

Occurrence and fate of *Ascaris lumbricoides* ova in biosolids in Victoria, Australia: a human health risk assessment of biosolids storage periods

Nicholas A. O'Connor, Aravind Surapaneni, David Smith and Daryl Stevens

ABSTRACT

Reuse of sewage biosolids in Victoria, Australia, typically involves mesophilic anaerobic digestion followed by air-drying and long-term storage to ensure removal of ova of soil-transmitted helminths (STH) such as *Ascaris lumbricoides*. Long-term storage degrades the biosolids' agronomic quality due to the loss of key plant nutrients and takes up large areas of storage space. The impact of varying biosolids holding times and other processes on STH using *Ascaris* as the reference STH pathogen was examined in this study using a quantitative risk analysis approach. Risk modelling of the potential human health impacts from the presence of *Ascaris* ova in biosolids was undertaken for discrete holding periods of 1, 2 and 3 years. Modelling showed that to meet the WHO $1 \mu\text{DALY}\cdot\text{person}^{-1}\cdot\text{year}^{-1}$ disease burdens guideline for limiting exposure category, a biosolids storage period of 1.24 years or 2.1 years would be required, depending on the data source of ova shedding rates per worm (Bangladesh or Nigeria, respectively). The soil exposure and salad/root vegetable consumption models included a number of variables with moderate to high degrees of uncertainty. Monte Carlo simulation was used to assess the effect of uncertainty in model input variables and to assist in highlighting areas for further research.

Key words | *Ascaris lumbricoides*, Australia, biosolids, DALY, disease burden, helminth ova, Monte Carlo simulation, risk modelling, soil transmitted helminths

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ABBREVIATIONS

LRV log reduction value
WHO World Health Organization
DALY disability adjusted life year
MAD mesophilic anaerobic digestion
EPAV Environment Protection Authority Victoria
STH soil transmitted helminths
MPN most probable number

PFU plaque-forming units
NLAR nutrient loading application rate

HIGHLIGHTS

- The health risk to soil workers and vegetable crop consumers from *Ascaris* in biosolids was modelled.
- Different biosolids holding periods were modelled for different exposure categories.
- Compliance with the WHO μDALY guideline was achieved for some modelled scenarios.
- A key assumption was choice of country (Bangladesh or Nigeria) for ova shedding rates per worm.
- Longer biosolids holding periods are required if basing model estimates on Nigerian data.

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INTRODUCTION

Background/problem formulation

Sewage biosolids are a by-product of the sewage treatment process. They can be used for a variety of purposes, but are most commonly used as a fertiliser and soil conditioner for the purposes of growing cereal, pasture and vegetable crops. Biosolids require treatment processes to control pathogens and these processes can be broadly divided into either thermal processes that rapidly kill off the pathogens, or stockpiling and storage until such a time that the pathogens die off. In developed countries, the combination of stringent public health regulations and limited demand for biosolids has seen an emphasis on lower cost treatment processes such as air-drying and long term storage (e.g. >3 years). Ambient temperature anaerobic digestion is also commonly used.

The use of biosolids in Australia is regulated at a state level and various state biosolids guidelines apply under each state's environmental protection legislation. These guidelines are harmonised at a national level through compliance with the National Water Quality Management Strategy (NWQMS) (NRMMC 2004; DoE 2015). In the State of Victoria, biosolids use must comply with the requirements of the Environment Protection Authority Victoria (EPAV) *Guidelines for Environmental Management. Biosolids Land Application* (EPA Victoria 2004).

The Victorian biosolids guidelines describe three treatment grades: T1, T2 and T3 (graded according to descending microbiological quality). The treatment grades are primarily based on satisfying three key criteria: (i) the adoption of a prescribed treatment process with minimum performance criteria (for example temperature/time); (ii) microbiological limits to demonstrate that the defined treatment processes are operating effectively; and (iii) measures for controlling bacterial regrowth, vector attraction (for example insects, birds, vermin) and generation of nuisance odours (EPA Victoria 2004).

If the treatment process is not a prescribed process under the guidelines, classification is based on a verification program for the process to justify its addition to the EPAV list of prescribed treatments, or alternatively, intensive batch testing to demonstrate pathogen removal. Grant *et al.* (2012) point out that gaining independent pathogen removal data for verification for many regional treatment systems has proven difficult, particularly with the focus on ensuring removal of ova of parasitic worms such as *Ascaris lumbricoides* and *Taenia* spp. to produce T1-grade biosolids suitable for unrestricted use. As a result, the most common biosolids production process in Victoria is mesophilic anaerobic digestion

(MAD) followed by air-drying and long-term storage for at least 3 years (Grant *et al.* 2012). Rouch *et al.* (2011) have shown that long-term storage (>3 years) degrades the biosolids' agronomic quality due to the substantial loss of key plant nutrients. This decline in agronomic quality is believed to be a factor in the low rates of biosolids use in Victoria, where more than two million tonnes of biosolids are stored in long-term stockpiles (Grant *et al.* 2012). Regulatory controls also mean that the overall capacity of the existing sludge stockpile areas will need to be increased to meet population growth.

To be added to the EPAV list of prescribed treatment processes, the default performance objectives under realistic worst case process conditions are:

- >3 log reductions in enteric viruses (i.e. a 1,000-fold reduction);
- >2 log reduction in *Ascaris ova* (i.e. a 100-fold reduction);
- <1 *Salmonella*/50 g (dw – dry weight);
- <100 *Escherichia coli* MPN/g (dw);
- ≤1 enteric virus PFU/100 g; plus
- additional requirements for minimum sample volumes and number of samples.

Ascaris ova are by far the longest lived of the soil-transmitted helminths (STH) and can remain infective in soil for several years (Feachem *et al.* 1983). For this reason and their tendency to settle in sewage sludge, they are an appropriate reference pathogen for STH.

While *Salmonella*, *E. coli* and virus testing of treated biosolids is routinely performed, testing for *Ascaris ova* in Victoria is problematic as infection rates in the community are so low the ova are rarely found in raw sludge or even in the raw sewage entering the sewage treatment plant (STP) (AECOM 2015). Thus demonstration of the specified performance targets (i.e. the required 2 log reductions) is not possible based on such testing. An alternative approach proposed by the WHO (WHO 2010) is to derive a health outcome target such as a tolerable burden of disease measured using disability-adjusted life years (DALYs). This approach is known as a health-based target (WHO 2011). For drinking water, WHO suggests a tolerable burden of disease as an upper limit of 10^{-6} DALY per person per year (or $1 \mu\text{DALY}\cdot\text{person}^{-1}\cdot\text{year}^{-1}$) (WHO 2010, 2011). This is considered an appropriate target for developed countries where the overall burden of disease by multiple exposure routes (e.g. water, food, air, direct personal contact, etc.) is very low (WHO 2010). The WHO $1 \mu\text{DALY}\cdot\text{person}^{-1}\cdot\text{year}^{-1}$ tolerable disease burden target has also been adopted by the Australian Guidelines for Water Recycling (NRMMC,

EPHC & AHMC 2006) and is readily applicable to the health risks posed by *Ascaris* and other STH.

The current minimum holding period for T1 biosolids in Victoria is 3 years. In this paper, risk modelling is used to determine the potential health impacts of the 3-year and shorter holding periods in terms of μ DALYs and to assess the extent of compliance with the WHO 1μ DALY \cdot person $^{-1}\cdot$ year $^{-1}$ target.

Risk modelling

For risk modelling, an exposure pathway for *Ascaris* in biosolids was developed based on the common approach for biosolids production and storage in Victoria (Figure 1). Settled solids from the MAD process are mechanically spread across large drying pans and once the moisture content is low enough, they are excavated and stockpiled

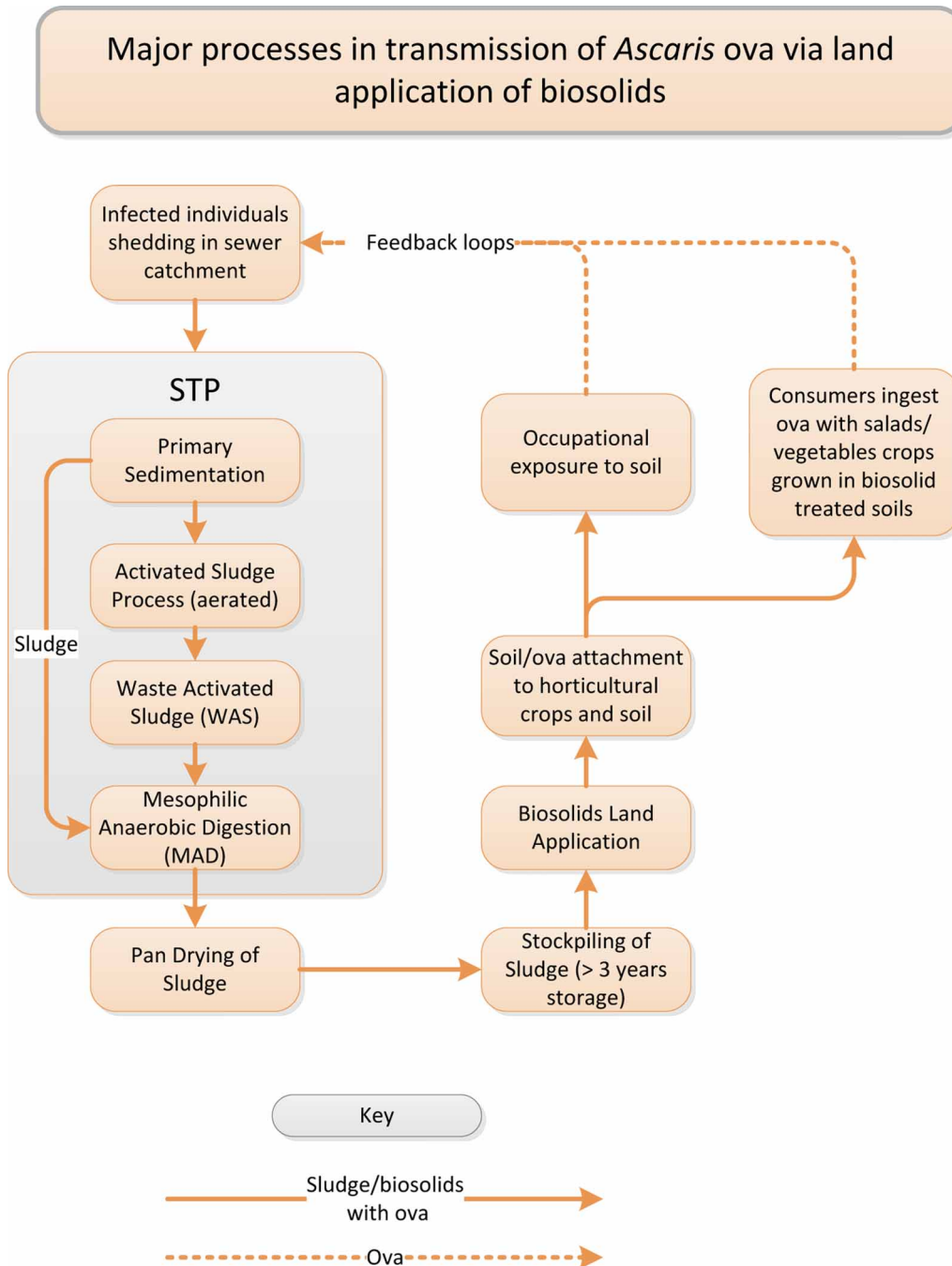


Figure 1 | Major processes in the transmission of *Ascaris ova* with sewage biosolids. The feedback loops are not expected to be significant in developed countries with modern sanitation infrastructure. STP = Sewage Treatment Plant – commonly an activated sludge plant in Victoria.

for prescribed periods prior to use. When applied to land to fertilise crops, surviving *Ascaris ova* are transmitted to crops (via adhering soil), to humans as agricultural workers and as consumers of crops grown in the biosolids amended soil. The highest rates of exposure via the consumption of crop products is considered to be associated with salad and vegetable crops, hence they were considered in the current risk assessment.

There is the possibility of a feedback loop via infected agricultural workers and consumers to the sewer catchment; however, this is thought to be insignificant due to the very low prevalence of *Ascaris* in the Victorian community and is not considered further here. Should prevalence rates increase, this assumption would need to be reviewed.

Until recently in Victoria, after a minimum of 3 years of storage, biosolids that also meet all other treatment, microbiological and chemical criteria could be released for beneficial use in agriculture. In late 2015, EPAV approved a biosolids storage period of 1 year for two regional STPs; Boneo and Somers, on the provision that additional measures were undertaken including statistically valid monitoring to check for changes in the risk profile of helminth ova, third party certification of biosolids production, storage and handling (e.g. Hazard Analysis at Critical Control Points (HACCP) certification); and end point testing of biosolids for pathogenic bacteria, viruses and *Ascaris ova*. EPAV approval was based on the verification programme undertaken by South East Water Corporation to test if a 1-year storage period achieved the required reduction in a range of enteric pathogens and parasites (Irwin et al. 2017).

The aim of this study is to quantify the potential human health impacts from the STH reference pathogen, *Ascaris lumbricoides*, of different stockpiling periods for pan-dried biosolids produced using the low cost MAD process. To do this we used quantitative risk analysis to identify the key process steps that determine risks to human health and assessed this risk against a health-based target.

With an improved understanding of *Ascaris ova* fate in the biosolids production and storage process, it may also be possible to reduce reliance on costly and relatively imprecise end-point testing and focus instead on the effectiveness of critical process steps to produce safe quality biosolids for land application.

MATERIALS AND METHODS

The following equations were developed for the current study to assist in identifying the key variables that determine

the risks to human health from *Ascaris ova* in biosolids when used in agricultural production and via ingestion of salad and vegetable produce.

The concentration of *Ascaris ova* in the stored biosolids, C , was determined using Equation (1).

Ascaris ova concentration in biosolids

$$C = \left(\overbrace{\omega \phi a \left(\frac{\chi}{2}\right)^{(b+1)}}^{\text{ova loading}} \cdot \overbrace{10^{-(I_T + I_{PD} + (I_S \theta))}}^{\text{ova inactivation}} \right) / \underbrace{\kappa \lambda}_{\text{biosolids loading}} \quad (1)$$

where the elements of the equation are:

C = Concentration of *Ascaris ova* in stored biosolids (ova·g⁻¹)

ω = Proportion of population in the sewer catchment that are shedders

ϕ = Faecal load per person (g·day⁻¹)

a = Shedding rate per worm a-coefficient

b = Shedding rate per worm b-coefficient

χ = Average no. of worms per infected person (shedder) (worms·person⁻¹)

I_T = Inactivation due to treatment process (MAD) (log reduction value)

I_{PD} = Inactivation due to sludge pan drying (log reduction value)

I_S = Inactivation due to storage (log reduction value·year⁻¹)

θ = Number of years stored (years)

κ = Solids production rate per L raw sewage (g·L⁻¹)

λ = Discharge of sewage (wastewater) per person per day (L·person⁻¹·day⁻¹)

The equation consists of three components: (i) the load of ova to the STP arising from the shedding of ova by infected individuals in a sewer catchment (ova loading), (ii) the sum of environmental factors that act to inactivate the ova (ova inactivation) – these are broken down by treatment process steps, and (iii) quantity of biosolids received at the STP (biosolids loading).

The quantity of *Ascaris ova* ingested per day due to exposure to biosolids-amended soils is given in Equation (2). Standard soil ingestion rates were derived from guidance published by the US EPA (US EPA 2016, citing US EPA 1991) or EnHealth (2012). Biosolids application rates were based on the maximum of the nutrient loading application rate (NLAR) for nitrogen, as this was always greater than the NLAR for phosphorus. For the soil exposure risk modelling, the NLAR was based on spinach as an appropriate example of a leafy vegetable crop. However, a root

vegetable could also be used. The NLAR value varies locally depending on soil conditions and crop type.

Ascaris ova ingested per day through exposure to biosolids amended soils

$$E_S = \frac{(\epsilon \eta_S C \cdot 10^{-5})}{\zeta d_p} \tag{2}$$

where:

- E_S = *Ascaris* ova ingested per day (ova·day⁻¹)
- ϵ = Soil ingestion rate (mg·day⁻¹)
- η_S = Biosolids application rate – spinach, (max NLAR tonnes·ha⁻¹)
- C = Concentration of *Ascaris* ova in stored biosolids (ova·g⁻¹) (Equation (1))
- ζ = Soil bulk density (g·cm⁻³)
- d_p = Depth of biosolids-amended soil (cm)

The annual disease burden per person per annum due to exposure to biosolids-amended soils is given in Equation (3). This is based on the work of Navarro et al. (2009) on *Ascaris* ova dose-response and on the disease burden per case derived for this study.

Annual disease burden per person per annum through exposure to biosolids amended soils

$$D_S = 10^6 \cdot \beta \delta \left\{ - \left(\frac{E_S (2^{1/\alpha} - 1)}{\gamma_s} + 1 \right)^{-\tau_s \alpha} + 1 \right\} \tag{3}$$

where:

- D_S = Annual disease burden per person (soil) per annum, (μDALY·person⁻¹·year⁻¹)
- 10^6 = Conversion factor DALY to μDALY
- β = Disease burden per case, (DALY·case⁻¹)
- δ = Fraction of consumers susceptible to infection
- E_S = *Ascaris* ova ingested per day (ova·day⁻¹) (Equation (2))
- α = Beta-Poisson Dose-response coefficient
- γ_s = N₅₀ dose (soil) (ova·day⁻¹)
- τ_s = Frequency of ingestion per year (soil) (days·year⁻¹)

The quantity of *Ascaris* ova ingested per day due to consumption of uncooked salad or vegetables grown in biosolids-amended soils is given in Equation (4). The equation assumes that both leafy (e.g. spinach) and root (e.g. carrot) vegetables are consumed and sums the exposure across each crop type. The equation accounts for the transfer of helminth ova with soil to the salad leaf or root vegetable and the loss of ova due to washing (a standard procedure in the harvesting and packaging process in Australia). Larger carrots are also frequently peeled; however, it is assumed here that the carrots are unpeeled.

Ascaris ova ingested per day by a person consuming fresh, uncooked salad or vegetables grown in biosolids amended soils

$$E_P = \frac{\overbrace{C}^{\text{Ova concentration in soil}}}{\zeta d_p} \left(\underbrace{(\eta_C \xi_{rw} A_C P_C O_C \cdot 10^{-(5+\psi)})}_{\text{carrot}} + \underbrace{(\eta_S \xi_S P_S O_S \cdot 10^{-(2+\psi)})}_{\text{Spinach}} \right) \tag{4}$$

where:

- E_P = *Ascaris* ova ingested per day (ova·day⁻¹)
- C = Concentration of *Ascaris* ova in biosolids (ova·g⁻¹) (Equation (1))
- ζ = Soil bulk density (g·cm⁻³)
- d_p = Depth of biosolids amended soil (cm)
- η_C = Biosolids application rate – carrot: use max NLAR, (tonnes·ha⁻¹)
- ξ_{rw} = Soil adherence to root vegetable, (mg·cm⁻²)
- A_C = Surface area of carrot (cm²)
- P_C = Carrot ingestion rate, (g·serve⁻¹)
- O_C = Serving frequency – carrot (non-leafy veg) (serves·day⁻¹)
- ψ = Ova removal due to crop washing (log reduction value)
- η_S = Biosolids application rate – spinach: maximum NLAR (tonnes·ha⁻¹)
- ξ_S = Soil adherence to spinach (%)
- P_S = Spinach/lettuce ingestion rate (g·serve⁻¹)
- O_S = Serving frequency – lettuce/spinach (leafy veg) (serves·day⁻¹)

The annual disease burden per person per annum due to consumption of uncooked salad or vegetables grown in biosolids-amended soils is given in Equation (5). This is based on the work of Navarro et al. (2009) on *Ascaris* ova dose response and on the disease burden per case derived for this study.

Annual disease burden per person per annum through consumption of fresh, uncooked salad or vegetables grown in biosolids amended soils

$$D_V = 10^6 \cdot \beta \delta \left\{ - \left(\frac{E_P (2^{1/\alpha} - 1)}{\gamma_v} + 1 \right)^{-\tau_v \alpha} + 1 \right\} \tag{5}$$

where:

- D_V = Annual disease burden per person (vegetables), (μDALY·person⁻¹·year⁻¹)
- 10^6 = Conversion factor DALY to μDALY

β = Disease burden per case, DALY·case⁻¹
 δ = Fraction of consumers susceptible to infection
 E_p = *Ascaris ova* ingested per day (ova·day⁻¹) (Equation (4))
 α = Beta-Poisson Dose-response coefficient
 γ_v = N₅₀ dose (salad/vegetables), ova·day⁻¹
 τ_V = Frequency of salad and vegetable consumption per year, days

The values used in the equations and their information source are given in Tables 1–5. Four exposure categories were modelled (Table 3). The first three categories were

soil exposure categories as follows: Outdoor Worker, Outdoor Recreator (sporting field), and Domestic Gardener. The fourth category was a consumer of salad and vegetable crops that were grown in biosolids-amended soil.

The ova shedding rate per worm is a potentially significant factor in determining total ova load in biosolids. Hall & Holland (2000) provide power functions fitted to data on worm burdens and ova per gram counts from Bangladesh and Nigeria (Table 2). To derive the power function coefficients (a, b), Hall and Holland plotted the relationship between the number of ova per gram of faeces and female

Table 1 | Model inputs to soil exposure and ingestion of salad and vegetable crop models

Model inputs	Units	Symbol	Value	Data source
<i>Inputs common to soil exposure and ingestion of salad and vegetable crops models</i>				
Daily faecal load per person	g·day ⁻¹	φ	128.3	Average of male (m) and female (f) means f: 125.7 d ⁻¹ , m: 130.9 g d ⁻¹ , wet weight (Wyman <i>et al.</i> 1978), 126 ± 95 g d ⁻¹ wet weight; (high income countries) (Rose <i>et al.</i> 2015).
Average no. of worms per infected person (shedder)	Worms·person ⁻¹	χ	2	Average worm burden across infected villagers in Venezuela was 7.5 worms per infected person (Morales <i>et al.</i> 1999). However, in a modern developed country with high quality sanitation, worm burdens of infected persons who are also shedders is expected to be close to the minimum (1 male, 1 female) possible to sustain shedding. This is because the ingestion of infective ova is considered to be a rare and isolated event in such populations.
Proportion of population that are shedders	dimensionless	ω	0.0002	Incidence of infection in population = 20 per 100,000 in Denmark, 2005 (Arnbjerg-Nielsen <i>et al.</i> 2005). Supported by monitoring of raw sewage at South East Water STPs, which shows no ova in many 1 L samples.
Discharge sewage (wastewater) per person per day	L·person ⁻¹ ·day ⁻¹	λ	180	EPA Victoria (1997), Table 2 - Daily Flow: established residential housing 125–180 L·person ⁻¹ , new subdivision schemes 150–200 L·person ⁻¹ , 'A' rated flats and units 125–180 L·person ⁻¹ . (180 = toilet 15 L, bathroom 50 L, laundry 45 L, other uses 40 L).
Solids production rate per L raw sewage	g·L ⁻¹	κ	0.220	South East Water wastewater engineers report an average total suspended solids concentration of 0.310 g·L ⁻¹ for raw sewage at regional STPs (e.g. South East Water's Somers plant). Solids digestion through the plant is estimated at 71% for a 20 day sludge age in winter. So the resultant solids production rate per litre of raw sewage is 0.310*0.71 = 0.2201 g·L ⁻¹ .
Inactivation due to treatment process (MAD) LRV	dimensionless	I _T	0	Currently set at zero due to lack of relevant local data. In a Mexican study of waste stabilisation ponds, Nelson & Darby (2002) reported an inactivation curve characterised by an initial lag phase, a period of roughly first-order inactivation, and a tailing region. During the first year, 50 to 60% of the eggs were inactivated (LRV = ~0.3), after which the rate decreased.
Inactivation due to sludge pan drying LRV	dimensionless	I _{PD}	0	Currently set at zero due to lack of relevant local data.

(continued)

Table 1 | continued

Model inputs	Units	Symbol	Value	Data source
Inactivation due to storage (rate per annum) LRV	LRV·y ⁻¹	I _s	1.62	Based on Pecson <i>et al.</i> (2007) at 20 °C, 2 LRV can be expected after 450 days at pH 7. On a pro rata basis, after 1 year the LRV is 1.622. Colls <i>et al.</i> (2012) reported an average annual soil temperature at 10 m depth for Melbourne of 18.5 °C. Closer to the surface, the temperature variation is more extreme and varies at 10 cm between 6 °C and 30 °C. These figures apply to normal soil. It is expected that biosolids stockpiles will be much warmer than 20 °C due to the significant self-heating caused by heat released by decomposition (Aganetti <i>et al.</i> 2016).
Soil bulk density	g·cm ⁻³	ζ	1.3	Lindeburg (2015).
Depth of biosolids amended soil	cm	dp	10	Industry standard for crops on Australian soils (D. Stevens personal communication).
Beta-Poisson dose-response coefficient	dimensionless	α	0.104	Navarro <i>et al.</i> (2009).
Disease burden per case	DALY·case ⁻¹	β	0.002968	Calculated using Severity Weight from Pullan <i>et al.</i> (2014) and Prevalence from Brooker & Pullan (2013) and Dold & Holland (2011).
Fraction of consumers susceptible to infection	dimensionless	δ	1	Navarro <i>et al.</i> (2009).
Available Biosolids Nitrogen, Year 1	kg·tonne ⁻¹	B _N	8.06	Based on biosolids nitrogen concentrations from South East Water's Boneo STP 2011 to 2014 (stockpiles ages of 1, 2 and 3 years).
Reduction rate in available biosolids nitrogen per annum	kg·tonne ⁻¹ ·yr. ⁻¹	B _{N,Yr}	1.06	
Nutrient uptake, carrots (nitrogen)	kg·ha ⁻¹	C _N	250	Westerveld <i>et al.</i> (2006). Average nitrogen uptake on organic soil = 250 kg·ha ⁻¹ N. Based on results from 2 cultivars measured on organic and mineral soils at different N application rates. Average for Idaho carrots (mineral soils = 109 kg·ha ⁻¹ , organic soils = 229 kg·ha ⁻¹), Fontana carrots (mineral soils = 94 kg·ha ⁻¹ , organic soils = 271 kg·ha ⁻¹).
Nutrient uptake, lettuce/spinach (nitrogen)	kg·ha ⁻¹	LS _N	145	Spinach: Heinrich <i>et al.</i> (2013). Average 104.2 kg·ha ⁻¹ (converted from lb·acre ⁻¹). Lettuce: Bottoms <i>et al.</i> (2012). Mean N uptake 145 kg·ha ⁻¹ , critical uptake 116 kg·ha ⁻¹ . Lettuce value used for consistency.
Carrots (nitrogen)	tonnes·ha ⁻¹	A _{CN}	31.00	=C _N /B _N , example for 1-year-old biosolids shown.
Lettuce/spinach (nitrogen)	tonnes·ha ⁻¹	A _{SN}	17.98	=LS _N /B _N , example for 1-year-old biosolids shown.
Biosolids application rate – carrot	tonnes·ha ⁻¹	η _c	Table 6	The maximum NLAR. Calculated according to the formula C _N /(B _N ·(B _{N,Yr} *θ) + 1). Values used are shown in Table 6.
Biosolids application rate – lettuce/spinach	tonnes·ha ⁻¹	η _s	Table 6	As above, calculated according to the formula: LS _N /(B _N ·(B _{N,Yr} *θ) + 1).
Years stored	No. years stored	θ	Modelled scenario	Scenarios were 1 year, 2 years or 3 years.
<i>Inputs for salad and vegetable crop model only</i>				
Soil adherence to root vegetable	mg·cm ⁻²	ξ _{rv}	0.446	US EPA Exposure Factors Handbook (US EPA 2011). Assuming the same rate of adherence to vegetable skin as to an agricultural worker's hands.

(continued)

Table 1 | continued

Model inputs	Units	Symbol	Value	Data source
Surface area of carrot	cm ²	A_C	169.646	Assuming a standard carrot is a truncated cylinder with $L = 18$ cm, $R = 2$ cm and $r = 1$ cm, $= 2\pi ((R + r)/2) L$, where $R =$ radius at top, $r =$ radius at bottom and $L =$ length of carrot.
Soil adherence to spinach	%	ξ_s	0.1	Assumes a 10% transfer of ova to spinach leaves for a given ova·g ⁻¹ of total biosolids amended soil. Here it is assumed that the transfer rate is identical to that used by Williams <i>et al.</i> (2012) for lettuce leaves and is based on the study by Jimenez <i>et al.</i> (2006).
Carrot ingestion rate	g·serve ⁻¹	P_C	37.2	Australian Health Survey: Nutrition First Results – Foods and Nutrients, 2011–12. Australian Bureau of Statistics (ABS 2014).
Spinach/lettuce ingestion rate	g·serve ⁻¹	P_S	24.6	As above.
Serving frequency – carrot (non-leafy veg)	serves·day ⁻¹	o_c	2	Australian Guidelines for Water Recycling (NRMCC, EPHC & AHMC 2006, p 92, Table 3.3, note b). Cites Australian Bureau of Statistics data stating that vegetables other than lettuce are eaten at twice the rate of lettuce.
Serving frequency – lettuce/spinach (leafy veg)	serves·day ⁻¹	o_s	1	As above.
LRV due to crop washing	LRV	ψ	1	WHO (2006). pages 25–26.
N ₅₀ dose (vegetables)	ova·day ⁻¹	γ_v	859	Navarro <i>et al.</i> (2009).
Frequency of ingestion per year	days	τ_v	140	Australian Guidelines for Water Recycling (NRMCC, EPHC & AHMC 2006, p 92, Table 3.3, note b). Food crop ingestion 140 serves per year (uncooked produce). Consumption rate for cooked vegetables will be higher, but it is assumed that cooking will kill <i>Ascaris</i> ova.
<i>Inputs for soil worker exposure model only</i>				
N ₅₀ dose (soil)	ova day ⁻¹	γ_s	35	Navarro <i>et al.</i> (2009).

Table 2 | Model input scenarios: Ova shedding rate per worm a and b power function coefficients derived from data from two different source countries

Inputs	Units	Symbol	Bangladesh	Nigeria
Ova shedding rate per worm, a-coefficient	dimensionless	a	291	9,802
Ova shedding rate per worm, b-coefficient	dimensionless	b	-0.2737	-0.4994
Size of data set (N) used to derive the coefficients	dimensionless		$N = 1,365$	$N = 563$

Data source: Hall & Holland (2000).

worm burdens between 1 and 50 and fitted a polynomial curve of the form $y = ax^b$. Note that the b coefficients in Hall & Holland (2000) include some typographical errors that were corrected in our application.

Sensitivity analysis – Monte Carlo simulation

Since many of the variables making up the risk model are associated with some level of uncertainty, Monte Carlo simulation was used to explore overall model uncertainty.

The effect of certain key model factors that have variable ranges was assessed by choosing discrete values as scenarios to model. These factors were: θ , the number of years biosolids were stored before use (1, 2 or 3 years), a combination of soil ingestion rates for different worker exposure categories, ε , and soil ingestion rates τ_s (Tables 3 and 4) (three occupational exposure categories), a fourth exposure category for consumers of salad and vegetable crops grown in soil with applied biosolids, and the source of data used to determine ova shedding rates per female *Ascaris* worm. Of the remaining 27 model input variables, 18 continuous variables were allocated probability distribution functions (PDFs) (Table 6), while the remaining

Table 3 | Model input scenarios: soil ingestion rate and frequency of ingestion per year for four reference exposure categories

Inputs	Units	Symbol	Outdoor worker	Outdoor recreator (sporting field)	Domestic gardener	Data source
Soil ingestion rate	mg soil·day ⁻¹	ϵ	100	100	50	Derived from data in Table 4
Frequency of ingestion per year	days	τ_s	225	24	4	Derived from data in Table 4

Table 4 | Soil ingestion rate data sources

Inputs	Units	Symbol	Value	Data source
Soil ingestion rate – outdoor worker / farmer / composite worker	mg soil·day ⁻¹	ϵ_O	100	Default exposure parameters table in: Preliminary Remediation Goals for Radionuclides (PRG). PRG User's Guide (US EPA 2016, citing US EPA 1991).
Soil ingestion rate – adult	mg soil·day ⁻¹	ϵ_A	50	EnHealth (2012) Chapter 4. Soil Ingestion.

Table 5 | Disease burden calculations

Symptom	Proportion with symptom	Severity weight	Duration	Standardised duration (years)	Disease burden per case (DALY) ^a
Symptomatic infection (heavy infection)	0.92% ^b	0.0296 ^c	1 month ^d	0.083	2.272×10^{-5}
Wasting (heavy infection)	0.79% ^e	0.1245 ^c	1 month ^d	0.083	8.188×10^{-5}
Mild abdominopelvic problems (medium infection)	4.63% ^b	0.0108 ^c	0.5 years ^f	0.5	2.502×10^{-4}
Death	0.00037% ^g	1 ^h	80 years ⁱ	80	2.962×10^{-4}
Total <i>Ascaris</i> illness (DALY)					6.511×10^{-4}

^aDisease burden per case (DALY) = Proportion with symptom × severity weight (DALY) × standardised Duration (years).

^bProportion with symptoms from Table 3 of de Silva et al. (1997).

^cDisability weights for Ascariasis from Table 3 of Pullan et al. (2014).

^dEstimated – assuming that illness will result in seeking medical treatment within a month of onset of symptoms.

^eProportion with symptoms calculated from data in Tables 13.2 and 13.4 of Brooker & Pullan (2013).

^fEstimated – assuming that milder illness will result in seeking medical treatment up to 0.5 years of onset of symptoms.

^gProportion with symptoms from calculation after Brooker & Pullan (2013).

^hWHO (2011), p. 39, box 3.1 Severity for death = 1.

ⁱAustralian Guidelines for Water Recycling Phase 1, Box 3.1 (NRMCC, EPHC & AHMC 2006).

nine factors (Table 7) were considered to vary little and thus unlikely to have much influence on overall model variability.

For the 18 continuous factors, no data was identified that could be used to construct PDFs for any of the variables, with the exception of the volume of wastewater discharged to sewer per person per day, which was based on some limited data published by Melbourne Water (Melbourne Water 2017). Consequently, BetaPERT distributions, commonly used in modelling expert estimates, were constructed for each variable. The BetaPERT distribution requires estimates of minimum, most likely and maximum

values. In each case, the most likely value was the value given in Table 1 with maxima and minima plus and minus 10% of the most likely value.

Note that for the continuous variable ϕ , Faecal loads per person, Table 1 contains estimates of within population variability. However, since our models were constructed at the population level, within population variability is not relevant. Nevertheless, there may be some variation between populations due to socio-economic (e.g. diet) and biological factors (e.g. average person size, etc.) so such variability was modelled using minima and maxima plus or minus 10% of the most likely value.

Table 6 | Variables selected for use in Monte Carlo sensitivity analyses

Variable	Units	Symbol	Minimum	Likeliest	Maximum
Faecal load per person	g·day ⁻¹	φ	115.5	128.3	141.1
Proportion of population that are shedders	%	ω	0.00018	0.00020	0.00022
Wastewater discharge to sewer per person per day	L·person ⁻¹ ·day ⁻¹	λ	125.0	166.0	200.0
Solids production rate per L raw sewage	g·L ⁻¹	κ	0.198	0.220	0.242
Inactivation due to storage (rate per annum)	LRV	I_S	1.46	1.62	1.78
Soil adherence to root vegetable (using same as farmer hands adherence rate)	mg·cm ⁻²	ξ_{rv}	0.401	0.446	0.491
Soil adherence to spinach	%	ξ_s	0.09	0.10	0.11
Surface area of carrot	cm ²	A_C	152.7	169.7	186.6
Carrot ingestion rate	g·serve ⁻¹	P_C	33.5	37.2	40.9
Spinach/lettuce ingestion rate	g·serve ⁻¹	P_S	22.1	24.6	27.1
LRV due to crop washing	LRV	ψ	0.90	1.00	1.10
Beta-Poisson dose-response coefficient	d'm'less	α	0.09	0.10	0.11
N ₅₀ dose (soil)	ova·day ⁻¹	γ_s	31.5	35.0	38.5
N ₅₀ dose (vegetables)	ova·day ⁻¹	γ_v	773.1	859.0	944.9
Frequency of ingestion (salad & vegetables)	days·yr ⁻¹	τ_v	126.0	140.0	154.0
Available biosolids nitrogen, 1 year	kg·tonne ⁻¹	B_N	7.25	8.06	8.87
Carrots nitrogen uptake	kg·ha ⁻¹	C_N	225.0	250.0	275.0
Lettuce/spinach nitrogen uptake	kg·ha ⁻¹	LS_N	130.5	145.0	159.5

The PDF for each variable was a BetaPERT distribution assuming each input value is a most likely value and with minima (–) and maxima (+) 10% respectively, except for λ which as a BetaPERT distribution based on published data (from Melbourne metropolitan data for 2015/16 as reported on Melbourne Water's website Feb 2017 (Melbourne Water 2017)).

Table 7 | Variables excluded from Monte Carlo sensitivity analyses and reason for exclusion

Variable	Units	Symbol	Value	Reason for exclusion from sensitivity analyses
Avg no. of worms per infected person (shedder)	No. of worms	χ	2	Assume to be fixed at 2 worms per infected person, rarely greater (so not included in simulation assessment).
Inactivation due to treatment process	LRV	I_T	0	No data to assess this effect so conservatively modelled at zero.
Inactivation due to pan drying	LRV	I_{PD}	0	No data to assess this effect so conservatively modelled at zero.
Soil bulk density	g·cm ⁻³	ζ	1.3	Very conservative parameter – unlikely to vary greatly.
Depth of biosolids amended soil	cm	d_p	10	Unlikely to vary greatly in the Australian context due trades offs between cost and fertiliser efficiency.
Serves/day – carrot (non-leafy veg)	serves·day ⁻¹	o_c	2	Population-based statistic from the Australian Bureau of Statistics. Unlikely to vary significantly between surveys.
Serves/day – lettuce/spinach (leafy veg)	serves·day ⁻¹	o_s	1	
Disease burden per case	DALY·case ⁻¹	β	0.000651	Calculated from expert classification of disease burden at the population level, so no further need for sensitivity analysis.
Fraction of consumers susceptible to infection	d'm'less	δ	1	Due to the very low rates of infection in the population, it is reasonable to assume zero immunity.

Three additional model factors, the reduction rate in available biosolids per annum, $B_{N,Yr}$, and the NLAR for carrots and spinach, A_{CN} , and A_{SN} respectively, were derived by calculation from other input variables and thus were not allocated their own PDFs.

For Monte Carlo simulations, modelled scenarios were iterated 100,000 times using Oracle Crystal Ball, a simulation add-in software package to Microsoft Excel, and the results tabulated and reported as 5th, mean and 95th percentiles.

RESULTS

The exposure category with the highest disease burden was the outdoor worker, which is consistent with the fact that this category also has the highest exposure to soil. Using the Bangladesh ova shedding rates per worm the predicted disease burdens were slightly above the $1 \mu\text{DALY}\cdot\text{person}^{-1}\cdot\text{year}^{-1}$ guideline after 1 year of storage (Tables 8 and 9). To meet the guideline for outdoor worker exposure (the limiting exposure scenario) the biosolids storage period would need to be around 1.24 years (mean model estimate).

All other exposure categories were below the $1 \mu\text{DALY}\cdot\text{person}^{-1}\cdot\text{year}^{-1}$ guideline after 1 year of storage with reference to Bangladesh ova shedding rates.

Using ova shedding rates per worm based on Nigerian data gave a 32-fold increase in disease burden estimates and would require biosolids storage periods of around 2.1 years to achieve compliance with the guideline (mean model estimate).

The variation in model predictions between 5th and 95th percentile predicted disease burdens was relatively

narrow, lying well within one order of magnitude. This is not unexpected as, firstly, the continuous variables selected for Monte Carlo simulation would be at unrealistic values if permitted to vary more than one order of magnitude; secondly we constrained the PDFs for each variable to no more than $\pm 10\%$ of the most likely value, as we felt this to be a reasonable level of variation given the uncertainty associated with each variable.

Sensitivity analysis

The relative influence of each continuous model variable on model μDALY predictions was assessed by calculating correlation coefficients between each PDF variable and each model prediction (Figures 2 and 3). For the soil exposure models (Figure 2), variables with the greatest influence were the Beta-Poisson dose response coefficient, α , the inactivation log reduction value (LRV) due to storage, I_S , and the daily wastewater discharge to sewer per person, λ . Note that the results in Figures 2 and 3 are the same for the Nigerian and Bangladeshi data, since only the correlation between the input variable and the predicted disease burden is being graphed.

For the salad and vegetable consumption exposure models (Figure 3), variables with the greatest influence were the Beta-Poisson dose response coefficient, α , the inactivation LRV due to storage, I_S , the LRV due to crop washing, ψ , and the daily wastewater discharge to sewer per person, λ .

Interestingly, for each model, the LRV due to crop storage changes from the second most influential variable at

Table 8 | Predicted disease burdens for worker exposure scenarios in μDALY per person per year using ova shedding rates per worm from different source countries

Scenarios	Storage duration								
	1 year			2 years			3 years		
	5th	Mean	95th	5th	Mean	95th	5th	Mean	95th
Bangladesh rates									
Outdoor worker	1.40	2.34	3.63	0.04	0.06	<i>0.11</i>	0.00	0.00	0.00
Outdoor recreator (sporting field)	0.15	<i>0.24</i>	<i>0.39</i>	0.00	0.00	<i>0.02</i>	0.00	0.00	0.00
Domestic gardener	0.02	0.02	0.04	0.00	0.00	0.00	0.00	0.00	0.00
Nigeria rates									
Outdoor worker	46.29	75.73	115.75	1.12	2.24	3.91	0.02	0.07	<i>0.13</i>
Outdoor recreator (sporting field)	5.03	8.34	12.92	<i>0.11</i>	<i>0.24</i>	<i>0.41</i>	0.00	0.00	0.02
Domestic gardener	<i>0.41</i>	<i>0.69</i>	1.08	0.02	0.02	0.04	0.00	0.00	0.00

Values greater than 1 exceed the WHO and AGWR tolerable burden of disease guideline and are shown in bold font. Values less than one but greater than 10% of the guideline are shown in italics, while values less than 10% of the guideline are shown in normal font.

Table 9 | Predicted disease burdens for ingestion of salad and vegetable crops in μ DALY per person per year using ova shedding rates per worm from different source countries

Scenarios	Storage duration								
	1 year			2 years			3 years		
	5th	Mean	95th	5th	Mean	95th	5th	Mean	95th
Bangladesh rates	0.41	0.73	1.16	0.02	0.02	0.04	0.00	0.00	0.00
Nigeria rates	14.03	24.03	38.06	0.34	0.69	1.23	0.00	0.02	0.04

See legend for Table 7 for further explanation.

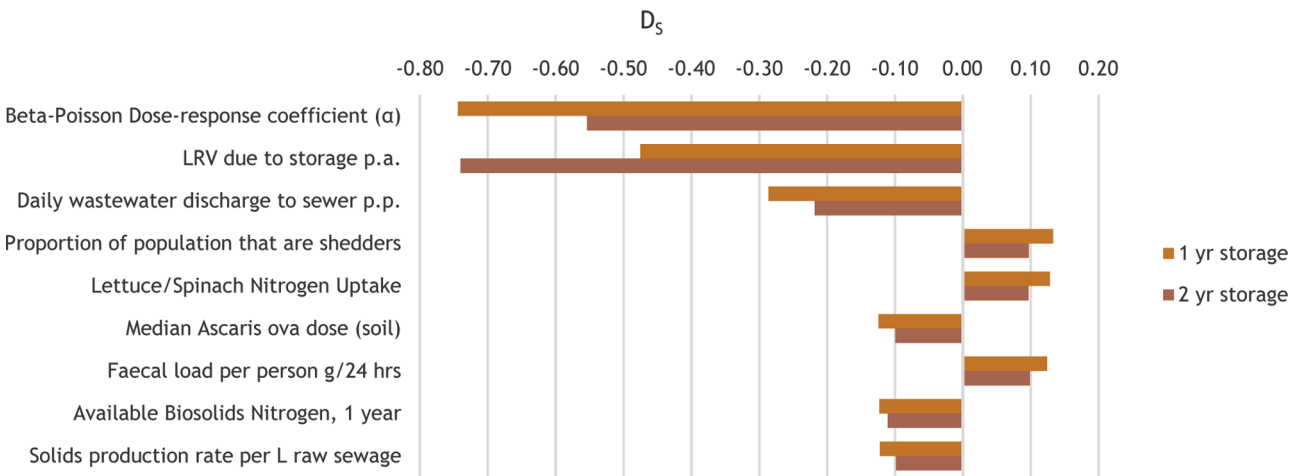


Figure 2 | Sensitivity analysis correlation between selected modelled input variables and predicted annual disease burden per person (soil), D_s , using the Outdoor Worker limiting risk category for soil exposure. Variables with correlation coefficients less than an absolute value of 0.1 are not shown. The relative influence of the LRV due to biosolids storage, L_s , increases greatly between years and becomes the most influential variable over 2 years. The effect is slightly greater over 3 years (not shown).

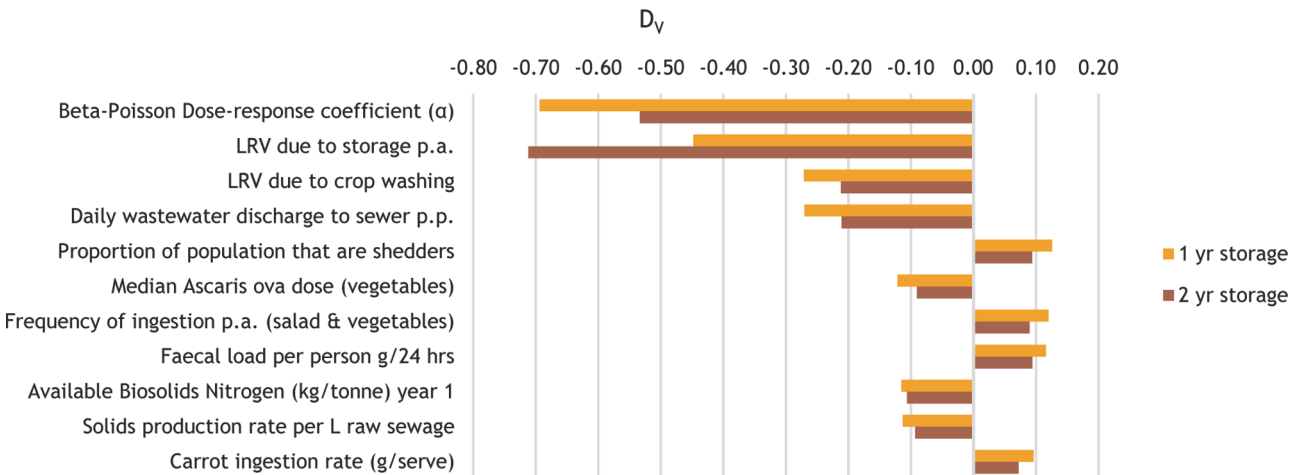


Figure 3 | Sensitivity analysis for modelled input values using the salad/vegetable crop consumption risk category correlation between selected modelled input variables and predicted annual disease burden per person (salad/vegetable crop consumption), D_v . Variables with correlation coefficients less than an absolute value of 0.1 are not shown. The relative influence of the LRV due to biosolids storage, L_s , increases greatly between years and becomes the most influential variable over 2 years. The effect is slightly greater over 3 years (not shown).

1 year of storage, to the most influential variable with 2 or more years of storage (Figures 2 and 3), because there is more time for the variable to exert an influence in the 2 years and greater storage scenarios, whereas the other factors do not change between scenarios.

DISCUSSION

Risk modelling of the potential human health impacts from the presence of *Ascaris lumbricoides* ova in biosolids was undertaken for discrete holding periods of 1, 2 and 3 years. Using ova shedding rates per worm derived from Bangladeshi data, risk modelling showed that the predicted disease burden for a 1-year holding period slightly exceeded the WHO $1 \mu\text{DALY}\cdot\text{person}^{-1}\cdot\text{year}^{-1}$ guideline for the outdoor worker category; 1.24 years would be required to meet the guideline. This outdoor worker category has the greatest occupational exposure to biosolids amended soil. Using ova shedding rates per worm derived from data from Nigeria indicated holding periods of 2.1 years would be required to protect workers exposed to biosolids amended soils.

Using Nigerian data, holding periods of around 1.9 years (~23 months) would be required to protect consumers of fresh, uncooked salad and root vegetable crops grown in biosolids-amended soils. Clearly the choice of data for ova shedding rates per worm has a major bearing on the model results. Possible reasons for the difference in ova shedding rates per worm between the countries are differences in laboratory recovery rates and the impact of average worm burdens on ova shedding rates. In the latter case, the presence of other *A. lumbricoides* in the gut acts to inhibit the production of eggs. This is known as density-dependent fecundity and results in the number of eggs shed per female worm decreasing in proportion to the worm burden in the gut (Walker *et al.* 2013). In any event, since the ova shedding rate of worms infecting south-eastern Australian hosts is not known, the conservative default assumption is to assume the higher shedding rate.

Areas for further research

Sensitivity analyses showed the following variables had the greatest influence on the soil exposure and salad/root vegetable consumption models: the Beta-Poisson dose response coefficient, α , the inactivation LRV due to storage, I_S , the LRV due to crop washing, ψ , and the daily wastewater discharge to sewer per person, λ . In addition, the choice of

ova-shedding rate per worm and the level of exposure to soil, or consumption of salad and vegetable crops grown in biosolids-amended soil are also influential variables.

Two potentially influential variables were set to zero (i.e. no effect) in the models due to lack of information on their values. These were *Ascaris ova* inactivation LRV due to MAD, I_T , and due to sludge pan drying, I_{PD} . It is possible that a combined LRV of 1 to 2 or more could be applied to the model if *Ascaris ova* inactivation through these processes was appropriately characterised.

The inactivation rate due to storage was based on an assumed average biosolids temperature of 20 °C; however, the effect of high diurnal and seasonal temperature fluctuations experienced by south eastern Australian biosolids plus the heat generated by decomposition means that a higher inactivation rate is likely to be in effect. A better understanding of short term exposures to higher and lower temperatures on *Ascaris ova* inactivation through field studies could clarify this issue.

The soil and produce exposure variables (see Tables 1, 3 and 4), along with λ are relatively well understood in the Australian context and can be considered a lower priority for research.

In summary, the following factors can be considered as a high priority for future research: α , I_S , I_T , I_{PD} , ψ , and choice of country upon which to base the ova-shedding rate per worm coefficients a and b. These variables should be the focus of future research to improve model accuracy.

Background *Ascaris* infection rate in the population

The proportion of the population that carry an *Ascaris* infection, and thus shed *Ascaris* ova, was based on data for Denmark (Arnbjerg-Nielsen *et al.* 2005). In the current model a proportion of 0.02% of the population (1 shedder per 5,000 as per the Danish study) gives an estimated biosolids ova concentration of just under 0.2 ova-g⁻¹ dry weight, which is the current analytical detection limit available from commercial pathogen laboratories in Australia. Since most of the ova testing of data for biosolids in south eastern Australia yields zero results, either the analytical recovery rates are overstated, ova inactivation through the biosolids production process is greater than modelled, or the true proportion of shedders in the Australian STP sewage catchments is less than that reported for Denmark.

In relation to the proportion of shedders in the Australian STP sewage catchments, two key factors are of importance: (i) modern sanitation and (ii) the practice in Australia of administering anti-helminthic medicines to

new migrants and refugees. Modern sanitation acts to impede the natural life cycle of *Ascaris* and other STH. With respect to *Ascaris lumbricoides*, the ideal habitat is a rural village where villagers defecate in the open near their dwellings or, if using a basic latrine, the nightsoil is reused to fertilise crops. *Ascaris* ova must undergo a period of soil conditioning before becoming infective. In developed countries, the presence of flushing toilets is nearly universal and acts to remove the opportunity for *Ascaris* ova to encounter a new host, or to receive soil conditioning around the dwelling and become infective. Unless reinfection occurs within a couple of years, the worms reach the end of their life span – around 1.5 years (Feachem *et al.* 1983) - and are ejected in the host's faeces. Pathways for reinfection are therefore limited.

New migrants who are refugees typically come from refugee camps which often are constrained environments with poor sanitation. In the Australian context, such refugees are administered the anti-helminthic medicine albendazole as part of the predeparture medical assessment conducted on behalf of the Australian Government (Hanieh *et al.* 2016). The medication is particularly effective for controlling nematode infections (Swanson *et al.* 2012) and such practices also act to limit the prevalence of most STH.

CONCLUSIONS

The risk modelling has highlighted the key variables that determine the risk of transmission of *Ascaris* through biosolids use for growing human edible horticultural crops. It is argued that in the absence of a strong justification for using other data sources, the Nigerian data for ova shedding rate per worm should be preferred to guide responses to the risk modelling. The limiting risk is outdoor worker soil exposure and, based on the Nigerian data, an additional LRV of 1.6 is required in biosolids ova concentration from raw sewage to biosolids to meet the WHO μ DALY guideline for a 1-year holding period based on most likely model inputs. The risk models used in this study are considered conservative, and it is feasible that improvements in the characterisation of several key input variables could readily demonstrate improved *Ascaris* ova LRVs and lower predicted disease burdens to the degree that a 1-year storage period for biosolids produced by the MAD process meets the WHO μ DALY guideline.

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