except for the P11 monitoring station. The positive samples were detected throughout the year (77%) and the maximum concentration of 27.5 eggs/L was found only once at the P11 station. Also, the density of 23 eggs/L was found twice during the months of August and October 2009 at the same monitoring station.

Table 2. Intestinal parasitic nematode monitoring results in the surface waters

Monitoring stations	No. of samples tested	Number and percentage of samples by nematode concentration range				Maximum concentration
		<2.5*	2.5-5.0	5.1-10.0	>10.0	
Ascaris lumbricoides	eggs/L					
P1	13	6 (46.2)	3 (23.1)	4 (30.8)	0	10
P2	13	7 (53.8)	4 (30.8)	1 (7.7)	1 (7.7)	17.5
P6	13	6 (46.2)	3 (23.1)	2 (15.4)	2 (15.4)	20
P10	13	8 (61.5)	4 (30.8)	0	1 (7.7)	15
P11	13	3 (23.1)	2 (15.4)	4 (30.8)	4 (30.8)	27.5
All stations	65	30 (46.2)	16 (24.6)	11 (16.9)	8 (12.3)	-

*<2.5 represents sample limit of detection (SLOD)

Ascaris infection risk from accidental ingestion of contaminated water was estimated highest for children (0.013%), followed by adults (0.006%) and least in secondary recreators (0.001%) and followed the same trend in all five different statistical methods used for censored data. When looking at the comparative infection risk, based on the D-R models (β-Poisson and β-binomial model) for the mean single exposure risk in population exposed to contaminated surface water, the β-binomial had the highest infection risk caused by recreational exposure in the river for children (0.37%), followed by adults (0.14%) and secondary recreators (0.06%). The annual infection risk (n=209 days for recreational use) for β-binomial showed highest risk in children and ranged from 53% estimated annual risk in children to 11% probability of infection in 2^0 recreators from incidental ingestion of contaminated waters. The β-binomial D-R model showed high infection risks varying from 0.01% to 0.08% in incidental ingestion of contaminated irrigated water by vegetable growers. The estimated annual mean risk of infection in vegetable growers due to accidental ingestion of irrigated water was 0.01% from the β-Poisson D-R model compared to 0.62% from the β-binomial D-R model. The estimated mean single exposure risk from ingestion of vegetables spray-irrigated with surface water using different D-R models was determined. The mean single exposure infection risk was less for cucumber than for the other vegetables for all scenarios (~1 order of magnitude lower). The mean single exposure risks were quite similar in all different scenarios and did not vary much. The levels of risks posed by broccoli, lettuce and different cultivars of cabbage were similar, although they tended to be a little bit higher for winter head cultivar (cabbage). Water-cress had the highest risk of infection in all scenarios for a single exposure event as well as annual risk. The risk levels were higher in the β-binomial D-R model as compared to the β-Poisson D-R model and differences were typically in the range of 1 to 2 orders of magnitude. Water -cress showed a highest mean risk in the β-binomial D-R model as (0.908%) whereas the estimated mean for β-Poisson D-R model was 0.0155%.

It can be seen that changing the "*r*" value in the exponential D-R model created a shift in the graph on a log dose versus response plot (Figure 2). As the "*r*" value decreases, the graph shifts to the right of the log dose scale, which showed the lower probability of infection at a given dose.

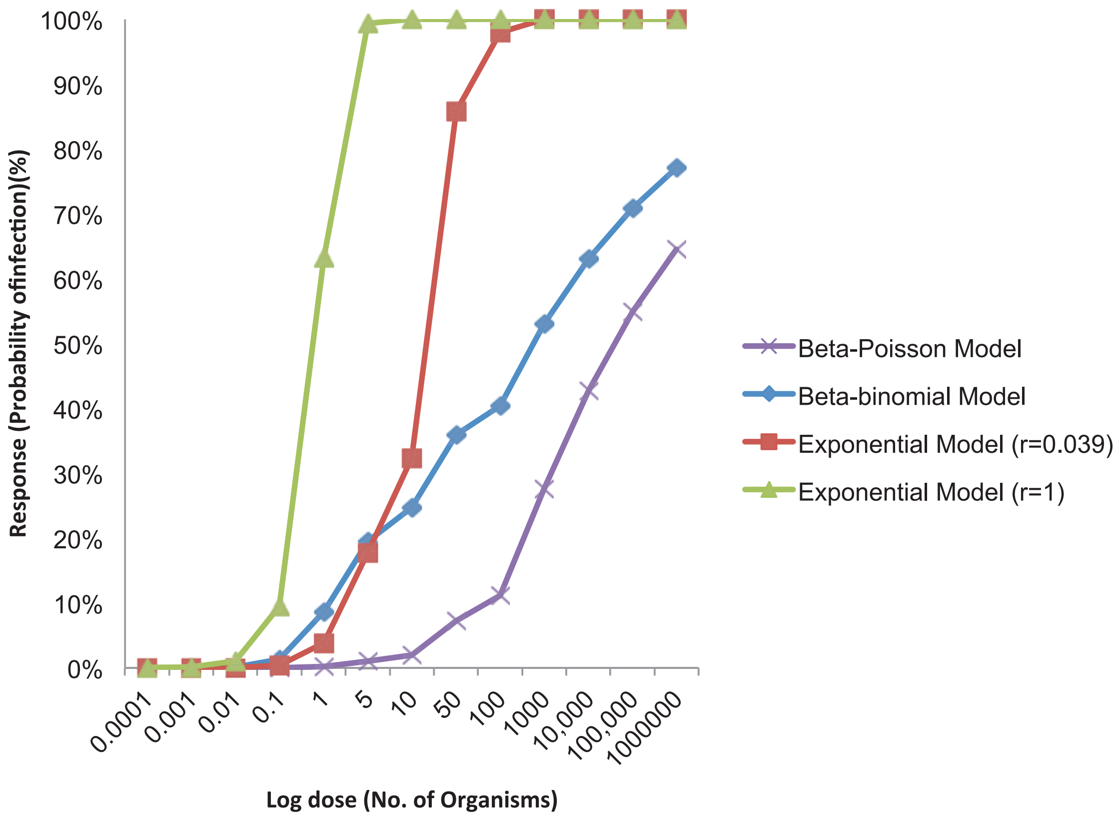

Figure 2. Comparison of different dose-response (D-R) models (beta-Poisson, beta-binomial, exponential model at r =1 and r = 0.039).

Dose-response parameters did not have a profound impact on the estimated risks from intestinal nematode and had least correlation coefficient (Figure 3).

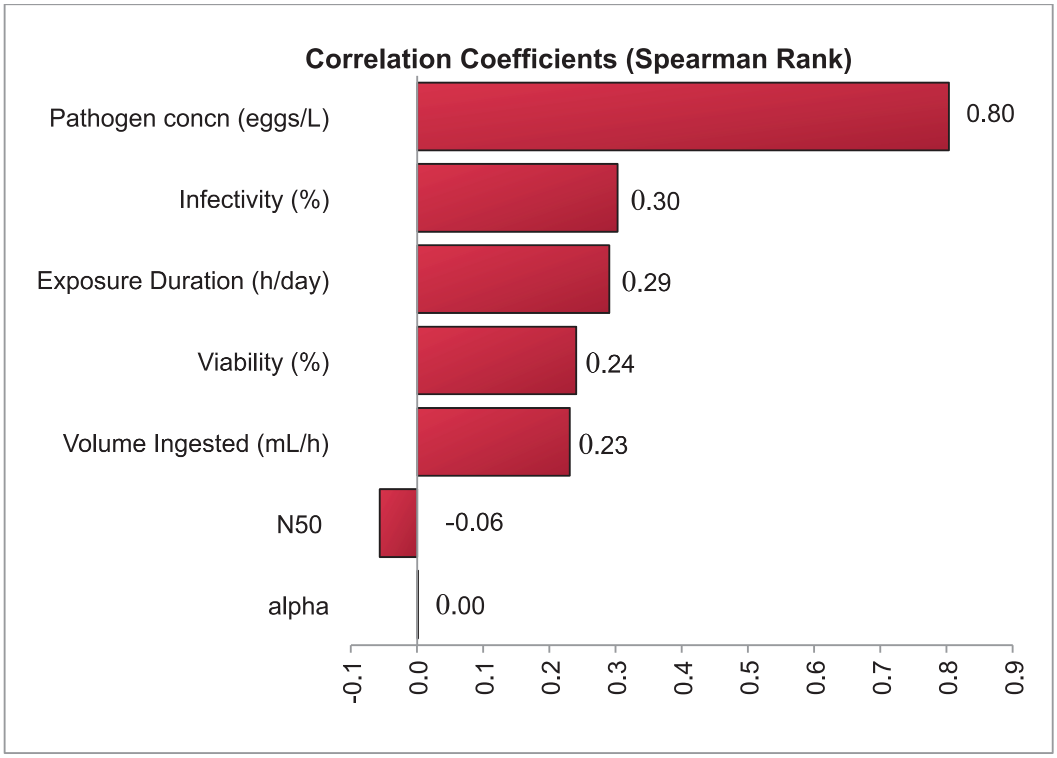

Figure 3. Sensitivity analysis of the variables in the children risk model, resulted from the Monte Carlo simulation

4. DISCUSSION

Haas et al. (1999) developed D-R models for micro parasites (viruses, protozoa and bacteria) to quantify the infection risks. There is some dissimilarity in infection mechanism between microparasites and macroparasites (helminths). There is no multiplication of helminths inside the host and each worm is the result of a separate infected egg. Thus, individual eggs are the unit of transmission and transmission rate is a function of the intensity of infected eggs ingested, whereas in microparasites, a single successful infection leads to colonization of the host, which is a therefore the principal unit of transmission (Bundy, 1988). In addition, microparasite-induced infections constitute an all-or-nothing event and there is more direct correspondence between infection and disease, whereas it is not a direct relationship in helminthiases. We compared different D-R models as well as two different r values for the exponential D-R model. The exponential model was the most conservative model among all the different D-R models. The probability of infection was significantly reduced as expected when we set the r-value at 0.039 at a given dose. In addition, the β-Poisson model tended to be more flexible in its ability to describe data, primarily as a result of the additional parameters. However, the β-Poisson model gave the lowest risk estimates as compared to other D-R models. McBride et al. (2005) showed the mathematical derivation and proved high-risk estimates using the β-binomial D-R model in comparison with the β-Poisson D-R model.

The total uncertainty is comprised of two components, variability and uncertainty, where variability is defined as the inherent characteristics of the phenomenon and uncertainty is defined as the lack of knowledge about the parameters (Hamilton et al., 2006). Improved sampling can reduce the uncertainty, but not the variability. We captured the overall uncertainty in different scenarios. It was observed that the concentration of pathogens played a major role in the risk estimates for incidental ingestion of water from recreational activities in different groups for surface waters of the Arias-Arenales river: primary contact by adults, primary contact by children and secondary recreators regardless of age. The consumption rate of different vegetables contributed the highest uncertainty in the final risk estimates followed by concentration of nematodes in the irrigated water. These results are very similar to those obtained by Hamilton et al. (2006) and showed that, in fact, the uncertainty associated with consumption data accounted for much of the overall uncertainty. The D-R parameters and body mass had only a small effect on the overall uncertainty of the final risk estimates.

5. CONCLUSION

Even though helminth ova are a major health concern in developing nations, there is a dearth of information related to the viability and infectivity of the helminth eggs in the surface water. Therefore, there is an urgent need for more research in this field. Rapid monitoring techniques like molecular methods should be utilized to enumerate viable and infectious *A. lumbricoides* helminth eggs as compared to current techniques of enumeration that may take more than one month. Health risks depend on the type of ingested vegetable. The results indicated that the annual risk was highest from the consumption of watercress and lowest from the consumption of cucumber. However, the consumption rate of watercress was highest among all considered vegetables (100g/day). Risks of ascariasis decreased drastically with using only a fraction of viable (mean 50%) and infectious (mean 30%) helminth eggs as compared to 100% viable and infectious eggs, the annual mean estimated infection was one order of magnitude lower in children when these fractions were considered viable and infectious. This research showed the impact of changing traditional D-R models often applied in microbial risk assessment. Consideration of conditional D-R models increased in higher risk estimates resulting in more conservative estimates of extreme risks as compared to β-Poisson D-R model. Annual intestinal parasitic infection risks for 75 active days of daily exposure to accidental ingestion of irrigated surface water were higher than tolerable level of risks for vegetable growers (1.2×10^{-2}). Estimated single exposure and annual infection risks were also a function of the level of intestinal nematode in the surface water as well as the ingestion rate/consumption rate of the vegetables. The consumption rate and concentration of the intestinal nematode had a significant effect on the final estimated risks and introduced major uncertainty in the final risk estimates. More research is needed regarding occurrence and prevalence of helminth in the irrigated water as well as on crops.

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