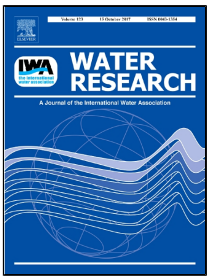
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Helminth log reduction values for recycling water from sewage for the protection of human and stock health

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PII:	S0043-1354(17)30720-0
DOI:	10.1016/j.watres.2017.08.069
Reference:	WR 13192
To appear in:	*Water Research*
Received Date:	16 March 2017
Revised Date:	15 July 2017
Accepted Date:	24 August 2017

Please cite this article as: Daryl P. Stevens, Aravind Surapaneni, Rachna Thodupunuri, Nicholas A. O'Connorc, David Smith, Helminth log reduction values for recycling water from sewage for the protection of human and stock health, *Water Research* (2017), doi: 10.1016/j.watres.2017.08.069

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Highlights

- Two human disease burdens were defined for exposure to HE from *A. lumbricoides*
- An LRV of 3 for HE achieved health base targets (HBT) for a non-endemic region
- 3 LRV for Helminth Eggs (HE) achieved an HBT of 100 μDALY in an endemic region
- A log reduction value (LRV) of 3 for HE related to cattle and pigs was acceptable
- Design equations for HE removal were derived for ASPs and lagoons

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Helminth log reduction values for recycling water from sewage for the protection of human and stock health.

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Abbreviations

AGWR	Australian Guidelines for Water Recycling
ASP	Activated sludge plant
DALY	Disability adjusted life year
epg	eggs per gram
HE	Helminth egg
HRT	Hydraulic retention time
HBT	Health-based target
LRV	Log reduction value
PDF	Probability density function
QMRA	Quantitative microbial risk assessment
STP	Sewage treatment plant
WHO	World Health Organisation
YLD	Years lived with disability
YLL	Years life lost
pppy	per person per year
SE	South-east

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Graphical abstract

See attached.

Highlights

See attached

Abstract

The LRVs required to decrease HE concentrations in raw sewage to an acceptable level to manage the risk to human and livestock health were determined. An LRV of 3.0 was required to meet the HBT of 1 µDALY pppy in SE Australia where human helminth infections are not endemic. In comparison, a similar exposure volume and LRV in endemic regions would result in a HBT of 100 µDALY pppy.

The risks posed by cattle- and pig-related helminths were also managed acceptably with the treatment of sewage providing an LRV of 3.0.

New design equations were derived to determine LRVs based on hydraulic residence times (HRTs) in an activated sludge plant (ASP) and lagoons. The new equation for lagoons indicated that an LRV of 3.0 could be achieved with a HRT of 18 days or less.

Keywords: QMRA, Helminth Guidelines, Log Reduction Value, recycled water, raw sewage, *Ascaris lumbricoides, Taenia spp.*, *Fasciola hepatica,* Eggs (Ova)

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1 INTRODUCTION

There is a strong demand for recycled water in many countries due to population growth and limited new supply options from natural water sources. Typically, water recycled from sewage is used to substitute potable water in non-potable applications. In Australia, water recycled from sewage is supplied for a number of uses including the irrigation of a broad range of crops and pasture for livestock. These uses involve significant exposure of humans and animals to the recycled water, which must therefore be treated to reduce pathogen concentrations to safe levels.

The major pathogen groups, bacteria, viruses, protozoa and helminths, differ in the extent to which they can be removed by different treatment processes. While certain types of filtration can be effective for all groups (e.g. membrane filtration), the process is relatively expensive. Consequently, lagoon detention (in sewage oxidation ponds) followed by primary disinfection with chlorine, UV or both is a common treatment arrangement for pathogen reduction in Australian sewage treatment plants (STPs). However, HEs are relatively thick and multilayered and resistant to chlorine and UV disinfection (Guadagnini et al., 2013; Wharton, 1983). Therefore, the Australian Guidelines for Water Recycling (AGWR) specify a lagoon HRT of ≥ 25 days, or equivalent treatment to achieve a HE LRV of 4.0 in order to manage helminth risks to human and livestock health (NRMMC et al., 2006). This guideline is predominantly based on international guidelines and historical experience in Australia.

The WHO guideline for the safe use of wastewater in agriculture specifies a limit of ≤ 1 HE/L (arithmetic mean) and recommends additional measures to protect the health of children under 15 years, i.e., ≤ 0.1 HE/L or chemotherapy (Navarro and Jiménez, 2011; WHO, 2006). The guideline indicates that the required LRV should relate to the HE concentration of HE in the raw sewage. For example, if HE concentrations in the raw sewage are < 100 HE/L, an LRV of 2 would be required to achieve < 1 HE/L. A key point to note is that the WHO guidelines are based on epidemiological data from countries where helminths are endemic. In many countries with good sanitation systems (e.g., Australia) helminth infections are not endemic in humans and HEs are not commonly detected in raw sewage (Grant et al., 2012; Irwin et al., 2017). In these countries the mean concentration of HEs in raw sewage is expected to be lower than 1 HE/L, which meets the limit specified in the WHO guideline in the absence of any additional treatment. This finding suggests that: (i) the AGWR treatment requirements and those in other countries where helminths are not endemic are too conservative, (ii) the LRV required for HEs is much less than 4.0, and (iii) the lagoon HRTs could be reduced from 25 days and still achieve the limit specified in the WHO guideline.

In this study, we explore the relationship between HE concentrations in raw sewage, LRVs through the sewage treatment process and guidelines for the protection of human and livestock health. Based on this analysis, the objective was to determine the minimum HE LRV for the treatment of raw sewage to ensure that the health risks to humans and livestock from HE meet the relevant guidelines. Consideration is also given to the different HE

concentrations expected in raw sewage between countries with and without signficant helminthic disease burdens and to the different guidelines that may apply.

2 Methods

2.1 Risk posed by Helminth Eggs in recycled water

Sedimentation in lagoons is one of the main processes to remove HEs as they are resistant to disinfection processes. Filtration processes are not commonly used in Australia and other parts of the world, however, their efficacy in removing HEs is otherwise well established. To assess HE removal in ASPs and lagoons, a suitable reference pathogens is required that present a worst-case combination of high prevalence in sewage, high pathogenicity, low removal in treatment and long survival in the environment (NRMMC et al., 2006).

In the South East (SE) Australia context, appropriate reference helminths are the cestodes *Taenia saginata* and *Taenia solium* (beef and pork tapeworms, respectively), the trematode, *Fasciola hepatica* (liver fluke), which is common in cattle in SE Australia, and the nematode *Ascaris lumbricoides* (human roundworm). Although the nematode *Trichuris trichiuria* (human whipworm) is endemic in some remote parts of northern Australia, it is believed to be absent from SE Australia and we have excluded this species from further consideration.

The pathways of exposure and potential risk assessed in this paper are outlined in Table 2-1. Although this study was focused on helminth risks in SE Australia where human helminth-related diseases are not endemic, for comparison, risks were also evaluated for *A. lumbricoides* in endemic countries.

The nature of the risk assessment varied depending on the reference helminth. The disease burden was calculated in Disability Adjusted Life Years (DALYs) using the published dose-response model for *A. lumbricoides* (Navarro et al., 2009; Navarro and Jiménez, 2011) and compared against accepted HBTs. This assessment is consistent with the standard approach for quantitative microbial risk assessment (QMRA) (WHO, 2011). For the other reference helminths (i.e., *Taenia spp.* and *Fasciola spp.*), no human or livestock dose-response models were available and it was not possible to calculate health impacts in DALYs. For these reference pathogens, the compliance target was the concentration of HE/L as recommended by the WHO guideline for wastewater reuse in agriculture (<0.1 HE/L) (WHO, 2006) and achieving acceptable LRVs. These assessments are considered to be qualitative rather than quantitative. If the LRV required to meet the HBT for human-to-human exposure, also results in the appropriate HE concentrations in recycled water by using acceptable LRVs for all exposure pathways listed in Table 2-1, then the stock and human health risks via these exposure pathways can be considered to be controlled.

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Table 2-1 Overview of helminth, pathway and assessments used

Helminth	Exposure pathway	Risk	Infection	Assessment	Typical	Scenario[A]
Ascaris lumbricoides	Human → Human	Human health	Ascariasis	QMRA	Yes	1
Taenia saginata	Human → Cattle	Cattle meat and health	Cysticercosis	Qualitative	Yes	1
	Human → Cattle → Human	Human health	Taeniasis	Qualitative	Yes	1
Taenia solium	Human → Pig	Pig meat and health	Cysticercosis	Qualitative	Yes	1
	Human → Pig → Human	Human health	Taeniasis	Qualitative	Yes	1
	Human → Pig → Human → Human	Human health	Cysticercosis	Qualitative	Yes	n/a
Fasciola hepatica	Cattle → Cattle	Cattle health	Fascioliasis	Qualitative	Yes	2

QMRA = Quantitative Microbial Risk Assessment, n/a = not assessed. [A]described in Section 2.1.3.

2.1.1 Human to human exposure route – *A. lumbricoides*

2.1.1.1 Sensitivity analyses

Since many of the variables making up the risk model are associated with some level of uncertainty, sensitivity analyses were conducted using Monte Carlo simulation. The simulations were conducted using @Risk (Professional version 6.1.1) and 10,000 Latin hypercube sampled iterations (Palisade P/L., 2012). Pert Probability Density Functions (PDFs), commonly used in modelling expert estimates, were constructed for each variable. The Pert distribution requires estimates of minimum, most likely (mode) and maximum values for each variable considered (with the range determined from the relevant literature; Table 2-2 and Table 2-3). Output values calculated from the simulations were recorded as the median value with the error range in parentheses (5^{th} and 95^{th} percentiles; Table 2-2).

2.1.1.2 DALY per case

The DALY per case (DALYc), or disease burden per case, for *A. lumbricoides* was estimated as described in the AGWR and WHO guidelines (NRMMC et al., 2006; WHO, 2011), using the following equations:

$$DALYc = YLD + YLL$$

Equation 1

Where:

YLD = years lived with a disability or illness
YLL = years of life lost

$$YLD \text{ or } YLL = S \times D \times F$$

Equation 2

Where:

S = Severity (Disability weighting or degree of health impact)
D = Duration (years)
F = Frequency (Portion of population that show each symptom with a disability weighting)

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2.1.1.3 Years lived with a disability (YLD)

Two regions are outlined in Table 2-2, these are reasonable worst-case values for: (i) regions without endemic helminths (e.g., SE Australia), and (ii) endemic countries. The duration (D) mode used for the PDF assumed that symptoms would be treated relatively quickly in developed countries (e.g. Australia) where medication is readily available, compared to endemic developing countries where medical resources are limited (Table 2-2).

Frequency (F) values represent a worst-case for non-endemic countries where medical treatment is readily available (e.g., Australia). In this case, the frequency would be expected to be less given that moderate infection could be treated and this would also minimise the frequency of heavy infections.

To span the significant variation in the literature, Pert PDFs were used with the minimum and maximum set at 50% of the mode (S) or 50% lower and 100% higher (D, F) (Brooker and Pullan, 2013; Chan et al., 1994; de Silva et al., 1997; Pullan et al., 2014).

Table 2-2 Possible symptoms for *A. lumbricoides* and the related Severity (S), Duration (D) and Frequency (F) values used for probability density functions

Symptom [A]	Infection intensity	Region	Severity (S)	Duration (D)[B]		Frequency (F) *A. lumbricoides*	DALYc calculation (S x D x F)[C]
			A. lumbricoides	(days)	(years)		
Mild abdomino-pelvic problems	Medium	SE Australia	0.011 (0.005, 0.0162)	183 (91.3, 365)	0.5 (0.25, 1.0)	0.0463 (0.0231, 0.0927)	$2.83x10^{-4}$
		Endemic region		548 (274, 1095)	1.5 (0.75,3.0)		$8.49x10^{-4}$
Symptomatic infection	Heavy	SE Australia	0.030 (0.015, 0.044)	30 (15, 60)	0.08 (0.04,0.16)	0.0092 (0.0046, 0.0184)	$2.53x10^{-5}$
		Endemic region		60 (30,120)	60 (0.08,0.33)		$5.07x10^{-5}$
Wasting	Heavy	SE Australia	0.125 (0.062, 0.187)	30 (15, 60)	0.08 (0.04,0.16)	0.0079 (0.0039, 0.0158)	$9.14x10^{-5}$
		Endemic region		60 (30,120)	60 (0.08,0.33)		$1.83x10^{-4}$
Death		SE Australia	1	29,200[E]	80[F]	$3.7x10^{-6}$ ($1.9 x10^{-6}$, $7.4 x10^{-6}$)	$3.15x10^{-4}$
		Endemic region		25,550[E]	70[F]	$5.4 x10^{-6}$ ($2.7 x10^{-6}$, $1.1 x10^{-5}$)	$4.60x10^{-4}$
References for DALYc		SE Australia	(Brooker and Pullan, 2013; Pullan et al., 2014)	(Albonico et al., 1999; Belizario et al., 2003; Khandelwal et al., 2008)		(Brooker, 2010; Brooker and Pullan, 2013; Chan et al., 1994; de Silva et al., 1997; Pullan et al., 2014)	$7.22x10^{-4}$
		Endemic region					$1.54x10^{-3}$

Values are conservative mode estimates for non-endemic countries; parentheses indicate the minimum and maximum values used in Pert PDFs (Section 2.1.1.1). [A]Sequelae and disabling consequences to humans, [B]For *A. lumbricoides* with the assumptions that medication is readily available and accessed once symptoms are identified and that disease rates would be lower due to early treatment and good sanitation preventing reinfection, [C]Median values calculated from probability density functions, [F]Duration of life was not varied for the sensitivity analysis (NRMMC et al., 2006).

2.1.1.4 Years life lost (YLL)

Severity (S) of death was defined as 1 and a duration (D) of 80 years was assumed as the life expectancy in Australia versus 70 years in developing countries (typical of endemic regions) (NRMMC et al., 2006; UNDP, 2016).

Frequency (F) estimates of death caused by *A. lumbricoides* were previously reported as 10,000 deaths per 1.274 billion infected ($F=7.85x10^{-6}$) (de Silva et al., 1997). However, a more recent estimate has been used here for Australia ($F=3.7x10^{-6}$) and an average for endemic countries ($F=5.4\ x10^{-6}$) (Table 2-2). Mortalities due to intestinal nematodes are usually only associated with heavy infection in countries where helminths are endemic. In contrast, no deaths due to helminth infections were recorded among the 1.425 million deaths reported in Australia from 2005 to 2014 (ABS, 2016).

Much of the information in Table 2-2 was derived from total populations, so it includes impacts on children and adults. However, no weighting was given specifically to consider children separately (de Silva et al., 1997).

2.1.1.5 Dose equivalent to meet DALY health-based target (HBT)

The dose equivalent (DALYd) was calculated using the logic from the AGWR (NRMMC et al., 2006) (Equation 3), but with a modification (Equation 4) to overcome limitations of a simplification of the dose response curve in Equation 3 (FAO UN and WHO, 2003):

$$DALYd = DALY \times \frac{\beta}{\alpha} \div R_{i:f} \div DALYc \div S_{fr}$$

Equation 3

Where:

DALY = acceptable DALY HBT
α and β = constants in the beta-Poisson dose-response model (Table 2-3)
$R_{i:f}$ = The ratio of illness:infection (Table 2-3)
DALYc = DALY per case (disease burden) (Equation 1)
S_{fr} = Susceptibility fraction (Table 2-3)

To overcome dose response curve limitations and allow a higher HBT to be considered, as proposed by Mara and Sleigh (2010) (e.g., acceptable DALYs of 1.0×10^{-4} or 1.0×10^{-5} pppy), the dose-response curve (Equation 5) was incorporated into Equation 3 (as Pinf = DALYd x α/β) to derive:

$$\mathrm{DALYd} = \left\{\left[\frac{1}{\left(1-\left(\mathrm{DALY}/\left(R_{i:f} \times \mathrm{DALYc} \times S_{fr}\right)\right)\right)}\right]^{\frac{1}{\alpha}} - 1\right\} * \beta$$

Equation 4

Where:

DALYd = Dose equivalent to the acceptable DALY
DALY = acceptable DALY (10^{-4}, 10^{-5} and10^{-6})
$R_{i:f}$ = The ratio of illness:infection (Table 2-3)
DALYc = DALY per case (disease burden) (Equation 1)
S_{fr} = Susceptibility fraction (Table 2-3)
α and β = constants in the Beta-Poisson dose-response model (Table 2-3)

2.1.1.6 Ratio of illness:infection ($R_{i:f}$) and Susceptibility fraction (S_{fr})

The illness:infection ratio ($R_{i:f}$) for *A. lumbricoides* is commonly assumed to be 1, although it can range between 0.1 to 1.0 (Jimenez, 2008; Mara et al., 2010). The susceptibility fraction (S_{fr}) was also set at 1 (i.e., 100%) (Table 2-3).

Typical ingestion volumes of recycled water used were the largest volume defined in the AGWR (NRMMC et al., 2006) (720 mL/yr from dual reticulation and municipal irrigation).

Helminth egg viability in sewage was not explicitly included in the equations. It was assumed to be 100% although there have been reports of it being 80% to 90% (Gupta et al., 2009).

Table 2-3 Summary of other distributions used in the calculation of log reduction requirements

Parameter	Abbreviation	Mode (minimum, maximum)	Distribution	Section
Ratio of illness:infection	$R_{i:f}$	0.9 (0.1,1.0)	Pert	2.1.1.6
Susceptibility fraction	S_{fr}	1.0	Single value	
Constants in the beta-Poisson dose-response model	α	0.104 (0.104, 0.83)	Pert	2.1.1.7
	β	1.1 (0.8, 7.0)		
Helminth eggs in raw sewage: SE Australia	HE/L	1.0 (0.5, 10.0) (Median 2.1)		2.1.1.9 and 3.1
Helminth eggs in raw sewage: Endemic country		500 (50,1000) (Median 506)		Estimate from (Jiménez et al., 2007)

2.1.1.7 Dose-response curve

The approximate beta-Poisson dose-response curve was derived for *A. lumbricoides* based on outbreak data and epidemiological studies where produce (spinach) was irrigated with recycled water (Equation 5) (Navarro and Jiménez, 2011).

$$P_{inf} = 1 - \left(1 + \frac{D}{\beta}\right)^{-\alpha}$$

Equation 5

Where:

P_{inf} = Probability of infection if exposed to a dose of *A. lumbricoides* eggs
D = Dose of *A. lumbricoides* eggs
α and β = constants in the approximate beta-Poisson dose-response model (Table 2-3)

A degree of caution is required to adopt this dose-response curve (Equation 5) since it was not derived from a typical controlled dose-response experiment. Navarro and Jiménez (2011) recognised that it was difficult to obtain outbreak data of helminth disease in countries where helminths are endemic and the epidemiology is complicated by the delay often experienced between exposure and the symptoms. They assumed the main exposure routes were the consumption of uncooked vegetables (irrigated with untreated wastewater) and the accidental ingestion of soil from land irrigated with recycled water (Navarro et al., 2009; Navarro and Jiménez, 2011). Despite the lack of controlled experiments on humans, the proposed dose-response curve remains the best estimate currently available.

To account for uncertainty in the derivation of the dose-response curve, values for α and β were substituted in model calculations with Pert PDFs (Table 2-3). The PDFs were used to provide ± 1 order of magnitude for a dose of ≤ 0.1 HE (Figure 2-1 left) (Haas et al., 2014) and provide a conservative estimate for higher HE concentrations (Figure 2-1 right). Figure 2-1 shows the variability of the dose-curve considered in (Equation 5) from the ranges of α and β and

compares this to ranges typically covered by dose-response relationships for QMRAs for a diverse range of pathogens. The results indicate that the curve developed for HE is consistent with those for viral, bacterial and protozoan reference pathogens. The limitations of the approximate beta-Poisson dose-response model (Equation 5) were recognised as estimates of the exact beta-Poisson model (Brouwer et al., 2017; FAO UN and WHO, 2003). However, as the approximate model was used to describe the original dose-response, it was considered appropriate to use in this study.

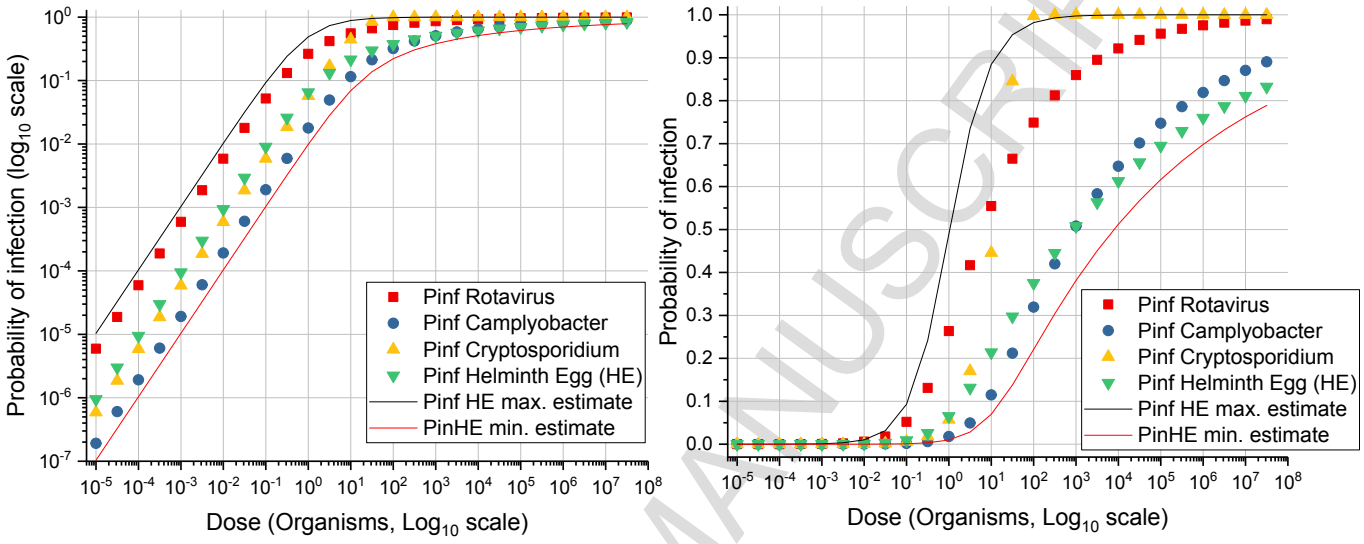


Figure 2-1 Probability of Infection from doses of helminth eggs and range of variation of α and β across dose-response curves in the AGWR for rotavirus, *Campylobacter* and *Cryptosporidium* (NRMMC et al., 2006) (Scale: Left log-log and right log-normal).

2.1.1.8 Log Reduction Value (LRV)

The LRV for *A. lumbricoides* required to achieve the acceptable DALY was calculated using the methodology described in the AGWR (NRMMC et al., 2006):

$$LRV = Log_{10}\left[\frac{HE \times E \times N}{DALYd}\right]$$

Equation 6

Where:

LRV = Log Reduction Value required to meet the acceptable DALY (pppy)
HE = Helminth eggs per L (HE/L) (Table 2-3)
E = Exposure per event (L) (Table 3-1)
N = Number of events per year (Table 3-1)
DALYd = Equation 3

2.1.1.9 Helminth egg concentrations in raw sewage

To obtain an estimate of HE concentrations in raw sewage for SE Australia, raw sewage samples (n=169) were collected as 24-hr flow-weighted composite samples from four STPs SE of Melbourne, Victoria, Australia (Nov. 2014 to Jan. 2017) and tested for HEs. Sampling times were devised to capture every weekday as well as summer and

winter months from each STP. The number of eggs of *Ascaris spp.* and *Taenia spp.* in the samples were determined in the laboratory using a modification of the method of Bowman *et al.* (2003; Shahsavari et al., 2017). The limit of detection was 1 HE/L. *Ascaris lumbricoides* and *Taenia spp.* were quantified while *F. hepatica* and *T. trichiura* were assessed for presence. All HEs detected were considered viable for this investigation. *Ascaris suum* eggs were used to determine the recovery rate, the value of which ranged from 50 to 87%.

2.1.2 Stock related exposure route – *Taenia spp.* and *Fasciola*

Humans and livestock

A qualitative approach was used for the human to livestock to human exposure route as there were no dose-response curves or HBTs identified for *Taenia spp*. The qualitative approach considered the frequency of occurrence, life cycle analysis of *Taenia spp.* and control measures in place to minimise the risk posed to cattle, pigs and humans via this route. These risks and HE concentrations were then compared to currently accepted control measures which include: removal via sewage treatment processes (i.e., ≥ 25-day lagoon HRT or equivalent), cooking of meat, hand hygiene, meat inspection, availability of medication, and the prohibition on the supply of recycled water to pigs directly or indirectly via crops irrigated with recycled water.

The eggs of *Taenia spp*. in human faeces were estimated for Scenario 1 (Section 2.1.3) as they are usually not counted due to the intermittent nature of expelling of proglottids. Estimations assumed 100,000 HEs per proglottid for *T. saginata* (50% less for *T. solium*), approximately six proglottids are released per day and humans are usually parasitized by a single tapeworm (i.e., 600,000 HE/day/person) (CDC, 2016; Murrell, 2005).

Livestock only life cycle

The exposure pathway for *F. hepatica* (typical of stock related helminths) is from cattle faeces via water to cattle; thus, *F. hepatica* HEs would typically not be found in human sewage.

2.1.3 High pathogen load scenarios in the sewerage catchment

Two scenarios, each rare hypothetical events, were devised to assess the impact of higher HE loads in a small sewer catchment (Table 2-4). The scenarios allowed consideration of abnormal variation in HE load to the STP and the types of extremes for which the LRV should provide appropriate protection. The scenarios were:

1. Travellers returning home infected with *A. lumbricoides or Taenia* spp. (Table 2-4). Australian citizens or permanent residents returning home was the focus of this scenario as helminth loads from immigrants or migrants entering Australia should be minimal given the prerequisite health check requirements of the Australian Department of Immigration and Border Protection (DIBP, 2017; Hanieh et al., 2016).

2. Livestock faeces entering the sewer, where cattle graze a pasture that has been irrigated with recycled water and therefore may be exposed to HE (e.g., *F. hepatica*) (Table 2-4). This scenario assumed that wash-down from livestock yards and trucks was subject to only limited on-site treatment before discharge to the sewer (Typical of SE Australia).

Table 2-4 Assumptions used for rare, high pathogen load scenarios

Scenario 1 – Infected human group			Scenario 2 – Cattle faeces entering sewer		
Parameter	Value	Reference and assumptions	Parameter	Value	Reference and assumptions
People	48 (Bus load)	Assumes equivalent of a bus load of infected people live in same sewer catchment	Cattle yards	3400 cattle, all infected	Maximum capacity of livestock yard in sewer catchment
Faeces load	5,000 epg (*A. lumbricoides*)	(Montresor et al., 1998) assuming infection is light before symptomatic and treated	Cattle manure (530 kg animal)	31,800 g/manure/day	(QDAF, 2016)
	4,688 epg (*T. saginata*)	(CDC, 2016; Murrell, 2005)	Portion of manure entering sewer	5% of daily total from in cattle yards and 5% in truck washdown[A]	(Gregory et al., 2000; Pethick, 2006)
	2344 epg *T. solium*)			10% enters sewer	Due to trade waste management[B]
Human stool (average)	128 g/person/day	Average stool (Rose et al., 2015)	HE in cattle faeces	4 HE/g (*F. hepatica*)	(Brockwell et al., 2014; Vercruysse and Claerebout, 2001)
HE base load[C]	0.77, 0.72 and 0.36 HE/L for Faeces load, respectively	HE already entering the sewage before rare event	HE base load	0.0 HE/L	Single source entering sewer
Sewage catchment	30,000 equivalent people	Typical of smaller sewer catchments in SE Melbourne	Sewage catchment	30,000 equivalent people	Typical of smaller sewer catchment in SE Melbourne
Raw sewage inflow to STP	5,000 KL/day	Actual flow into STP from catchment above	Raw sewage inflow to STP	5,000 KL/day	Actual flow into STP from catchment above

[A]Assumes average cattle live weight of 530 kg produces 31.8 kg of manure (faeces plus urine), an average of 4 hours in livestock yard, truck washing and cattle yards waste are combined (conservative as some stockyards only discharge truck wash-down to sewer post-pretreatment) and stock are fasted for 24 hours before transport to market (Gregory et al., 2000; Pethick, 2006). [B]Confirmed as conservative, given average total suspended solids in volumes leaving sale yard in sewer catchment. [C]Assumes 1 out of 5,000 people in sewer catchment already infected. HE = Helminth eggs. epg = eggs per gram

The calculations to determine the concentration of HEs in the raw sewage of the STP from the two scenarios were as follows:

Scenario 1:
$$HE_{RS} = HE_B + \frac{NI \times FP \times HE_A}{V_{RS}}$$

Equation 7

Scenario 2:
$$HE_{RS} = HE_B + \frac{NI \times FP \times HE_A \times 0.01}{V_{RS}}$$

Equation 8

Where:

HE = Helminth egg concentration (HE/L),
HE_{RS} = HE in raw sewage,
HE_B = HE base load
NI = Number of people or stock infected
FP = Faeces or manure produced (g/animal/day)
HE_A = HE concentration in human or animal faeces from scenario (HE/g faeces)
V_{RS} = Volume of raw sewage (L/day).

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312 0.01 considers losses and portion of manure entering the sewer.

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2.2 Helminth egg removal in ASPs and lagoons

2.2.1 Helminth egg removal in ASPs

Suitable data (n=9) for assessment of HE removal in ASPs were identified from Tunisia, Australia (seeded), USA, India and Egypt (Ben Ayed et al., 2009; Cram, 1943; Stadterman et al., 1995; Stott, 2003; Tyagi et al., 2011; Wen et al., 2009).

2.2.2 Helminth egg removal in lagoon systems

Suitable data for HE removal from lagoon systems was sourced from Brazil, India, Morocco, Spain, Burkina Faso, Egypt and Kenya (Gambrill *et al.*, 1992; Ouazzani *et al.*, 1995; Grimason *et al.*, 1996; Mandi *et al.*, 1996; Kouraa *et al.*, 2002; Shalaby *et al.*, 2003; Tyagi *et al.*, 2008; Reinoso *et al.*, 2011; Tyagi *et al.*, 2011; Konaté *et al.*, 2013). These were all endemic countries with relatively high HE concentrations. All data for lagoon LRVs weres taken from 1992 onwards, to avoid repeating any data used in the 1992 study (Ayres et al., 1992). There were also some data in the literature recognised as being from lagoons with poor design (stated or interpreted where possible). These data were shown to indicate HE removal from poorly designed or maintained lagoons (Verbyla et al., 2013). However, these were not used for the linear regression. Poor lagoon design was identified as large single lagoons, compromised hydraulic flows and non-ideal inlet/outlet structures.

The 1992 lagoon HE removal data points (n=21) were extracted from Figure 1 in Ayres *et al.* (1992).

3 Results and discussion

3.1 Risks posed by helminths

3.1.1 *Ascaris lumbricoides* – Human to human exposure route

Human to human risk – Typical

The HE concentration measured in the raw sewage samples from the SE Australian STPs were all < 1 HE/L. This equates to < 2 HE/L if a 50% recovery rate is used (Section 2.1.1.9). The QMRA for *A. lumbricoides* used a Pert PDF with a conservative HE median of 2.1 HE/L (Table 2-3). If all the data was consolidated for each of the STPs, then the actual HE concentration was < 0.02 HE/L (after adjusting for 50% recovery).

Due to the higher duration of symptoms and higher death frequency in endemic regions, the DALYc for endemic regions was more than twice that calculated for SE Australia (Table 3-1).

An LRV of 2.6 was required for the treatment of HEs in sewage to meet the DALY HBT (95th percentile, Table 3-1) for recycled water. With improved detection limits and greater certainty in variables this LRV is expected to be lower. In

contrast, in endemic regions where the HE concentration in raw sewage is higher, the same exposure volumes required a much greater LRV (5.2). However, when a lower acceptable DALY was used (1.0×10^{-4}), as suggested by Mara et al., (2010), the LRV required was much lower (3.1, Table 3-1). The 95th percentile LRV is used throughout this study to be conservative, given the variation and uncertainty in the data.

Table 3-1 Summary of DALYd, LRV and Ascaris concentrations to achieve health targets (DALY) for typical helminth concentration in raw sewage in south-east Australia and endemic countries.

Parameter	South-east Australia			Endemic region		
Median HE/L raw sewage[A]	2.1			506		
Exposure volume (mL/yr)	720					
DALY/case or disease burden (DALYc)	0.00072 (0.00051, 0.00101)			0.00153 (0.00104, 0.00233)		
Acceptable DALY (pppy)	1.0×10^{-4}	1.0×10^{-5}	[B] 1.0×10^{-6}	1.0×10^{-4}	1.0×10^{-5}	[A] 1.0×10^{-6}
DALYd	3.115 (0.742, 20.502)	0.172 (0.055, 0.537)	0.016 (0.005, 0.049)	1.003 (0.280, 4.025)	0.077 (0.025, 0.243)	0.008 (0.002, 0.024)
LRV	-0.3 (-1.2, 0.4)	0.9 (0.2, 1.6)	2.0 (1.3, 2.6)	2.5 (1.8, 3.1)	3.6 (3.1, 4.2)	4.7 (4.1, 5.2)
Ascaris lumbricoides (HE/L)	4.062	0.233	0.022	1.324	0.105	0.010

[A] Using Pert from Table 2-3. [B]Generally accepted HBT of 1.0 µDALY. Parentheses indicate 5th and 95th percentiles of the median.

The tornado figures for the calculation of the LRV showed that the most sensitive input parameters were the HE concentration in the raw sewage (HE/L), the constants in the beta-Poisson dose-response model (α and β), and the ratio of illness:infection ($R_{i:f}$) (Figure 3-1). The use of 95th percentiles for recommended LRVs was considered to account for such uncertainty. Interestingly, chronic mild disease symptoms exhibited similar influence to death; this was because of the much longer duration of chronic symptoms (Figure 3-1).

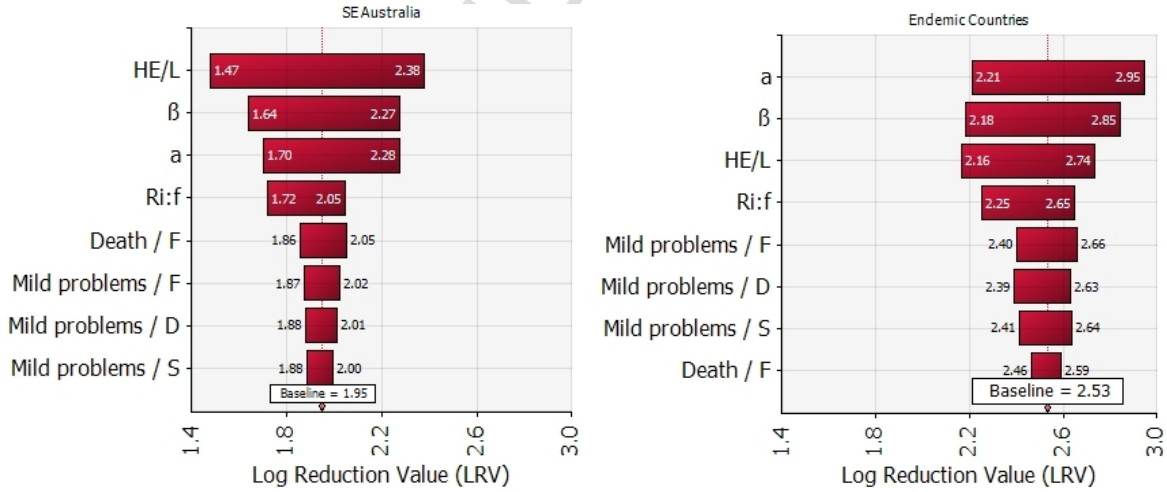


Figure 3-1 Tornado diagram indicating most sensitive parameters for calculation of log reduction values (LRV) for *A. lumbricoides*. HE = Helminth eggs, α and β = constants in the beta-Poisson dose-response model, Ri:f = ratio of illness to infection, D = Duration, F = Frequency and S = Severity. Left Australia, right endemic countries, DALY = 1.0×10^{-6}.

The LRV_{95} (95th percentile) was calculated for *A. lumbricoides* (SE Australia) using the HBT of 1.0 µDALY (Table 3-1) and a range of exposure volumes:

$$LRV_{95} = (0.4343 \times \ln(V_{rw.y})) - 0.2332$$

Equation 9

Where:

LRV_{95} = LRV required to meet the HBT 95th percentile
$V_{rw.y}$ = Volume of recycled water ingested in a year (mL/yr)

The DALYc estimates of ascariasis in SE Australia of 7.22×10^{-4} and endemic countries of 1.54×10^{-3} (Table 2-2) for *A. lumbricoides* were within the ranges reported by Chan (1997) who calculated a DALYc for ascariasis of $9.7x10^{-4}$ (Pre-school), $1.7x10^{-3}$ (School age) and $1.6x10^{-4}$ (Adults). The DALYc for SE Australia was considered a conservative estimate and likely to be typical of non-endemic countries with good medical care. This view was supported by the observation that: (i) death from ascariasis is unlikely in Australia; and (ii) most infected people in Australia are likely to be asymptomatic due to limited reinfection and therefore a lower worm burden (Albonico et al., 1999; Khandelwal et al., 2008).

Using the medians from these calculations for SE Australia, the HE concentration required to meet the 1 µDALY pppy HBT was 0.023 HE/L. For the WHO (2006) guideline limit of 0.1 HE/L in recycled water used for agriculture the µDALY was calculated to be 4.5 pppy, which supports the application of this limit to protect human health.

Human to human risk – Scenario 1

For this scenario (involving *A. lumbricoides*) there would be a maximum of 6.9 HE/L in the raw sewage entering the STP (Table 2-4). A mean of 2.5 (2.0, 3.0) LRV was required to achieve an HBT of 1 µDALY (Table 3-2). This 95th percentile of 3.0 was higher than the LRV of 2.6 (Table 3-1) for typical exposure. Therefore, accounting for model uncertainty, an LRV of 3.0 is recommended for the protection of human health in SE Australia. This is less than the conservative 4 LRV recommended in the AGWR (NRMMC et al., 2006). Compared to the WHO (2006) guideline value of <0.1 HE/L for wastewater reuse in agriculture, a 3.0 LRV for raw sewage containing 2.0 HE/L would achieve 0.002 HE/L in the recycled water. This concentration is much lower than <0.1 or <1.0 HE/L in the WHO guidelines and the LRV of 3.0 is typically conservative while managing risk from rare events (WHO, 2006).

3.1.2 Taenia saginata and T. solium – Risk to cattle or humans (SE Australia)

Risk to cattle or humans (human to cattle to human) – Typical

The frequency of *T. saginata* infection of cattle and *T. solium* in pigs in Australia are rare (Table 3-2). The inherent risk to animals and humans from *Taenia spp.* in SE Australia is low as there should not be any significant source releasing HEs to the sewer (Table 3-2). Control measures used in Australia also provide additional barriers of safety (Section 2.1.2).

Risk to cattle or humans (human to cattle to human) – Scenario 1 (Table 2-4)

Current local controls manage typical exposure in Australia. However, it is possible for people to visit, immigrate, or return from overseas, where infected meat may have been ingested. Faeces of humans infected with *Taenia* spp. could contain up to 4,688 eggs per gram (epg) (Table 2-4), which equate to up to 6.5 HE/L in raw sewage (Table 3-2).

Considering the life cycle of *T. saginata*, there should be no HE in faeces of cattle or pigs, as humans are the definitive host (HEs are found in human faeces). Hence, the risk from *Taenia spp.* exposure to cattle or pigs in Scenario 2 (cattle to cattle) does not warrant further consideration (Table 3-2).

Table 3-2 HE concentrations in raw sewage and recycled water post application of LRVs (SE Australia)

Helminth and risk pathway	Typical HE concentration		Scenario HE concentration		
	Raw sewage (measured)	Recycled water (2.6 LRV)	Raw sewage (Estimate)	Recycled water (2.6 LRV)	Recycled water (3.0 LRV)
	Typical HE concentration		**Scenario 1 [C] – infected group of humans**		
Ascaris lumbricoides					
Risk to Humans (HE/L) (Human to human)	<2.0 (likely 0.02)	0.0053 (0.0001)	6.9 (max)	0.0173	0.0069
LRV requirement for HBT	2.0 (1.3, 2.6)		2.5 (2.0,3.0)		
Taenia spp.					
Risk to cattle or humans (*T. saginata*) (Human to cattle to human)	Unlikely[A] to be significant source in sewer, risk low		6.5	0.0163	0.0065
Risk to pig or humans (*T. solium*) (Human to pig to human)	Unlikely[B] to be significant source in sewer, risk low		3.3	0.0083	0.0033
	Typical HE concentration		**Scenario 2 [D] – Infected cattle**		
Fasciola hepatica (an example of a stock helminth)					
Risk to cattle (Cattle to cattle)	No source in sewer, risk low		0.9	0.0023	0.0009

HBT = Health target of 1.0 µDALY. [A] Frequency in cattle of 0.00005 (Pearse et al., 2010). [B]Frequency of *T. solium* in Australian pigs is considered rare (Gardner et al., 2013). Helminth egg (HE) concentrations calculated using Equation 7Equation 7[C] and Equation 8 Equation 8[D].

For Scenario 1, the concentration of *Taenia spp.* eggs in recycled water post 3.0 LRV treatment was less than 0.0065/L (Table 3-2). Therefore, the recycled water concentration would be an order of magnitude lower than the strictest limit of <0.1 HE/L and should be considered acceptable for protection of human health (WHO, 2006).

There are no published guideline concentrations of HE in units of HE/L for the protection of stock health. However specific treatment methods have been proposed (e.g., such as sand filtration) for the production of recycled water for agricultural use (Helper, 2007; Murrell, 2005). Sand filtration is an acceptable alternative method for lagoon treatment for agricultural uses of recycled water (Helper, 2007; Murrell, 2005). The initial research used to determine this acceptability estimated an average LRV of 3.3 (range 2.4 to 3.9 assuming a detection limit of 1 HE/100 mL; Newton et al., 1949). However, data was limited in this initial research (n=4) and more recent literature suggests a maximum LRV of 3.0 would be appropriate for sand filters (Jiménez et al., 2000; Latrach et al., 2016; Rose et al., 1996; WHO, 2006). Therefore, an LRV of 3.0 was assumed to provide equivalent protection of stock.

3.1.3 *Fasciola hepatica* – Risk to cattle (SE Australia)

Risk to Cattle (cattle to cattle) – Scenario 2 (Table 2-4)

Eggs of *F. hepatica* were not identified in any of the samples examined. However, based on Scenario 2, the concentration of the HE entering the STP was estimated to be 0.9 HE/L (Table 2-4). Treatment capable of a 3.0 LRV would provide a concentration of HE in recycled water of 0.0009 HE/L (Table 3-2). The direct cattle to cattle transmission of *F. hepatica* is a much greater risk than indirect transmission through recycled water. Especially as *F. hepatica* in livestock can be controlled by drenching (Boray, 2007).

3.2 Helminth egg removal in ASP and lagoon systems

3.2.1 ASP removal of helminth eggs

Acceptable data for the LRV of HE in ASPs available in the literature was limited to nine data points (Figure 3-2). A linear regression analysis identified a significant relationship between HRT and LRV ($p<0.005$).

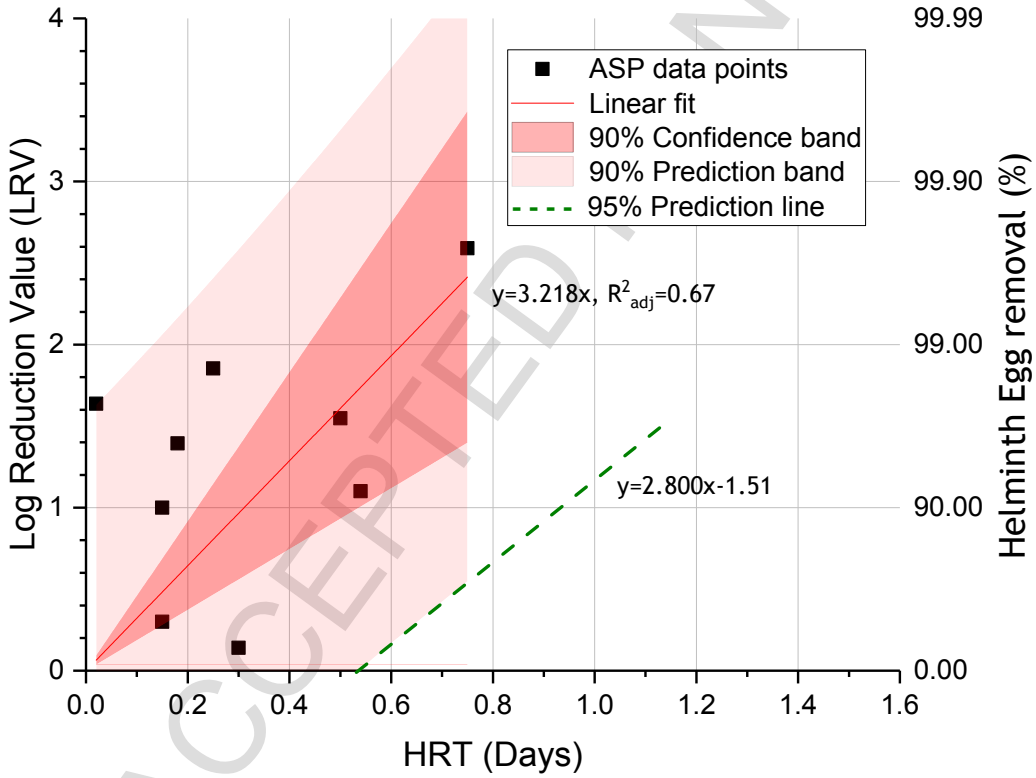


Figure 3-2 Helminth egg removal from sewage effluent via activated sludge plants (ASPs). Includes all suitable data (n=9). HRT = Hydraulic retention time.

The proposed design equation for removal of HEs through ASPs suffered from high variability and was based on limited data (Figure 3-2). The removal of suspended solids through ASPs was expected to be associated with the concomitant removal of HE as the eggs are sticky and often attached to suspended solids (Jiménez, 2007a;

Sengupta et al., 2012a). The WHO guidelines (WHO, 2006) indicate that the sewage treatment processes of activated sludge and secondary sedimentation could achieve a 1 to < 2 LRV, supporting the results of this study.

The 95% prediction line was recommended as a conservative calculation of the LRV that can be achieved for ASPs (LRV_{ASP}) that are well operated, maintained and controlled (Figure 3-2). Additional research is required to reduce the confidence interval and improve the practical use of the 95% prediction line as ASPs can have less than half a day HRT (Figure 3-2).

3.2.2 Lagoon removal of helminth eggs

All high-quality data (Ayers *et al*., 1992) and suitable data identified post-1992 (2016 data) were regressed and a 95% prediction line determined (Equation 10).

$$LRV_{LAGOON} = 0.224HRT - 1.038 \quad \text{(From Figure 3-3).}$$

Equation 10

Where:

LRV_{LAGOON} = Log Reduction Value = $-Log_{10}$ (lagoon Inlet concentration/outlet concentration)
HRT = mean hydraulic retention time (days)
Note: this is the design equation recommend by Ayres *et al.* (1992)

A few data points were identified as outliers (Studentized residuals >2.0) and were not used in the regression analysis, as these most likely represented highly inefficient or highly efficient lagoon systems. Six data points were discarded because they were from lagoon systems identified in the literature as having poor hydraulics or design (Figure 3-3). To account for parameter uncertainty, the 95% prediction line equation is recommended for determining the HE LRV for well-designed and well-maintained lagoon systems (Figure 3-3 and Equation 10).

The updated design equation for lagoon systems is based on appropriate data (e.g., HRT and detection limits stated) sourced from several countries and a variety of conditions. The variability in the data highlights that HE removal via lagoon systems can be influenced considerably by design, operation and the dominant removal mechanisms (Figure 3-3).

Several removal mechanisms have been suggested as acting during lagoon retention (e.g., flocculation and entrapment). However, the major mechanism is settling, which relies on gravitational force described by Stokes Law and provides a theoretical constant settling rate of >0.3 m/hr (Jiménez, 2007b; Stott, 2003). Settling rates will vary depending on the HE size, the specific density of the egg, the specific density of the liquid, the dynamic velocity of the liquid, and assumptions of a spherical shape and laminar flow. Resuspension could also occur in lagoons, depending on climatic conditions and flow rates. However, the lagoon sludge sediment layer stabilises with time and settled HEs are gradually buried and less likely to resuspended (Sengupta et al., 2012b, 2012a).

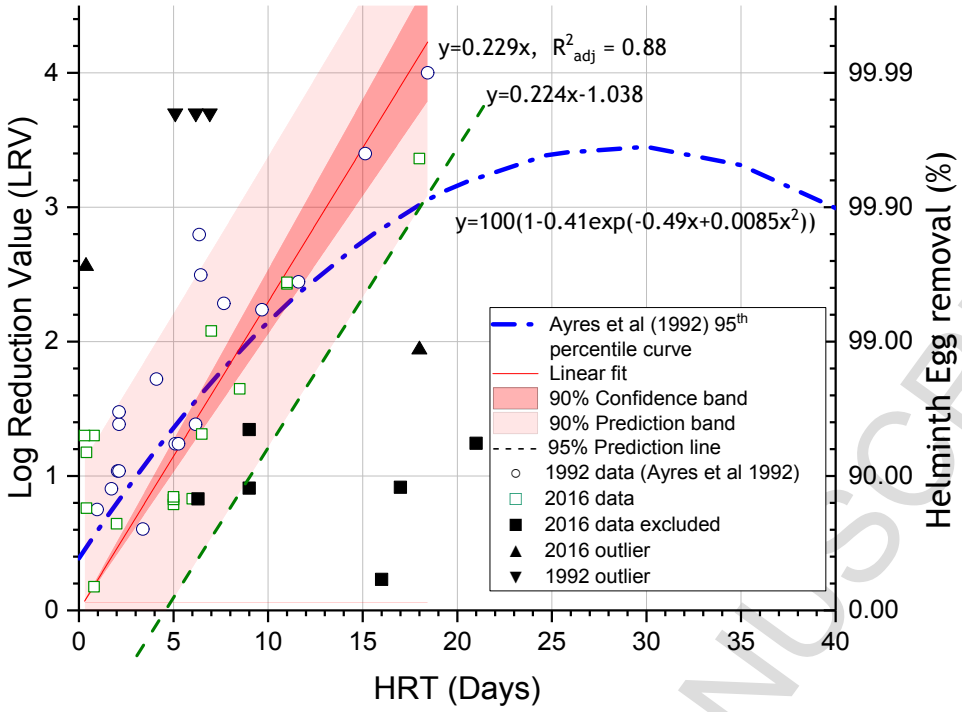


Figure 3-3 Helminth egg removal from sewage effluent via lagoons with a combination of aerated, anaerobic and facultative ponds (n=39, n=6 excluded, for reasons of data exclusion refer to text). HRT = Hydraulic retention time. The line of best fit (solid line) fitted to all data points except excluded (Black filled square) and outliers (Black filled triangles).

The proposed equation for removal of HEs in lagoons (Equation 10) was derived from data that cover a range of climatic conditions and helminth species. Therefore, it provides a relatively robust analysis. Nevertheless, lagoons must have a good design to maximise HE removal efficiency and reliability (e.g., some smaller ponds in series, with appropriate inlet/outlet structures minimising hydraulic short-circuiting). They should also be well maintained and consider sludge build up in the calculated HRT (Ayres et al., 1992; Verbyla et al., 2016).

4 Conclusions

A 3.0 LRV of HE in raw sewage is required to achieve the HBT of 1 µDALY to protect human health associated with the use of recycled water in SE Australia. This LRV was also found to provide acceptable protection for cattle and pigs.

The calculated LRV of 3.0 is based on several conservative assumptions that should be typical, if not over-protective, for many countries where helminths are not endemic. However, for endemic regions a 3.0 LRV for the treatment of raw sewage would only achieve a human HBT of 100 µDALY with similar exposure.

Well designed and efficiently operated ASPs and lagoons system should achieve the proposed 3.0 LRV of HE in 18 days or less.

Funding and acknowledgements

The authors gratefully acknowledge financial support from South East Water for the funding and ALS Water for helminth analysis. Constructive comments from Dr Jonathan Schmidt and the reviewers are also acknowledged.

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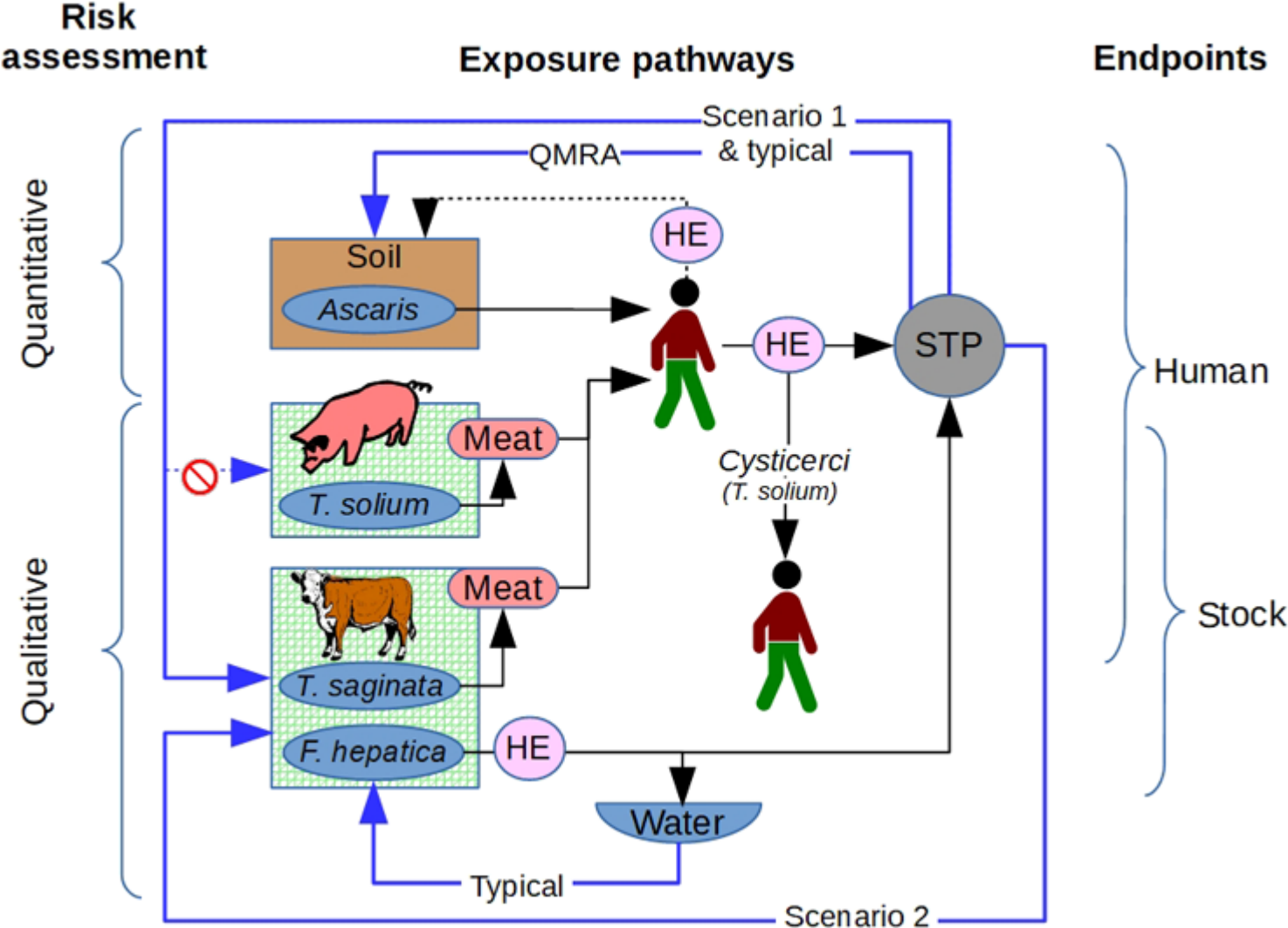
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Risk assessment
Exposure pathways
Endpoints
Quantitative
Qualitative
Scenario 1 & typical
QMRA
Soil
Ascaris
HE
HE
STP
Human
Meat
T. solium
Cysticerci (T. solium)
Meat
T. saginata
F. hepatica
HE
Stock
Water
Typical
Scenario 2