Taenia eggs in a stabilization pond system with poor hydraulics: concern for human cysticercosis?

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ABSTRACT

The objective of this study is to compare the removal of *Taenia* eggs to the removal of *Ascaris* eggs in a wastewater stabilization pond system consisting of three ponds in series, where the hydraulic residence time distribution has been characterized via a tracer study supported by computational fluid dynamics modeling. Despite a theoretical hydraulic retention time of 30 days, the peak dye concentration was measured in the effluent of the first pond after only 26 hours. The smaller-sized *Taenia* eggs were detected in higher concentrations than *Ascaris* eggs in the raw wastewater. *Ascaris* eggs were not detected in the pond system effluent, but 45 *Taenia* eggs/L were detected in the system effluent. If some of these eggs were of the species *Taenia solium*, and if the treated wastewater were used for the irrigation of crops for human consumption, farmers and consumers could potentially be at risk for neurocysticercosis. Thus, limits for *Taenia* eggs in irrigation water should be established, and precautions should be taken in regions where pig taeniasis is endemic. The results of this study indicate that the theoretical hydraulic retention time (volume/flow) of a pond is not always a good surrogate for helminth egg removal.

Key words | computational fluid dynamics, dye tracer study, helminth eggs, short-circuiting, water reuse, WHO guidelines

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INTRODUCTION

The World Health Organization recommends (WHO 2006) concentrations of <1 helminth egg/L in wastewater used for irrigation, and considers eggs of four specific nematode species (*Ascaris lumbricoides*, *Trichuris trichiura*, *Ancylostoma duodenale* and *Necator americanus*) to be the most important (Mara 2007). In wastewater ponds, the main removal mechanism for helminth eggs is sedimentation. Ayres *et al.* (1992) proposed an equation that relates nematode egg removal to the theoretical hydraulic retention time (HRT) of a pond, and the WHO (2006) suggested that theoretical HRT (volume/flow) can be used as a surrogate for helminth egg removal in pond systems. However, equal-sized ponds with different configurations may have different residence time distributions (RTD) despite having similar theoretical HRTs (Persson 2000). While Torres *et al.* (1999) concluded that the hydraulic behavior of three facultative ponds in Spain was similar to that of ideal completely mixed reactors, Macdonald & Ernst (1986) reported a mean HRT that was 25% lower than the theoretical value in a system consisting of four ponds in series in Australia, and detected dye passing through all four ponds at times less than 5% of the theoretical HRT. The percentage of influent water passing through a pond in this initial short-circuiting path may influence helminth egg removal efficiency to a greater extent than the mean or the theoretical HRT.

Taenia eggs are smaller in size and settle at a velocity that is reportedly similar to (Sengupta *et al.* 2011) or possibly lower than (Feachem *et al.* 1983; Murrell *et al.* 2005) the settling velocities of eggs from the nematode genera *Trichuris* and *Ascaris*. Early work on wastewater reuse (Shuval *et al.* 1986) listed *Taenia* in the highest risk category due to the health risk of taeniasis, which is listed in the Environmental Disease Classification system as a Category D disease (Mara & Feachem 1999), characterized by latency, persistence, and very high infectivity, with an cow or pig intermediate host. Human cysticercosis, however, which results from the direct ingestion of *T. solium* eggs, is not listed in this classification system (Mara & Feachem 1999), despite the fact that it is the

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leading cause of acquired epilepsy in developing countries and affects 5–6 million people in Latin America alone, resulting in 50,000 deaths per year (Bern *et al.* 1999). Unlike taeniasis, human cysticercosis is more closely related to Category A diseases (non-latent; no intermediate host), and *Taenia* eggs are more persistent in soil than other non-latent Category A disease agents. *Taenia* eggs have been detected on market vegetables in Mexico (Sorvillo *et al.* 2011) and Libya (Abougrain *et al.* 2010), and human cysticercosis is endemic in many regions of Latin America, Africa, and Asia (Murrell *et al.* 2005), with prevalence as high as 23% in one region of rural Bolivia (Bern *et al.* 1999). Human cysticercosis has also been reported in almost all major cities of Bolivia, with one-fourth of epilepsy patients from one sample manifesting cerebral calcifications typical of neurocysticercosis (Barragán *et al.* 1986). Nevertheless, it has received little attention in international guidelines for wastewater use in agriculture (WHO 2006).

Given the importance of human cysticercosis to global public health and the theoretically low settling velocity of *Taenia* eggs, the purpose of this study is to compare the removal of *Taenia* eggs to the removal of *Ascaris* eggs in a wastewater stabilization pond system where the hydraulic RTD has been characterized with a tracer study supported by computational fluid dynamics (CFD) modeling.

METHODS

The pond system studied serves 780 people from a small town in the Yungas region of Bolivia, and consists of a facultative pond and two maturation ponds in series. Based on 24-hour flow measurements taken on six different occasions in the month of June in 2007 and 2012, the average daily flow rate entering this system is 91.5 m^3/day (SD = 27.5), which corresponds to an overall theoretical HRT of 37 days (SD = 13). However, the average flow rate entering the system was measured to be almost twice as high in 2012 as it was in 2011 (Table 1). Each pond has one inlet and one outlet pipe, located near the center of the pond width (for the facultative pond) or at opposite corners (for the maturation ponds). The inverts of the 15 cm inlet and outlet pipes in the facultative pond are 1.1 and 1.0 m above the bottom of the pond respectively. Sludge depths in the facultative pond were measured in June 2011 using the white towel method and the total volume was estimated to be 169 m^3 using the Kriging method (see Lizima 2012). Other aspects of this system, including its performance for physical–chemical parameters, have been previously described (Verbyla *et al.* 2013).

Helminth egg analysis

Composite 24-hour wastewater samples were collected at the influent and effluent of the facultative pond and at the effluent of the second maturation pond on three different occasions in 2011 and 2012, with sample volumes ranging from 2–32 L. Samples were processed for the analysis of helminth eggs in accordance with the protocol of the Centro de Aguas y Saneamiento Ambiental at the Universidad Mayor de San Simón in Cochabamba, Bolivia. This protocol follows a slightly modified version of the Mexican standard method (Secretaria de Comercio y Fomento Industrial 1999). Briefly, helminth eggs were isolated from samples using centrifugation, flotation, and biphasic separation. The main difference between the protocol used for this study and the Mexican standard is that magnesium sulfate is used instead of zinc sulfate for flotation. Representative aliquots of the concentrated samples were placed on a Neubauer counting chamber, where eggs were identified and enumerated under a light microscope.

Dye tracer study

The dye tracer study was carried out in June 2011 for the facultative pond using Rhodamine Water Tracing dye

Table 1 | Dimensions and theoretical HRTs of the stabilization ponds

			2011		2012	
Pond	**Dimensions (L × B × D/m)[a]**	**Volume (m^3)**	**Avg. flow (m^3/day)**	**Theoretical HRT (days)**	**Avg. flow (m^3/day)**	**Theoretical HRT (days)**
Facultative pond	50 × 27.5 × 1.8	2,000	66	30	121	17
Maturation pond 1	39 × 13 × 1.5	550	61	9	118	5
Maturation pond 2	39 × 13 × 1.5	550	59	9	117	5
Totals			–	48	–	27

[a]L = length, B = breadth, D = depth.

(Bright Dyes, Miamisburg, OH, USA). A 300-ppm solution of dye, prepared with distilled water, was diluted with stabilization pond water to obtain a 300-ppb solution. A volume of 2.17 L of this solution was mixed with 3 L of untreated wastewater entering the stabilization pond, and the mixture was poured into an influent distribution box. A handheld fluorometer (Equipco, Concord, CA, USA), calibrated with pond water and the 300-ppb solution of dye, was used to measure fluorescence of samples collected in triplicate at the pond effluent. Fluorescence readings were collected for 35 time points during a period of 12 days. Each time samples were collected, the flow rate was measured, and the cumulative mass of dye that had passed through the pond was estimated by multiplying the dye concentration by the flow rate and the time since the last reading. Due to limitations in the field, the duration of this study was only 12 days. Therefore, the results of the field study were supported by CFD modeling.

Computational fluid dynamics modeling

Two-dimensional (2D) CFD models were developed to simulate flow conditions and the transport of tracer in the facultative pond at the pond surface level and at the level of the inlet pipe invert. Gmsh software was used to create the fine-element mesh (Geuzaine & Remacle 2009). The accumulated sludge was treated as an obstacle to flow. OpenFOAM software (the OpenFOAM® Foundation) was used to display the velocity distribution in the pond, based on the Navier–Stokes equation, and ParaView software (Kitware, Inc.) was used for post-processing of the model and visual image generation. The models were run for a simulated period of 90 days, using a flow rate of 66 m^3/day, the average 24-hour flow rate measured during the week of the field tracer study. A three-dimensional (3D) CFD model was also created and run for a simulated period of 12 days, to confirm the pattern of short-circuiting.

RESULTS AND DISCUSSION

Table 2 shows the concentrations of *Taenia* and *Ascaris* eggs detected at the different points in the system. Globally, *Ascaris* eggs are generally more widely distributed in raw wastewater than *Taenia* eggs (Jiménez 2007), but *Taenia* eggs have been detected in concentrations higher than eggs of other helminth species in France (Cabaret *et al.* 2002). In raw wastewater from the present study, *Taenia* eggs were detected at higher concentrations than eggs from other helminth species. The prevalence of taeniasis in this Bolivian community is unknown, but was roughly estimated based on the observed concentrations of eggs in the wastewater, the influent flow rates at the time of sampling, the population connected to the system, and the number of eggs released per day by adult tapeworms (Feachem *et al.* 1983). Based on these estimates, the prevalence in this community could be 3–47%. The lower portion of this range is consistent with reports of taeniasis in endemic regions of Bolivia (Barragán *et al.* 1986; Carrique-Mas *et al.* 2001) and Peru (Murrell *et al.* 2005). Cabaret *et al.* (2002) has reported taeniasis prevalence to be as high as 36% in some regions of Dagestan.

Table 2 | Observed removal of *Taenia* and *Ascaris* eggs

Sample	Concentration of *Taenia* spp. (eggs/L)			Concentration of *Ascaris* spp. (eggs/L)		
	6/13/11	6/17/12	6/20/12	6/13/11	6/17/12	6/20/12
Raw wastewater	306	3,006	1,167	306	N.D.	N.D.
Outlet of facultative pond	–	365	N.D.[a]	–	487	N.D.
Outlet of 2nd maturation pond[b]	–	45	N.D.	–	N.D.	N.D.

[a]N.D. = none detected.
[b]The minimum limit of detection for this sample was 22 eggs/L.

In the present study, despite an overall theoretical HRT of 27 days in 2012, *Taenia* eggs were detected in the system effluent (45 eggs/L), while *Ascaris* eggs were not. The minimum limit of detection for samples collected at the system effluent was 22 eggs/L, so *Ascaris* eggs may have been present in the effluent, but at lower concentrations. The observed removal of *Taenia* eggs in this system is still less than the predicted removal of nematode eggs by Ayres *et al.* (1992). The observed removal of *Taenia* eggs in this system was also limited compared to several previous reports of helminth egg removal in other full-scale pond systems (Ayres *et al.* 1993; Madera *et al.* 2002; Oakley 2004). However, many of these papers do not report minimum limits of detection, and simply report the theoretical HRT of the system. Thus it is difficult to compare our results with these without understanding the pond hydraulics in these systems. The locations of inlets and outlets, the length-to-width ratio, and the accumulation of sludge in ponds can significantly influence their hydraulic performance and pathogen removal efficiency (Shilton & Harrison 2003). Furthermore, many of the pond systems where better helminth egg removal was reported have anaerobic ponds. The system from the present study does not have an anaerobic pond or a grit removal chamber. The absence of a grit removal chamber or an anaerobic pond will likely affect sludge accumulation in the subsequent pond, which in turn may affect helminth egg removal efficiency.

This study is not the first report of helminth eggs in the effluent of pond systems with high theoretical HRTs. Ellis *et al.* (1993) detected as many as 690 hookworm eggs/L in the effluent of a system of five ponds in series with an overall theoretical HRT of 23 days. Ben Ayed *et al.* (2009) reported 52 *Taenia* eggs/L in the effluent of a 20-day pond system, although *Ascaris* eggs were also detected. Mara & Silva (1986) reported *Ascaris* and hookworm eggs at concentrations as high as 13 eggs/L in the effluent of a five-pond system with a nominal HRT of 17 days. The variable removal of helminth eggs with respect to theoretical HRT or even estimated mean HRT of different ponds is likely to result from differing hydraulic efficiencies and RTDs of those ponds.

The measured concentrations of dye tracer in the effluent of the facultative pond are displayed in Figure 1. The highest concentration was measured after only 26 hours, and it was estimated that 8 and 12% of the dye mass passed through the pond in less than 48 and 72 hours respectively. These times can be expressed as the 8th and 12th percentiles (i.e. $T_8 = 2$ days, $T_{12} = 3$ days). If the retention times associated with these percentiles are compared to the theoretical HRT in 2011 (30 days), it is evident that the RTD is positively skewed. The results from the 2D CFD models also confirmed this, estimating the mean HRT to be half of the theoretical HRT.

Evidence of hydraulic short-circuiting is also supported by the 3D CFD tracer simulation. The time of the peak dye concentration estimated by the 3D CFD simulation matched the time of the highest concentrations of dye measured in the field. The magnitude of the peak may have even been underestimated in the field study, meaning that the T_8 and T_{12} values may be overestimated; the actual times may be even lower. Furthermore, the RTD estimated by the field study and the CFD modeling is based on the flow rate measured in 2011. In 2012, when the flow rate was almost twice as high, the portion of water (and helminth eggs) passing through the pond in this short-circuiting path likely reached the effluent in an even shorter amount of time.

Flow velocities in this short-circuiting path are likely to affect helminth egg removal efficiency. Figure 2 shows flow velocity contours estimated by the 3D CFD model. At a flow

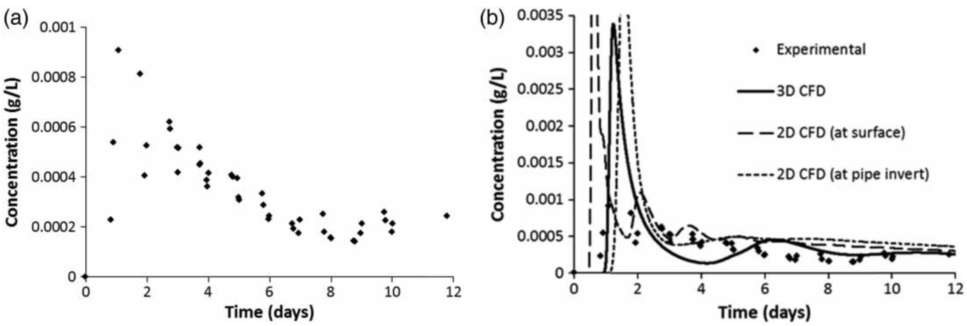

Figure 1 | Results of (a) the dye tracer field study, superimposed with (b) the results from the CFD simulations.

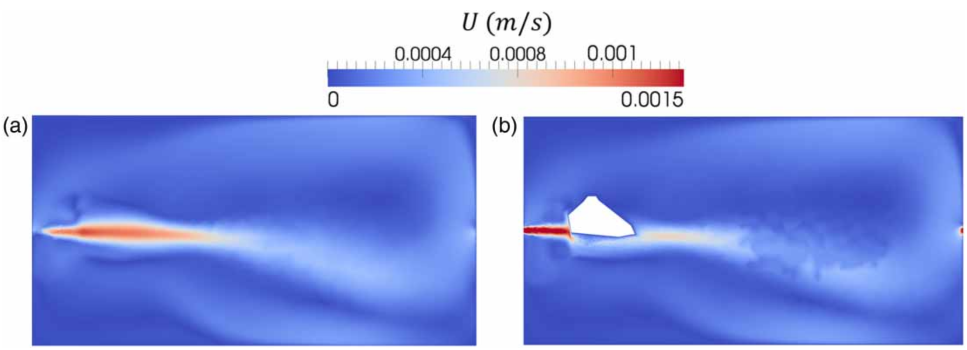

Figure 2 | Flow velocity contours based on 3D CFD model (a) at 0.1 m and (b) at 0.7 m below the water surface (1.7 m and 1.1 m above the bottom of the pond, respectively). Note: the blank portion near the inlet at 0.7 m below the water surface represents the extent of accumulated sludge at that level.

rate of 66 m^3/day, simulated velocities generally ranged from negligible to 0.0015 m/s, but reached a maximum of 0.0038 m/s. At twice the flow rate, velocities would be even higher. Assuming that *Taenia* eggs settle in the pond at 0.1 m/h (Murrell *et al*. 2005), the eggs that pass through the pond via this short-circuiting path are exposed to high velocities and may not have enough time to settle to the bottom of the pond. Theoretical settling velocities would also only govern helminth egg settling under laminar flow conditions with no vertical mixing. In reality, flow conditions are turbulent, especially at the pond influent or where sludge has accumulated. This turbulence, as well as wind effects or thermal inversion, may cause objects in the pond to settle more slowly or become resuspended. In this pond, the difference in water temperature at the surface and the bottom varied from negligible at night to ~8 °C in the afternoon.

CONCLUSIONS

- The facultative pond in this system exhibits signs of hydraulic short-circuiting with the peak dye concentration measured in the effluent after only 26 hours. Furthermore, 2D CFD models predict that the mean HRT is only half of the theoretical HRT for this pond. The theoretical HRT of a stabilization pond is not always a good surrogate for helminth egg removal. The percentage of water passing through the pond during the first short-circuiting peak and flow velocities in this short-circuiting path are likely more important. The helminth egg removal design equation might be improved by using an estimated 10th percentile value (T_{10}) based on pond dimensions, inlet/outlet configurations, etc., and by relating helminth egg removal to the ratio of T_{10} to the theoretical HRT. To improve removal efficiency, engineers and plant operators should attempt to increase the ratio of T_{10} to theoretical HRT by changing the configuration of the inlet structures, installing baffles or considering wind influence, as described by Shilton & Harrison (2003).
- *Taenia* eggs were the most common type of helminth eggs detected in the raw wastewater from this system, and were also the only eggs detected in the system effluent. While humans can only acquire taeniasis by consuming undercooked pork or beef infected with *T. solium* or *T. saginata* cysts, the direct ingestion of *T. solium* eggs by humans can cause human cysticercosis. The unitary classification system for water- and excreta-related diseases (Mara & Feachem 1999) should be updated to include human cysticercosis in Category A, and local authorities implementing guidelines for crop irrigation with wastewater should establish limits for *Taenia* eggs, especially where taeniasis is endemic.
- Future research on pathogen removal in pond systems should also include tracer studies or simulations to understand the hydraulic efficiency of the particular pond configuration. For helminth egg analysis, minimum limits of detection should always be reported, especially if no eggs are detected in the pond effluent.

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