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# Simulation of enteric pathogen concentrations in locally-collected greywater and wastewater for microbial risk assessments

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ABSTRACT

As decentralized water reuse continues to gain popularity, risk-based treatment guidance is increasingly sought for the protection of public health. However, efforts to evaluate pathogen risks and log-reduction requirements have been hindered by an incomplete understanding of pathogen occurrence and densities in locally-collected wastewaters (*i.e.*, from decentralized collection systems). Of particular interest is the potentially high enteric pathogen concentration in small systems with an active infected excreter, but generally lower frequency of pathogen occurrences in smaller systems compared to those with several hundred contributors. Such variability, coupled with low concentrations in many source streams (*e.g.*, sink, shower/bath, and laundry waters), has limited direct measurement of pathogens. This study presents an approach to modeling pathogen concentrations in variously sized greywater and combined wastewater collection systems based on epidemiological pathogen incidence rates, user population size, and fecal loadings to various residential wastewater sources. Pathogen infections were modeled within various population sizes (5-, 100-, and 1,000-person) for seven reference pathogens (viruses: adenoviruses, *Norovirus*, and *Rotavirus*; bacteria: *Campylobacter* and *Salmonella* spp.; and protozoa: *Cryptosporidium* and *Giardia* spp.) on each day of 10,000 possible years, accounting for intermittent infection and overlap of infection periods within the population. Fecal contamination of fresh greywaters from bathroom sinks, showers/baths, and laundry, as well as combined greywater and local combined wastewater (*i.e.*, including toilets), was modeled based on reported fecal indicators in the various sources. Simulated daily infections and models of fecal contamination were coupled with pathogen shedding characteristics to generate distributions of pathogen densities in the various waters. The predicted frequency of pathogen occurrences in local wastewaters was generally low due to low infection incidence within small cohort groups, but increased with collection scale (population size) and infection incidence rate (*e.g.*, *Norovirus*). When pathogens did occur, a decrease in concentrations from 5- to 100- and from 100- to 1,000-person systems was observed; nonetheless, overall mean concentrations (*i.e.*, including non-occurrences) remained the same due to the increased number of occurrences. This highlights value of the model for characterizing scaling effects over averaging methods, which overestimate the frequency of pathogen occurrence in small systems while underestimating concentration peaks that likely drive risk periods. Results of this work will inform development of risk-based pathogen reduction requirements for decentralized water reuse.



## Introduction

Limited water resources and the rising awareness of conservation potential has led to an increased interest in water reuse. On-site or local collection, treatment, and reuse of household wastewater or greywater offers the practical opportunity to provide water savings while minimizing the cost and liability of centralized infrastructure, particularly when coupled with energy recovery (Xue et al., 2015). However, compared to municipal sewage these waters experience large variations in quality due to lack of wastewater dilution, sporadic pathogen occurrences, and variability in user behavior, and their pathogen content is poorly characterized (O'Toole et al., 2014; Schoen and Garland, 2015). This has precluded the

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**Table 1**
Reported enteric reference pathogen measurements in greywater (GW).

| Pathogen genus | Sample type | Sample size | Occurrence | Concentration range | Reference |
|---|---|---|---|---|---|
| *Campylobacter* | Laundry | Not stated | Not detected | Not detected | Christova-Boal et al. (1996) |
| *Cryptosporidium* | Laundry | Not stated | Not detected | Not detected | Christova-Boal et al. (1996) |
| *Giardia* | Laundry | Not stated | Not detected | Not detected | Christova-Boal et al. (1996) |
| *Norovirus* | Laundry | 75 | 13% | Qualitative only | O'Toole et al. (2012) |
| *Rotavirus* | Laundry | 75 | 1% | Qualitative only | O'Toole et al. (2012) |
| *Salmonella* | Laundry | Not stated | Not detected | Not detected | Christova-Boal et al. (1996) |
| *Campylobacter* | Shower/bath | Not stated | Not detected | Not detected | Christova-Boal et al. (1996) |
| *Cryptosporidium* | Shower/bath | Not stated | Not detected | Not detected | Christova-Boal et al. (1996) |
| *Giardia* | Shower/bath | Not stated | Not detected | Not detected | Christova-Boal et al. (1996) |
| *Norovirus* | Shower/bath | 36 | 8% | Qualitative only | O'Toole et al. (2012) |
| *Rotavirus* | Shower/bath | 36 | Not detected | Not detected | O'Toole et al. (2012) |
| *Salmonella* | Shower/bath | Not stated | Not detected | Not detected | Christova-Boal et al. (1996) |
| *Campylobacter* | Bathroom sink | 3 | Not detected | Not detected | Birks et al. (2004) |
| *Cryptosporidium* | Bathroom sink | 3 | 67% | 0.4–1.2 oocysts·$L^{-1}$ | Birks et al. (2004) |
| *Giardia* | Bathroom sink | 3 | 67% | 0.6–1.2 cysts·$L^{-1}$ | Birks et al. (2004) |
| *Salmonella* | Bathroom sink | 3 | Not detected | Not detected | Birks et al. (2004) |
| *Campylobacter* | Combined GW | 9 | Not detected | Not detected | Winward et al. (2008) |
| *Campylobacter* | Combined GW | 8 | Not detected | Not detected | Birks and Hills (2007) |
| *Cryptosporidium* | Combined GW | 8 | Not detected | Not detected | Birks and Hills (2007) |
| *Giardia* | Combined GW | 8 | 63% | 0.5–1.5 cysts·$L^{-1}$ | Birks and Hills (2007) |
| *Salmonella* | Combined GW | 13 | Not detected | Not detected | Winward et al. (2008) |
| *Salmonella* | Combined GW | 9 | Not detected | Not detected | Benami et al. (2015) |
| *Salmonella* | Combined GW | 8 | 13% | Not stated | Birks and Hills (2007) |
| *Salmonella* | Combined GW | Not stated | Not stated | 5400 CFU·100 $mL^{-1}$ | Kim et al. (2009) |

development of pathogen-based treatment standards for decentralized water reuse, resulting in existing standards that specify treatment parameters that are routine to measure yet lacking a demonstrated relationship to pathogen risk (NSF/ANSI, 2012). Indeed, a recent assessment of greywater reuse potential by the National Academy of Sciences concluded that household or multi-residential scale systems can offer cost-effective reductions in water demand, but that expansion is hindered by lack of risk-based reuse guidelines. Better understanding of pathogen occurrence and fate in these systems is necessary for determination of fit-for-purpose treatment requirements (NAS, 2016).

Attempts to measure enteric pathogens directly in greywater have been largely ineffective, often experiencing non-detects (Benami et al., 2015; Christova-Boal et al., 1996; Winward et al., 2008) (Table 1). To a large extent, these non-detects may occur due to intermittent infection incidence among smaller population sizes (*e.g.*, single households, apartment buildings/subdivisions, or blocks/districts as opposed to entire cities). Conversely, as the population size increases, wastewater dilution effects result in a more stabilized low pathogen concentration; with the large contributing population of municipal wastewater, *Norovirus* concentrations during outbreak conditions remain comparable to those that are routinely observed (Hellmér et al., 2014; Pouillot et al., 2015). Given quantitative limits of detection for any method, this interplay creates difficulty in measurement and interpretation of non-detect results; pathogens may indeed be present but below detection limits, or present at other times not captured by the sampling campaign. The few studies containing positive detections (Table 1) have either been non-quantitative (O'Toole et al., 2012) or too limited (system-specific or insufficiently reported) for broad applicability (Birks et al., 2004; Birks and Hills, 2007; Kim et al., 2009).

Others have attempted to estimate pathogen content based on the ratio of pathogens to fecal indicators in municipal wastewater (Deere et al., 2006; NRMMC-EPHC-AHMC, 2006; Maimon et al., 2010), but poor correlations and scaling effects limit the ability of such methods to accurately characterize onsite waters (O'Toole et al., 2014). An alternative approach is the use of indicators to determine fecal contamination of the water and epidemiological data to estimate pathogens shed in that feces (Barker et al., 2013a; Ottoson and Stenström, 2003; Schoen et al., 2014; Fane et al., 2002). These models have often determined pathogen concentrations using the number of infections averaged over an annual (Ottoson and Stenström, 2003), seasonal (Mok et al., 2014), or monthly basis (Barker et al., 2013a). However, this simplification results in a small number of fractional infections on each day, an impossible description of actual conditions. Implicitly, such models assume that pathogens are always present but in low concentrations, and neglect scaling effects as infections are averaged over the same population in which dilution occurs. In order for (low) annual infection incidence rates to be correct, the requirement of whole numbers of infections implies that in small populations there are often days in which no infection occurs. No models have been developed for a comprehensive suite of fecal pathogens and source water types while accounting for scaling effects.

The objective of this work was to simulate enteric pathogen occurrence and concentrations in various local wastewaters (source-separated greywaters, combined greywater, and total domestic wastewater) as a function of collection scale (population size). This was accomplished by coupling literature review of fecal contamination of fresh greywater from bathroom sinks, showers/baths, and laundry, as well as of local wastewater from all sources including toilets, with a model of infection occurrence in small populations and reported pathogen shedding characteristics (durations and fecal densities). These results are intended to support the development of risk-based treatment guidance for the safe reuse of human-impacted wastewaters (Schoen et al., 2016).

## Methods

Enteric pathogen densities within locally-collected wastewater and greywater (*i.e.*, from decentralized collection systems) were modeled using an epidemiology-based approach (Barker et al., 2013a; Ottoson and Stenström, 2003; Fane et al., 2002; Mok et al., 2014; Barker et al., 2013b). The epidemiology-based approach consisted of three separate phases: 1) estimation of fecal load in the collected water (wet g feces per L water), determined by the ratio of fecal indicator density in the water to that in human feces (both freshly collected); 2) simulation of enteric pathogen infections in the selected population; and 3) estimation of pathogen concentration in the water based on modeled fecal load and the