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Quantitative microbial risk assessment of outdoor aerosolized pathogens in cities with poor sanitation

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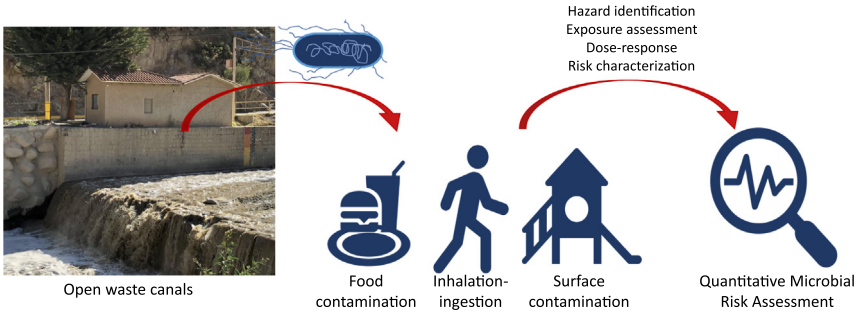
HIGHLIGHTS

- Infection risk of fecal bioaerosols near open waste canals (OWCs) was assessed.
- A Quantitative Microbial Risk Assessment (QMRA) model and a web app were developed.
- Fecal bacterial aerosols near OWCs pose non-negligible risks of infection.
- Bioaerosols near OWCs may not be a major cause of diarrheal disease in La Paz.
- The web application allows users to conduct QMRA in different contexts.

GRAPHICAL ABSTRACT



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ABSTRACT

The aeromicrobiological transmission pathway of enteric pathogens in places with unsafe sanitation services is poorly understood. In an attempt to partly fill this knowledge gap, we assessed the potential public health impact of bioaerosols near open waste canals (OWCs) using Quantitative Microbial Risk Assessment (QMRA). We used data acquired in La Paz, Bolivia to characterize the risk of disease that aerosolized enteric pathogens may pose through food, fomites and inhalation (all followed by ingestion). Three reference pathogens were selected to conduct the assessment: enterotoxigenic *Escherichia coli* (ETEC), *Shigella flexneri*, and *Campylobacter jejuni*. Inhalation followed by ingestion had the highest median infection risk per event i.e. 3×10^{-5} (3 infections for every 100,000 exposures), compared to contaminated food e.g. 5×10^{-6} and fomites e.g. 2×10^{-7}, all for *C. jejuni* infections. Our sensitivity analysis showed that bacterial fluxes from the air were the most influential factor on risk. Our results suggest that fecal bacterial aerosols from OWCs present non-negligible risks of infection in La Paz, with median annual infection risks by *C. jejuni* being 18 (food), and 100 (inhalation) times greater than the EPA's standard for drinking water (1×10^{-4}). We included two of the QMRA models presented here in a novel web application we developed for user-specified application in different contexts.

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1. Introduction

Access to safe drinking water, sanitation, and hygiene continues to be limited in many areas of the world, accounting for 60% of the global diarrheal deaths in 2016 (800,000) (Prüss-Ustün et al., 2019). While many studies have been conducted on the fecal-oral route for transmission of enteric diseases, studies on aerosol transmission of enteric pathogens in places with poor sanitation and associated risk of infection are limited in the literature. Numerous studies found enteric microorganisms in aerosols emitted from land application of biosolids (Dungan, 2014; Viau et al., 2011), concentrated animal feeding operations (Jahne et al., 2015; Millner, 2009), and toilet flushing (Johnson et al., 2013; Wilson et al., 2020). Our recent work detected multiple enteric pathogens in aerosols sampled near open waste canals (OWCs) (Rocha-Melogno et al., 2020; Ginn et al., 2021). In the present analysis, we aimed to quantify the risk of disease that these aerosolized pathogens near OWCs may pose through food, fomite, and inhalation routes using Quantitative Microbial Risk Assessment (QMRA) and previously published data (Rocha-Melogno et al., 2020; Ginn et al., 2021). The steps involved in this risk assessment were: hazard identification, exposure assessment, dose-response model selection, and risk characterization.

Biological aerosols (bioaerosols) result from wastewater aerosolization, bubble bursting, human waste handling, droplet impaction, and many other processes (Farling et al., 2019; Kim et al., 2019; Rocha-Melogno et al., 2020; Schmale and Ross, 2015; Wéry et al., 2017). Some studies, e.g., at wastewater treatment plants (WWTPs) found positive associations between working with sewage and flu-like symptoms (Douwes et al., 2001) and gastrointestinal symptoms (Thorn and Kerekes, 2001). However, a common limitation involving recall bias and over-reporting illness when odors were present has been identified (Viau et al., 2011). These limitations led to risk assessment studies sampling bioaerosols at WWTPs and characterizing the risk they pose on the plant's workers, i.e., estimating a risk of illness of 14×10^{-2} (infections/exposure events) after a 3 min exposure to aerosolized human adenovirus (HadV) (Carducci et al., 2018). Similarly, annualized risk of gastrointestinal illness from exposure to aerosolized rotavirus and norovirus at WWTPs have been estimated to be of 5.25×10^{-3} to 5×10^{-1} and 1.77×10^{-1} to 5×10^{-1} (infections/annual exposure events), respectively (Pasalari et al., 2019). A study in France used urinary biomarkers and aerosol samples at WWTPs and found that sewage workers were exposed to airborne genotoxicants. These workers had higher genotoxicity in urine samples than their office counterparts who were exposed to lower concentrations of genotoxicants (Al Zabadi et al., 2011). Others have looked at the impact of aeration systems as sources of bioaerosols in Spain, finding that mechanical agitation aerosolized 10 to 100 times more coliform bacteria at WWTPs compared to air diffusers (measured with a single stage impactor), potentially increasing exposure risks (Sánchez-Monedero et al., 2008). However, a 2016 literature review of atmospheric dispersion modelling of pathogenic bioaerosols identified the lack of QMRAs being conducted, limiting studies to qualitative observations about the risk that pathogenic bioaerosols present, and with most studies concentrating on high income countries and agricultural settings (Van Leuken et al., 2016). There is a great need in developing a better understanding of the risks posed by pathogenic bioaerosols in low and middle income countries.

Open waste canals such as the Choqueyapu River in La Paz, Bolivia, were channelized to collect the city's wastewater (Vega et al., 2017). This OWC has cascades throughout its course given the city's steep slopes, and flowrates averaging 14,400 m^3/h during the rainy season (Medina et al., 2021). This causes sewage aerosolization, potentially contaminating nearby food stands, playgrounds, and households. Indeed, we recently found fecal bioaerosols near these OWCs (Rocha-Melogno et al., 2020), including pathogens and antibiotic resistant coliforms (Medina et al., 2021; Salazar et al., 2020). These bioaerosols may pose an exposure risk to populations which live near heavily polluted streams found in cities from Africa, Latin America as well as South and East Asia (Peal et al., 2014). We hypothesized that aerosolized enteric pathogens from the Choqueyapu River pose an exposure risk for the population of La Paz.

Therefore, the overall objective of this research was to assess the potential public health impact from bioaerosols near OWCs. We used QMRA and data we have acquired in Bolivia as a case study. We previously collected fecal indicator bacterial fluxes through passive bioaerosol sampling, and detected human pathogens through active bioaerosol sampling and RT-PCR (Ginn et al., 2021; Rocha-Melogno et al., 2020). This earlier work identified the need for tools to characterize the risk of exposure to bioaerosols from OWCs and facilitating their use through user friendly platforms. For this reason, we added the current food and fomite QMRA to a web-based application we developed, called Aerosol-Mediated Infectious Disease Risk Assessments, or AMIDRA (Rocha-Melogno et al., 2021). The website https://rapidqmra.shinyapps.io/Rapid_QMRA/ is a collection of aerosol QMRAs that facilitates their dissemination, allowing interested parties such as public health professionals to conduct QMRAs for their specific application. The web application uses the methodology presented in this paper with user inputted data, fitting the data to gamma or lognormal distributions and runs 10,000 stochastic simulations (using the Monte Carlo method) to estimate the risk of infection and gastrointestinal illness for different exposure scenarios. See Figs. S1 and S2 for examples of the application's user interface.

2. Materials and methods

We used QMRA (Haas et al., 2014) to estimate the risk of infection and gastrointestinal illness from three relevant scenarios: contaminated surfaces (fomites) in playgrounds, contamination of food sold in food stands, and inhalation near the OWCs. This selection was made after observing co-location of human activity with a higher concentration of viable *Escherichia coli* and total coliforms immediately next to OWCs in La Paz, which run through the center of the city collecting untreated sewage from ~800,000 citizens (Rocha-Melogno et al., 2020). We did not measure all viable culturable pathogens, but instead, we used indicator organisms as bacterial pathogen surrogates (Owusu-Ansah et al., 2017; Petterson et al., 2016). Aerosol samples analyzed through real time PCR using a TaqMan Array Card (TAC) (Liu et al., 2013) indicated the presence of *Giardia* spp., *Cryptosporidium parvum*, *Yersinia* spp., *Salmonella* spp., *Enterococcus faecium*, enterotoxigenic *Escherichia coli* (ETEC, heat-stable enterotoxin), *Shigella* spp., enteroinvasive *Escherichia coli* (EIEC, ipaH gene), enteroviruses (all serotypes with the enterovirus genus), adenoviruses (serotypes 40/41), norovirus GII, and astroviruses (all human serotypes) (Ginn et al., 2021). See Tables S1 and S2 in Ginn et al. 2021 for further details on the TAC and ddPCR targets (Ginn et al., 2021), and Table A2 in Rocha-Melogno et al. 2020 for CFU flux data (Rocha-Melogno et al., 2020).

Upon subsequent droplet-digital PCR (ddPCR) confirmatory and quantitative tests, we decided to develop the QMRA presented here using ETEC and *Shigella flexneri*. We chose these bacterial pathogens because we detected them in 12% and 42% of our samples, respectively, and because of their genetic similarity to the *E. coli* spp. (Vieira et al., 2007) we enumerated through culture methods (Ginn et al., 2021; Rocha-Melogno et al., 2020). The quantities of the pathogens used in this QMRA averaged 8 genome copies/m^3 for ETEC, 7 genome copies/m^3 for *Shigella* spp., and 18,130 genome copies/m^3 for *E. coli* ybbw gene (the latter was used to estimate the ratio between pathogens and *E. coli* as the indicator organism). We did not sequence our samples to know the microbial composition of the aerosol samples we took. However, in Ginn et al. (2021) we provide detailed data of pathogen presence/absence via multiplex RT-PCR and genome copy quantification via ddPCR.

In addition, although it was not detected on our aerosols samples, we included *Campylobacter jejuni* as a reference pathogen (Bivins et al., 2017) given its low median infectious dose (N50) (Black et al., 1988), thus yielding more conservative risk estimates. These pathogens (ETEC, *S. flexneri*, and *C. jejuni*) were also selected because of the existence of reliable dose-response data that could be used for the QMRA.

2.1. Pathogen's viability estimation

In our previous study we used a selective growth medium, Aquatest (Bain et al., 2015), to enumerate culturable fecal coliforms and *E. coli* that deposited onto three to six 100 mm Petri dishes over the course of 2 h (Rocha-Melogno et al., 2020). We enumerated these bacterial targets as colony forming units (CFUs). We assumed that the reference pathogens used in this QMRA were culturable as well as viable. We acknowledge that not all viable pathogens are culturable (Oliver, 2005), and even those that are culturable may not be viable at the time of exposure (Haas, 2020). It is known that aerosols are a hostile micro-environment for bacteria which are subject to multiple killing stresses, e.g., desiccation, ultraviolet radiation (UV) exposure, and nutrient deprivation (Chang et al., 2017). Newer techniques are available and in development to assess a pathogen's viability i.e. assays with ethidium monoazide, propidium monoazide (Elizaquível et al., 2014) or azide intercalators (Leifels et al., 2019). However, it is not known which of these assays would provide the most accurate data for risk assessments (Haas, 2020). Considering the low resource context of our work, culture methods that are widely available may allow the advancement of our understanding of the risk that bioaerosols near OWCs pose despite methodological limitations.

2.2. Hazard identification

2.2.1. Selection of reference pathogens and deposition flux

For this QMRA we selected ETEC, *S. flexneri* and *C. jejuni* as reference pathogens. ETEC is the most common bacterial cause of traveler's diarrhea, commonly found in places with unsafe access to water and sanitation (Black, 1990; Hill and Beeching, 2010). It is an organism that produces heat labile or stable enterotoxins and colonizes the small intestine (Wolf, 1997). It is frequently detected in children with diarrhea; symptoms in response to infection by ETEC vary from mild to severe (Qadri et al., 2005). ETEC's median incubation period is estimated to be 42 h, with a median duration of illness of 3 days (Clayton et al., 2011; Dalton et al., 1999) and a rate of 0–20% of asymptomatic cases in children (Qadri et al., 2005).

Shigella spp. are also fecal-oral transmitted pathogens, being able to easily adapt and reproduce in the colonic epithelial cells (Thomas and Keusch, 1996), causing ~165 million cases of shigellosis disease yearly, mostly in low and middle income countries (LMICs) (99%) and in children (69%) (Kotloff et al., 1999). They are considered endemic in places with poor sanitation (Thomas and Keusch, 1996) and have an incubation period of 1–4 days, and a duration of illness of 5–7 days (Dekker and Frank, 2015). *S. flexneri* in the most abundant serogroup, identified in ~60% of *Shigella* isolates in LMICs (Kotloff et al., 1999). It has been shown that *Shigella* spp. (including *S. flexneri*) have low median infectious doses compared to pathogenic *E. coli* spp., (Enger, 2015; Haas et al., 1999) having low dose-responses in people exposed to *Shigella* spp. (DuPont et al., 1989). That said, *Shigella* spp. are genetically very similar to EIEC, making them hard to differentiate experimentally (Vieira et al., 2007). This is the reason why we previously reported detections of both *Shigella* spp./EIEC, finding *Shigella* spp./EIEC genes in 11 out of 26 (42%) of our aerosol samples collected in La Paz during the rainy and dry seasons (Ginn et al., 2021).

Campylobacter spp. infections have risen in the last decade, affecting developed and developing countries and being commonly transmitted through the fecal-oral route, raw meat and water (Kaakoush et al., 2015). This organism is known to cause gastroenteritis in humans, with an incubation period of 2 to 5 days (Horn and Lake, 2013), though symptoms appear 24–72 h after ingestion, and last 6 days on average (Kaakoush et al., 2015). Infections by *C. jejuni* can also lead to autoimmune disorders (Guillain-Barré syndrome and Miller Fisher syndrome) (Man, 2011). *C. jejuni* also has a low median infectious dose compared to *E. coli* spp. (Black et al., 1988; Enger, 2015; Haas et al., 1999), and several studies have reported its growing resistance to antibiotics, increasing the risk of prolonged disease and mortality (Kaakoush et al., 2015), making it an important pathogen in settings with limited sanitation services. For these reasons, it was included as a reference pathogen in this study.

We collected 60 flux samples within 10 m of the OWC in 5 locations (~1.6 km from each other) and 5 other locations 100–1000 m away from each site between 8 am and 6 pm during the rainy season of 2019 (Rocha-Melogno et al., 2020). For this QMRA we used the complete data set because 1) the data set is small and collected over the course of 2 weeks, and 2) we did not conduct microbial source tracking experiments to confirm that the OWCs were the source of the indicator bacteria or pathogens, although the OWCs are the likely the major source. Considering that the rainy season had higher CFU fluxes compared to the dry season, we used the rainy season culture-based data in this QMRA to evaluate the worst-case scenario (Rocha-Melogno et al., 2020).

Experimentally, we were not able to differentiate with certainty *E. coli* from total coliforms in our previous study, given the damage to the growth medium caused by sunlight exposure when conducting passive sampling on site (Rocha-Melogno et al., 2020). Therefore, we assumed that our deposition flux (expressed in CFU m^{-2} h^{-1}) reported previously was composed solely of *E. coli* spp. as a conservative approach. We did not need to include bacterial decay in the model because the experimentally determined fluxes (based on culturable organisms) already included decay caused by environmental stress (UV light, low relative humidity) (Rocha-Melogno et al., 2020). We used averaged pathogen genome copies (GC) to *E. coli* GC ratios (Table 1) to determine the fraction of pathogenic organisms present in our samples, an approach previously used in drinking water QMRAs (Bivins et al., 2017; van Lieverloo et al., 2007). Such ratios were estimated from GC concentrations sampled during the rainy season in 2019 using a BobCat Dry Filter Continuous Air Sampler (InnovaPrep, Drexel, MO, USA) with a flow rate of 200 L_{air}/min. Downstream molecular analysis post extraction to quantify GC was done via Droplet Digital PCR (ddPCR; QX200 Droplet Digital PCR System, BioRad, Hercules, CA) (Ginn et al., 2021).

As discussed later (see Study limitations and recommendations section), we acknowledge that the correlation between indicator organisms and pathogens can vary greatly. We highlight that the bacterial flux data were the same for all scenarios, having the same starting point for the models.

2.3. Exposure assessment

We considered three independent possible exposure pathways likely to happen next to OWCs (within 10 m from the presumed source). Consideration of locations close to OWCs is supported by the observation of higher concentrations of indicator organisms close to OWCs compared to measurements taken at >10 m, and having observed people eating, recreating in playgrounds or commuting by foot within 10 m of the OWCs. Lower concentrations at distances greater than 10 m from the OWC were likely due to aerosol dispersion and dilution in the air, and bioaerosol inactivation due to environmental stressors, e.g., UVB irradiance (Rocha-Melogno et al., 2020). Therefore, we considered proximity to OWCs being of greater risk of exposure. The three independent exposure pathways were a) deposition on food followed by ingestion, b) deposition on surfaces followed by hand to mouth contact and ingestion, and c) inhalation of bioaerosols followed by ingestion, all near OWCs. Each scenario was modeled independently. Lacking behavioral data sets, our models combine assumptions and exposure parameters from published literature listed in Table 1. Our analyses do not consider bacterial regrowth, inactivation, re-aerosolization or acquired host immunity. We calculated the doses for the scenarios using Eqs. (1), (2) and (3).

$$\mathrm{Dose_{food}} = \mathrm{F_{[CFU\ m^{-2}h^{-1}]}} \times \mathrm{PA_{[m^2]}} \times \mathrm{T_{[h]}} \times \mathrm{PR_{[\%]}} \quad (1)$$

$$\mathrm{Dose_{fomite}} = \mathrm{F_{[CFU\ m^{-2}h^{-1}]}} \times \mathrm{HA_{[m^2]}} \times \mathrm{TE_{[\%]}} \times \mathrm{T_{[h]}} \times \mathrm{TEb_{[\%]}} \times \mathrm{Co_{[contacts\ h^{-1}]}} \times \mathrm{Tb_{[h]}} \times \mathrm{PR_{[\%]}} \quad (2)$$

$$\mathrm{Dose_{inhalation}} = \mathrm{C_{[CFU\ m^{-3}]}} \times \mathrm{IR_{[m^3h^{-1}]}} \times \mathrm{Ti_{[h]}} \times \mathrm{DF_{[\%]}} \times \mathrm{PR_{[\%]}} \quad (3)$$

Where F = flux, PA = plate area, T = food or fomite contamination time, PR = pathogen: *E. coli* ratio, HA = hand area, TE = fomite-to-hand

Table 1
Model parameters included in the QMRA. In selecting these parameters, we used references as close as possible to the modeled applications.

Model	Input parameter	Units	Distribution and/or value	Source
Food contamination	*E. coli* flux (F)	CFU × m^{-2} × h^{-1}	Gamma[a] Shape: 1.55×10^{-1} Rate: 2.83×10^{-4}	(Rocha-Melogno et al., 2020)
	Food contamination time (T)	h	Truncated normal Mean: 1 Std. deviation: 0.5 Range: 0.17 - 2	
	Plate area (PA)	m^2	6×10^{-2}	(Sharp and Sobal, 2012)
Fomites in playgrounds (includes flux, contamination time)	Children's hand area (HA)	m^2	Truncated normal Mean: 8×10^{-3} Std. deviation: 2×10^{-3} Range: 4×10^{-4}–13×10^{-3}	(Agarwal and Sahu, 2010)
	Fomite contamination time (T)	h	Truncated normal Mean: 1 Std. deviation: 0.5 Range: 0.17–2	(Rocha-Melogno et al., 2020)
	Transfer efficiency: fomite to hand (TE)	%	Uniform distribution Range: 6–33	(Mattioli et al., 2015)
	Transfer efficiency: hand to mouth (TEb)	%	Truncated normal Mean: 41 Std. deviation: 25 Range: 0.01–0.9	
	Hand to mouth contacts (Co)	Number of contacts/h	Weibull Shape: 0.75 Scale: 12.59	
	Time spent at playground (Tb)	h	Truncated normal Mean: 1 Std. deviation: 0.5 Range: 0.17–2	(Rocha-Melogno et al., 2020)
Inhalation followed by ingestion	*E. coli* concentration (C)	CFU × m^{-3}	Gamma[a] Shape: 1.78×10^{-1} Rate: 1.18×10^{-3}	(Heyder, 2004; Rocha-Melogno et al., 2020)
	Inhalation rate (IR)	m^3 × h^{-1}	Uniform Range: 0.65–1.74	(EPA, 2011)
	Time spent walking near OWC (Ti)	h	Truncated normal Mean: 1 Std. deviation: 0.5 Range: 0.17–2	(Rocha-Melogno et al., 2020)
	Aerosol deposition fraction that will be ingested (DF)	%	65	(Heyder, 2004)
Food, fomites and inhalation followed by ingestion	Measured pathogen to *E. coli* ratio (PR)	Ratio average	4.20×10^{-4} (ETEC) 3.90×10^{-4} (*Shigella flexneri*) 4.05×10^{-4} (assumed for *Campylobacter jejuni*)	(Bivins et al., 2017; Ginn et al., 2021)
	Morbidity rate	%	(*S. flexneri*) Uniform Range: 22–58 (*C. jejuni*) 30	(Dupont et al., 1972) (Bivins et al., 2017)
	Dose-response parameters (ETEC, *S. flexneri*, and *C. jejuni*)	Multiple	$\alpha = 1.78 \times 10^{-1}$ $N_{50} = 8.60 \times 10^{7}$ $\alpha = 2.65 \times 10^{-1}$ $N_{50} = 1.48 \times 10^{3}$ $\alpha = 1.51 \times 10^{-1}$ $N_{50} = 1.69 \times 10^{3}$	(Haas et al., 1999) (Dupont et al., 1972; Rose et al., n.d.) (Bivins et al., 2017)

[a] Distributions were fitted from data reported in the source using MLE.

transfer efficiency, TEb = hand-to-mouth transfer efficiency, Co = number of hand-to-mouth contacts, Tb = time spent at the playground, C = concentration, IR = inhalation rate, Ti = time spent walking near an OWC, DF = deposited fraction in upper respiratory airways followed by ingestion. We limited the time of exposure to be the maximum time of sampling (2 h) to avoid over-estimation of bacterial viability over time. See Fig. 1 for diagrams depicting the different exposure scenarios that were assessed.

We back-calculated the CFU airborne concentrations converting our flux data set by dividing the fluxes (CFU × m^{-2} × h^{-1}) by the deposition velocity of a 6 μm diameter aerosol in still air (3.6 m × h^{-1}). We chose this particle size to model the worst case inhalation followed by ingestion scenario, as previous research showed that these aerosols can deposit in the extrathoracic airways with a maximum of 65% deposition efficiency (Heyder, 2004). We assumed inhalation rates for light and moderate intensity activities for children and adults (EPA, 2011).

We then fit our left-censored flux and concentration data using recommended maximum likelihood estimation (MLE) methods (Canales et al., 2018) available in the 'fitdistrplus' package (Delignette-Muller and Dutang, 2015) in RStudio. This allowed the replacement of values below detection limit (<1 CFU) with values obtained from the cumulative distribution function. A gamma distribution was chosen instead of a lognormal distribution after assessing goodness of fit with Akaike's Information Criterion (ΔAIC: 22.564).

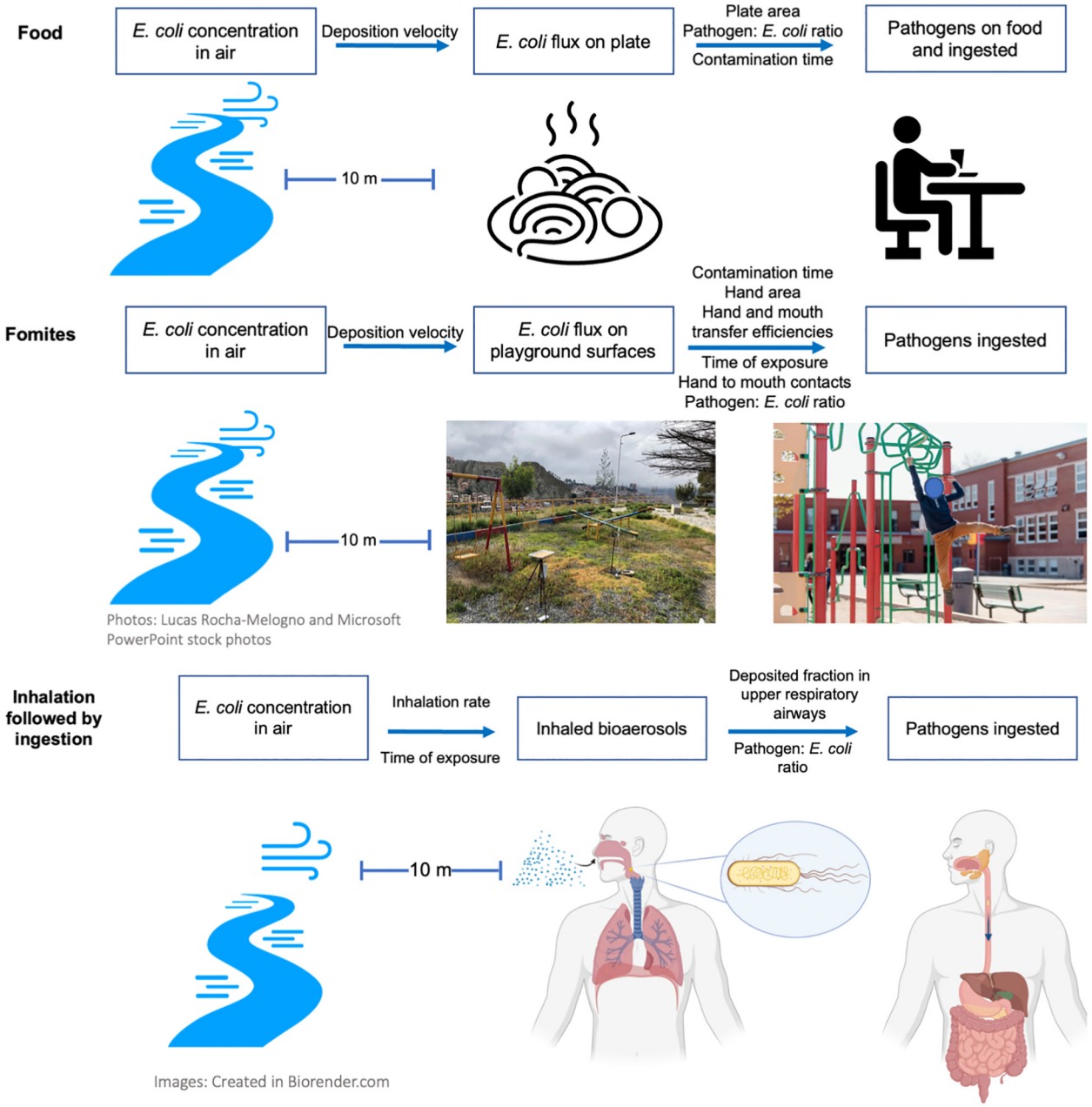

Fig. 1. Diagrams of exposure scenarios.

2.4. Dose-response and risk characterization

We estimated the per-exposure probability of infection upon ingestion using pathogen-specific dose-response functions, assuming the events happened once per day. We used a Beta-Poisson function for all reference pathogens, (Bivins et al., 2017; Haas et al., 1999; Rose et al., n.d.) shown in Eq. (4) where N_{50} is the median infectious dose and α is a dimensionless infectivity constant (Deepnarain et al., 2020), included in Table 1 for each microorganism. The health endpoint (response) for ETEC is diarrheal disease (not requiring a morbidity rate) (Haas et al., 1999), and the endpoint for *S. flexneri* and *C. jejuni* is positive detection and isolation in stool (Bivins et al., 2017; Black et al., 1988; Rose et al., n.d.). We assumed a morbidity rate of 22–58% (uniform distribution) for *S. flexneri* (Dupont et al., 1972), and a 30% morbidity rate for *C. jejuni* (Bivins et al., 2017).

$$P = 1-\left[1+\left(\frac{dose}{N50}\right)\left(2^{\frac{1}{\alpha}}-1\right)\right]^{-\alpha} \quad (4)$$

We calculated the per-exposure probability of infection and illness by conducting stochastic Monte Carlo simulations in RStudio, drawing 10,000 values at random from the variables considered in the exposure pathways. This resulted in a distribution of probability of infection and illness for each of the reference pathogens. We conducted a sensitivity analysis of our models using Spearman's rank correlations (Hamilton et al., 2018) between the input variables and calculated risks to identify the most relevant inputs and uncertainty sources in our models. We also estimated the annual risk of infection using Eq. (5) which assumes independent daily exposure, applicable for low-probability events (Haas et al., 2014, 1999). A daily exposure was chosen given the probability of recurrence of the events considered (playground, food).

$$P_{ann} = 1-(1-P)^{365} \quad (5)$$

2.5. Web application development

The AMIDRA web application was developed using RShiny version 1.5.0 and is available at https://rapidqmra.shinyapps.io/Rapid_QMRA/. Two of the current exposure scenarios are included under the "Data Fitting", "Food Contamination", and "Playground Contamination" tabs. Simplified modifiable parameters (Table 1) allow users to replicate the current research as well as introduce their own data and examine the effects of different parameter values on the probabilities of infection and illness. Users may upload their own flux data in the form of a .csv file which the application fits to a distribution using the R package 'fitdistrplus', or the user may choose to input pre-fitted data. This allows maximum flexibility for users with a variety of input data types. The parameters shown in Table 1 are the inputs of our models used in this QMRA.

3. Results and discussion

3.1. Fecal bacterial aerosols from OWCs present non-negligible risks of infection

We estimated the per-exposure risk of infection and illness for each reference pathogen for the aforementioned scenarios: food contamination from bioaerosol deposition, transmission from fomites in playgrounds and bioaerosol inhalation followed by ingestion. We caution that the health endpoint (response) for ETEC was illness in the following results. The highest median risk (as a measure of the worst-case scenario) of infection for food contamination was linked to *C. jejuni*, followed by *S. flexneri* and ETEC (Table 2). We observed a similar trend in the estimated risk of infection through fomites in playgrounds near OWCs, and this scenario presented a lower per-exposure risk of infection overall. Inhalation followed by ingestion had the highest median infection risk compared to contaminated food and fomites. Table 2 summarizes our per-exposure and annual risk estimates by scenario.

A 10^{-4} annual probability of infection (1 infection for every 10,000 exposures), or 2.7×10^{-7} daily probability of infection (27 infections for every 100,000,000 exposures) are commonly used as benchmark for tolerable risk of infection from drinking water (Fewtrell et al., 2001; Hamilton et al., 2019; Schoen et al., 2017). *C. jejuni* and *S. flexneri* exceeded this threshold through food contamination and inhalation followed by ingestion, but did not through fomites, assuming a single exposure event per day. ETEC did not exceed the aforementioned risk threshold in any of the assessed exposure scenarios.

We include the estimated probability of illness per day for all scenarios in Fig. 2. The median risk of illness was 6×10^{-11} (food), 2×10^{-12} (fomites), and 3×10^{-10} (inhalation) for ETEC; 5×10^{-7} (food), 2×10^{-8} (fomites), and 2×10^{-6} (inhalation) for *S. flexneri*; and 2×10^{-6} (food), 5×10^{-8} (fomites), and 8×10^{-6} (inhalation) for *C. jejuni*. These estimates suggest that the number affected individuals would be below the limit of detection of epidemiological studies given their sample size limitations.

3.2. Variability in risks by different pathogens is attributable to their specific dose-response functions

The average genome copies (GC) ratio to *E. coli* was of 4.20×10^{-4} for ETEC and 3.90×10^{-4} for *Shigella* spp., which we assumed to be for *S. flexneri* using the data we collected during field sampling (Ginn et al., 2021). Lacking GC data for *C. jejuni*, we used the average ratio of ETEC and *Shigella* spp. for *C. jejuni* (4.05×10^{-4}). These ratios are highly variable in feces, wastewater and raw water but the ratio of *Campylobacter* spp. to fecal coliforms (*E. coli* and *Klebsiella* spp.) is typically 1×10^{-4} in the aforementioned matrices according to the WHO (WHO, 2017). Despite these ratios being in the same order of magnitude in our aerosol samples compared to those in wastewater (WHO, 2017), the estimated risks of infection differed between *C. jejuni* and ETEC by five orders of magnitude, and so did for *S. flexneri* and ETEC in the food contamination scenario. We found a similar trend in the fomite and inhalation scenarios.

3.3. Sensitivity analysis

We assessed the sensitivity of our Monte Carlo models to their input variables using Spearman's rank correlation coefficients. These coefficients are a measure of the relationship of two variables (where 1 is equivalent to a perfect positive correlation, 0 indicates no correlation and -1 is a perfect negative correlation). The most important predictive factors were the bacterial fluxes (coefficient of 0.99 in the food scenario and inhalation scenario, 0.90 in the playground scenario), being the only location-specific parameter. The number of hand-to-mouth contacts had the second highest coefficient (0.35), included only in the playground scenario. Exposure time was second in importance in the inhalation model (0.11), and third in relevance in the playground model (0.18) and the food model (0.11). Inhalation rates had the lowest correlation coefficient (0.07). Tornado plots for visualization of the sensitivity analysis are available in Fig. 3. We acknowledge that all other parameters were generalized from published literature in absence of site-specific exposure data. Our results agree with previous QMRAs that found pathogen measurements through culture and molecular methods were one of the largest contributors to risk estimation variability (Canales et al., 2018; Crank et al., 2019; Julian et al., 2009). These results underline the importance of continuous and spatially resolved monitoring of microbial indicators and human pathogens through active or passive aerosol sampling to improve future QMRAs accuracy.

3.4. Comparison of risk estimates to available regional epidemiologic data

Lacking observational data regarding population behavior near OWCs, we estimated the number of people eating or commuting by foot near OWCs in La Paz using available reports online (Cooperación Suiza en Bolivia, 2015; El Deber, 2019). It is estimated that 25% of the urban population of Bolivia commute by foot (Cooperación Suiza en Bolivia, 2015), and 36.5% of the population of La Paz eat street food daily (El Deber, 2019). Considering the population of La Paz city (800,000) (Rocha-Melogno et al., 2020), there are 200,000 commuters by foot and 292,000 people eating street food every day.

Table 2
Summary of per-exposure and annual median infection risk estimates by scenario with 90% confidence intervals in parentheses.

Scenario	Pathogen	Per-exposure probability of infection	Annual probability of infection	Greater than EPA's annual risk threshold?
Food contamination	*C. jejuni*	5×10^{-6} ($2 \times 10^{-10} - 4 \times 10^{-4}$)	2×10^{-3} ($7 \times 10^{-8} - 14 \times 10^{-2}$)	Yes
	S. flexneri	1×10^{-6} ($5 \times 10^{-11} - 9 \times 10^{-5}$)	4×10^{-4} ($2 \times 10^{-8} - 3 \times 10^{-2}$)	Yes
	ETEC	6×10^{-11} ($2 \times 10^{-15} - 4 \times 10^{-9}$)	2×10^{-8} ($7 \times 10^{-13} - 1 \times 10^{-6}$)	No
Fomite transmission at playground	*C. jejuni*	2×10^{-7} ($5 \times 10^{-12} - 3 \times 10^{-5}$)	7×10^{-5} ($2 \times 10^{-9} - 1 \times 10^{-2}$)	No
	S. flexneri	4×10^{-8} ($1 \times 10^{-12} - 8 \times 10^{-6}$)	1×10^{-5} ($4 \times 10^{-10} - 3 \times 10^{-3}$)	No
	ETEC	2×10^{-12} ($1 \times 10^{-16} - 4 \times 10^{-10}$)	7×10^{-10} ($4 \times 10^{-14} - 1 \times 10^{-7}$)	No
Inhalation followed by ingestion	*C. jejuni*	3×10^{-5} ($4 \times 10^{-9} - 1 \times 10^{-3}$)	1×10^{-2} ($1 \times 10^{-6} - 3 \times 10^{-1}$)	Yes
	S. flexneri	6×10^{-6} ($8 \times 10^{-10} - 3 \times 10^{-4}$)	2×10^{-3} ($3 \times 10^{-7} - 4 \times 10^{-6}$)	Yes
	ETEC	3×10^{-10} ($4 \times 10^{-14} - 1 \times 10^{-8}$)	1×10^{-7} ($1 \times 10^{-11} - 4 \times 10^{-6}$)	No

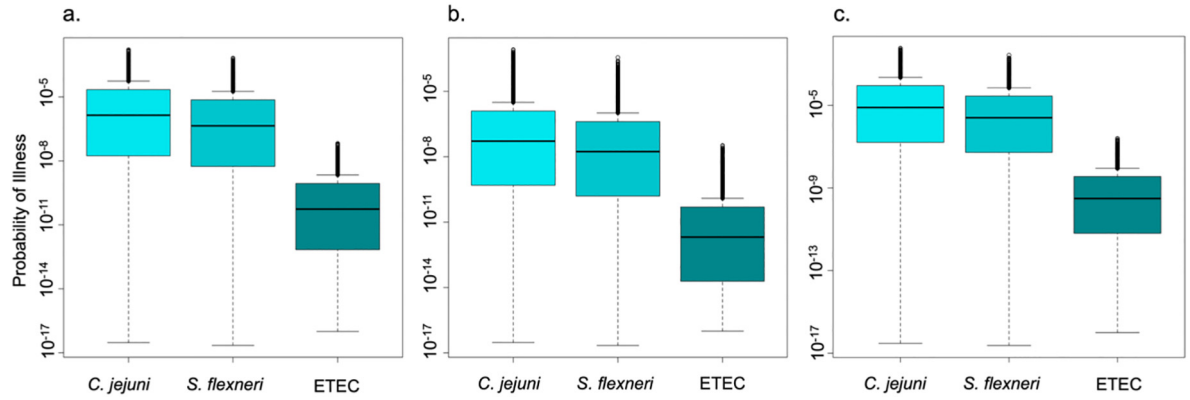

Fig. 2. Probability of illness per exposure to aerosolized enteric pathogens a) contaminating street food, b) contaminating surfaces in playgrounds near OWCs, and c) being inhaled and then ingested.

Assuming that 1 out of 10 citizens commute or eat near OWCs, this yields 20,000 daily commuters and 29,200 people eating street food near OWCs. We assessed each exposure pathway independently, without adding exposures through inhalation and contaminated food. We assumed that commuters were the population at exposure risk through inhalation only, and people that eat street food as the population at exposure risk through food contamination only. Using our highest median annual risk estimates, we estimated 200 annual infection cases from inhaling and ingesting *C. jejuni*, and 58 annual infection cases from food contamination. These numbers suggest that bioaerosols near OWCs present a non-negligible annual risk of infection for the current population of La Paz that commutes by foot or consumes street food. Around 100,000 cases of diarrheal disease were registered in La Paz in 2016 (Página Siete, 2017). Assuming a similar prevalence in 2020, the estimated cases attributable to bioaerosols near OWCs would represent <1% of the total number of cases of gastrointestinal illnesses registered in 2016. This could increase in the future as the city's population continues to grow and more fecal waste is disposed in OWCs. As more data becomes available, this model can be made more robust serving as a framework for looking at risks in these contexts.

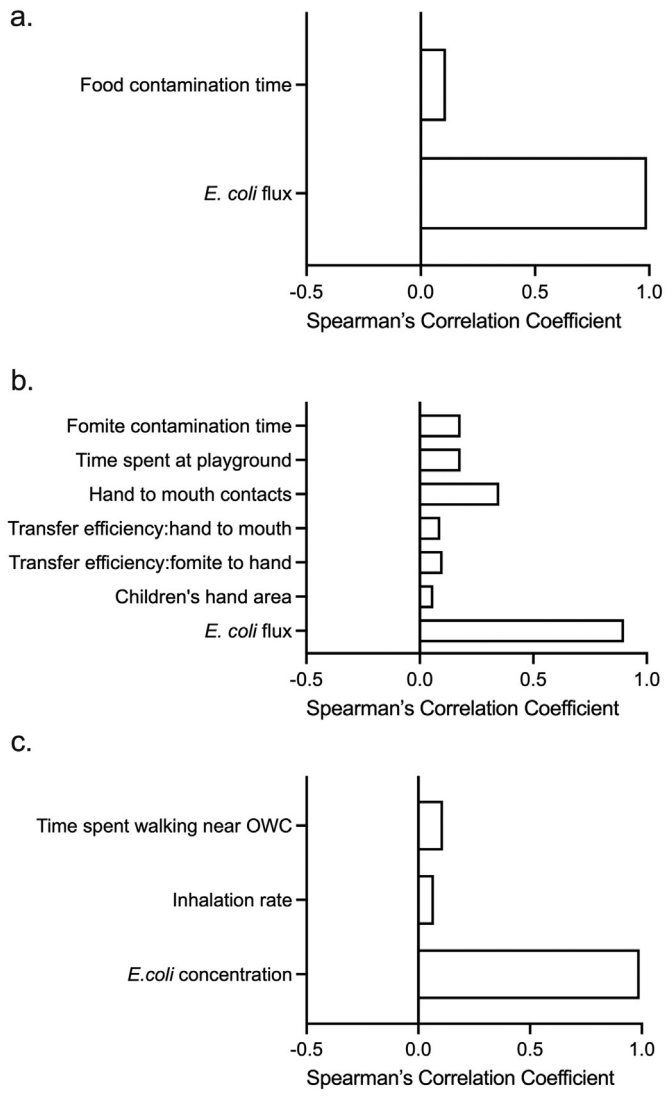

Fig. 3. Sensitivity analysis results for *Campylobacter jejuni*. Spearman's rank correlation coefficients are shown for stochastic parameters in the three scenarios that were modeled, a) contaminating street food, b) contaminating surfaces in playgrounds near OWCs, and c) inhalation followed by ingestion.

For the playground scenario, we considered children under 10 years old, who are 19% of the total population in La Paz (Bolivian National Institute of Statistics, 2020), or 152,000 children in 2020. Assuming 1 out of 10 children uses a playground near an OWC, this amounts to 15,200 children exposed to fecal bioaerosols. Using our highest median annual risk estimates from *C. jejuni* exposure through fomites, we estimated 1 annual infection case despite the assumed number of children at exposure. These numbers suggest that fomites contaminated with bioaerosols present a low risk of infection for the children of La Paz that use the playgrounds near OWCs, compared to the 10^{-4} annual risk threshold aforementioned (Fewtrell et al., 2001; Hamilton et al., 2019; Schoen et al., 2017). The Bolivian National Institute of Statistics estimated a diarrheal disease prevalence of 13.5% in children under 5 in La Paz in 2016 (Bolivian National Institute of Statistics, 2018). Assuming a similar prevalence in 2020 for children under 10 years old, the cases attributable to bioaerosols in playgrounds near OWCs would represent 0.005% of the total diarrheal disease prevalence. The Bolivian Ministry of Health and Sports estimates that 70% of diarrheal diseases are caused by contaminated food in Bolivia (Bolivian Ministry of Health and Sports, 2015). In this context, our findings suggest that bioaerosols near OWCs are not a major cause of diarrheal disease in La Paz. That said, we recommend not placing playgrounds near OWCs to prevent exposure to enteric pathogens given previous overflows of the OWCs in La Paz (Hardy, 2009; Página Siete, 2020). Alternatively, we recommend implementing containment measures to prevent aerosol release from OWCs until underground sewers connected to wastewater treatment plants are constructed.

Given uncertainty in select model parameters, we can rapidly evaluate alternative exposure conditions using our AMIDRA web application. For example, assuming that a person in La Paz eats street food that has been exposed to the environment for 1 h near an OWC, and increasing the ratio between *C. jejuni* and *E. coli* to 1×10^{-3}. Then, the median risk of infection for that event would be of 2×10^{-5} (one order of magnitude higher than

our previous estimate using a ratio of *C. jejuni* to *E. coli* of 4.05×10^{-4}). In the context of La Paz, this would result in 212 infection cases per year, i.e., 4 times greater than our previous estimate and 70 times greater than the EPA's annual risk standard for drinking water (1×10^{-4}). This highlights the importance of acurate pathogen detection and quantification, as slight changes in the pathogen to *E. coli* ratios can have a noticeable effect on risk estimates.

3.5. Study limitations and recommendations

Our current QMRA models have the following limitations that should be considered to avoid oversimplification of their results.

First, the models depend heavily on sampling data which have a direct impact on risk variability and thus are a major source of uncertainty. Large or longitudinal data sets of bacterial fluxes or bioaerosol concentrations are currently scarce, but would serve to reduce model uncertainty.

Second, the lack of pathogen-specific viability data increased model uncertainty. Because of this, we used pathogen to *E. coli* GC ratios to estimate potential viable pathogens present in the samples. We acknowledge that the correlation between indicator organisms and pathogens varies greatly (Wu et al., 2011). However, we justify the use of indicator organisms given the low cost, wide availability and low technical skill requirement for their monitoring in resource-constrained settings (Rocha-Melogno et al., 2019).

Third, our data sets were collected in cross-sectional studies with small sample sizes. Therefore, we qualify our risk estimates being at the screening level, requiring validation using longitudinal data.

Fourth, microorganisms could be viable but non-culturable (Oliver, 2005), and our culture-based sampling methodology will have missed these organisms. This can result in risk underestimation, highlighting the importance of continuous monitoring to have larger data sets to work with.

Fifth, we had to make multiple assumptions for the exposure assessment, adding uncertainty to our risk estimates. Further observational studies characterizing behaviors that result in exposure will reduce such uncertainties and the need for assumptions. Additional experimental studies are needed to better understand the inhalation exposure to pathogens and their consequent deposition in the upper respiratory airways followed by ingestion. Previous studies have estimated a 50% retention and deposition of aerosolized *Legionella* bacteria in lower respiratory airways of guinea pig models (Hamilton and Haas, 2016). This deposition rate may not vary widely by pathogens, as it is dictated by the size of the aerosol in which pathogens were transported into the respiratory tract (Heyder, 2004; Heyder et al., 1986; Thomas, 2013). However, the infection site will be pathogen-dependent due to their infection mechanism, sensitivity to immunological responses, or resistance to stomachal acidity (Thomas, 2013).

Sixth, the dose-response functions we used were derived from human feeding studies in high income countries, not being specific to the population of Bolivia. The risk of infection could be lower or higher in a population that may have acquired immunity through chronic exposure or have compromised immune systems, respectively (Korpe and Petri, 2012), or when we consider secondary transmission of infections.

QMRA models can, and have been compared to epidemic curves, generating significant fits to real outbreak data (Burch, 2019; Enger et al., 2012; Gupta and Haas, 2004; Prasad et al., 2017) and are an acceptable risk assessment tool being used by the U.S Environmental Protection Agency (EPA, 2010) and WHO (WHO, 2016). Our estimates could improve in accuracy with more aerosol and fomite measurements and epidemiological studies that incorporate the aerosol transmission pathway, secondary transmission, and dose-response models specific to the inhalation mode of exposure. This would allow model (re)calibration, particularly in the dose-response and exposure assessment. To our knowledge, there are no epidemiological studies characterizing the aerosol exposure pathway to fecal or enteric pathogens in cities with poor sanitation. Although our study provides an initial insight into the likely low health risks associated to fecal aerosols from OWCs, we recommend aerosol sampling as part of monitoring efforts of sanitation technology implementation and city infrastructure development.

Seventh, a significant limitation was not including viruses in our assessment, estimated to cause ~60% of human respiratory and enteric infections (Boone and Gerba, 2007). We did not test viral viability and did not extrapolate the observed bacterial viability for the viruses we detected through PCR because bacteria and viruses respond differently to environmental and sampling conditions (Tang, 2009). This limited our ability to characterize the risk of viral infections near OWCs.

3.6. Conclusions

Despite a noticeable increase in QMRA scientific literature (Haas et al., 2014; Owens et al., 2020), practical tools for its larger implementation are scarce. With the web application (AMIDRA) we developed, researchers and public health practitioners can translate bioaerosol monitoring data into potential health outcomes without having to develop QMRAs in statistical software that requires significant training. These web applications are designed to increase accessibility, speed, and transparency of QMRA processes (Crank et al., 2019; Weir et al., 2017). The simple models we developed can also serve as a starting point for professional risk assessors, with the caveat that QMRA's complexity and inclusion of multiple variables has not been found to be related with increased certainty of risk estimation (Owens et al., 2020). The results of our sensitivity analysis suggest that certainty of risk estimates would benefit more from continuous monitoring, providing larger pathogen's datasets of the most relevant input and uncertainty source. The correct handling of fluxes or concentrations data when having values below detection limits could also improve risk estimation certainty (Canales et al., 2018). Including PCR or pathogen-specific culture data to estimate pathogen to indicator ratios or concentrations in the environment could further reduce uncertainty in risk estimates, at higher costs and technical skill requirements. Our exploratory risk assessment indicates that aerosol transmission of enteric pathogens in cities with poor sanitation deserves further investigation.

CRediT authorship contribution statement

Lucas Rocha-Melogno: Conceptualization, Methodology, Software, Resources, Formal analysis, Investigation, Data curation, Writing – Original Draft, Writing – Review & Editing, Visualization, Project Administration, Funding Acquisition. Katherine Crank: Methodology, Software, Investigation, Writing – Original Draft, Writing – Review & Editing. Olivia Ginn: Methodology, Investigation, Data curation, Writing – Review & Editing. Michael Bergin: Writing – Review & Editing, Supervision. Joe Brown: Writing – Review & Editing, Supervision, Funding Acquisition. Gregory Gray: Writing – Review & Editing, Supervision, Funding Acquisition. Kerry Hamilton: Methodology, Writing – Review & Editing, Supervision. Kyle Bibby: Writing – Review & Editing, Supervision. Marc Deshusses: Conceptualization, Methodology, Resources, Writing – Original Draft, Writing – Review & Editing, Supervision, Project Administration, Funding Acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2022.154233. The QMRA web interface AMIDRA can be accessed at https://rapidqmra.shinyapps.io/Rapid_QMRA/

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