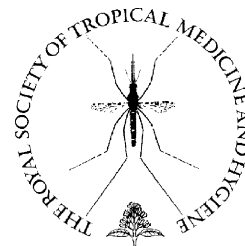




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MINI-REVIEW

Quality of reclaimed waters: a public health need for source tracking of wastewater-derived protozoan enteropathogens in engineered wetlands

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Summary Demand for high-quality drinking and recreational water rises exponentially owing to global demographic growth in the human population, reinforcing an urgent need for microbiologically safe reclaimed water. However, constructed wetlands, implemented into municipal wastewater treatment, may not provide substantial remediation for human protozoan enteropathogens such as *Cryptosporidium*, *Giardia* and human-virulent microsporidia. Improving reclaimed water quality by lowering faecal coliforms is not a sound solution for these pathogens. Current advances in molecular technology can benefit public health in developing and developed countries by changing the conceptual research framework for wastewater-receiving wetlands from 'pathogen removal' to 'pathogen source tracking' efforts.

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Cryptosporidium parvum, *Giardia lamblia* and human-virulent microsporidia (i.e. *Enterocytozoon bienersi*, *Encephalitozoon intestinalis*, *Ent. hellem* and *Enc. cuniculi*) are human anthrozoönotic enteropathogens that inflict considerable morbidity on healthy people, particularly children, and can cause mortality (e.g. *Cryptosporidium* and microsporidia) in immunosuppressed individuals (Graczyk et al., 2007a). *Cryptosporidium* and *Giardia* are very frequently transmitted via water, which is also involved in the epidemiology of microsporidian spores (Graczyk et

al., 2007a). The transmissive stages, i.e. oocysts, cysts and spores, are environmentally robust and therefore ubiquitous in aquatic habitats (Graczyk et al., 2007a). These enteropathogens are category B biodefense agents on the National Institutes of Health list, and microsporidian spores are on the Contaminant Candidate List of the US Environmental Protection Agency because spore identification, removal and inactivation in drinking water is technologically challenging. Water resources utilised for drinking and recreational purposes are commonly found in the same watersheds where treated effluents from wastewaters are discharged (Davison et al., 2005). Drinking, recreational and wastewaters are not monitored for these pathogens, despite considerable evidence demonstrating environmental contamination derived directly and indirectly from

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wastewaters. Constructed wetlands, of either vertical or horizontal (i.e. free surface or subsurface) flow, are increasingly being used worldwide for secondary or tertiary treatment of municipal sewage (Davison et al., 2005). In many developing and developed countries, these reed bed systems discharge to waters used for drinking water abstraction or recreational purposes (Davison et al., 2005).

It is presumed that human protozoan enteropathogens identified in reclaimed waters receiving effluents from wastewater treatment operations through constructed wetlands originate from those wastewaters (Thurston et al., 2001). However, a recent study demonstrated that this may not be the case (Graczyk et al., 2007a). Reed bed discharges can contribute to microbial build up in reclaimed water, but these pathogens may not necessarily originate from the wastewater received by these wetlands (Graczyk et al., 2007a). Whilst constructed wetland systems facilitate nutrient removal (Davison et al., 2005), they may not provide substantial protozoan pathogen remediation, despite reported removal efficiencies (Thurston et al., 2001). This is because constructed wetlands can act as reservoirs supporting both propagation and transmission of zoonotic protozoan enteropathogens entering these wetlands (Graczyk et al., 2007a). Sizing reed bed systems for a residence time of 5 days has become standard practice (Davison et al., 2005), leaving plenty of time for propagation and spreading of these anthropozoonotic protozoa in wetland habitats via a wide variety of wildlife species (Graczyk et al., 2007a).

Current advances in molecular epidemiology technology permit genotyping of human protozoan pathogens, which allows precise reservoir host identification (Jiang and Xiao, 2003). This considerably enhances the pathogen source-tracking efforts throughout the wastewater treatment system, i.e. primary and secondary, reed bed polishing and effluent-receiving reclaimed waters. Source tracking is commonly assumed to relate to bacterial overload and waterborne transport. However, faecal coliform source-tracking technology is not easily adoptable by wastewater facilities because of its sophistication. In addition, multiple studies have already shown the inadequacy of standard faecal coliforms (i.e. *Escherichia coli*, enterococci, faecal and total coliforms) as indicators of contamination of drinking, recreational and wastewaters with human protozoan pathogens.

Sewage wastewater discharges are worldwide risk factors for the introduction of human protozoan enteropathogens into surface waters. Pathogen source-tracking research in sewage treatment wetlands is deficient owing to the lack of available molecular technology in the past. None of the relevant studies used molecular genotyping of *Cryptosporidium*, *Giardia* or human-virulent microsporidia (Thurston et al., 2001).

Current technology allows for multiplexed species-specific identification, enumeration, viability assessment and source tracking of human protozoan pathogens (Graczyk et al., 2007a, 2007b; Jiang and Xiao, 2003). Such advances can benefit public health in developing and developed regions of the world by changing the conceptual research framework for constructed wetlands from

'pathogen removal' to 'pathogen source tracking' efforts. 'Removal' assumes that 'survived pathogens' originate from pathogens delivered to that wetland from the sewage treatment process (Thurston et al., 2001), whilst 'source tracking' evaluates the complexity of pathogen ecological interactions in engineered wetlands as well as its seasonal dynamics.

The demand for microbiologically safe reclaimed waters grows exponentially owing to the global demographic rise of the human population. Improvements in reclaimed water quality by lowering faecal coliform counts is not a sound solution for human protozoan enteropathogens. This reinforces a need for better water quality indicators or, alternatively, for testing the constructed wetlands discharging to drinking or recreational water reservoirs for *Cryptosporidium*, *Giardia* and human-virulent microsporidia.

Provision of safe quality, reclaimed waters is the outcome of partnerships between engineering, environmental health and epidemiological sciences, reinforced by regulatory bodies. The compiling evidence of this significant research gap, related to wetland discharges of treated wastewaters, needs to be appropriately addressed by the research-sponsoring agencies concerned with water reuse.

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