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Application of Helminth ova infection dose curve to estimate the risks associated with biosolid application on soil

I. Navarro, B. Jiménez, E. Cifuentes and S. Lucario

ABSTRACT

Helminth ova (HO) are the main biological concern when reusing sludge for agricultural production. Worldwide sludge regulations consider a permissible range of 0.25–1 HO/gTS. Such limits are unaffordable to most developing countries, due to high helminth ova content in sludge, and the lack of viable technology to inactivate them as needed. The quantitative microbial risk assessment (QMRA) is a useful tool to estimate the risk of treated sludge, considering feasible and viable limits. QMRA, however, has not been applied before for HO because no dose-infection curve was available. Therefore, the objectives of this paper are: to build up a risk-based model designed for untreated wastewater exposure (i.e., land irrigation) using *Ascaris lumbricoides* eggs as indicators for HO, and apply the results to assess health risk (i.e., *Ascaris lumbricoides* infection) associated with consumption of crops grown on biosolid-enriched soil. Data showed that it may be feasible to update HO threshold in biosolids from developing countries without significantly increasing risks. To reduce health risk from HO, it may be wiser to achieve feasible and evidence-based standards, than to set unaffordable limits in these countries. QMRA data suggested additional protection measures, such as biosolid application rates, crop restriction, and produce better washing practices.

Key words | agriculture, biosolids, dose response curve, helminth ova, quantitative microbial risk assessment, sludge

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INTRODUCTION

Helminth infection is a major public health concern in developing countries, especially in tropical and subtropical regions of Africa, Asia, Central and South America. The combination of poverty, malnutrition, socio-cultural habits and climate, results in increased transmission. The most important soil-transmitted helminth infections are Ascariasis and Trichuriasis, while the most important water-based helminth infection is Schistosomiasis. These diseases together infect over 2.6 billion people around the world, inflicting health injuries ranging from simple allergic reactions to severe life-threatening health conditions (WHO 1990).

Helminth ova (HO) are considered the main biological health risk when applying biosolids on agricultural soils (WHO 2006). Due to poor sanitation standards, helminth ova are commonly detected in sludge in developing countries in greater concentrations (70–735 HO/gTS) than in developed ones (<1–13 HO/gTS, Jimenez & Wang 2006). Furthermore, in many developing countries sludge disposal practices consist of direct sludge discharge into sewers and rivers or simply land disposal in uncontrolled sites (Jimenez *et al.* 2004). Wastewater treatment interventions, on the other hand, pose a series of concerns regarding sludge produced, as well as the necessary conditions to stabilize this sludge, prior to disposal and safe reuse. Additionally, an increasing number of developing countries are setting up regulations to treat and reuse biosolids based on the limits established for

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helminth ova by the U.S. EPA Part 503 rule (1993) or WHO (2006), namely 0.25 and 1 HO/gTS, respectively. Even in those places where the sludge recommended treatment methods are affordable (i.e., excluding pasteurization and gamma radiation due to their cost), results show HO inactivation efficiencies of 70–95% (Jimenez & Wang 2006) involving final contents of 4–37 HO/gTS in treated sludge from developing countries. In other words, the above-mentioned limits are not fulfilled. Therefore, it is important to evaluate the risks resulting from using sludge of such quality.

U.S. EPA limits and WHO guidelines for helminth ova were established based on limited epidemiological evidence and by the performance of different sludge treatment methods, and not by estimating the actual health risk. In addition, neither of them based their considerations on results of a dose response-curve "because methodologies had not been developed sufficiently enough to make such calculations" (U.S. EPA 1995; WHO 2006). In fact, of the three different methods used to evaluate microbial risks, microbiology laboratory analysis, epidemiological studies and QMRA, only the first two have been applied to helminth data (Tellez *et al.* 1997; Howard *et al.* 2002; Berhe *et al.* 2004). In contrast, a number of human dose–response relationships have been developed for bacteria, viruses, and protozoa (Regli *et al.* 1991; Rose *et al.* 1991; Haas *et al.* 1993, 1996, 1999*b*; Crockett *et al.* 1996; Fazil 1996; Medema *et al.* 1996; Teunis *et al.* 1999). From a literature survey, it seems that human dose–response relationships for *Ascaris* or *Taenia* (types of helminth) have not been developed yet (NRC 2002). Therefore, a first step in addressing this gap is to provide a risk-based analysis for helminth ova, as has been done for standards and guidelines for other human pathogens.

Quantitative microbial risk assessment (QMRA) is emerging as a useful tool for developing standards for human exposure to pathogens (Haas 2002). QMRA has shown its effectiveness in assessing the transmission of water and food-borne infections. The best probabilistic estimates have been obtained using epidemiologic data from actual outbreak studies conducted mostly in developed countries. As an example, Rose *et al.* (1991), Teunis *et al.* (1999) have developed infection models for *Salmonella*, *Giardia* and *Cryptosporidium*. When applying QMRA, the first step is to define the best distribution model fitting observed infection rates as a function of pathogen exposure doses. The most commonly used models are the exponential and the Beta-Poisson, although others can also be used. QMRA, like any risk assessment, has uncertainties that need to be considered, and when possible, quantified. Major sources of uncertainty involve hygiene-related behaviour and consumption patterns (i.e., water or food) for a targeted population and the actual pathogen doses under consideration.

Considering QMRA has not been applied to assess helminth risks through exposure to fecally polluted water, sludge or soil, and the lack of dose-infection curve, the aim of this research is to make a contribution to fill this gap. Thus, the objective of this research was to build up a risk-based model for *Ascaris lumbricoides* (*A. lumbricoides*) infection designed for untreated wastewater exposure (i.e., land crop irrigation), and apply this model to assess human risks associated with helminth (*A. lumbricoides*) exposure from crops grown in biosolid-enriched soil.

For the development of the *A. lumbricoides* infection dose–response relationship, exposure scenarios were selected based on actual exposure conditions observed in the Mezquital Valley, Mexico, a site where for 100 years flooding using untreated wastewater has been standard irrigation practice for approximately 90,000 ha. *Ascaris* ova were selected because they are considered an indicator of the presence of other helminth ova due to their high environmental resistance (Feachem *et al.* 1983). Concerning the exposure pathway in assessment of human risk from crops grown in biosolid-enriched soil, the consumption of two types of crops, raw spinach and carrots, was considered based on the available data.

METHODOLOGY

This research used results obtained from three previous studies. The first was an epidemiological study which establishes the prevalence of *A. lumbricoides* in the Mezquital Valley, Mexico (Cifuentes *et al.* 1991, 1993; Blumenthal *et al.* 1996). The second source of data was a wastewater quality study assessing the *A. lumbricoides* content in the wastewater used to irrigate the valley

(Jimenez *et al.* 1992). And the third study consisted of experimental research relating the *A. lumbricoides* content in crops grown in biosolid-enriched soil (Jimenez *et al.* 2006).

Dose–response assessment

The dose–response model was developed using epidemiological data on *A. lumbricoides* infection rates from the Mezquital Valley, Mexico (Cifuentes *et al.* 1991, 1993; Blumenthal *et al.* 1996) as well as the water quality data of the wastewater used for crop irrigation in the valley. Two scenarios were considered in order to obtain the best dose–response model for *A. lumbricoides*: the consumption of uncooked vegetables irrigated with untreated wastewater and the accidental ingestion of soil from land irrigated with wastewater.

For the development of the dose–response relationship, the fitness of data for the exponential and Beta-Poisson models was analyzed considering the following equations (Haas *et al.* 1999*a*):

$$\boldsymbol{P}(\boldsymbol{d}) = 1 - \exp(-\boldsymbol{rd}) \quad \text{(Exponential model)} \tag{1}$$

$$\boldsymbol{P}(\boldsymbol{d}) = 1 - \left(1 + \left(\frac{\boldsymbol{d}}{\boldsymbol{N}_{50}}\right)\left(2^{1/\alpha} - 1\right)\right)^{-\alpha} \quad \text{(Beta-Poisson model)} \tag{2}$$

$$\boldsymbol{P}_{\mathrm{I(A)}}(\boldsymbol{d}) = 1 - [1 - \boldsymbol{P}(\boldsymbol{d})]^{n} \quad \text{(Annual risk of infection)} \tag{3}$$

In these equations $\boldsymbol{P}(\boldsymbol{d})$ is the risk of infection in an individual due to the ingestion of an average number of organisms in a dose, $\boldsymbol{P}_{\mathrm{I(A)}}$ is the annual risk of infection in an individual from n exposures per year to pathogen dose; $\boldsymbol{d}$ is the total number of organisms in a known consumed amount of either vegetables or soil, based on *A. lumbricoides* concentration for each exposure scenario; $\boldsymbol{N}_{50}$ is the median infective dose, and $\boldsymbol{r}$, α and β are model parameters.

To define the best-fit dose–response relationship for *A. lumbricoides* infection, the chi-squared goodness of fit test was applied, and the following assumptions were made to estimate the exposure doses for crops and soil ingestion scenarios.

Epidemiological data and target population

The epidemiological data developed by Cifuentes *et al.* (1991) consists of rates of *A. lumbricoides* infection determined through stool parasitological tests from the following population groups: children under the age of 5, children between 5 and 15 years old, and older than 15, from different communities in the Mezquital Valley. Cifuentes *et al.* (1991) found that the groups at higher risk were children under 15 for all sites, with a high annual *A. lumbricoides* prevalence rate of 10–17% exposed to untreated wastewater used for irrigation, and derived from a large population sample. Therefore, children under 5 and between 5 and 15 years old were selected as representing the target population in developing the dose response model (Table 1). In fact, these two groups represent the most vulnerable population in a region where ascariasis is an endemic infection.

The population groups selected by Cifuentes *et al.* (1991) correspond to different exposure sites (communities) due to the change in helminth ova content in wastewater as it flows through the valley as a consequence of sedimentation in reservoirs. The sites selected in that study were identified as: (a) raw wastewater, (b) the Endho reservoir, and (c) the Rojo Gomez reservoir. Considering the objectives of this research, we selected infection rates for groups of children under 15, exposed to raw wastewater during the rainy and dry seasons as well as the rates found for Rojo Gomez reservoir where it is partially treated after its passage through two reservoirs (Table 1). As a consequence, the selected data enabled us to assess the most vulnerable population based on different infection rates associated with different exposure levels as a result of differences in the *Ascaris* content of the wastewater used to irrigate in the region.

Ascaris lumbricoides content in crops and soil

Data on the *A. lumbricoides* content and viability in wastewater developed by Jimenez *et al.* (1992) was used to estimate the content in crops and soil. The *A. lumbricoides* content and viability was measured in that study using a U.S. EPA (1992) analytical method for quantification and incubation. In total, 24 values of *Ascaris* content (k) were

Table 1 | Data*,†,‡ of prevalence of *A. lumbricoides* infection in the Mezquital Valley used in this research

Age group	Sites	Population sample size (n_i)	*Ascaris* prevalence Population infected (p_i)	%
0–4 years	Raw wastewater§	341	34	10.0
	Rojo Gomez reservoir	335	46	13.7
	Raw wastewater‖	396	59	14.9
5–14 years	Raw wastewater§	759	94	12.4
	Raw wastewater‖	817	132	16.2
	Rojo Gomez reservoir	698	115	16.5

*Cifuentes *et al.* (1991).
†Blumenthal *et al.* (1996).
‡Cifuentes *et al.* (1993).
§Dry season prevalence infection.
‖Rainy season prevalence infection.

used. The *Ascaris* content varied between 33 and 73 *A. lumbricoides*/5 L with 52–93% viability (Figure 1). Therefore, different exposure levels to wastewater of varying quality were considered instead of using only the mean *Ascaris* content, as is frequently done when estimating the dose–response curve.

To estimate the content of *A. lumbricoides* in crops, the variable pathogen content in the irrigation water (see Figure 1) was considered and it was assumed that 10 ml of wastewater remains in 100 g of produce (Shuval *et al.* 1997). This assumption implied that the *Ascaris* content in crops varied from 0.42 to 1.15 *Ascaris* per 100 ml of water in the crop (Figure 2). On the other hand, due to lack of pathogen content data in soil from the Mezquital region, the concentration of *A. lumbricoides* in soil irrigated with untreated wastewater was estimated considering the water retention index in soil and the *Ascaris* content in irrigation water. The water retention index was estimated as a function of the irrigation flow rate, the soil active layer, the number of irrigations per year and the mean soil density in the region (0.99 g/cm^3). The results for these estimates are illustrated in Figure 2. The *Ascaris* content in soil varied from 0.85 to 2.33 *Ascaris*/100 g; these results are consistent with the preliminary data from ongoing research which found 0.25–4.5 *A. lumbricoides*/100 g of soil in five different sites in the Valley.

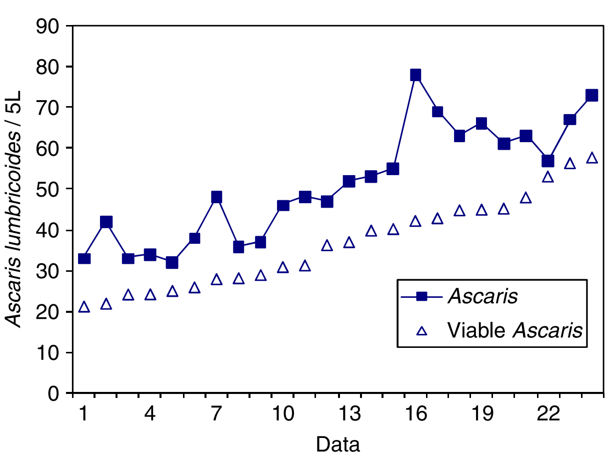

Figure 1 | *A. lumbricoides* content in wastewater used for irrigation in the Mezquital Valley.

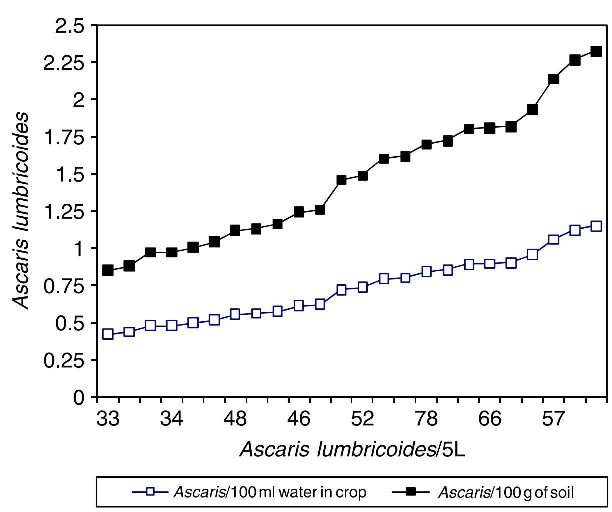

Figure 2 | Estimate of *A. lumbricoides* content in water-crops and soil.

Crops and soil ingestion rates

Population from the Mezquital Valley consumes some crops cultivated using untreated wastewater of varying degrees of quality. Because of lack of data on type of crops and the amount of produce consumed, a consumption of 100 g of raw crops per week per child during one year was assumed.

For the child accidental soil ingestion scenario, a mean ingestion rate of 100 mg/d was used following U.S. EPA recommendation (1997), and it was assumed an exposure frequency of two times per week during one year for both groups, children under 5 and children older than 5 and younger than 15 years.

Quantitative risk estimate

Results from an experimental study performed to measure the total amount of helminth ova content, including the *A. lumbricoides* percentage in carrots and spinach grown in soil fertilized with biosolids were used for the QMRA. A detailed description of the data was published elsewhere (Jimenez *et al.* 2006). In that study, the helminth ova (HO) rates applied in biosolids experiments were equivalent to 0.25 gHO/TS, 1 HO/gTS, 4 and 37 HO/gTS. Biosolids were land-applied once at different application rates in order to simulate the desired contamination rate. Crops grown under such conditions were harvested and the amount of helminth ova (and *Ascaris* ova) remaining in carrots and spinach was measured to obtain crop pollution curves as a function of biosolids applied. The analytical method used was also that of the U.S. EPA (1992). Results from that research as well as the best infection model for *A. lumbricoides* developed with the data described in the dose–response assessment were used to estimate the probability of infection from eating raw vegetables, carrots and spinach, with the following assumptions:

- Exposure group of concern: children under 15 years old.
- Use of four different initial helminth ova contents in biosolids (HO/gTS) (Tables 2 and 3): the international standards of 0.25 HO/gTS (U.S. EPA 1993) and 1 HO/gTS (WHO 2006), and the minimum and maximum concentration values expected in affordable sludge treatment in developing countries, 4 and 37 HO/gTS respectively.
- Application to agricultural land of four different helminth ova rates (HO/cm^2), corresponding to vegetable nutrient requirements in the region of study (Tables 2 and 3).
- An *A. lumbricoides* content measured as 90% of the total helminth ova (HO/g) content in carrots (C_{carrot}) (Table 2) and in spinach ($C_{spinach}$) (Table 3).
- The carrot consumption (IR_{carrot}) variability was addressed assuming a mean intake range estimate of 28–38 g per event for children under 15 years in the USA (U.S. EPA 2002), as well as the mean, 90th and 99th percentile of carrot intake distribution (U.S. EPA 1997) (Table 4).
- Due to limited data in the literature concerning spinach consumption ($IR_{spinach}$), data for lettuce was used with a mean intake range estimate of 30–54 g per event for children under 15 years in the USA (U.S. EPA 2002) as well as the mean, 90th and 99th percentile of lettuce intake distribution (U.S. EPA 1997) (Table 4).
- Child consumption of raw crops once a week.
- A 1 (WHO 2006) and a 2 log (as a best case scenario) *A. lumbricoides* reduction due to crop washing.

Table 2 | Helminth ova content in biosolids, on land-application rates and *A. lumbricoides* content in carrots, data from Jimenez *et al.* (2006)

Biosolids (HO/gTS)	Land application (HO/cm^2)	Carrots (HO/g)	(*Ascaris**/g) C_{carrot}
0.25	0.003	0.004	0.003
1	0.011	0.015	0.013
4	0.042	0.059	0.053
37	0.390	0.546	0.491

*90% content of HO/g for carrots.
C_{carrot} is the *A. lumbricoides* content in carrots after harvesting.

Table 3 | Helminth ova content in biosolids, on land-application rates and *A. lumbricoides* content in spinach, data from Jimenez *et al.* (2006)

Biosolids (HO/gTS)	Land application (HO/cm^2)	Spinach (HO/g)	(*Ascaris**/g) $C_{spinach}$
0.25	0.005	0.072	0.065
1	0.021	0.285	0.257
4	0.080	1.066	0.959
37	0.780	3.390	3.051

*90% content of HO/g for spinach.
$C_{spinach}$ is the *A. lumbricoides* content in spinach after harvesting.

Table 4 | Ingestion rates for carrot and spinach

	IR_{carrot} (g)	$IR_{spinach}$* (g)
Minimum†	28	30
Maximum†	38	54
Mean‡	43	65
90th‡	100	140
99th‡	183	270

*Lettuce consumption rates were used due to the lack of spinach consumption data.
†Mean range of produce quantities consumed by children per eating event (U.S. EPA 2002).
‡Consumption data per eating event in three days (U.S. EPA 1997).
IR_{carrot} = carrot consumption rates used for QMRA.
$IR_{spinach}$ = equivalent spinach consumption rates used for QMRA.

RESULTS

Dose–response relationships for *Ascaris lumbricoides*

The exponential and Beta-Poisson models were used for fitting the prediction of *A. lumbricoides* infectivity using the epidemiological data from Cifuentes *et al.* (1991) in a study performed in the Mezquital Valley, Mexico. Data was fitted to a dose–response relationship describing the observed infectivity as a function of dose. In the exponential (Equation (1)) and the Beta-Poisson (Equation (2)) models it is assumed that the dose is Poisson distributed and that one organism is sufficient to cause infection. The Beta-Poisson model takes into account the variations which exist in pathogen-host interactions. The parameters of the models were calculated using the maximum likelihood method (MLE). The values of the parameters which minimize the deviance ($Y_{\min}$) are the maximum likelihood estimates. If the optimum value of the deviance $Y_{\min}$ is less than the tabulated chi-squared value χ^2 at k–j degrees of freedom (k is the number of doses, 24 in this study, and j the number of parameters in the model-one for the exponential and two for the Beta-Poisson), then the fit is considered acceptable (Haas *et al.* 2000). Tables 5 and 6 show the parameters obtained for both dose–response models. From theses tables, it can be observed that the fit to the exponential dose–response model fails a goodness of fit test to data ($Y_{\min} > \chi^2$) for both exposure pathways (Table 5), while the fit to the Beta-Poisson relationship is acceptable (Table 6).

For the accidental soil ingestion scenario, the Beta-Poisson model required an extremely low β parameter (0.044) to adjust to the data (Table 6), even though it complies with the chi-squared goodness of fit test. However, for the crop ingestion pathway the Beta-Poisson model with $\beta = 1.1$ and $\alpha = 0.104$ parameters (Table 6) was the best approximation found. It actually complies with the criterion that the model is acceptable if the p-value (0.999) > 0.05, $\beta > 1$ and $\alpha < \beta$ (Haas *et al.* 1999*a*; Chen *et al.* 2006) and it satisfies as well the chi-squared goodness of fit test ($Y_{\min} < \chi^2$). The infections predicted with this model, i.e., annual consumption of crops once-a-week, are shown in Table 7 and Figure 3.

Therefore, the probability of infection due to the ingestion of carrots and spinach grown on biosolid-amended

Table 5 | Exponential model parameters for the two exposure pathways

Exposure pathway ingestion	*A. lumbricoides* content*	r	$Y_{\min}$	χ^2
Crops	0.42–1.15 *Ascaris l.*/100 ml	0.039	43.289	35.17
Soil	0.85–2.33 *Ascaris l.*/100 g	0.965	43.289	35.17

*See Figure 2 for estimate of *A. lumbricoides* content in water-crops and soil.
r: model parameter; $Y_{\min}$: Minimum deviance; χ^2: chi-square value at 23 degrees of freedom.

Table 6 | Best-fit Beta-Poisson model parameters for the two exposure pathways

Exposure pathway ingestion	*A. lumbricoides* content*	N_{50}	α	β	$Y_{\min}$	χ^2
Crops	0.42–1.15 *Ascaris l.*/100 ml	859	0.104	1.096	5.074	33.924
Soil	0.85–2.33 *Ascaris l.*/100 g	35	0.104	0.044	5.074	33.924

*See Figure 2 for estimate of *A. lumbricoides* content in water-crops and soil.
α, β: model parameters; N_{50}: median infectious dose; $Y_{\min}$: Minimum deviance; χ^2: chi-square value at 22 degrees of freedom.

Table 7 | Predicted infectivity for *A. lumbricoides* using Beta-Poisson model

Dose (d_i) (*Ascaris*/week-year)	Population Sample size (n_i)	Infected (p_i)	Annual prevalence Predicted (%)
2.196	341	34	10.7
2.271	341	34	10.9
2.505	341	34	11.5
2.511	341	34	11.5
2.596	759	94	11.7
2.687	759	94	12.0
2.895	759	94	12.4
2.920	759	94	12.5
3.001	335	46	12.7
3.205	335	46	13.1
3.245	335	46	13.2
3.764	335	46	14.2
3.840	396	59	14.3
4.134	396	59	14.9
4.176	396	59	14.9
4.380	396	59	15.3
4.449	817	132	15.4
4.652	817	132	15.7
4.668	817	132	15.7
4.695	817	132	15.8
4.980	698	115	16.2
5.513	698	115	16.9
5.853	698	115	17.3
5.998	698	115	17.5

soil and eaten raw was based on the following model developed (Equation (4)).

$$P(d) = 1 - \left(1 + \left(\frac{d}{859}\right)\left(2^{1/0.104} - 1\right)\right)^{-0.104} \quad (4)$$

Risk estimate for carrots and spinach eaten raw

The risk calculations associated with a single week of exposure to carrots and spinach eaten raw, estimated with the Beta-Poisson model developed (Equation (4)), are shown in Tables 8 to 11. Based on these estimates, the expected risk of *A. lumbricoides* infection has been determined where spinach carries a higher risk than carrots

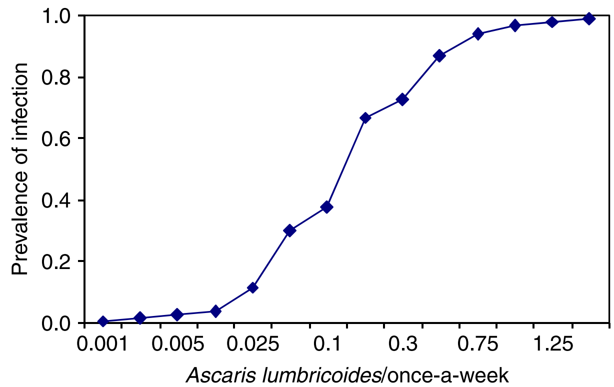

Figure 3 | Beta-Poisson prediction of *A. lumbricoides* infection for annual crop consumption.

Table 8 | Risk estimate based on U.S. EPA and WHO standards for once-a-week exposure to carrots

Biosolids HO/Gts	$IR_{carrots}$ (g/d)	Carrots Risk 2 log	Infection (%) 2 log	1 log
0.25	28	9.0×10^{-5}	0.009	0.09
0.25	43	1.4×10^{-4}	0.014	0.14
0.25	100	3.2×10^{-4}	0.03	0.32
0.25	183	5.9×10^{-4}	0.06	0.57
1	28	3.5×10^{-4}	0.04	0.35
1	43	5.4×10^{-4}	0.05	0.53
1	100	1.3×10^{-3}	0.13	1.19
1	183	2.3×10^{-3}	0.23	2.07

IR_{carrot} = carrot consumption rates used for QMRA.

Table 9 | Risk estimate using U.S. EPA and WHO standards for once-a-week exposure to spinach

Biosolids HO/gTS	$IR_{spinach}$ (g/d)	Spinach Risk 2 log	Infection (%) 2 log	1 log
0.25	30	1.8×10^{-3}	0.18	1.68
0.25	65	3.9×10^{-3}	0.39	3.32
0.25	100	5.9×10^{-3}	0.59	4.70
0.25	270	1.5×10^{-2}	1.52	9.43
1	30	7.0×10^{-3}	0.70	5.37
1	65	1.5×10^{-2}	1.46	9.15
1	100	2.2×10^{-2}	2.16	11.76
1	270	5.0×10^{-2}	4.95	18.67

$IR_{spinach}$ = equivalent spinach consumption rates used for QMRA.

Table 10 | Risk estimate from once-a-week exposure to carrots eaten raw with biosolids *A. lumbricoides* content in developing countries

		Carrots		
Biosolids HO/gTS	IR_{carrot} (g/d)	Risk 2 log	Infection (%) 2 log	1 log
4	28	1.4×10^{-3}	0.14	1.31
4	43	2.1×10^{-3}	0.21	1.94
4	100	4.9×10^{-3}	0.49	4.00
4	183	8.7×10^{-3}	0.87	6.36
37	28	1.2×10^{-2}	1.22	8.09
37	43	1.8×10^{-2}	1.81	10.55
37	100	3.8×10^{-2}	3.77	16.19
37	183	6.0×10^{-2}	6.02	20.59

IR_{carrot} = carrot consumption rates used for QMRA.

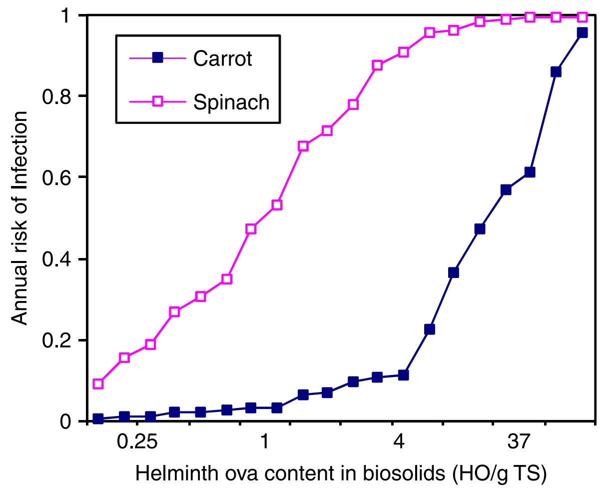

Figure 4 | Annual risk estimates for carrot and spinach (52 d/y and 2 ova log reduction due to crop washing).

(Figure 4). The risk assessment of consuming uncooked carrots grown on biosolid-amended soil is in the range of 6×10^{-2} to 9×10^{-5} per week, while in the case of spinach it is in the range of 2×10^{-1} to 1.8×10^{-3}, considering 2 ova log reductions due to produce washing. The difference in the risk of infection is expected because the *A. lumbricoides* content in spinach after harvesting was greater by one order of magnitude, compared with carrots for equivalent initial helminth ova content in biosolids (Tables 2 & 3), but using different application rates due to nutrient requirements. This shows that infection risks depend on both the application rate and the type of crop and not only on the helminth ova content in sludge.

Table 11 | Risk estimate from once-a-week exposure to spinach eaten raw with biosolids *A. lumbricoides* content in developing countries

		Spinach		
Biosolids HO/gTS	$IR_{spinach}$ (g/d)	Risk 2 log	Infection (%) 2 log	1 log
4	30	2.4×10^{-2}	2.39	12.51
4	65	4.6×10^{-2}	4.56	17.91
4	100	6.3×10^{-2}	6.31	21.06
4	270	1.2×10^{-1}	11.83	28.31
37	30	6.1×10^{-2}	6.10	20.72
37	65	1.0×10^{-1}	10.16	26.39
37	100	1.3×10^{-1}	12.90	29.48
37	270	$2.0 \times 10^{-!}$	19.94	36.25

$IR_{spinach}$ = equivalent spinach consumption rates used for QMRA.

Additionally, the type of vegetable (root and non-root crops) is more important to infection risk than the sludge rate itself, because the probability of infection from spinach eaten raw is one or two orders of magnitude greater than that caused by the ingestion of carrots.

Risk estimate for U.S. EPA and WHO criteria

For the study region (the Mezquital Valley) the model showed that if the U.S. EPA standard or the WHO criteria (0.25 and 1 HO/gTS, respectively) were applied, the overall risk of infection could be greater than 10^{-4} and the *A. lumbricoides* infection rate for carrots eaten raw would be 0.009 to 0.23% (see Table 8), while the rate would be 0.18 to 5.0% for spinach (Table 9) for the ingestion rates assumed, and if the washing procedure is enhanced to remove 2 ova log. These rates are considerably lower than those actually observed (10–17%).

Risk estimate for affordable treatment in developing countries

The prediction of *A. lumbricoides* infection may be greater than 10^{-3} if the minimum (4 HO/gTS) and maximum (37 HO/gTS) content of HO expected in affordable sludge treatment in developing countries were used. The *A. lumbricoides* infection rate for carrots eaten raw varied from 0.14 to 6% (Table 10) and for spinach from 2.4 to 20% (Table 11), if the washing procedure is enhanced to remove

2 ova log, which is considered viable from unpublished results.

When the application of a sludge with 4 HO/gTS was considered (Table 10), the model showed that the infection risk rate was around 0.9% for carrots and 12% for spinach, for the maximum crop consumption rate ($IR_{carrot} = 183$ g/d). This situation, although not ideal, would improve local health conditions. Produce washing also had an important effect on the infection risk rate. For instance, the carrot infection rate with 4 HO/gTS in sludge is 6% if washing is performed in such a way that only 1 ova log is removed, but it can be reduced to 0.9% if the washing procedure is enhanced to remove 2 ova log.

The infection risk observed with 37 HO/gTS in sludge is notably higher than the cases analyzed; it varied from an acceptable 1% up to 36% (Table 11). As observed in previous cases, the health risk is highly dependent on the consumption rate and the efficiency of washing procedure. If the mean consumption rate is considered representative for ingestion among children (28–43 g/d), then the infection rates vary from 1 to 11% for carrots, assuming 1 and 2 ova log reduction respectively. This result is within the range obtained for 4 HO/gTS content in sludge. But in the case of spinach the infection rate (21–26%) is greater than the observed prevalence when reduction of only 1 ova log is estimated for the mean consumption rates (30–65 g/d). It could be improved (6–10%) if the washing procedure is enhanced to remove 2 ova log. Moreover, if the consumption rate of 100 g/d for both vegetables is considered as a reasonable maximum exposure in a week for children the prediction for the *A. lumbricoides* infection rates (4% for carrots and 13% for spinach) would correspond to the observed prevalence, if a reduction of 2 ova log is obtained by produce washing.

DISCUSSION

Confidence on dose–response model for *Ascaris lumbricoides* infection

The U.S. EPA and WHO guidelines for helminth ova were not developed using a risk-based framework, nor were they intended to be. The lack of a risk-assessment approach means that there is no explicit delineation of acceptable helminth ova risk concentration for biosolids. Moreover, the use of below detection criteria in some defined amount of biosolids, <1 HO/4gTS for EPA and <1 HO/gTS for WHO guidelines might be unnecessarily stringent, as has been suggested in the case of reuse of wastewater for agricultural purposes (Blumenthal *et al.* 2000). Therefore, a risk-assessment approach may provide numerical limits to achieve a defined threshold for human health protection, among other control measures.

In the absence of a dose–response relationship for *A. lumbricoides* in the literature, we decided to develop one with the best available data instead of using a relationship for another pathogen, as is frequently reported. That is, the dose–response relationship developed predicts children (under 15 years old) *A. lumbricoides* infection from crops eating raw (irrigated with wastewater). The statistical procedure used in this research, maximum likely estimate of parameters, is identical to that used to fit exponential and Beta-Poisson models by Haas *et al.* (1999*a*). The most essential component of the risk model described in this paper is the dose–response curve, which focuses on infection prevalence rather than on illness or disease. The data used to develop the dose–response model were derived from accurate data describing the infection prevalence, the pathogen content in wastewater, and the irrigation practices in the study region.

Regarding the dose–response relationship obtained from available infection data (Cifuentes *et al.* 1991), it was derived from *A. lumbricoides* prevalence using stools from a large sample of children, in a population in which Ascariasis is an endemic infection resulting in a certain degree of immunity. Then, the dose response-curve developed applies only for *A. lumbricoides* infection and may not be representative of other helminthiases such as Tricuriasis, Schistosomiasis, etc. In the case of helminthiases in developing countries, there may be at least three species, *A. lumbricoides*, *Trichuris trichiura,* and *Schistosoma* (Jimenez 2007) with differences regarding infectivity and severity of illness which need to be considered and, ideally, a dose–response relationship for each particular species should be obtained. On the other hand, the developed Beta-Poisson model might not be directly applied to a healthy population considering that the immune response to infection by different populations is uncertain. However, this approach may be applied to estimate the risk to on-site agricultural workers.

Once the dose–response model for *A. lumbricoides* infection has been established, the model should be validated using credible epidemiological research. A convenient and simple method for determining the plausibility and consistency of the dose–response model developed is to compare the model estimates with human outbreak information. Unfortunately, in the case of Ascariasis, consistency is hard to demonstrate due to the endemic nature of the disease in developing countries, as well as the absence of appropriate health data. In fact, information from epidemiological studies, well documented in literature, may help estimate illness for the population in developing countries but is inadequate for estimating attributable population risk (Craun & Calderon 2006). Therefore, it is necessary to obtain outbreak information when possible with the following well-documented characteristics: i) vehicles of infection, ii) attack rates, and iii) measurements of *A. lumbricoides* levels in the incriminated sources.

Assuming the dose–response relationship derived from reported data (Cifuentes *et al.* 1991) is representative for children younger than 15, living near agricultural sites irrigated with raw wastewater, we may be overestimating risk based on the assumption that all viable *A. lumbricoides* ova in both wastewater and crops are species which infect humans. In addition, consumption rates of 100 g of vegetables or 100 mg of soil assumed may represent an overestimate of exposure, depending on individual consumption and behaviour.

Although both infection prevalence as well as wastewater quality may vary (i.e., variable exposure to *A. lumbricoides* in crops) in this study, we considered the development of dose–response relationship in which uncertainties were assumed (e.g., values for crops concentration and crops ingestion rate). In fact, one of the main sources of uncertainty in the dose–response relationship proposed is the crops consumption rate. The consumption rate considered, 100 g/week-year, comes from literature from a different country with different exposure conditions. Any other quantity, as well as the 100 g assumed will introduce variations in the risk estimate. Appropriate data for the specific study region regarding types of crops, consumption intake and frequency is needed to reduce those uncertainties and increase the confidence on infection predictions.

Even though the model for the soil ingestion scenario failed a goodness of fit for the data developed, there are some improvements that should be noted. For example, the use of current *A. lumbricoides* data for soils, instead of the indirect estimate conducted. Nevertheless, as mentioned before, the values selected do not appear to be far off the actual values, as field preliminary data indicates. This improvement, as well as alternative data compilation on the amounts of accidental child ingestion of soils in the region, or from similar rural areas, would reduce uncertainties regarding predicted infectivity of that model. However, in this case, the soil ingestion rate of 100 mg almost certainly contributes to underestimating the risk to children less than 5, because exposure frequency and the amount of soil could be greater than the values assumed.

Helminth ova content in biosolids and control measures

A risk assessment approach was developed to obtain a comparative risk analysis of helminth ova biosolid content i.e., agricultural applications in developing countries. The most essential components of the quantitative microbial risk model described in this paper are: the dose–response curve, the relationship between the initial or proposed regulated helminth ova content in biosolids and the effective content in the biosolid-dose applied to agricultural soils.

The results show that the risk from carrots and spinach eaten raw depends not only on the land application rate of biosolids with specific helminth ova content, but also on the type of crop (root and non-root crops). These two variables have a great influence on *A. lumbricoides* content in crop produce, which reflect the main factors determining infection risk. Additionally, in all the cases analyzed in this research, the QMRA showed that the risk of infection is highly dependent on consumption rates and on the efficiency of produce washing.

An important issue regarding the infection rates obtained from consuming uncooked carrots and spinach growing on biosolid-amended soil meeting the U.S. EPA and WHO standards is that, even if the risk of infection is greater than 10^{-4}, the infection rates (0.009–5%) are considerably lower than those actually observed (10–17%). However, these standards could only be achieved in developing countries if high-priced sludge treatments are applied, and if consumer washing practices are improved to remove 2 ova log. On the other hand, even though higher expected infection rates were estimated (0.9–12%) for the application of 4 HO/gTS content in sludge, this scenario may induce a gradual improvement in population health conditions. Healthier population would produce sludge with a lower helminth ova content, which could be treated largely through affordable and already installed sludge treatment processes. Moreover, for concentration range expected in affordable sludge treatment in developing countries (4 to 37 HO/gTS), the higher infection rates estimated (6–28% and 21–36% respectively) for both vegetables might be reduced (0.9–12% and 4–13% respectively) to the prevalence rates observed if the washing practice is enhanced to remove 2 ova log, and if the maximum consumption rate for carrots and spinach is less than or equal to 100 g/d.

These results suggests that U.S. EPA and WHO standards for biosolid application in agricultural production are too strict and the threshold could be higher by an order of magnitude, as has been suggested for WHO guidelines for restricted irrigation (Ayres *et al.* 1992). The change of such standards for cropland application would allow developing countries to reuse a valuable resource more economically, particularly where the upgrading of existing sludge treatment systems to meet the standards is financially impractical.

Consequently, regulations targeting biosolid reuse in developing countries should address the challenge of deciding first acceptable infection risk and, second, putting in place an integral framework for risks management, involving additional health protection measures. Factors to be considered include: (a) an affordable treatment process, (b) crop restriction policy, (c) different sludge application rates, and (d) efficient produce washing methods, among others.

Confidence on QMRA for biosolids

Assessing risk associated with the reuse of biosolids in agricultural production need to take into consideration that pathogen exposure may not be homogeneous. The results of this research indicated that this could be due to several factors such as the type of sludge treatment process used, nutrient requirements for enriching agricultural soil, the rate of biosolid application, the crop category and the harvesting-consumption time. Some of these factors, as well as produce washing and consumption patterns (amount and frequency, cooked or non-cooked produce) among the concerned population have all temporal dimensions, while others have spatial ones. Therefore, there is a notable difference in the level of exposure to pathogens for each specific situation.

The real scenario analyzed -children aged 15 or younger who comprise one of the more sensitive subpopulations (farmer and their families) involved in wastewater land irrigation- confirmed that differences in the level of exposure to pathogenic organisms may arise, after all, by variations in helminth ova concentrations in vegetables and in consumption patterns. To improve confidence on the distribution of risks obtained, those factors leading to increased variability need to be better characterized in order to reach permissible helminth ova limits for biosolids when applying them on agricultural production in developing countries.

Variability in consumption behaviour is known to be relevant in QMRA used in wastewater irrigation scenarios (Hamilton *et al.* 2006). The selection of some vegetable consumption rates (Table 4) allowed us to account for our lack of knowledge about this parameter estimate (uncertainty) in Mexico, and to include inherent natural variation (variability) in the risk assessment conducted. Therefore, it is uncertain if the mean and upper percentiles values (43, 100 and 183 g for carrots and 65, 140 and 270 g for spinach, respectively) used for the risk estimation represent a reasonable maximum exposure level for children under 15 years old in the Mezquital Valley. Thus, the infection risk could have been overestimated by using such conservative assumptions.

Regarding the assumption made in the mean range of produce quantities consumed by children per eating event of 28–38 g for carrots and 30–54 g for spinach taken from U.S. data available (U.S. EPA 1997, 2002), we think these estimates may be appropriate for the Mexican case analyzed herein since these were considered as within the range of possible weekly consumption amounts expected for a rural area. Therefore, there is more confidence on the predicted infections obtained from these consumption rates.

However, it should be highlighted that health risks associated with spinach consumption were based on consumption rates for lettuce due to the lack of spinach consumption data, which in turn added uncertainty to risk estimates.

Besides the actual HO content in biosolids applied on agricultural soil and the type of crop grown (root and non-root crops), another factor influencing HO concentrations in vegetables includes the irrigation practice. The model developed for this research did not considered the irrigation method, although it was implicit in the development of the dose–response model. Flooding being the current irrigation practice in the Mezquital Valley, a different dose–response curve could be observed if another type of irrigation practice (for example drip or sprinkler systems) is used. This limitation can be overcome if the actual content of *A. lumbricoides* in crops is monitored.

The underestimation of risk may be of greater concern, due to underestimation of exposure reflected by the inefficiencies of the methods for detection of helminth ova and *A. lumbricoides* both in biosolids and in vegetables. It is well known that the assessment of exposure, and in particular pathogen concentration, relies upon the adequacy of microbial methods. In general, these methods are often expensive (several hundred US dollars per sample), tedious and time consuming, and may not be perfect with respect to specificity (the ability to detect only the organism of interest), sensitivity (the ability to detect any amount of the organism of interest), and assessment of viability. As a consequence, an important source of concern if the model developed in this research is applied to other regions is the lack of an international standardized analytical technique. Additionally, the analytical results used in this research were obtained from the application of a commonly-accepted technique; therefore a different dose–response curve could be obtained if another analytical procedure is used.

Finally, to improve overall QMRA conducted for bisolids reuse in agriculture, it is necessary to obtain ascariasis epidemiological information, which correlates attack rates of infectivity with measurements of *A. lumbricoides* content in vegetables eaten raw, and have been grown on biosolid-amended soil. These considerations will provide more confidence on dose–response relationship for *A. lumbricoides*. There is no doubt that a sensitivity analysis needs to be achieved for the biosolids model in order to identify parameters contribution to infection variability and uncertainty prediction, mainly for those describing consumption rates. However, any effort to gather accurate and current data on vegetable consumption rates would enhance consumption behaviour parameters.

CONCLUSIONS

The contribution made in this paper consisted of integrating epidemiological and environmental data to evaluate the risk using the QMRA approach. It shows that multidisciplinary work is needed to help decision makers define evidence-based strategies for effectively protecting human health under different circumstances. Our data indicated that it would be possible to use higher helminth ova content standards than those proposed by U.S. EPA and WHO in biosolids from developing countries without significantly increasing the risks. To improve local health conditions, it may be wiser to have achievable guidelines and fulfil them, rather than to set unaffordable standards that will never be met. Our study also pointed out that health risk is not only associated with the helminth ova content in sludge, but also with a wide variety of factors such as biosolid application rates, category of crop, produce washing and crop consumption rates. These considerations may allow policymakers to define different cost-benefit and control strategies.

Regarding the main source of concern expressed in this paper (lack of certain data) we consider that these limitations can be overcome if current measurements are made to be replaced in the developed procedure. Finally, considering that there are different helminth species, one of which is *Ascaris*, and that they entail different infectivity factors and illness symptoms, it is important to perform a QMRA for each species, by developing the respective dose–response curve. A methodology similar to the one presented here could help to do this.

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