Research Paper

Risk of adenovirus and *Cryptosporidium* ingestion to sanitation workers in a municipal scale non-sewered sanitation process: a case study from Kigali, Rwanda

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ABSTRACT

Sanitation workers provide essential services that protect public health, often at the cost of their own health and safety. In this study, we evaluate occupational exposure to fecal pathogens at each stage in a non-sewered sanitation process. Bulk fecal waste samples were collected during waste collection and waste processing tasks and analyzed for *Cryptosporidium*, adenovirus, *E. coli*, and total coliforms using quantitative polymerase chain reaction and culture methods. Structured observations of worker hand-to-mouth behavior were conducted, and worker hand- and glove-rinse samples were collected and analyzed for *E. coli* and total coliforms. A Monte Carlo simulation was used to model the dose of pathogen ingested and the risk of disease across two waste collection and processing tasks. The model results show that the probability of disease was highest from exposure to adenovirus during collection. Our analysis highlights that pathogen-to-indicator ratios are useful for predicting the risk to adenovirus which has a high detection rate. On the other hand, the use of pathogen-to-indicator ratios to predict *Cryptosporidium* concentration is fraught due to variable detection rates and concentration.

Key words | occupational exposure, quantitative microbial risk assessment, resource recovery, Rwanda, sanitation workers, urban sanitation

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HIGHLIGHTS

- This study outlines methods for conducting quantitative microbial risk assessments in low-resource settings.
- Pathogen-to-indicator ratios specific to this study are constructed from primary data in order to minimize model uncertainty.
- The probability of pathogen infection and disease was estimated for sanitation workers at each stage in a municipal scale fecal waste collection and waste-to-fuel process.

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INTRODUCTION

In the absence of a sewer network, non-sewered sanitation systems rely on workers to physically handle fecal sludge, a practice that can increase the risk of contracting enteric diseases (Stenström 2011). Despite this, risk assessments on occupational exposures to workers within non-sewered sanitation systems are lacking (World Bank ILO WaterAid and WHO 2019).

Prior risk assessments along fecal waste management processes have been conducted largely in the context of the agricultural land application of recycled fecal sludge (Mara *et al.* 2007; Seidu *et al.* 2008; Silverman *et al.* 2013; Amha *et al.* 2015). Fewer studies have looked at the pathogenic risks to workers in upstream processes such as waste collection and waste treatment (Fuhrimann *et al.* 2016; Mackinnon *et al.* 2018; Bischel *et al.* 2019). To our knowledge, there are no existing studies which examine risks to workers exposed to fecal sludge during waste collection and treatment processes.

In the absence of direct pathogen measurements in low-resource settings, health risk assessment often relies on the use of pathogen-to-indicator ratios – the number of pathogens in an environmental sample is calculated by multiplying the concentration of indicators measured by a pathogen-to-indicator ratio from a known source of fecal contamination (Oragui *et al.* 1987; Rose *et al.* 1996; Howard *et al.* 2007; Mara *et al.* 2007; Bivins *et al.* 2017). The use of such ratios introduces uncertainty into models when ratios derived from contamination sources in one geographic and population context are used to estimate pathogen concentrations in another. However, this widespread use of pathogen-to-indicator ratios in contexts different from their origin has been called into question. Relative concentrations of different organisms are known to be driven by context-dependent factors such as the source of the fecal input, pathogen endemicity in the population, concentrations of other naturally occurring organisms, and exposure of the medium to environmental factors which drive differential die-off rates (Cutolo *et al.* 2012; Silverman *et al.* 2013; Keuckelaere *et al.* 2015).

In this study, we develop a sampling and analysis framework for measuring pathogen-to-indicator ratios and quantifying pathogen specific risk of ingestion to workers in a fecal sludge collection and processing operation. In addition to the framework developed, the findings from this study can help governments, process designers, and occupational hygienists target resources toward controlling high-risk occupational exposures during waste collection and processing.

METHODS

Study site

This study is a case study of a fecal waste collection and waste transformation process operating in Kigali, Rwanda, a city with no central sewer network (NIOSR 2014). When pit latrines and septic tanks fill, they are emptied by service providers who pump out the waste and transport it by truck to a waste-to-fuel facility which converts the fecal sludge into solid industrial-grade fuel.

Recruitment and ethics

Twelve workers were sampled while performing one of two types of work tasks in the waste-to-fuel process: (1) waste collection tasks and (2) waste processing tasks at the waste-to-fuel facility. See Supplementary Material for further description.

All workers consented to participating in the study, and all study protocols were approved by the UC Berkeley Committee for Protection of Human Subjects prior to the conducting of this research (CPHS/OPHS Protocol ID: 2017-06-10016). Potential candidates for inclusion were all workers performing jobs in the waste collection and transformation process over the age of 18. All workers who participated in the study were provided with information about reducing workplace exposures. All information was provided in Kinyarwanda, and written informed consent was collected from each participant prior to data collection activities. Our methodology combined a participatory research approach with capacity-building strategies which were designed to overcome knowledge gaps in microbial

sampling, exposure assessment, and occupational health (Cahill 2007). This approach enabled the local project team to continue carrying out occupational and environmental monitoring beyond the scope of this study.

Model framework

A quantitative microbial risk assessment (QMRA) model was developed to estimate pathogen ingestion by workers due to contaminated hand- and glove-to-mouth contact events over an 8-h work period (Haas *et al.* 2014). Three types of inputs were measured directly in this study: (1) concentration of indicator bacteria on worker hands and gloves, (2) ratio of pathogens and indicator organisms in bulk sludge samples, and (3) frequency of hand-to-mouth activity during a work task. The model also incorporates other factors that influence the number of pathogens transferred from hands and gloves to mouth: (1) the transfer efficiency between hand or glove, and mouth and (2) the surface area of the hand or glove in contact with the mouth. The sampling framework used to collect primary microbial data is shown in Figure 1.

Total coliform and *E. coli* in bulk fecal sludge samples

The concentration of pathogens and indicators was measured in three types of bulk fecal sludge samples: (1) sludge from the waste collection trucks, (2) sludge from the waste transformation facility, and (3) the final fuel product.

Two cross-sectional sampling campaigns were carried out between June–August 2017 ($N = 15$) and January 2018 ($N = 27$).

All samples were analyzed using a membrane filtration system with MI media (BD Difco, Franklin Lakes, NJ) and incubated at $35 \pm 0.5\ ^{\circ}C$ for 24 h according to the United States Environmental Protection Agency method 1604 for enumerating *E. coli* and total coliforms (USEPA 2002). Additional information on total coliform and *E. coli* limit of detection (LOD) in bulk samples is available in the Supplementary Material.

Pathogens in bulk samples

Two pathogens, adenovirus and *Cryptosporidium* spp., referred to as *Cryptosporidium* throughout this paper, were chosen for this study based on the following criteria: (1) detection and high prevalence in stool samples of diarrheal patients in Rwanda (Kabayiza *et al.* 2014), (2) published dose-response curves (Teunis *et al.* 1996), and (3) available molecular detection methods.

DNA was extracted from bulk samples and then analyzed for *Cryptosporidium* and adenovirus genes using quantitative polymerase chain reaction (qPCR). Additional information on DNA extraction and PCR protocols is available in the Supplementary Material.

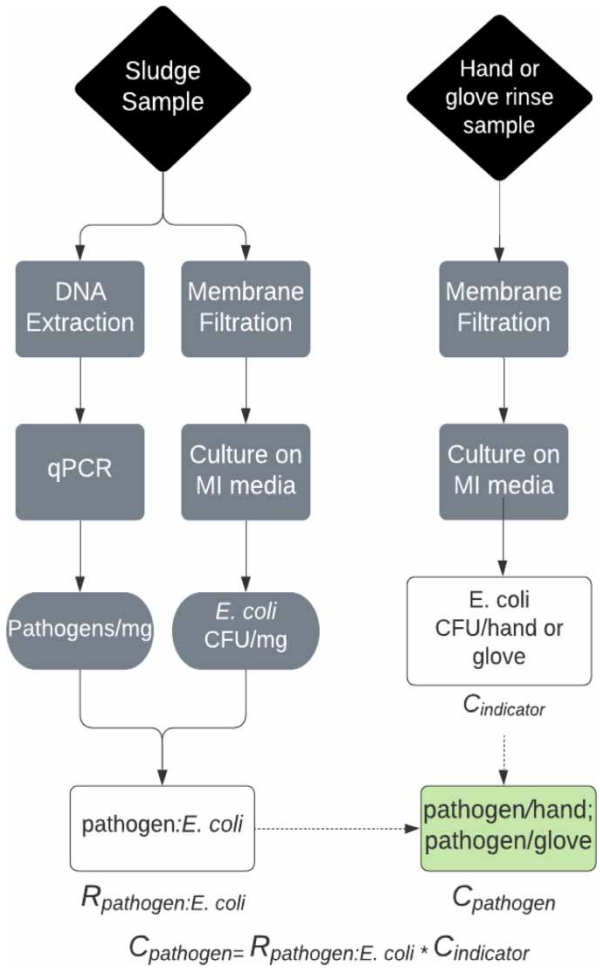

Figure 1 | Sampling framework. Bulk samples were collected of the sludge at different stages in the process and analyzed for pathogens and indicators using qPCR and culture methods. Hand- and glove-rinse samples were collected from workers in the two task groups and processed for indicator bacteria using culture methods. The ratio of pathogens to indicators in bulk samples was determined and used to estimate pathogen concentrations in hand-rinse samples.

Pathogen-to-total coliform ratio in bulk samples

Concentration ratios of pathogens-to-total coliforms in bulk samples, $R_{\text{path:}tc}$, were constructed as lognormal probability distributions fit to the empirical data of pathogen and indicator concentrations, $C_{\text{pathogen}\,jg}$ and $C_{\text{indicator}\,jg}$. Concentrations lower than the LOD were replaced by a value equal to half the detection limit prior to fitting the lognormal distributions.

Indicator bacteria in worker hand- and glove-rinse samples

Hand-rinse samples of ungloved hands (prior to glove application) and gloved hands were obtained from workers in collection and facility tasks using the USEPA method 1604 and a field protocol developed by Pickering *et al.* (USEPA 2002; Pickering *et al.* 2011). For each task that was observed, four rinse samples were collected – a glove- and hand-rinse sample before the task started, and a glove- and hand-rinse sample when the task was completed. Hand washing in the middle of a task, or work shift, was not observed.

All hand-rinse samples were analyzed using a membrane filtration system with MI media (BD Difco, Franklin Lakes, NJ) and incubated at 35 ± 0.5 °C for 24 h according to USEPA method 1604 (USEPA 2002). If the measured concentrations were lower than the LOD, then half the detection limit of 1 CFU/filter was assumed as the concentration. If the concentrations were too numerous to count (>500 CFU/filter), then the concentration of bacteria in the sample was calculated assuming 500 CFU/volume filtered. Additional information on the LOD and the full dataset of indicator bacteria results from hand- and glove-rinse samples is available in the Supplementary Material.

Worker hand-to-mouth activity

One worker from the waste-to-fuel facility was trained to observe fellow workers and enumerate the number of hand-to-face activities observed, f_{jg}, while wearing gloves (g = gloves) or using ungloved hands (g = hand) during collection (j = collection) and facility tasks (j = facility) (Gorman Ng *et al.* 2016; Kwong *et al.* 2016). Each observation was limited to a single observer recording the hand-to-face activities of a worker doing a single task. We sought to minimize observers by hiring a familiar staff member to conduct observations.

Dose estimation

The total dose of pathogens, $d_{\text{pathogen}\,j}$, ingested from both worker hand- and glove-to-mouth activities in a given task j, was calculated as follows:

$$d_{\text{pathogen}\,j} = \sum_{\text{g=hand}}^{\text{g=glove}} (C_{\text{pathogen}\,jg} * FSA * TE * \max(f_{jg})^* v) \quad (1)$$

where $d_{\text{pathogen}\,j}$ is the lognormal distribution of the pathogen ingested over an 8-h period in a given task type. $C_{\text{pathogen}\,jg}$ is the lognormal distribution of pathogen concentration in the hand- or glove-rinse samples derived as a product of the indicator concentration measured in a rinse sample, $C_{\text{indicator}\,jg}$, and the lognormal distribution of the pathogen-to-indicator ratio for the sludge in each task group, R_j. FSA is a point value representing the fractional surface area of the hand, glove, or phone coming into contact with the perioral area (Beamer *et al.* 2012, 2015). TE is a constant representing the fraction of pathogens transferred between the hand and the mouth. The frequency of glove or hand-to-mouth contacts observed in each task type is represented by $\max(f_{jg})$. The viability of detected *Cryptosporidium* and adenovirus is represented by v. As qPCR does not distinguish between viable and nonviable organisms, we made the health-protective assumption that 100% of the organisms were quantified are viable.

We assume that the transfer efficiency of all hand- or glove-to-face activities is equivalent to the 33%, the transfer efficiency reported for viruses between fingertips to lips, given the dearth of the literature on more specific transfer efficiencies (Nicas & Best 2008).

Risk of infection and disease

A 10,000-trial Monte Carlo simulation was used to estimate a worker's probability of disease, highly credible gastrointestinal disease (HCGI), from exposure to *Cryptosporidium* or adenovirus over an 8-h work period. For each 8-h exposure

simulated in either $j = collection$ or $j = facility$ task, the model randomly selects a set of values from probability distributions describing the input parameters' natural variability and uncertainty. A full list of the parameters used is available in Supplementary Table S1. When parameter distributions could not be confidently constructed or sourced from the literature, a point value was used.

A previously published beta-Poisson dose–response relationship was used to calculate adenovirus risk while an exponential dose-response model was used to calculate the *Cryptosporidium* risk. Additional information on both models is available in the Supplementary Material.

All simulations were conducted in R (R-3.2.4 version, R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Concentration of indicators and pathogens in bulk fecal sludge samples

The concentration of total coliforms detected was above the LOD in the vast majority of sample types, unlike *E. coli* which was below the LOD in 33% of samples (Table 1). Surprisingly, the average concentration of total coliforms in facility samples was an order of magnitude higher than the concentrations in both collection and fuel samples. The average total solids in each type of sample are shown in Supplementary Table S2.

Adenovirus was detected in 88% of the bulk samples. A high adenovirus detection rate was found in the samples from the collection (89%) and facility (100%) samples compared with the fuel samples (67%) (Table 1). Within collection samples, the geometric mean of adenovirus samples above the detection limit (7.6×10^4) was an order of magnitude higher than that in the facility (9.10×10^3) and fuel samples (3.4×10^3).

Cryptosporidium was detected in 16% of bulk samples from all stages of the process. Most samples of *Cryptosporidium* did not amplify within the quantifiable range and those that did exhibited a very large spread. The variation in the concentration within the detectable range was far higher than the variation of the other indicators and pathogens, especially in the facility and fuel samples. Information on the pathogen-to-total coliform ratios, $R_{\text{path:}tc}$, derived from concentrations observed in the bulk samples can be found in Supplementary Table S3.

Table 1 | Concentration of indicator bacteria and pathogens measured (CFU/g total solids) in bulk fecal sludge samples

Organism	Task type	*N*	> LOD	% >LOD	Range	GM (GSD)
Total coliform	Collection	15	14	93	5.2×10^4–1.5×10^7	1.2×10^6 (8.1)
	Facility	2	2	100	8.7×10^6–1.7×10^7	1.20×10^7 (1.6)
	Fuel	10	10	100	2.1×10^5–4.7×10^7	4.2×10^6 (5.1)
	*LOD TC: 3.3×10^4 CFU/g-collection, 3.0×10^3 CFU/g-facility, 1.1×10^3 CFU/g-fuel					
E. coli	Collection	15	13	86	4.2×10^4–5.2×10^6	2.9×10^5 (5.9)
	Facility	2	0	0	–	–
	Fuel	10	5	50	5.3×10^4–2.1×10^6	3.9×10^5 (6.4)
	*LOD *E. coli*: 3.3×10^4 CFU/g-collection, 3.0×10^3 CFU/g-facility, 1.1×10^3 CFU/g-fuel					
Adenovirus	Collection	18	16	89	2.9×10^3–2.6×10^6	7.6×10^4 (5.7)
	Facility	16	16	100	3.8×10^2–1.2×10^6	9.1×10^3 (8.3)
	Fuel	9	6	67	4.5×10^2–1.5×10^4	3.4×10^3 (5.0)
	*LOD *Cryptosporidium*: 1 copies/g-collection; 49 copies/g-facility; 215 copies/g-fuel					
Cryptosporidium	Collection	18	3	17	1.7×10^4–4.8×10^4	2.9×10^4 (1.7)
	Facility	16	2	13	1.2×10^3–3.5×10^5	2.1×10^4 (5.4×10^1)
	Fuel	9	2	22	9.6×10^2–6.3×10^5	2.5×10^4 (9.8×10^1)
	*LOD *Cryptosporidium*: 11 copies/g-collection; 156 copies/g-facility; 346 copies/g-fuel					

GM, geometric mean; GSD, geometric standard deviation.

Concentration of indicator bacteria in worker hand-rinse samples

The concentration of *E. coli* and total coliform concentrations measured in worker hand- and glove-rinse samples are shown in Table 2. The concentration of total coliform and *E. coli* measured in rinse sample of hands and gloves taken prior to the start of the work task was noticeably high, indicating a baseline contamination level at the start of the work shift. The geometric mean concentration was higher in after-task samples across both indicator and task types, except for *E. coli* measured on hands of facility workers, indicating a general accumulation of bacteria on both hands and gloves over the course of a work task.

Pathogen dose and risk estimation

The dose of *Cryptosporidium* and adenovirus was estimated based on observed worker hand-to-mouth activity (see Supplementary Table S4) and the measured concentrations of indicators in hand-rinse samples. The geometric mean dose of *Cryptosporidium* ingested by workers in the facility task group was an order of magnitude higher than in the collection task group, while the geometric mean dose of adenovirus was higher in the collection tasks (Table 3).

The risk of contracting gastrointestinal disease resulting from hand-to-mouth exposure events during an 8-h period is shown for both pathogens in two task groups. Between the two pathogens in both task groups, adenovirus posed a higher risk of gastrointestinal disease. Between the two task types, facility tasks posed a higher risk of gastrointestinal disease from both pathogens (Table 3).

DISCUSSION

The results of this risk assessment suggest that interventions should focus on reducing exposure for workers performing fecal waste collection and waste processing tasks. Of the pathogen task combinations explored in this study, the probability of disease was highest from exposure to adenovirus during collection tasks.

A high degree of contamination in the glove-rinse samples taken before the task suggests poor storage or poor sterilization of gloves in between tasks or workdays. Storage of gloves in areas that are protected from airborne contamination as well incorporating regular handwashing and hand-sanitizing practices into standard operating procedures may encourage better decontamination of hands and gloves before, during, and after work tasks. Proper cleaning and storage of all personal protective equipment (PPE) is necessary to prevent other exposure pathways that were not explored in this study. Contaminated uniforms, eyewear, and boots may also elicit exposure pathways if they are improperly cleaned or stored.

The high levels of hand contamination seen in the hand-rinse samples taken after the task suggest that the gloves worn did not provide an adequate exposure barrier. This

Table 2 | Concentration of *E. coli* and total coliform measured per hand and glove in worker hand- and glove-rinse samples

				Before task			After task		
Sample type	**Task type**	**Org.**	***N*[a]**	**> LOD**	**Range**	**GM (GSD)**	**> LOD**	**Range**	**GM (GSD)**
Hand-rinse	Collection	*E. coli*	9	6	1.3×10^{2}–4.0×10^{3}	7.9×10^{2} (4.5)	9	2.5×10^{2}–4.8×10^{3}	9.9×10^{2} (3.1)
	Collection	TC[2]	9	9	1.0×10^{3}–1.2×10^{4}	5.1×10^{3} (2.7)	9	3.2×10^{3}–1.7×10^{4}	8.4×10^{3} (1.7)
	Facility	*E. coli*	26	20	1.3×10^{2}–5.7×10^{3}	7.9×10^{2} (3.8)	18	1.3×10^{2}–1.6×10^{4}	5.6×10^{2} (4.05)
	Facility	TC[2]	26	25	1.3×10^{2}– 1.6×10^{4}	5.4×10^{3} (2.7)	26	2.5×10^{2}–1.2×10^{5}	7.2×10^{3} (2.9)
Glove-rinse	Collection	*E. coli*	9	9	2.50×10^{2} $^{-}4.3\times10^{3}$	1.8×10^{3} (2.7)	9	5.0×10^{2}–8.5×10^{3}	2.8×10^{3} (2.42)
	Collection	TC	9	9	4.2×10^{3}–1.7×10^{4}	9.6×10^{3} (1.57)	9	7.0×10^{3}–1.5×10^{4}	1.1×10^{4} (1.3)
	Facility	*E. coli*	26	22	1.3×10^{2}–9.0×10^{3}	1.3×10^{3} (4.15)	22	1.3×10^{2}–1.3×10^{4}	1.6×10^{3} (4.05)
	Facility	TC	26	26	5.0×10^{2}–1.8×10^{4}	8.7×10^{3} (2.11)	26	2.2×10^{3}–1.25×10^{5}	1.1×10^{4} (2.03)

[a]*N* refers to the number of before- and after-task sample pairs.
GM, geometric mean; GSD, geometric standard deviation.
LOD: 125 CFU/hand.

Table 3 | Modeled distributions of *Cryptosporidium* and adenovirus dose

		Dose		Risk	
		Range	GM (GSD)	Range	GM (GSD)
Cryptosporidium	Collection	1.1×10^{-4}–2.1×10^{2}	3.2×10^{-1} (5.8)	6.1×10^{-7}–4.8×10^{-1}	9.3×10^{-4} (5.9)
	Facility	9.3×10^{4}–2.0×10^{4}	1.5 (7.3)	4.1×10^{-6}–7.0×10^{-1}	4.6×10^{-3} (6.9)
Adenovirus	Collection	9.4×10^{-1}–2.3×10^{5}	5.0×10^{2} (5.3)	9.1×10^{-2}–5.0×10^{-1}	5.0×10^{-1} (1.1)
	Facility	1.9×10^{-2}–2.3×10^{3}	4.3 (4.8)	8.7×10^{-2}–5.0×10^{-1}	2.3×10^{-1} (2.3)

GM, geometric mean; GSD, geometric standard deviation.

could be due to the use of gloves that have been damaged, ill fitted, or improperly applied and removed.

The concentration of indicators measured in bulk samples varied widely across the different days sampled. Within the samples from the collection task, this variation may be due to the sludge age and moisture content, factors that are driven by the type of pit construction, frequency of emptying, geological features of different neighborhoods, or the temperature and humidity differences which may affect die off and regrowth (Nabateesa *et al.* 2017). Within the facility samples, recolonization events may occur and be influenced by factors such as wind and precipitation. During such events, dusts and aerosols of untreated fecal sludge may disperse and deposit in downstream parts of the facility (Zaleski *et al.* 2005).

The concentration of pathogens measured in bulk samples also varied widely across the different days sampled. One hypothesis to account for this variation is the neighborhood-specific prevalence of these two pathogens. For example, *Cryptosporidium* prevalence has been shown to vary as a function of the contamination of local water sources, malnutrition, poor environmental sanitation, and use of household sanitation facilities (Sarkar *et al.* 2014). These factors have been shown to vary by neighborhood in Kigali and may explain some of the variance in *Cryptosporidium* concentrations over different sampling days (Tsinda *et al.* 2013). Further research is required to better understand the drivers of spatial variation of pathogens in sludge collected from different neighborhoods.

The presence of a high number of non-detectable concentration values in the *Cryptosporidium* concentration measurements suggests that ratios may be best suited for estimating risks in areas where the incidence of cryptosporidiosis has already been established in the population. In general, using pathogen-to-indicator ratios for rare species is fraught with uncertainty that is driven by the high number of non-detects which are assigned a value of LOD/2 in order to proceed with the risk assessment analysis.

Although the process was designed to completely remove pathogens, we have several hypotheses to explain the detection of indicators and potentially viable pathogens in the final fuel product. If sludge is going through the process as non-uniformly sized cluster of particles, some of the sludge in the center of larger clusters may not completely dry or be exposed to the necessary heat to cause inactivation. An alternative hypothesis is a recolonization or recontamination event occurring at some point during the process.

There are several limitations to this study. While we intended to collect more samples, the facility from which the samples were collected unexpectedly closed during the second year of our research. Although the limitation in sample size introduces a high degree of uncertainty for the dose and risk estimate, it provided us with a mechanism to explore the impact of ratios and assumptions about ratios on the risk model. Thus, the ratios that are derived here should not be used for other QMRA studies. Rather, we view this study as establishing a methodological framework for future studies, identifying sources of faulty assumptions around commonly used QMRA methodology, and identifying priority areas for future research.

In this study, participatory research methods were used to recruit participants and collect samples in Kigali. Despite limited sample size due to resources spread between training, communication, and data collection, we highlight the importance of participatory methods in building a

framework for long-term exposure monitoring in low-resource settings.

CONCLUSION

Several interventions may be useful in controlling worker exposure to fecal sludge. Elimination and substitution are the most effective ways to reduce workplace hazards but are not applicable in this case. Therefore, engineering controls should be prioritized such as enclosure and automation of processes that require workers to handle or come into close contact with sludge. Administrative controls including education on the fecal-oral route or implementation of workplace hygiene programs can also prevent workplace exposures. Finally, protecting the worker with PPE that is properly worn, stored, and replaced at regular intervals can prevent exposure and disease to sanitation workers.

DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

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