

Review

Improvement of Log Reduction Values Design Equations for Helminth Egg Management in Recycled Water

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Abstract: Understanding and managing the risk posed by helminth eggs (HE) is a key concern for wastewater engineers and public health regulators. The treatment processes that produce recycled water from sewage at wastewater treatment plants (WWTPs) rely on achieving a defined log₁₀ reduction value (LRV) in HE concentration during the production of recycled water from sewage to achieve the guideline concentration of ≤ 1.0 HE/L. The total concentration of HE in sewage reaches thousands of HE/L in developing countries and therefore, an LRV of 4.0 is generally accepted to achieve a safe concentration in recycled water, as this will meet the guideline value. However, in many developed countries with good sanitation and public health standards, the HE concentration in sewage is generally < 10 HE/L. Therefore, validation of the sewage treatment process relied on to achieve an LRV of 4.0 can be difficult. Because of these limitations, design equations to predict LRVs from hydraulic retention times (HRT), which are geographically non-specific, are commonly relied on to ensure the production of safe quality recycled water with respect to HE. However, these design equations could be further refined by defining the design and management of the treatment process in greater detail and thus be used more effectively for determining the LRV required. This paper discusses the limitations and possible improvements that could be applied to LRV design equations for predicting HE removal at WWTPs and identifies the data requirements to support these improvements. Several options for LRV design equations are proposed that could be validated experimentally or via the ongoing operation of WWTPs. These improvements have the potential to assist the rationalization of the HE removal requirements for specific treatment options, exposure scenarios and use of recycled water in agriculture.

Keywords: helminth; egg; sewage; log reduction value; recycled water; treatment

1. Introduction

Due to climatic and demographic changes, there is an increasing demand around the world for finite freshwater resources. As a result, the recycling of water from a range of

endemic countries may increase the incidence of helminth-associated diseases in developed countries [10,11]. Furthermore, helminths, such as *Taenia solium* (pork tapeworm), *Taenia saginata* (beef tapeworm) not only infect humans but can cause infections in livestock which could result in significant economic loss [12].

Without filtration, reduction of pathogenic bacteria, viruses and protozoa is typically accomplished by oxidative disinfection (e.g., chlorine) or by ultraviolet (UV) disinfection, or both. The protozoan pathogen *Cryptosporidium* is commonly the limiting pathogen here since it is resistant to chlorine. Consequently, UV disinfection is used to kill *Cryptosporidium* oocysts. Unfortunately, due to their thickness and multilayered structure, HE are resistant to chlorine and UV disinfection [13,14]. Consequently, the preferred mechanism by which HE can be removed at WWTPs without filtration is via settling of the HE, which is typically achieved via lagoon hydraulic retention (i.e., retaining the water in the lagoon for several days or weeks to allow time for settlement of solids including HE). Since HE are generally larger than protozoan-encysted life stages and have a specific gravity greater than 1, they tend to settle out of the water column given sufficient time [9]. Using settlement for removal of HE has been acknowledged as an effective measure for HE control for decades [15]. The key question here is how long should the hydraulic retention time (HRT) be to provide safe quality recycled water? The answer depends on the concentrations of HE in raw sewage at the WWTP and the design and operation of the WWTPs. For example, settling of HE in lagoons is impacted by sludge accumulation and the associated change in HRT, pond depth, the length to width ratio, the configuration of the inlet and outlets, baffling, wind speed and direction, and stratification due to diurnal shifts in temperature [16]. Appropriate design and regular system maintenance and monitoring can ensure these impacts are minimized and the lagoon system is optimized.

Recently, the original design equations for HE LRVs based on HRTs in lagoons [17] were updated [12]. Two design equations, one for lagoon systems and one for activated sludge plants (ASPs), were derived from data from a range of operational WWTPs across the world [12]. For the data to be useful for such an approach, it required the following:

1. Quantification of HE concentrations in both the sewage and recycled water of the relevant treatment process in the WWTP (measured concentrations needed to be greater than the detection limit), and
2. The associated HRT.

Without such data, the LRV cannot be appropriately determined and used in conjunction with the HRT to derive a reliable design equation [12]. In situations where the HE concentration in sewage is already near the detection limit (typically 0.1 to 1.0 HE/L), the concentration in recycled water is often quoted as the limit of detection and hence the LRV cannot be accurately calculated. Consequently, design equations are difficult to validate for treatment processes in WWTPs where; (i) HE concentrations in the sewage are low, including lower than the available analytical detection limit, or (ii) the treatment process results in recycled water HE concentrations lower than the detection limit and thus the true LRV cannot be accurately determined.

The current HE removal design equation was first published 3 decades ago. Since then, there has been extensive research documenting the removal of HE from sewage, and several advances in identifying and measuring HE concentrations in sewage and recycled water. This review aims to determine if the management of HE removal from sewage can be improved and identify any future research needs to achieve this. Firstly, the review identifies the current management and associated risk posed by HE in sewage and the guidelines relevant for the management of HEs in sewage for the production of recycled water. Secondly, the possible limitations and improvements to the LRV design equations for HE removal from sewage for various treatment processes within WWTPs are determined. Finally, the research required verifying the LRV design equations in controlled conditions in the laboratory and in operational WWTPs is discussed, considering where the HE concentration in the sewage is low and complicates the calculation and validation of the LRV.

2. Helminth Management

2.1. Helminth Infection

In developed countries, the WHO recommends an upper limit of 10^{-6} disability-adjusted life years (DALY) per person per year (pppy) as a tolerable limit for the burden of the disease [9]. However, this is not achieved in many developing countries, where helminth infections are still endemic and the morbidity and sequelae they cause are higher due to the lack of prevention and treatment [17].

Since many helminths are transmitted via a fecal-oral route, high rates of exposure in developing countries due to limited sanitation present a public health challenge [6]. In such countries, high exposure rates are caused by factors, such as poverty, poor hygiene, limited treatment of sewage, higher prevalence of helminth eggs, and the lack of use of personal protective equipment when working with partially treated sewage [18,19].

A major source of infection for humans can be via exposure to sewage, and inadequately treated recycled water or biosolids allowing the completion of the helminth's life cycle. The number of HE in sewage has been reported to be as high as 5730 HE/L in some developing regions (Table 1). Wastewater treatment plant workers and farmers applying recycled water and biosolids for agriculture and aquaculture are particularly at risk as they may be directly exposed to HE present in the recycled water and biosolids during their work [20]. Large portions of the community can also be indirectly exposed to HE via handling and consuming products that are grown on farms that utilize recycled water and biosolids without adequate treatment (Figure 1).

Table 1. Reported concentrations of helminth eggs of human health significance in sewage from developing and developed nations.

Country	Economic Status ^A	Helminth Eggs in Sewage (HE/L)	References
Australia	Developed	≤ 1.0	[12]
France		9	[19]
UK		<1	[21]
USA		1–8	[21]
Bolivia	Developing	306–3006	[8]
Brazil		166–202	[8]
Jordan		300	[19]
Mexico		6–330	[19]
Morocco		840	[19]
Ukraine		60	[22]
Vietnam		5730	[23]

^A A developing country was defined as a country with a less developed industrial base and a low Human Development Index (HDI) relative to other countries [24].

2.2. Helminth Egg Loads Entering the Sewer

Helminth egg loads in sewage usually arise from the general population (although other sources, e.g., abattoirs, may be a source in some sewer catchments) and without sanitation, the life cycle of the helminth perpetuates. Where improvements to sanitation are difficult, there are large programs of mass drug administration designed to lower the helminth disease burden in endemic countries until sanitation can be improved. In non-endemic countries, the HE loads in the sewage system are reduced due to the lower disease burden in the population from good sanitation and access to medication. However, as the migration and movement of humans increases across the world, this could be reflected in variations in HE loads in sewage systems.

Zoonotic HE can also enter the sewer via licensed wastewater discharges from animal facilities (e.g., stock sale yards, abattoirs, boarding kennels, etc.) or via household disposal of fecal matter from domestic animals into the sewer. Stormwater from such facilities and urban areas can also be a source where it is connected to the sewer. This is particularly the case in jurisdictions with combined sewage and stormwater drainage systems.

For animal health, the helminth species of concern depends on the animal species in the catchment, the species exposed to the recycled water, and the helminth species endemic to the area. Because of these specificities, animal only helminths are not discussed in detail in this review, but they are important to consider for specific sites recognizing their potential sources discussed above.

The helminth taxa of most concern for sewage and protection of human health are typically *Ascaris lumbricoides* (human roundworm), *Trichuris trichiura* (human whipworm), *Ancylostoma duodenale* (human hookworm), *Necator americanus* (Human hookworm), *Taenia solium* (pork tapeworm) and *T. saginata* (beef tapeworm). Note that *Strongyloides stercoralis* (human roundworm) is excreted in feces as the larvae and therefore, readily removed by the WWTP.

The HE load from humans in sewage will be determined by the prevalence of helminth infections in the population and the presence of (a) mass drug administration (MDA) in endemic countries, and (b) population migration and movement between endemic and non-endemic countries. These two components are discussed below.

2.2.1. Mass Drug Administration

Mass HE loads in developing countries are expected to decrease due to preventive chemotherapy in the form of recent MDA programs where populations or sub-populations are offered treatment without an individual diagnosis [25]. The 2012 London declaration [26] committed to sustaining, expanding and extending drug access programs to ensure the necessary supply of drugs and other interventions to help control STHs by 2020. This declaration has seen many countries in Africa and Asia roll out MDA, especially among pre-school and school-aged children [27–30]. The WHO recommends MDA annually when the infection prevalence is between 20 and 50% and biannually when the prevalence exceeds 50% [31]. This approach is based on the current lack of sanitation systems and high reinfection rates in developing countries and is focused on these regions. For example, in a study conducted in India during 2001–2010, the cumulative impact of seven rounds of MDAs (especially albendazole and diethyl carbamazine) on STH infections in school children led to a decline in the prevalence of helminth infections from 60.4% to 12.5% [32].

2.2.2. Population Migration and Movement

The LRV and associated minimum lagoon HRT of 18 days was considered conservative for Australian conditions and likely to protect against outbreaks of helminthiasis from current baseloads in the country [12]. However, immigration and movement of people from endemic areas that are potentially infected pose a risk to these assumptions if HE loads in sewage are not monitored. It is most likely that imported cases of helminthiasis occur in most developed countries due to immigration from endemic regions [27] and business and tourist travelers returning from such regions.

Where there are large numbers of people moving from countries that are endemic with helminths to countries not endemic with helminths, there is a risk that the helminth loads to sewage systems in the non-endemic countries will increase. For example, in Australia, the population is projected to increase 61% from 23.3 million in 2020 to 37.6 million by 2050 with 60% of the new immigrants expected to be from endemic STH regions [33]. In limited cases, infections in immigrants in non-endemic areas have been detected more than 20 years after migration from an endemic area [10]. Maintaining modern sanitation and administration of anti-helminthic drugs to migrants and refugees in developed countries represent readily available options to limit the prevalence of helminth infections [34].

Other factors to consider are the presence of helminths in specific regions as climate change may have an impact on the spread of the pathogens as climates change across regions [10]. This could be related to shifting populations (hosts) or the life cycle of the helminth which may be temperature or moisture sensitive.

2.3. Controlling HE Movement into Non-Endemic Regions

Screening individuals returning to their country of origin from areas with endemic helminth infection could be used to control HE infection rates and thus HE loads to sewage. For example, in Australia people seeking residence as asylum seekers undergo health assessment on arrival at immigration detention facilities. However, screening focuses on the detection of diseases, such as acquired immunodeficiency syndrome (AIDS), and tuberculosis [10]. Since the diseases caused by STHs are not notifiable, there is a possibility that infections among Australians and travelers who enter Australia are neither identified nor reported. Surveys conducted among long-term immigrants from East Africa, Laos and Cambodia to Australia between 1997 and 2002 reveal that despite living in Australia for many years and having been subjected to immigration screening, there was a high prevalence of STH diseases among them [35,36]. Although the infections were generally asymptomatic, severe complications, such as eosinophilic pneumonia and malnutrition can occur [10]. Nevertheless, immigration screening for helminths and protozoa is not conducted upon entry into Australia [37], although refugees from refugee camps may be administered the anti-helminthic medicine albendazole as part of the predeparture medical assessment conducted on behalf of the Australian Government [38]. Since the migrant and refugee population in Australia is increasing, screening for helminth infections upon entry or within one month of arrival would appear appropriate.

Travelers returning home from overseas, particularly STH endemic areas may have also incidentally been infected with helminths. The only protection for this cohort is when the helminthiasis is identified, and medication is provided to treat the infection. Medication is generally considered effective [39].

One of the major sources for helminth infections in Australia is from returned service personnel, especially army veterans who have served in countries, such as Vietnam, Cambodia and other endemic regions of the world [40]. Since adult worms can persist for many years, there have been cases of long-term Australian residents who still test positive for STH infections [41].

2.4. Helminth Egg Removal from Sewage

2.4.1. Removal of HE via Activated Sludge Plants and Lagoons

As noted, the resistance of HEs to disinfection via chlorine or UV makes it difficult to inactivate or destroy them via conventional disinfection processes. Consequently, in many countries, removal of HE in WWTPs is achieved through ASPs and waste stabilization ponds (referred to as lagoons hereafter).

The wastewater treatment processes of activated sludge and secondary sedimentation (ASP) should achieve 1 to <2 LRV for HEs [5]. Various studies suggest that the effectiveness of HE removal rates for ASPs range between 91 to 97% (i.e., around 1 to 2 LRV) [20,42–44].

If well designed, constructed and maintained, a series of maturation lagoons are an accepted method for the efficient settling of HEs from the sewage [45]. However, there is some evidence of lagoons not achieving the required <1.0 HE/L, perhaps due to the poor design or management (e.g., sludge build-up and hydraulic short-circuiting) [21].

In developed countries, WWTPs still utilize lagoon systems frequently as part of the treatment process and to achieve the required HE removal. The lagoons can be cost-effective to build and with appropriate design, operation and maintenance, the removal efficiency of HE can be maximized [12]. Appropriate design refers to appropriate inlet/outlet structures and baffling which minimize hydraulic short-circuiting, orientation in relation to the prevailing wind, width/length ratio, and depth.

2.4.2. Removal of HE via Sand Filtration

Early research reporting the removal of HE using sand filtration which suggested a possible 3 to 4 LRV has been moderated to a more widely accepted view [46–49] that sand filtration can achieve 1 to 2 LRV of HE, and 2 to 4 LRV of HE with coagulation. These LRVs depend on the process type and parameters used and require assessment of the sand filtration method for validation of the LRV achieved. However, validation is complicated where the HE concentration in sewage is <10 HE/L and the limit of detection is 1 HE/L (i.e., only 1 LRV can be validated).

One common and conservative solution to allocating an LRV to a filtration system in water recycling schemes using more advanced treatment systems than just ASPs and/or lagoons has been to adopt the LRV associated with protozoan parasites (usually *Cryptosporidium* oocysts) and simply assume that HE behave similarly [7]. Since HE are larger than *Cryptosporidium* oocysts and generally less numerous, they are less likely to be the limiting health risk compared to *Cryptosporidium* oocysts [50].

2.4.3. Other Filtration Approaches

Membrane filtration methods, such as microfiltration with pore sizes of filters ranging from 0.1 to 10 µm are effective in the removal of HE. The pathogens are removed by size exclusion where HE with sizes greater than the pore size are retained [51]. A study conducted using woven monofilament filter cloths (disc filter) of different pore sizes revealed that filter cloths with a pore size of 20 µm were not effective in the removal of *Trichuris trichiura* eggs (22 to 60 µm) and recommended the use of material with much smaller pore sizes [52]. In contrast, *A. lumbricoides* eggs were not detected in the filtrate following passage through a material with pore sizes ≤37 µm (fertile eggs range from 45 to 75 µm in length [53]).

Another filtration method is based on the use of HydroTech Discfilter with a pore size of 10 µm for the removal of HE as these filters are postulated to exhibit a high retention capacity. Others reported that these filters were able to retain all HE in the influent water in a Spanish WWTP resulting in an effluent devoid of HE [54]. Another study supports these findings [55]. However, despite being low maintenance, these filters are costly and may not be affordable for use in WWTPs in developing countries.

Other membrane filtration methods, such as nanofiltration and reverse osmosis (RO) that are highly effective in the removal of viruses and protozoan cysts may also be utilized for removing HE, especially in the production of high-quality recycled water for irrigation. RO can be expected to achieve a high LRV of >6 for HE [3,7,9].

Although membrane filtration methods have several advantages in the removal of HE, equipment costs can be high and adequate maintenance is required. Therefore, defining HE reductions via ASP and lagoons remains an important practical component of HE management.

3. Guidelines for Helminth Eggs in Recycled Water

3.1. International Guidelines for Human Health

The WHO has played a major role in supporting safe wastewater recycling for agriculture by releasing guidelines and recommendations for the prevention of helminthiasis [56]. Guidelines for the use of wastewater, excreta and greywater in agriculture were published by WHO in 1989 and revised in 2006 (Table 2) [9]. The WHO specifies a limit of ≤1 HE/L for the safe use of recycled water in agriculture (Table 2) [9]. More stringent measures are recommended to protect/prevent pre-school and school-aged children from helminth infections where there may be higher exposure. The recommendations by the WHO for higher exposure environments are the application of MDA programs, the use of personal protective equipment (PPE), such as gloves and shoes, or to achieve the limit of ≤0.1 HE/L for recycled water.

Table 2. Helminth-based targets for agriculture.

Exposure Scenario for Unrestricted Irrigation	Log ₁₀ Pathogen Reduction Required ^A	Number of Helminth Eggs (HE/L)
Lettuce	6	≤1.0
Onion	7	≤1.0
Restricted irrigation		
Highly mechanised	3	≤1.0
Labour intensive	4	≤1.0
Localised (Drip Irrigation)		
High-growing crops	2	No recommendation, no crops to be pick up from soil
Low-growing crops	4	≤1.0

Source: WHO [9]. ^A based on a health-based target of 10⁻⁶ DALY per person per year.

The WHO guidelines [9] cite [57] who state that “although *Taenia* eggs have been known to survive for several months on grazing land, the risk of bovine cysticercosis is greatly reduced by ceasing wastewater application at least two weeks before cattle are allowed to graze” [57]. This comment infers that the guideline of ≤1.0 HE/L also permits sufficient management of *T. saginata* transmission to humans, given that the other controls mentioned are implemented. However, additional management measures may be required in some countries for controlling bovine cysticercosis in cattle. For example, *T. saginata* transmission can also be controlled by appropriate meat inspection procedures at the abattoir and freezing or cooking of the meat before consumption [58].

Based on the LRVs for the protection of human health published in the WHO guidelines (Table 3) [9], a concentration of ≤1.0 HE/L in sewage would not require any further LRV to achieve the recommended guideline limit of ≤1.0 HE/L in recycled water. However, this does not provide a buffer in the event of increases in HE loads in the sewage due to future outbreaks or importations of HE. Thus, what is required is either an LRV that is protective of potential outbreaks or a monitoring program using the HE concentrations as a trigger point for further investigation. For the monitoring option to be effective there is a need for easy, low-cost, real-time monitoring of HE concentrations in sewage, a technology that is currently not available. Therefore, a protective LRV approach is required to provide protection from any outbreak of helminthiasis in the population.

Table 3. Options for the reduction of helminth eggs in sewage and verification concentrations.

Number of Helminth Eggs in Sewage (HE/L)	Required HE Reduction by Treatment (log ₁₀ Units)	Verification Monitoring for Recycled Water (HE/L)	Comments
1000	3	≤1.0	Treatment should be shown to achieve this concentration reliably
100	2	≤1.0	
10	1	≤1.0	
≤1.0	none, see comments column	≤1.0	The target of ≤1.0 HE/L is automatically achieved

HE = Helminth egg, adapted from [9].

3.2. Australian Guidelines for Helminth Management in Sewage

The Australian Guidelines for Water Recycling (AGWR) specify a lagoon-of ≥25 days, or equivalent treatment to achieve an HE LRV of 4.0 if the recycled water is to be in contact with cattle [7]. This 25 day HRT is predominantly based on international guidelines to protect for high concentrations of HE in sewage (Table 1) and historical experience based on the fact

that lagoon systems have typically achieved a retention time of 25 days and there was no evidence of sewage-related helminth issues with the recycled water. The 25 day requirement was taken from the approximation that 4.0 LRV equates to a 25 day HRT, extrapolating the relationship described previously [45], a relationship recently improved [12] (discussed below and later). There is also no mention of a target concentration value in the AGWR, such as that specified by the WHO Guidelines of ≤ 1.0 HE/L (Section 3.1).

A recent review of the relationship between HE concentrations in raw sewage and recycled water (i.e., LRV), relative to the HRT of the treatment process in ASPs and lagoons, has led to the derivation of improved design equations to determine HE LRVs based on HRT [12]. This review concluded that for developed countries where helminth infections are not endemic; (a) the AGWR treatment requirements and those in other developed countries [59,60], may be too conservative as the guidelines were based on epidemiological data from developing countries where helminth infections are prevalent, (b) the LRV required for helminth eggs (HEs) to achieve the DALY of 10^{-6} is less than 4.0 (i.e., LRV of 3.0), and (c) well designed and efficiently operated ASPs and lagoons system should achieve the proposed 3.0 LRV of HE in 18 days or less. Further validation and verification of the LRV proposed design equations for cattle exposure to *T. saginata* have also indicated that an LRV of 1.5 to 3.5 would be adequate, depending on WWTP size and the exposure scenario [61]. These LRVs were determined for a range of HE concentrations and the quantitative microbial risk assessment of bovine cysticercosis for the protection of cattle and meat products. For the higher LRVs, the validation will require lower detection limits or the use of reliable surrogates.

The low infection rates within non-endemic countries make it difficult to monitor the presence of HE in wastewater as per the WHO guidelines. The AGWR also recommends a health-based target of 1 μ DALY per person per year (pppy) to protect human health associated with the use of recycled water in Australia [7]. Based on a quantitative risk assessment of the eggs of the highly persistent human roundworm (*A. lumbricoides*), it was concluded that an LRV of 3.0 for HE from raw sewage to recycled water was required to achieve the 1 μ DALY pppy [12]. This LRV was also found to provide acceptable protection for cattle from infection by beef tapeworm (*T. saginata*), and cattle from infection by liver fluke (*Fasciola hepatica*) for the limited scenarios modeled. Furthermore, detailed modeling indicated that the LRV requirement for the protection of cattle could range from 1.5 to 3.5, as discussed above [61]. All these LRV are difficult to validate when the HE concentration in raw sewage is ≤ 1 HE/L [12,61].

3.3. Animal Health

Livestock can be exposed to recycled water through uses such as livestock drinking water, direct grazing of grass, feeding of fodder irrigated with recycled water, and wash down of sheds or stockyards with recycled water. There is typically a species barrier where human pathogens are not a significant concern to the health of livestock. However, the life cycle of *Taenia* utilizes cattle and pigs as the secondary host and humans as a definitive host and therefore, the eggs of helminths (e.g., *Taenia solium* and *T. saginata*) may be present in the sewage from human feces, but can pose a risk to livestock [62]. Other origins of stock-only related HE that could infect livestock are typically from industrial wastewater sources, such as abattoirs or livestock sale yards (e.g., *F. hepatica*) [7,62,63].

One of the major limitations in the risk quantification of livestock health from exposure to HE present in recycled water is that data for dose-response models for animal infections are rare; dose-response models are even rare for humans [22]. Previous methods for determining LRVs required have assumed specific exposure events or outbreaks and achieved acceptable comparative HE concentrations in recycled water [12]. The quantification is also confounded by assessing the impact on the cattle, as the DALY concept cannot be used, and the impact of products needs to be quantified. The measures for cattle are related to the production rate, amount and quality of the milk and meat products. Recently, background

detection of *Cysticercus bovis* in cattle has been used as a benchmark for not increasing the risk to a country's cattle population [61].

For stock to stock related helminths infections, such as *F. hepatica*, the infection is generally transmitted via cattle to cattle within the property; therefore, drenching of the animal is commonly used to manage the risks of *F. hepatica* transmission in livestock [64]. The use of inadequately treated recycled water can lead to transmission between farm properties. If there are livestock holding yards or abattoirs that provide a source of livestock helminth entering sewage, there is a risk of animal to animal infection of helminths through the use of recycled water. While there is some guidance to minimize this exposure pathway, there are no specific recommendations and guidelines for HE concentrations in recycled water for the protection of livestock health. Consequently, this leaves the risk assessor no option other than to compare the background risk of exposure to HE of the farm to exposure via the recycled water pathway. The risk is then assessed by determining if the recycled water exposure pathway “significantly” increases the risk (e.g., [61]).

4. Limitations to the LRV Design Equation

The LRVs for microbes in sewage ensure that recycled water is fit for the intended use. Helminth eggs are one of the key microbial parameters where a design equation for LRV is typically used to indicate sufficient HE removal from the sewage to ensure that the recycled water is fit for the intended use. However, interpretation of the LRV and associated design equations is restrictive and complicated by several factors: (i) detection limits and recovery of HE, (ii) data limitations, (iii) analytical methods relying on visual identification, (iv) ASP and lagoon maintenance and management, (v) helminth egg viability assessment, (vi) use of surrogates, (vii) sedimentation of helminth, (viii) the helminth species of interest, and (ix) reliance on the assumed HE concentration of sewage. These complications are discussed below.

4.1. Detection Limits and Recovery of HE

The detection limit of HE in sewage and recycled water is typically ≤ 1.0 HE/L or ≤ 0.1 HE/L. Recovery of HE from wastewater typically involves the application of flotation and sedimentation techniques. A commonly used method, the Tulane method, incorporates both sedimentation and flotation processes. Sedimentation results in separation based on particle size, with the large particles removed with sieves of different mesh sizes. Flotation procedures on the other hand involve the use of solutions that have greater specific gravity than the eggs so that the eggs float and are thus separated from other heavier particles which settle out. The floating eggs are subsequently harvested from the supernatant using sieves of appropriate mesh size and counted via visual identification under a microscope. To test viability, the collected *Ascaris* eggs can be incubated at 25 °C for up to 28 days, which are optimal conditions for larval development which is ascertained by microscopic examination [11,65].

The application of the Tulane method has led to egg recovery rates of 60–76% from sludge samples [65,66] and this method is commonly used to recover and enumerate HE in WWTPs in Australia. A study indicated the HE rate of recovery for soil analysis was 75.5% with a precision of 32.5% [67]. Another study conducted with 169 raw sewage samples from four sewage treatment plants in Australia indicated a limit of detection of 1 HE/L (probably because a 1 L sample was processed) and the recovery rate ranged from 50 to 87% [12]. Sewage samples with a high suspended solids concentration are suggested to trap the HE or attach to the eggs and may lead to lower HE recovery rates as floatation is used to concentrate the eggs [66]. Another study conducted at the Baxi Llobregat Water Reclamation Plant in Barcelona (Spain) resulted in egg recovery efficiencies of 80 to 90% when 2 million *Trichuris suis* eggs were added to recycled water, filtered using a HydroTech Discfilter, and the filtrate analyzed for recovery efficiency using a modified Bailenger method [54]. Their research indicated that recycled water may have a higher recovery rate than raw sewage as it contains negligible solid content. Due to such variation in the

HE recovery efficiency in raw sewage and recycled water, reported concentrations may lead to an underestimation of the LRV. Recently, researchers have documented improved methods for the recovery and detection of HE in wastewater and sludge, with recovery ranging from 70 to 80% [11,68]. Furthermore, with advanced molecular methods, such as recombinase polymerase amplification and lateral flow assays, 1 HE/L or up to 10–15 fg genomic DNA/ μL can be detected within 30 min [69]. These improvements will assist in the accuracy of LRVs.

4.2. Data Limitation

There is a lack of field data to compare HE removal against HRT for the WWTP process. For example, only nine suitable data sets were identified for ASP [12]. Additional assessment of the recent literature indicates HE removal through ASPs; however, the literature does not define the HRT (discussed below; Section 4.4.1). Other data sets that are reported in the literature for lagoon systems cannot be used to describe well-operated and maintained systems due to the poor design and/or poorly maintained lagoons identified. However, they do provide examples of HE removal in poorly designed, operated, or maintained lagoons.

4.3. Analytical Methods Relying on Visual Identification

The HE found in sewage varies considerably in size and shape although some species are similar to each other (Figure 2). Some HE are visually similar to pollen or other artifacts present in extracts from sewage or recycled water [44]. This can lead to misidentification, resulting in both false positives and negatives if the laboratory technicians are not well trained and diligent. Often laboratories will count the total species of concern for a specific test and not others if they have not been identified as being of concern. Then, the “of concern” term is lost in the fine print, leading to a misrepresentation of data which is quoted as being “total helminths”, rather than the total helminths of concern. If it is a requirement to provide a total HE count this should be clearly specified.

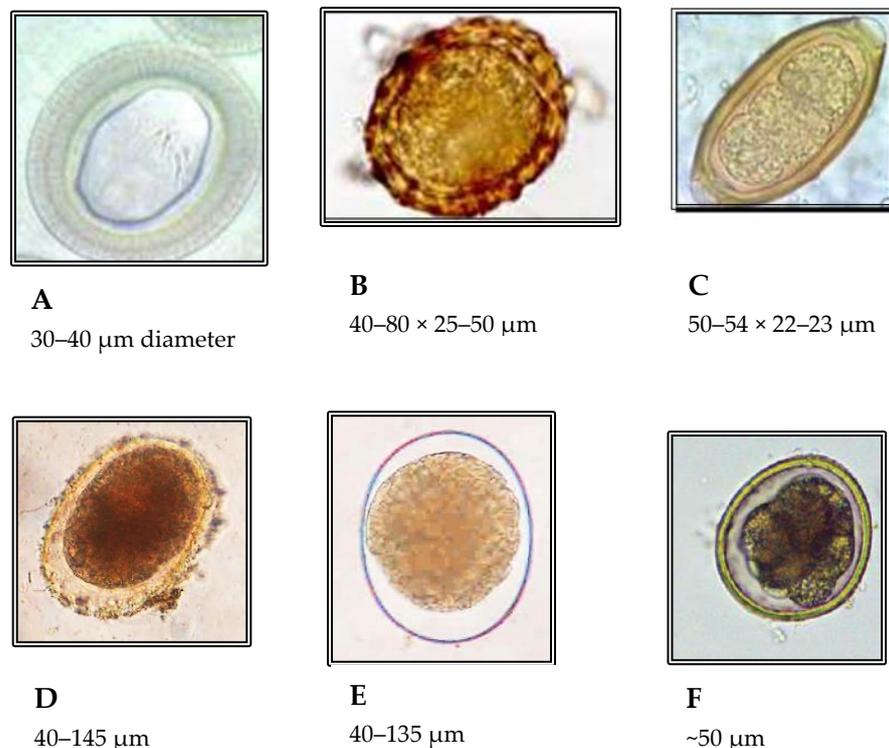


Figure 2. Electron micrograph images of *Taenia* spp. egg (A) [62], *Ascaris lumbricoides* egg (B) [70], *Trichuris trichiura* egg (C) [70], plant pod (D) [71], insect or spore ova (E) [71], *Toxocara cati* egg (F) [72].

4.4. ASP and Lagoon Maintenance and Management

One limitation to the design equation for helminth reduction in ASPs and lagoons is that there is no recognition of the design and management of treatment systems, which could potentially translate to lower and higher removal efficiencies for a particular process within a WWTP. For example, an early model predicted a 99.9% HE removal efficiency (>3 log) with an HRT of 20 days for lagoon treatment of sewage [45], thus adhering to the WHO guidelines of ≤ 1 HE/L (Table 3). The model took into account the size of the lagoon and the associated HRT but did not consider maintenance leading to sludge build-up, water depth and turbulence [67,73,74]. Occasionally, there are reported HE removal rates at various HRT that do not comply with the removal rates predicted by the early model [17,74]. It has been suggested that the reason for the variation between predicted (>4 log) and the actual values (1.7 log), may be due to excess accumulation of sludge resulting in a lowering of the HRT in the lagoons [67]. That is, LRV predictions can vary considerably if the design of the lagoons is compromised or they are not well maintained. A concept for recognizing a low or high level of design and management could be based on an LRV that 95% of ASPs or lagoons could achieve (Low) (i.e., the lower 90% prediction band), or 80% of ASPs or lagoons could achieve (High) (i.e., the lower 60% prediction band) (Figures 3 and 4). However, this concept requires validation in the laboratory and field to consider the degree that climatic variability (e.g., wind speed and temperature) can influence the LRV, compared to variability from design and management. This concept of LRV for low and high levels of design and management for ASPs and lagoons is detailed further below.

4.4.1. ASP Removal of HE

The 95% prediction line based on an HRT was suggested as a conservative calculation of the specific LRV that ASPs would achieve [12] (Figure 3). This equation could be revised so that 80% of ASPs would achieve a specific HE removal for a given HRT (long-dash red line, Figure 3). There are two reasons for this revision; firstly, the 80% protection line better represents the spread of the data, assuming a lower limit for well-managed ASPs. Secondly, an additional 30 data points extracted from the literature post-development of the original equation from 33 ASPs (blue data points in Figure 3) support this view [20,75,76]. The HRT was not defined in the 2018 data, which is a common problem with these data sets. However, by plotting the LRVs against the mean HRT (0.25 days) of the previous data (≤ 2017), the data points from 2018 literature provide an indication of what the 33 ASPs may have achieved relative to the 2017 data and the 80% LRV equation proposed (Figure 3). The proposed HRT limit (0.67 days using the 80% LRV equation) is based on the 95th percentile of HRT values from the 2017 data. It is important to note that the HE removal rate in an ASP is not only influenced by the HRT but also the sludge age, aeration type (diffused versus surface aeration) and rate, bubble size and temperature. Therefore, the HRT may not provide the most definitive LRV estimate and other options should be explored. However, the use of the HRT does provide a simple method for estimating LRV.

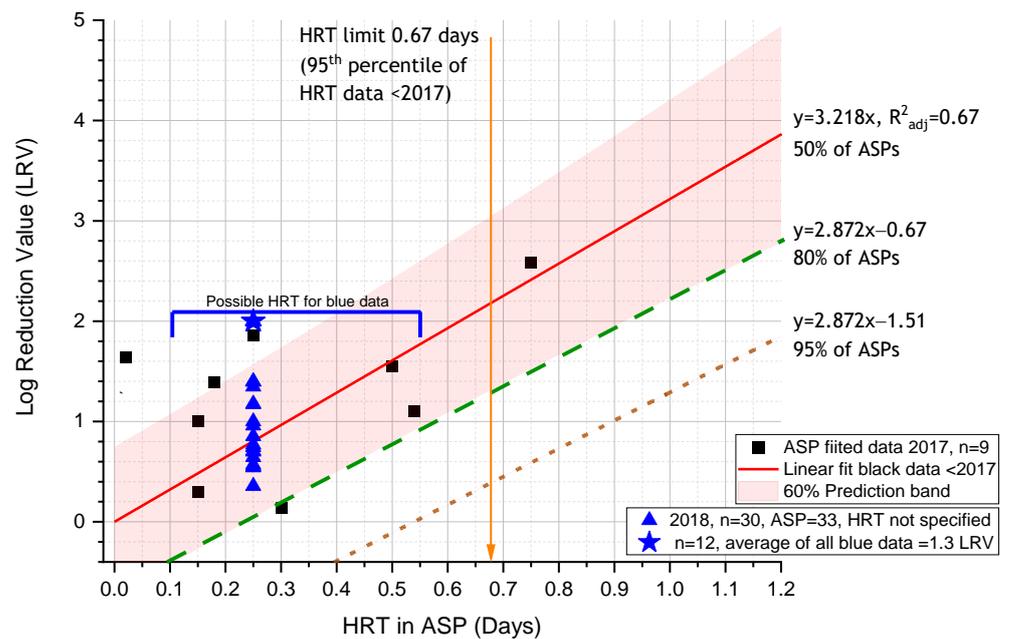


Figure 3. Log reduction value (LRV) design equation proposed for Activated Sludge Plants (ASPs). The green long-dashed line represents what 80% of ASPs would achieve (i.e., the lower 60% Prediction band) and is proposed for ASPs with a high level of design and management; data from [12]). The LRV equation for 95% of ASPs (red short-dashed line) was the removal equation originally proposed [12]. Blue data points are from the 2018 publications. Hydraulic retention time (HRT) limits are discussed in the text.

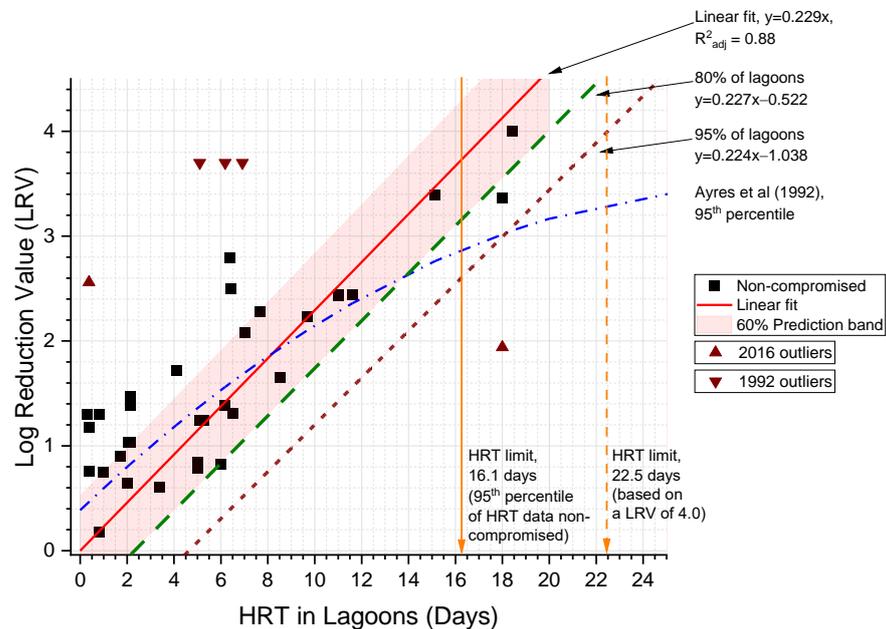


Figure 4. Log reduction value (LRV) design equation proposed for lagoons designed for wastewater stabilization. The green long-dashed line represents what 80% of lagoons would achieve (i.e., the lower 60% Prediction band) and is proposed for lagoons with a high level of design and management; data from [12]). The LRV equation for 95% of lagoons (brown short-dashed line) was the design equation originally proposed [12]. The blue dot-dash line is the 95th percentile design equation originally proposed [45]. Hydraulic retention time (HRT) limits are discussed in the text.

4.4.2. Lagoon Removal of HE

Similar to the assessment for ASPs above, the original 95% LRV equation was revised to improve the representation of optimized lagoons with a high level of design and management (80% LRV) (Figure 4). The revised equation uses the same data from [12] where they did not fit the line to the poor-quality data which they had identified from the literature and the outliers they identified statistically (studentized residuals > 2.0). Excluded data was where the literature noted poorly designed or maintained lagoons. In many cases, the systems needed to be compromised considerably for this to be noticed (i.e., obvious visual preferential flow through a lagoon system). To ensure the LRV calculation was not compromised by using the LOD [12], the only data used was from countries where HE concentrations were relatively high to ensure HE could be measured pre and post lagoon treatment, although for some post lagoon samples the detection limit of 1 HE/L was used. It is noted also that some lagoons in this data set would have had limited treatment before the lagoon system. However, this approach introduced a bias towards lagoons that may not be well operated and maintained, i.e., lagoons, such as those in developing countries where helminths are commonly endemic. Therefore, to better represent lagoons with a high level of designed and operation systems the lower 60% prediction band, representing 80% of the lagoons, is proposed as the design equation (green long-dashed line; Figure 4). The proposed HRT limit (16.1) is based on the 95th percentile of HRT values from the 2017 data, representing an acceptable range for this data (Figure 4). Another HRT limit is also indicated as an alternative, acknowledging that 4.0 LRV of HE is generally accepted for lagoons.

In many cases, the data points for the lagoon design equation for determining the LRV from the HRT is most likely an overestimate of the HRT (Figure 4). Lagoon LRV estimates are typically based on design volumes of the lagoons, not the measured volumes. There is no consideration of sludge build-up or compromised flow (exceptions were identified in the literature). Therefore, lagoon LRVs are usually calculated from data that are typically compromised to some extent, if not excluded where the literature has explicitly identified the lagoons as compromised (usually to a level where the fault is obvious). Most literature reviewed provided insufficient data to assess the magnitude of the overestimation of the HRT. In addition to this overestimation, if the LRV cannot be calculated because HE are not detectable in the lagoon treated water, these efficient lagoons cannot be included in the derivation of the design equation, adding bias to the LRV equation which is exacerbated by the coarse nature of lagoon HRT measurements. Therefore, based on the updated design equations which only apply to lagoons with a high level of design and management, HRTs of 16 and 20 days (approximately) are required to achieve LRVs of 3.0 and 4.0, respectively (Figure 4).

4.4.3. Conceptual Method for LRV Estimates Considering ASP and Lagoon Maintenance and Management

In summary, the reduction of helminth eggs from sewage is achieved through three main processes at WWTPs

1. Removal of solids with HE attached (e.g., ASP),
2. Settling, as HE have a greater density than water (e.g., lagoon system), and
3. Filtration, as HE are larger than most pathogens with a diameter > 25 μm .

Since HE are resistant to chlorine and ultraviolet (UV) disinfection these types of treatment are not effective at practical or economically viable dosing ranges. Both the activated sludge and lagoon processes effectively rely on the settling of sludge and HE and meeting HRT design requirements to achieve the required LRV. Combinations of the HRT and associated LRV for each process in the WWTP can be used to achieve the total LRV required for HE control. For simplicity, we have designated two levels of design and management; high and low (Table 4). For example, if an ASP with a high level of design and management can achieve 1.0 LRV (i.e., 0.6 day HRT) the downstream lagoon system which has a high level of design and management would need to achieve a 2.0 LRV

(i.e., 12 day HRT) for a combined 3.0 LRV of HE. An HRT of 18 days for lagoon systems was previously proposed to achieve an LRV of 3.0 (LRV 95%, Figure 4) [12].

Table 4. Log reduction values (LRV) credits proposed for hydraulic retention times (HRT) in lagoon systems and activated sludge plants (ASP).

Treatment Process	Level of Design and Management of the Treatment System Level		LRV Credit
	High ^A	Low ^B	
ASP	HRT (d)	HRT (d)	LRV
	0.41	0.70	0.5
	0.60	0.88	1.0
Lagoon system	HRT	HRT	LRV
	12	14	2.0
	14	16	2.5
	16	18	3.0
	18	21	3.5
	20	23	4.0

^A A high level of design and management would include a lagoon system designed and operated to promote HE removal. i.e., is not aerated, comprises a number of lagoons in sequence, appropriate weirs are present between lagoons, inlets and outlets are at a maximum distance apart, sludge build-up is limited, hence the operating volume is within 95% of design volume, well-controlled, managed and hydraulic retention times (HRT) are monitored, etc. For activated sludge plants (ASP), a high level of design and management should include continuous monitoring of operational performance to ensure operation within the design specifications, together with the use of critical control points. ^B A low level of design would be considered where the high level of design and management is not achieved or not well understood. Based on Figures 3 and 4.

4.5. Viability of Helminth Eggs

Helminth eggs present in wastewater matrices may be viable but not infective. To be infective, the HE need to be larvated which requires a temperature of approximately 25 °C and moisture content of at least 5% [49]. A count of total HE/L does not identify the portion of eggs that are viable. The viability of helminth eggs will impact the likelihood of infection and should be used with the LRV design equation to estimate concentrations of viable HE/L in recycled water. However, the measurement of viability is time-consuming, complex and expensive. The diverse methods available from conventional to molecular approaches are discussed below.

4.5.1. Conventional Methods

Microscope-Based Methods

Optical microscopy is still considered the gold standard to enumerate and identify viable HE in sewage and recycled water. It involves culturing HE for up to four weeks followed by microscope-based identification for the presence of larvated eggs to determine HE viability. The main drawback of this approach lies in the fact that it is laborious and time-consuming. Another approach is the direct counting method based on the morphology of HE. Non-viable eggs are confirmed only when the degeneration of egg membranes is observable which may require an extended period from weeks to months of incubation in growth media [45]. Others have observed distinct structural changes between viable (living) and non-viable (dead) eggs and concluded that culture-based methods were better than direct morphological methods for assessing egg viability, as there are distinct structural changes between viable (living) and non-viable (dead) eggs [15]. However, [66] indicated that the differential developmental stages of HE could interfere with viability determination. Additionally, the use of this morphological microscopy-based method can be challenging as it requires some experience and skill to recognize viable and non-viable eggs (observations of non-motile larvae are not a sign of dead eggs). Therefore, microscopy-based determination of HE viability lacks an objective standard.

Vital Stains

The determination of viability with incubation is time-consuming and the time lag for data may be discouraging, especially when utilized as a part of verification monitoring for wastewater reuse. However, changes in the permeability of helminth eggs can be exploited to differentiate between viable and non-viable eggs through the use of staining reagents [77]. Viable staining has the advantage that it is a simple and rapid method that can be completed within 10 min. However, evaluation of multiple dyes for use in the staining of eggs has been carried out with mixed results [78]. Dyes, such as potassium iodide solution and iodine were observed not to stain dead eggs. The application of another stain, trypan blue did not allow for precise detection of non-viable eggs. Other stains, such as thionine blue, eosin malachite green, methyl green, Sudan III, Congo red and neutral red were also unable to selectively stain living or dead eggs [78]. A solution of 0.05% methylene blue, applied for 5 min, stained dead eggs (larvae), however, did not stain viable *Ascaris* spp. eggs with larvae. The use of crystal violet gave mixed results and was found to be unreliable when eggs were in media with extreme pH values [78]. The viability determination with conventional incubation was observed to be 86% which was lower than that of stains, such as safranin (97%), crystal violet (92%) and methylene blue (87%) [79]. However, one of the potential disadvantages of such simple stains is their toxicity to viable HE and larvae resulting in leakage of internal components, thereby requiring rapid enumeration.

The use of fluorescent dyes in determining the viability of oocysts of protozoan parasites and eggs of helminths has also been explored in multiple studies. Immunofluorescence can be used to stain some microbes (e.g., *Giardia* and *Cryptosporidium*) and antibodies conjugated to fluorescein diacetate (FDA) were found to stain only viable cysts, resulting in green fluorescence as protozoan enzymes are degraded in dead oocysts [80,81]. Fluorescent stains, such as propidium iodide (PI) and 4', 6'-diamidino-2-phenyl-indole (DAPI) fluorescence stains viable helminth eggs blue (DAPI) and non-viable eggs red (PI) [82].

BacLight Bacterial Viability Stain

The Live/Dead[®] BacLight[™] bacterial viability kit is a nucleic acid fluorescence staining kit developed by Molecular Probes and is usually used to differentiate between living and dead bacteria.

The kit has two types of membrane-permeable DNA labelling dyes, namely Syto 9 and propidium iodide (PI). The first one, Syto 9 dye fluoresces green (maximum emission 498 nm) and the second dye, PI fluoresces orange-red (maximum emission 617 nm). Syto 9 penetrates live cells and stains their membrane green while PI enters cells that are dead or damaged and quenches Syto 9, thereby exhibiting red fluorescence (Figure 5) [77,79]. Therefore, the BacLight technique may be considered an alternative to traditional incubation-microscopy methods for the enumeration of HE, especially in wastewater matrices [77,79]. The kit has been successfully used in multiple studies to determine the viability of bacteria, protozoans and helminth eggs [77,79,83–86].

4.5.2. PCR-Based Methods

Different PCR primers and protocols have been developed for the detection and identification of helminths and parasites in wastewater and soil [20,34,87–89]. A number of studies have reported the use of propidium monoazide (PMA), a dye that permeates the membrane of non-viable eggs and intercalates with the DNA thus inhibiting its amplification when PCR is performed; however, PMA does not permeate intact membranes of viable HE. The viability of hookworm and *Ascaris* eggs has been determined using PMA-qPCR [77,85]. One study performed a comparative assessment of HE viability in wastewater using culture-based, BacLight Live/Dead staining and PMA-qPCR and suggested that the molecular approach was preferable to the other methods due to its accuracy, reduced subjectivity and speed [76]. Despite the fact that existing PCR methods are effective in detecting the eggs of *Ascaris* spp. in sewage and recycled water, they are less useful

in the detection of HE in sludge and biosolids due to the presence of PCR inhibitors and difficulty in breaking open the eggs to release the DNA [81].

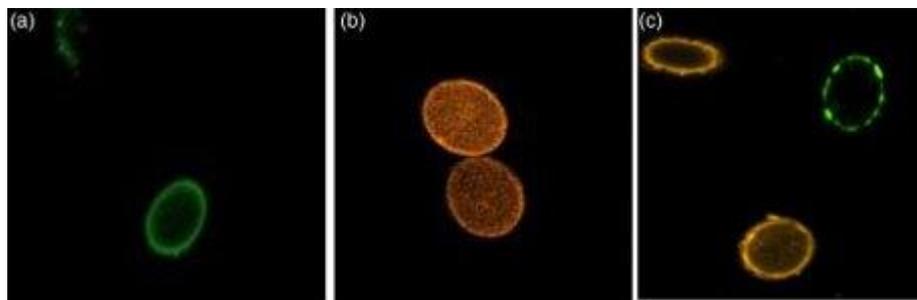


Figure 5. Confocal microscopy images of *A. suum* ova stained with Syto 9 (green) and propidium iodide (red) to determine the viability of ova: (a) viable ova, (b) non-viable ova, (c) both viable (green) and non-viable (red) ova. (Reproduced from [77]).

4.6. Surrogates

Surrogates can be used to dose sewage and estimate LRV by ensuring that the concentration in raw sewage is sufficient to allow HE detection in recycled water, based on the expected LRV through the WWTP. The use of surrogates as an alternative to infectious HE may be easier and safer to handle in spiking studies, as well as being cost-effective and rapid to assay [90]. Surrogates can be an organism or manufactured particles, such as microspheres that could act as a replacement for the target infectious pathogen without causing any potential health risks. However, the chosen surrogate must be able to achieve a reproducible correlation between the LRV of the target pathogen and the surrogate with similar treatment processes either via laboratory or field-based studies. Surrogates, such as phage (for viruses), *Escherichia coli* K12 (for pathogenic *E. coli*), aerobic bacterial spores (for *Cryptosporidium* oocysts) and microspheres (for helminth eggs) have been utilized in surrogate seeding studies [91–94]. A study conducted to determine the settling velocity of microspheres with varied surface coating type proteins in tap and wastewater in comparison to real helminth eggs (*Ascaris*, *Trichuris* and *Oesophagostomum*) revealed that there were no differences amongst the microsphere types used for settling in wastewater, concluding that microspheres could mimic the behavior of helminth eggs [94]. The use of non-viable (non-infective) HE could be a potential alternative for seeded experiments in laboratories or WWTPs to estimate LRV and the efficiency of the treatment processes. The use of surrogates will negate the risk of contaminating the laboratory or WWTP with viable HEs. However, the adhesion rates to suspended solids may differ between live and dead eggs resulting in differences in LRV. At present, there is a lack of data on the utilization of surrogates for validation testing in laboratories and operational WWTPs.

4.7. Sedimentation of Helminth Eggs

During wastewater treatment, most HE are removed by sedimentation in lagoon systems. Sedimentation of particles in water can be described by Stoke's Law which states that the settling velocity is based on size, the difference in densities between the HE and water and the viscosity of water [74,95]. Although the theoretical settling rate of HE varies with density and shape, based on Stokes' Law, the actual settling rate and removal of HE from sewage can vary based on other factors (Table 5). At the settling velocities presented in Table 5, removal in an unmixed 2 m deep lagoon would be approximately 9 h. The high specific gravity of *T. saginata* compared with *A. lumbricoides* does not translate to large differences in theoretical settling velocities. However, only limited data comparing the settling velocities of these helminth species in ASPs or lagoons in the sewage matrix is available making it difficult to quantify specific settling velocities for a range of HEs.

Table 5. Average helminth egg dimensions, specific gravity, surface theoretical and settling velocity.

Helminth	Helminth Egg Size ^A		Helminth Shape or Eccentricity	Specific Gravity (g/cm ³)	Surface	Settle Velocity (mm/s)		Reference
	Length	Width				Theoretical	Measured	
<i>Ascaris</i>	67.2 (52.2, 84.1) 75	55.4 (46.8, 64.6) 45	1.22	1.12	Mamillated	0.275	0.0612 (TW)	[74]
				1.23				[96] <i>A. suum</i>
	60	45		1.11		0.214 (20 °C)		[50]
	65	45		1.13		0.264		[78] <i>A. suum</i>
	55	40	Ellipse-shaped to round	1.11	Thick, rough albuminous outer wall	0.119		[78] <i>A. lumbricoides</i>
<i>Trichuris</i>	62.2 (54.2, 68)	30.8 (26.7, 38.3)	1.56	1.10	Smooth	0.129	0.149 (TW)	[74].
				1.15 to 1.23				
	50	22		1.15		0.203 (20 °C)		[50]
	52	22	Lemon-shaped, barrel-shaped	1.15	Thick shell with smooth surface,	0.133		[78]
<i>Taenia</i>	30	35			Radially-striated			[97]
				1.23				[96]
	40	30		1.23		0.200 (20 °C)		[50]
	40	35	Round,	1.3	Thick, smooth shell with radially striated embryophore	0.231		[78]

Eccentricity of 1 = spherical, as [74]. TW = Tap water. ^A (minimum, maximum).

The sedimentation of HE (*Ascaris suum*, *Trichuris suis* and *Oesophagostomum* spp.) in tap water and raw sewage using Owen tubes has been previously reported [74]. In this study, *A. suum* eggs settled more slowly (mean settling velocity 0.22 m/h) than *T. suis* (0.54 m/h) and *Oesophagostomum* spp. (0.45 m/h). However, in raw sewage, the mean settling velocity for *A. suum* eggs was reported to be faster (0.56 m/h) than for *T. suis* (0.16 m/h) and *Oesophagostomum* spp. (0.31 m/h). Consequently, the mamillated egg layer of *A. suum* may cause a turbulent flow, thus impacting the fluid layers to move in random irregular paths resulting in a decreased settling velocity [74]. In one study (unpublished data) we observed that *Ascaris* eggs form flocs with particles in raw sewage thus increasing the settling velocity, as speculated by [74] (Figure 6).

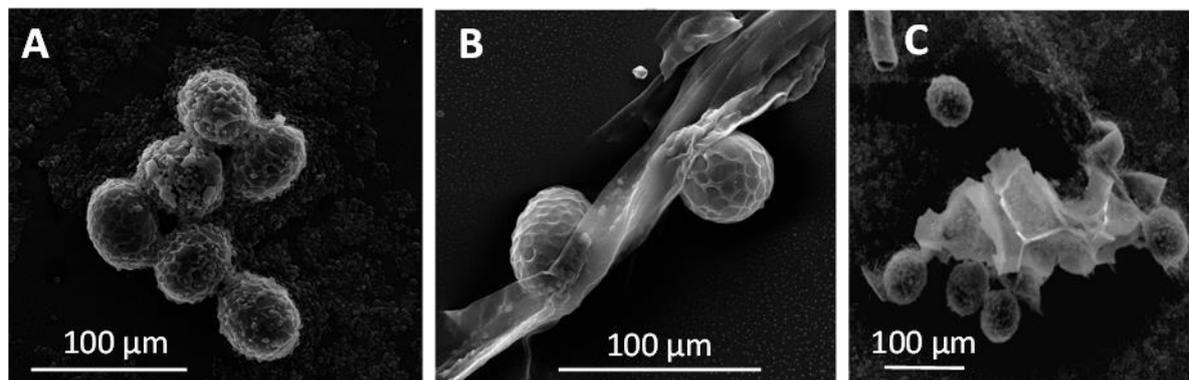


Figure 6. Scanning electron microscope images of *A. suum* eggs in tap and wastewater: eggs in clean tap water (A); the formation of floccules with suspended particles in wastewater (B,C).

Factors, such as depth, temperature, pH, turbulence and salinity can all influence the sedimentation rate of HE and may need to be taken into consideration when predicting HE removal as a function of HRT. The effects of temperature and salinity on HE settling velocities in wastewater have been investigated using granules with size and density larger than HEs [98]. This study revealed that settling velocities were indirectly proportional to salt concentrations; however, the lowest salt concentration used was 5,000 mg/L which is much higher than the salinity typically found in sewage. Another study conducted by Arthur et al. (2018) for the determination of microspheres (surrogates for HE) in chloride (Cl^-) salt solutions (30 mg NaCl, 7 mg KCl and 7 mg $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ /L) with wastewater particles demonstrated that the settling velocity decreased in wastewater samples seeded with chloride salt solutions compared to tap water [94]. Further studies regarding the impact of salt concentration, relevant to those found in sewage, on the settling velocity of HE in sewage are required. Another study, [98] carried out using granules from a laboratory-scale aerobic granular sludge reactor revealed an approximate two-fold increase in settling velocity with increasing temperature from 5 to 40 °C. The settling velocity of larger denser granules increased from 84 to 145 m/h while the settling velocity for the smaller and lighter granules increased from 35 to 63 m/h. A resuspension and settling study of HE in natural freshwater suggested that HE attach to the surface of the sedimented material which is then stabilized through time [95]. The study determined the erodibility (erosion rate and threshold) and settling velocity of *Ascaris* and *Trichuris* eggs as well as sediment cohesion sediment at different time points after being incorporated into the sediment. The erodibility of both helminth eggs and sediment decreased over time and this interaction increased settling velocity (since the contribution of resuspended ova was reduced). The mobility of HE decreased after incorporation into the sediment bed. Thus, the erodibility and settling velocity of HE could be determined by the mobility of the sediment. It was also shown that even at low flow velocities (0.07–0.12 m/s) new HE will be mobile in open irrigation channels [95]. However, we estimate the average theoretical velocity in a lagoon system (based on a 20 d retention time, a length:width ratio from 1 to

20, and a depth of 2 m) would range between 1.8×10^{-5} to 8.2×10^{-5} m/s. Lower velocity and increasing depth of a wastewater lagoon would be expected to minimize mobility and remobilization of sediment and the associated HE.

Environmental conditions that will adversely impact the settling of HE in wastewater lagoons (e.g., wind mixing, movement of fauna, and movement of algae that change buoyancy over the diurnal cycle) need to be considered in deriving HE LRV design equations.

4.8. Helminth Species of Interest

In many cases the use of HE of *Ascaris* spp. as the indicator of total helminth is appropriate, yet in some cases, it is not. For example, if assessing the risk of helminth egg exposure to cattle, the helminth of interest is *T. saginata*; yet the measurement of the total HE LRV will be biased towards *Ascaris* spp. because it is typically 1 or 2 \log_{10} higher in concentration [61,73,99]. Further, since the specific gravity of *T. saginata* eggs is slightly higher (approximately 1.22–1.3 g/cm³) than that of *A. lumbricoides* (1.11 to 1.13 g/cm³), settling of *T. saginata* eggs could be more effective in wastewater lagoons (Table 5). In this case the LRV of total HE may be too conservative as it will be biased towards the dominant HE, i.e., *Ascaris* spp. Although environmental conditions may override the variance discussed above, it is important to acknowledge that the removal of HE of *Ascaris* spp. has generally been used to infer the removal of all HE, yet many WWTPs could have different removal rates for different HE through various treatment processes.

4.9. Reliance on an Assumed Sewage Concentration of HEs

Numerous research studies indicate that the concentration of HE in sewage is variable but relatively consistent in being low in countries with modern sanitation and access to medication and high where this is not the case. If the LRV design equation is to be utilized on a finer scale for improving the management of WWTPs, the steady-state of sewage HE concentrations needs to be confirmed and maintained, to ensure the effectiveness of the LRV utilized. Increasing immigration from HE endemic countries, or the return of travelers from HE endemic to HE non-endemic countries, may likely threaten this steady state. A “protection buffer” of some sort could help ensure that helminthiasis does not exceed the recommended disease burden to humans or associated risk to livestock. What the magnitude of the protection buffer should be is a difficult question and relevant to the WWTP size and potential exposure. Historical concentration data can provide an indication of the likely variation based on historical loads to sewers and quantitative models can provide some indication of potential changes in the future. However, only measured concentrations in sewage and recycled water will ultimately provide verification of the HE concentrations.

5. Research Required to Improve the LRV Design Equation

In the short-term, an improved understanding of the LRV design equations relating to the removal of HE in both ASP and lagoon systems will allow the water industry to develop and operate WWTPs based on the revised LRV design equations with more flexibility while protecting human and livestock health. To advance our understanding and management of HE in sewage there are several improvements required to our current knowledge:

- A cost-effective method for the detection of viable HE concentrations is required with detection levels sufficient to estimate LRV of each relevant treatment process within WWTPs. Such a method should improve detection limits, improve recovery rates, decrease variability and assess viability, with a focus on cost-effective routine analysis.
- Quantification of the mechanisms and rate of HE removal through conventional sewage treatment processes under a range of operating conditions, with a view to deriving an improved LRV design relationship between key operational parameters and HE removal.

- Investigate the application of suitable surrogates for viable HE for application in estimating the settling velocity and impact of settling rates in controlled conditions and field dosing experiments.
- Challenge testing of lagoon systems with HE or a suitable surrogate to validate the LRV design equation. However, owing to the costs and complexity involved, it will be difficult to carry out experiments at the plant-scale. Consequently, bench-top or scaled-down pilot plants, that reflect full-scale WWTP function, may provide significant savings in costs and time.

In the long-term, for regions not endemic with helminths, a method for the continuous monitoring of HEs would facilitate the operation of critical control points in WWTPs where HE concentration baseloads change due to outbreaks or changes in population disease burdens. Accumulation of measured data that characterize outbreaks of HE in the sewage would also be beneficial.

With improved quantification and documentation of HE removal related to treatment plant operational parameters and HE removal, the LRV design equations can be improved to represent the operational characteristics of specific WWTP. Such improvement will also assist in more detailed risk assessments based on a more accurate assessment of concentrations of HE for relevant species of helminths.

6. Conclusions

Wastewater treatment plants rely on achieving a defined LRV of HE to produce recycled water with a concentration of ≤ 1.0 HE/L for agricultural use. In many developed countries with good sanitation and medication, the HE concentration in sewage is < 10 HE/L and can be consistently ≤ 1.0 HE/L. Due to the limits of analytical techniques for HE detection, the validation of specific treatment processes to achieve a defined LRV can be difficult. Consequently, the LRV design equation is commonly relied on to ensure HE management is achieved by the WWTP. However, it is now apparent that these design equations could be described in more detail and may not be currently used to their full potential. Research that allows more detailed analysis at lower HE concentrations in sewage and recycled water, and the treatment steps used to produce the recycled water, will improve our understanding of the design criteria for WWTPs and the management of HE in sewage. For well-managed and maintained WWTPs, this knowledge should translate to significant efficiencies, cost savings, and the production of consistently safe and qualified recycled water that, in terms of HE will not pose unacceptable risks to the health of humans and livestock.

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