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Assessing the microbial risk of faecal sludge use in Ugandan agriculture by comparing field and theoretical model output

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ABSTRACT

Reuse of faecal sludge in agriculture has many potential benefits, but also poses risks to human health. To better understand the potential risks, Quantitative Microbial Risk Assessment (QMRA) was performed for three population groups in Kampala, Uganda: wastewater and faecal sludge treatment plant workers; farmers using faecal sludge; and consumers of faecal sludge-fertilised vegetables. Two models were applied for farmers and consumers, one based on pathogen concentrations from field sampling of sludge, soils and vegetables, and one based on theoretical pathogen contribution from the last sludge application, including decay and soil to crop transfer of pathogens. The risk was evaluated for two pathogens (enterohaemorrhagic *E. coli* (EHEC) and *Ascaris lumbricoides*). The field data on sludge, soil and vegetables indicated that the last application of faecal sludge was not the sole pathogen source. Correspondingly, the model using field data resulted in higher risks for farmers and consumers than the theoretical model assuming risk from sludge only, except when negligible for both. For farmers, the yearly risk of illness, based on measured concentrations, was 26% from EHEC and 70% from Ascaris, compared with 1.2% and 1.4%, respectively, considering the theoretically assumed contribution from the sludge. For consumers, the risk of illness based on field samples was higher from consumption of leafy vegetables (100% from EHEC, 99% from Ascaris) than from consumption of cabbages (negligible for EHEC, 26% from Ascaris). With the theoretical model, the risk of illness from EHEC was negligible for both crops, whereas the risk of illness from Ascaris was 64% and 16% for leafy vegetables and cabbage, respectively. For treatment plant workers, yearly risk of illness was 100% from EHEC and 99.4% from Ascaris. Mitigation practices evaluated could reduce the relative risk by 30-70%. These results can help guide treatment and use of faecal sludge in Kampala, to protect plant workers, farmers and consumers.

1. Introduction

Inadequate sanitation, estimated to cause 432,000 deaths from diarrhoea annually, is a major factor in several neglected tropical diseases and contributes to malnutrition (Murray et al., 2012; Prüss-Ustün et al., 2014). In sub-Saharan Africa (SSA), 30% of the population has access to a basic sanitation facility, 18% has limited sanitation solutions, 31% has unimproved sanitation and 20% practise open defecation (JMP, 2020). Provision of sanitation facilities does not necessarily mean that human excreta are safely managed. It is estimated that in SSA, up to 80% of the total population is served by sanitation facilities that do not safely manage excreta, which in some cases are dumped in the surrounding environment (WHO/UNICEF, 2019).

In Kampala, Uganda, a small proportion (15%) of the population is served by a centralised sanitation system, while the rest relies on on-site technologies (Nimusiima et al., 2020, p. 39). Faecal sludge management in Uganda is still poorly developed (MWE, 2016). An excreta flow diagram created for Kampala in 2016 (Schoebitz et al., 2016) indicated that 78% of excreta ends up as faecal sludge, of which 22% is contained and treated. This suggests that there is significant scope for improving faecal sludge management.

Improving faecal sludge management through better containment and treatment can provide different benefits, from decreased disease transmission and protection of the natural environment to utilisation of resources in excreta as e.g. plant nutri-

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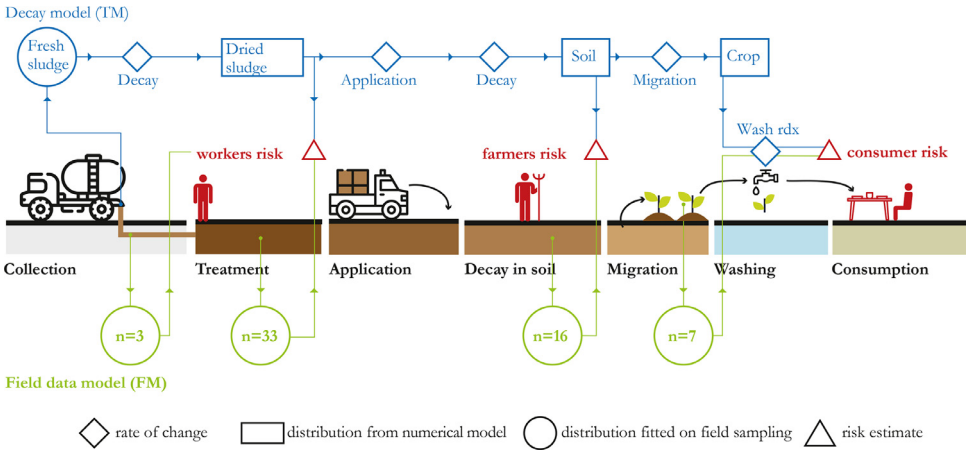

Fig. 1. Components of the two Qualitative Microbial Risk Assessment (QMRA) models applied: a field model (FM) using measured data (below, green, n=number of samples) and a theoretical model (TM) based on theoretically derived pathogen concentrations (above, blue).

ents (Guest et al., 2009; Peccia and Westerhoff, 2015). Material flow analyses show a negative soil nutrient balance in Uganda (Lederer et al., 2015; Nkonya et al., 2005; Sheldrick and Lingard, 2004; Wortmann and Kaizzi, 1998). Ugandan fertiliser policy has the target of applying 50 kg nutrients per ha and year to agricultural land by 2020 (Ministry of Agriculture Animal Industries and Fisheries, 2016).

Recirculating the plant nutrients in excreta, which originate from food, to agricultural land, coupled with other practices, could help overcome crop nutrient deficiency while decreasing the need for mineral fertiliser, thus decreasing use of fossil resources for fertiliser manufacture and exploitation of finite rock phosphate reserves. Based on FAO statistics on protein intake (2014-2017 average), the nitrogen and phosphorus content in excreta in Uganda comprises 98,200 tons and 14,800 tons, respectively, annually (Jönsson et al., 2004). This is almost 16-fold and 9-fold the current use of mineral fertiliser (FAOSTAT, average 2014-2017). Many factors could decrease the efficiency of nutrient recovery from excreta, but the high availability of these resources is promising.

Another driver for faecal sludge management could be financial opportunities through a reuse scheme. It has been proposed that agricultural reuse of faecal sludge could be the economic driver for uptake of such systems (Diener et al., 2014). Despite difficulties that need to be overcome to optimise faecal sludge reuse, studies show rather high acceptance for faecal sludge as fertiliser and willingness to pay for it among Ugandan farmers (Danso et al., 2017). This is evident in practice at the Lubigi wastewater and faecal sludge treatment plant (WWFSTP) in Kampala, where all the sludge produced is bought by small- and medium-scale farmers for use in agriculture (R. Sakaya, Lubigi plant engineer, personal communication, July 2019). Recirculating nutrients in human excreta to agriculture can increase crop production and food security. However, pathogens excreted from the human body could also be present in faecal sludge. Many pathogens in excreta are transmitted through the faecal-oral route and pose a microbial hazard for end-consumers of fertilised produce, whereas pathogens infecting through skin contact many be the dominant microbial hazard for workers along the faecal sludge treatment and reuse management chain.

The World Health Organisation (WHO) promotes reuse of nutrients and provides guidelines on safe management and reuse of wastewater, excreta and greywater (WHO, 2006a). These guidelines are based on health targets accepting a loss of 10^{-6} Disability Adjusted Life Years (DALYs) (Anand and Hanson, 1998, 1997). Quantitative Microbial Risk Assessment (QMRA) is recommended by the WHO to evaluate risk and support decision-making (WHO, 2006a). However, the QMRA approach may suffer from lack of data on pathogen contamination, setting specific dose-response relationships and validation of the estimated risks with epidemiological data (Van Abel and Taylor, 2018; WHO, 2006b). The aim of the present study was thus to assess quantitatively the microbial risk related to treatment (WWFSTP workers) and reuse (farmers and consumers) of treated faecal sludge from the Lubigi plant, based on pathogen concentrations measured along the faecal sludge management service chain. Two models were built for the analysis: a field model (FM) deriving risk estimates at different points along the management chain based on field sampling and a theoretical model (TM) deriving risk estimates for different points along the management chain based on a decay and transfer model, using pathogen concentrations in raw faecal sludge at the WWFSTP as initial input (Fig. 1).

2. Materials and methods

2.1. Location and process description

Lubigi WWFSTP (0.346939N, 32.544270E; ~1140 m asl) is managed by the Ugandan National Water and Sewerage Corporation and has been in operation since 2015. It has the capacity to treat 400 m^3 of faecal sludge and 5000 m^3 of wastewater per day. Faecal sludge from the city and surrounding areas is collected by vacuum trucks from on-site sanitation systems (Schoebitz et al., 2016). At the plant, the faecal sludge goes through a three-stage process: initial screening, sedimentation in two settling tanks (which operate alternately, one on duty and one on standby), and drying in covered sand beds. After a storage period intended to be at least six months, the faecal sludge is sold as agricultural fertiliser. However, due to high demand, customers often collect the dried sludge before the intended storage period.

2.2. Field sampling

A recent review of 22 QMRAs performed on countries in SSA identified gaps in data collection (Van Abel and Taylor, 2018). To overcome this limitation, in this study specific field sampling was performed for enumeration/determination of microorganisms. Samples of raw faecal sludge (n=3, 1 occasion), dried faecal sludge (n=33, 3 occasions), soil (n=22, 3 occasions) and crops (n=7, 2 occasions) were collected in July 2018, February 2019, July 2019 and January 2020. For faecal sludge samples, the sampling proce-

dure was in line with EPA standards (EPA, 1989). For each drying bed, three samples, each made of three pooled subsamples, were collected in zip-lock bags, stored in an ice-cooled container and transported to the laboratory at Makerere Microbiology for analysis. Two batches of unused dried faecal sludge found at one farm were also sampled. Soil samples were collected from five fields on three different farms. Three of the fields were fertilised with faecal sludge from Lubigi WWFSTP, one with NPK and one with chicken manure. Pooled samples of soil (4 subsamples, adding up to 50-80 g) were taken from the top layer (0-10 cm) in the field following a random location stratified using a regular grid, stored in zip-lock bags and kept at ambient temperature during transport to the laboratory. Three farms for which contact details were available, which were also willing to participate in the project, were all included in the sampling, due to their limited number.

Three sets of samples were collected from vegetable crops: from cabbages (*Brassica oleracea*) growing in a field fertilised with NPK (n=3), from *Amaranthus* spp. (amaranth, locally known as dodo) (n=3) (Mollee et al., 2017; Sogbohossou et al., 2015) and from red creole onion (*Allium cepa*) in a field fertilised with sludge (n=2). All sampled vegetables were randomly picked and stored in sterile plastic bags during transport to the laboratory for analysis. The data on field samples were used in the QMRA (Table 2).

2.3. Laboratory analysis

The field samples were analysed for *Escherichia coli, Enterococcus* spp., *Ascaris lumbricoides* and *Salmonella* spp. The *E. coli* and *Enterococcus* spp. concentrations in all matrices (faecal sludge, soil, vegetables) were determined on 10 g of material serially diluted 1:9 with buffered peptone water. Chromocult Coliform/EC Agar was used for the detection of *E. coli* and Bile Esculin Azide Agar for the detection of *Enterococcus* spp., by plating 0.1 mL (giving a detection limit of 100 cfu g^{-1} material) from three dilutions and incubating for 24±2 hours at 37°C. For Ascaris, the method proposed by the Water Research Commission of South Africa was used (Moodley et al., 2008), analysing eggs in 10 g of sludge. The method involves assessment of viable eggs by visual inspection under the microscope after 3 weeks of incubation. For detection of *Salmonella* spp., samples of dried sludge from one sampling occasion (n=15) were enriched (25 g in Rappaport Vassiliadis broth) and enumerated on Xylos Lysine Deoxycholate agar.

2.4. QMRA strategy/framework

This QMRA followed the conventional steps of hazard identification, exposure assessment, dose-response and risk characterisation (Haas et al., 2014; Karavarsamis and Hamilton, 2010; Mara and Sleigh, 2010; WHO, 2006b). The occupational risk caused by involuntary ingestion was estimated for workers on the faecal sludge line at the WWFSTP, farmers working in fields where faecal sludge is used as a fertiliser and end-consumers of raw vegetables grown in fields fertilised with faecal sludge (Table 1). To assess the contribution from faecal sludge reuse relative to the pathogen concentrations already present in the environment and the risks to farmers and consumers, field and theoretical models were applied (Fig. 1). Measured pathogen concentrations in faecal sludge, soil and vegetables were used in a field model (FM) to estimate risks, while measured pathogen concentrations in raw sludge were used as initial input in a theoretical model (TM) and literature values for decay and migration were used to derive concentrations in soil and on vegetables.

2.5. Hazard identification

Among infectious diseases transmitted through faecal contamination, diarrhoea accounts for most Disability-Adjusted Life Years (DALYs) in Uganda, mainly in children and the elderly, while soil-transmitted helminths are the most prevalent pathogens (Institute for Health Metrics and Evaluation (IHME), 2018). This study assessed the risk from enterohaemorrhagic *Escherichia coli* (EHEC) and *Ascaris lumbricoides,* pathogens posing risks for all three groups included in the QMRA. Together with rotavirus and calicivirus, pathogenic *E. coli* are the most frequently identified global cause of gastrointestinal illnesses in children below 5 years of age (Fletcher et al., 2013), responsible for more than 50% of all diarrhoeal deaths (Lanata et al., 2013; Abba et al., 2009). EHEC has been identified as a cause of diarrhoea in children aged below five years in Uganda, but is less common than other *E. coli* pathotypes (Masiga et al., 2020; Musiime et al., 2009; Tumwine et al., 2003). Due to the milder, more chronic nature of infection, *Ascaris lumbricoides* and other soil-transmitted helminths contribute less to DALYs compared with diarrhoea (Institute for Health Metrics and Evaluation (IHME), 2018). However, helminth infection can result in longer-term impairment of mental and physical development (Hotez et al., 2006; Jukes et al., 2002). Ascaris eggs can survive for months to years under severe environmental conditions (Dryzer et al., 2019; Stott et al., 2003; de Faria et al., 2017) and this pathogen is therefore suggested for QMRA in regions where it is prevalent (WHO, 2006b).

The risk assessment in the present study thus included one pathogen persistent to treatment (*Ascaris lumbricoides*), but which causes less severe disease, and one pathogen more sensitive to treatment (EHEC), but which can cause severe disease and which has a confirmed low infectious dose (Teunis et al., 2004; Tilden et al., 1996), stressing its importance as a bacterial pathogen. Samples of faecal sludge, soil and vegetables were analysed for *E. coli* as described above (Table 2) and it was assumed that 8% consisted of EHEC (Howard et al., 2006). In the Ascaris analysis, a recovery rate of 0.85 was assumed for sludge (Karkashan et al., 2015) and 0.37 for soil (Steinbaum et al., 2017).

2.6. Exposure assessment

2.6.1. WWFTTP worker scenario

There is growing concern about the health conditions of sanitation workers (World Bank et al., 2019). This scenario focused on the operational staff (11 individuals) at Lubigi WWFSTP, regarding their exposure during faecal sludge management. In previous research by Makerere University students, five different activities were identified as potentially hazardous (Table 3): offloading the raw faecal sludge from the truck, working at the grit removal station, moving the faecal sludge from drying beds to storage beds, loading the truck with dried faecal sludge, and eating near the drying beds (Kiffe and Wycliffe, 2016). Using literature data on occupational exposure (Schonning et al., 2007; U.S. Environmental Protection Agency, 2018, 2017, 1997; Westrell et al., 2004), involuntary ingestion during each activity was estimated. After site visits and interviews with the workers, these activities were linked to four duties performed by the WWFSTP workers: generic site supervision (events per person per year (pppy) n=43), working at the inlet (n=31 pppy), working at the grit and settling tanks (n=175 pppy) and working at the drying beds (n=91 pppy). Involuntary daily ingestion (I) of faecal sludge for each duty was estimated as the sum of sludge ingested during the activities performed during that day. Ingestion (mg day^{-1}) per duty type ($I_{g,i,s,d}$) was estimated using a normal distribution (Table 3).

In addition, reduced involuntary ingestion ($I_{gm,im,sm,dm}$) due to mitigation (wearing a mask and improved hygiene in eating (eating

Table 1
Parameters used in Qualitative Microbial Risk Assessment (QMRA)

Symbol	Parameter		Value	Comment
	Dose-response	*E. coli* O157:H7	Beta Poisson: α=0.248; β=48.80	Heterogeneous model, Teunis et al. (2008)
		Ascaris	Beta Poisson: α=0.04; N50 = 859	Navarro et al. (2009)
ω_e	Pathogenic fraction for *E. coli*		0.08	Ratio EHEC to *E. coli*, Howard et al. (2006)
ω_a	Pathogenic fraction for Ascaris		See Table 2	Values based on field sampling results
λ	Illness to infection	EHEC	0.35	Machdar et al. (2013)
		Ascaris	PERT(0.15, 0.27, 0.39)	Range based on Dold and Holland (2011) and Mara and Bos (2009).
	Ascaris recovery rate from sludge		0.85	Karkashan et al. (2015)
	Ascaris recovery rate from soil		0.37	Steinbaum et al. (2017)
Field model (FM)				
C_{rs}	Concentration in raw sludge (log_{10} g^{-1} / egg g^{-1}), used also in TM	*E. coli*	PERT(5.5, 5.6, 5.7)	Distributions fitted on 3 samples from
		Viable Ascaris eggs	PERT(28.9, 34.8, 38.4)	Lindberg and Rost (2018)
C_{ds}	Concentration in dried sludge (log_{10} g^{-1} / egg g^{-1})	*E. coli*	N(3.65, 1.69)	Distribution fitted on 33 samples over 2-year
		Viable Ascaris eggs	Log-N(1.7, 0.15)	period
C_{so}	Concentration in soil (log_{10} g^{-1} / egg g^{-1})	*E. coli*	2	Distribution fitted on 16 samples
		Ascaris	Weibull (1.4, 10.4)	
C_{cr}	Concentration on leafy vegetables (log_{10} g^{-1}/ /viable eggs g^{-1})	*E. coli*	PERT(2, 2.62, 3.91)	Distribution based on samples from leafy vegetables (n=4)
		Ascaris	PERT(9, 11, 14)	Distribution fitted on data from leafy vegetables (n=4)
	Concentration on cabbage (log_{10} g^{-1} / viable eggs g^{-1})	*E. coli*	0 (not detected)	Distribution based on samples from cabbages (n=3)
		Ascaris	PERT(0.13, 0.22, 0.47)	Distribution fitted on data from cabbage (n=3)
R_w	Washing reduction	Ascaris	PERT(0.4, 0.5, 0.6)	From Amoah et al. (2007) for light washing in a bowl.
		E. coli	PERT(0.11, 0.14, 0.18)	Based on Amoah et al. (2007), Duedu et al. (2014) and Uhlig et al. (2017)
Theoretical model (TM)				
C_{rs}	Same as for FM			
C_{ds}	C_{rs} with decay r_1 and time t_1			
C_{so}	C_{ds} with decay r_2 and time t_2 * R_{app}			
C_{cr}	C_{so} at day 100 after fertilisation * migration (R_m)			
r_1	Decay rate in sludge (log_{10} day^{-1})	*E. coli*	PERT(-0.041, -0.05, -0.0625)	T90= PERT(16,20,24) days (Table 3.5 WHO, 2006b)
		Ascaris	PERT(-0.00711, -0.0095, -0.011)	Based on (Pecson et al., 2007), calculation based on Fidjeland et al. (2015)
r_2	Decay rate in soil (log_{10} day^{-1})	*E. coli*	PERT(-0.032, -0.04, -0.052)	T90=PERT(19,25,31) (Table 3.5 WHO, 2006b)
		Ascaris	PERT(-0.002, -0.0028, -0.083)	A more conservative approach than WHO (2006, Table 3.9) with T90 (120,350,500) days
R_{app}	Application rate sludge to soil		PERT(0.01, 0.05, 0.1)	Range based on (Schonning et al., 2007; Jimenez et al., 2006) and field data, equal to 12.60 and 120 ton/ha
R_{m_l}	Egg ratio leafy vegetables crop: soil	Ascaris	0.7	Ratio mean concentration on leafy crop / mean concentration in soil
R_{m_c}	Egg ratio cabbage crop: soil	Ascaris	0.066	Ratio mean concentration on cabbage crop / mean concentration in soil
R_w	Washing reduction, same as FM			
Ingestion (FM and TM)				
$I_{g, d, l, s, gm, dm, sm, sm}$	Involuntary sludge ingestion WWFSTP workers (g day^{-1})		See Table 3	Based on Westrell et al. (2004), Mara (2007), USEPA (2017), Schonning et al. (2007)
I_{fh}	Farmers involuntary ingestion, intense work with soil (mg day^{-1})		PERT(0.0467, 0.0642, 0.0814)	Result of a Markov Chain, based on Vu (2018)), run 7, 9 and 11 times
I_{fl}	Farmers involuntary ingestion, light work with soil (mg day^{-1})		PERT(0.005, 0.0123, 0.0205)	Result of a Markov Chain based on Vu (2018)), run 2,3 and 14 times
I_c	Vegetable consumption rate (g $week^{-1}$)		N(30, 7)	US EPA (2017)
DALY				
Sev	Severity weight for gastroenteritis (mild, moderate, severe, fatal)		0.06, 0.2, 0.28, 1	Murray et al. (2012)
F	Frequency (mild, moderate, severe, fatal)	EHEC	0.9398, 0.05, 0.01, 0.0002	Katukiza (2013)
		Ascaris	0.95, 0.05, 0, 0	Brooker (2010)
D	Duration of illness (years) (mild, moderate, severe, fatal)	EHEC	0.015, 0.029, 0.044, 54	Katukiza (2013)
		Ascaris	0.095, 0.076, 0, 0	Brooker (2010)
Dis	Disability per event (mild, moderate, severe, fatal)	EHEC	0.0009, 0.0058, 0.012, 54	Calculated as Sev*D
		Ascaris	0.0057, 0.015, 0, 0	
DALY per event	mean DALY (log_{10})	EHEC	-1.92	Mean value of DALY per single case calculated as weighted average of Dis
		Ascaris	-2.22	

Table 2
Concentrations of *Escherichia coli, Enterococcus* spp. and *Ascaris lumbricoides* eggs in environmental samples, presented as positive samples in total samples with mean and standard deviation (sd) given for positive samples and range in brackets. The detection limit for bacteria was 100 cfu g^{-1} sludge and for Ascaris 1 egg 10 g^{-1}. Sludge was dried for 49-63 days before field sampling. Assumed recovery rate for Ascaris in sludge was 0.85 and in soil 0.37.

	E. coli $\log_{10}$cfu g^{-1} (field data)			*Enterococcus* spp. $\log_{10}$ cfu g^{-1} (field data)			Ascaris eggs g^{-1} (field data)			Ascaris viable eggs g^{-1} (field data)		
	pos/n	mean (min, max)	sd	pos/n	mean (min, max)	sd	pos/n	mean (min, max)	sd	mean (min, max)	sd	%
Raw sludge	3/3	5.6 (5.5, 5.7)	0.10	-	-	-	3/3	66 (45, 80)	18	34 (29, 38)	4.8	52%
Dried sludge	19/33	3.6 (<2.0, 6.4)	1.7	24/24	4.9 (3.0, 8.0)	1.1	32/33	21 (1.3, 90)	23	11 (0, 43)	10	53%
Dried sludge at farm	1/2	2.9 (<2.0, 3.7)	2.6	1/1	2.6 (2.6, 2.6)	-	2/2	27 (11, 43)	23	20 (3.8, 37)	23	75%
Soil - sludge	0/16	<2.0		12/16	2.6 (<2.0, 5.6)	2.0	16/16	15 (<0.1, 39)	12	9.5 (0, 22)	7.2	63%
Soil - manure	2/3	3.1 (<2.0, 3.7)	2.1	3/3	4.8 (4.3, 5.1)	0.42	2/3	6.8 (<0.1, 12)	6.0	2.9 (0, 4.6)	2.5	43%
Soil - NPK	1/3	2.7 (<2.0, 4.0)	2.3	2/3	2.6 (<2.0, 3.7)	1.9	1/3	5.2 (3.5, 7.6)	2.2	1.2 (0, 3.5)	2.0	23%
Cabbage	0/3	<2.0		3/3	3.8 (2.7, 4.4)	1.0	3/3	0.40 (0.20, 0.60)	0.24	0.30 (0.1, 0.5)	0.18	67%
Leafy vegetables	2/4	2.6 (<2.0, 3.9)	2.0	4/4	5.2 (4.2, 6.6)	1.2	4/4	17.6 (11, 24)	5.1	11 (9.0, 14)	1.9	65%

Table 3
Involuntary sludge ingestion (mg) as normal distributions given by mean (μ) and standard deviation (σ) for different activities and sum of ingestion during a duty performed by workers at Lubigi wastewater and faecal sludge treatment plant (WWFSTP), for the base case ($I_{g,b,l,s}$) and a case with mitigation measures ($I_{gm,bm,lm,sm}$)

Duty	#/yr	Ingestion during activities performed during each duty													
		Base		Eating		Offloading		Moving		Loading		Grit removal		Total, mg day^{-1}		Total, mg $year^{-1}$	
		μ	σ	μ	σ	μ	σ	μ	σ	μ	σ	μ	σ	μ	σ	μ	σ
Generic (I_g)	43	2.0		6.0	2.5									8.0	2.5	344	16
Generic mitigated (I_{gm})	43	2.0		3.0	1.3									5.0	1.3	215	8.2
Drying beds (I_d)	91	2.0		6.0	2.5			50	18	14	9.0			72	20	6552	193
Drying beds mitigated (I_{bm})	91	2.0		3.0	1.3			5.0	1.8	14	9.0			24	9.3	2184	88
Inlet (I_i)	31	2.0		6.0	2.5	142	89							150	89	4650	496
Inlet mitigated (I_{im})	31	2.0		3.0	1.3	14	8.9							19	9.0	589	50
Grit (I_s)	175	2.0										80	10	82	10	14350	132
Grit mitigated (I_{sm})	175	2.0										8.0	2.0	10	1.0	1750	13
Total ingestion base (g $year^{-1}$)																25.9	
Total ingestion mitigated (g $year^{-1}$)																4.738	

location and hand washing)) was assessed assuming that the measures reduced ingestion by 90% during working activities and 50% during eating. The daily pathogen dose, D_w (eggs or cfu day^{-1}), for a duty, *i.e.* worker on that shift, was calculated as:

$$D_w = \overbrace{I_{g,i,s,d}}^{ingestion} \times \overbrace{C_{rs,ds\}}}^{concentration} \times \overbrace{\omega_{a,e}}^{\substack{fraction\ of \\ infective\ pathogens}} \quad (1)$$

where C is concentration of organism (*E. coli* or viable *Ascaris* egg) in raw sludge (C_{rs}) (for duties at inlet and grit and settling tanks) or in dried faecal sludge (C_{ds}) (for generic duties and at the drying beds), and ω_e is the fraction of *E. coli* assumed to be EHEC.

2.6.2. Farmer scenario

This scenario focused on farmers using the faecal sludge as fertiliser and assessed the exposure from involuntary ingestion of contaminated soil. A general estimate of farmland affected by this practice was made considering that Lubigi WWFSTP is likely to sell 1450-2900 m^3 of faecal sludge from drying beds per year (personal communication, July 2019). This could be spread on an area of 67-136 ha of farmland, involving 700-1500 farmers, assuming a wet density of 1.4 kg L^{-1} (Strande et al., 2014), an average total solids rate of 30% (Lindberg and Rost, 2018) and 10 ton ha^{-1} application per year (in line with South African regulations) and an average of 11 farmers per hectare (Julien et al., 2019).

Farmers' activities were divided into two types: activities involving intense contact with soil performed in the first 10 days after fertilisation (I_{fh}) and activities involving less contact with soil performed on the other days (I_{fl}). The involuntary ingestion (mg day^{-1}) for the two activities was estimated with a distribution as PERT(0.0467, 0.0642, 0.0814) and PERT(0.005, 0.0123, 0.0205), respectively (Table 1). A mitigation scenario with farmers wearing a face cover during the first 10 days was also considered. This measure was assumed to produce a 90% reduction in involuntary ingestion.

The dose for the field data-based model (FM) was determined as:

$$D_{f\_FM} = \overbrace{C_{so\_FM}}^{\substack{concentration \\ in\ soil}} \times \overbrace{\omega_{a,e}}^{\substack{fraction\ of \\ infective\ pathogens}} \times \overbrace{I_{fh,fl}}^{ingestion} \quad (2)$$

For the theoretical model (TM), the concentration in soil at time t_2 was quantified as (where the concentration on the day of fertil-

ization is represented by the part in brackets):

$$C_{so}(t_2) = \overbrace{C_{fs}}^{\text{concentration in fresh sludge}} \times \overbrace{10^{r_1 t_1}}^{\text{decay in sludge}} \times \overbrace{R_{app}}^{\text{rate of application}} \times \overbrace{10^{r_2 t_2}}^{\text{decay in soil}} \quad (3)$$

The ingested dose was estimated as:

$$D_{f\_TM}(t_2) = \overbrace{C_{so}(t_2)}^{\text{pathogen concentration in soil}} \times \overbrace{\omega_{a,e}}^{\text{pathogenic fraction}} \times \overbrace{I_{fh,fl}(t_2)}^{\text{ingestion}} \quad (4)$$

where D_{f_FM} is daily dose of pathogens ingested per farmer, using field data (cfu or eggs day^{-1}), C_{so_FM} is concentration of organisms in soil from field sampling (cfu or eggs g^{-1}), ω is the pathogenic fraction (0.08 was assumed for *E. coli*, viable egg count was used for Ascaris), D_{f_TM} is daily dose of pathogens ingested per farmer estimated using the theoretical model (cfu or eggs day^{-1}), t_1 is time in drying beds at the WWFSTP (days), t_2 is time in soil (day) from previous fertilisation, r_1 is decay rate in faecal sludge ($\log_{10}$ cfu or egg day^{-1}), r_2 is decay rate in soil ($\log_{10}$ cfu or egg day^{-1}), R_{app} is sludge application rate (0 except on fertilisation days), C_{so} (t_2) is concentration of pathogens in soil as a function of days since fertilisation (cfu or egg g^{-1}), and $I_{h,l}$ (t_2) is involuntary ingestion on days with high-intensity soil work (10 days after fertilisation) and days with low-intensity soil work (remaining days) (g day^{-1}). For the TM in the farmer scenario, sludge drying age (t_1) of PERT(70, 77, 84 days) was used and farmers were assumed to fertilise twice a year (every 182 days) and to perform farming activities on all days of the year, thus being exposed to soil.

2.6.3. Consumer scenario

This scenario focused on consumers of raw products. Raw vegetables are not a fundamental part of the Ugandan diet (Kabwama et al., 2019) and a small proportion of the sludge will be used to fertilise crops eaten raw. Nevertheless, this risk assessment is worth considering and was stressed by farmers and the wastewater sector at stakeholder meetings.

The dose estimated for FM was calculated as:

$$D_{c\_FM} = \overbrace{C_{cr}}^{\text{concentration}} \times \overbrace{\omega_{a,e}}^{\text{fraction infective pathogens}} \times \overbrace{R_w}^{\text{washing reduction}} \times \overbrace{I_{c\}}}^{\text{ingestion}} \quad (5)$$

and for TM as:

$$D_{c\_TM} = \overbrace{C_{so}(100)}^{\text{concentration in soil at t=100}} \times \overbrace{\omega_{a,e}}^{\text{fraction infective pathogens}} \times \overbrace{R_m}^{\text{migration rate soil to crop}} \times \overbrace{R_w}^{\text{washing reduction}} \times \overbrace{I_{c\}}}^{\text{ingestion}} \quad (6)$$

where D_{c_FM} is ingested pathogens per event for the consumer, estimated using field data (cfu or eggs day^{-1}), C_{cr} is concentration of organisms found in crop from field sampling (cfu or eggs g^{-1}), R_m is migration rate from soil to crop (R_{m_l} for leafy vegetables and R_{m_c} for cabbages) modelled using field data, R_w is washing reduction rate, I_c is ingested amount of crop per event, D_{c_TM} is ingested pathogens per event by consumers estimated using the theoretical model (cfu or eggs day^{-1}) and C_{so} $^{(100)}$ is pathogen concentration in soil 100 days after fertilisation, i.e. the time of harvest when the main soil contamination was assume to occur. Table 1 presents the specific parameters for *E. coli*/EHEC and Ascaris. The sludge age (t_1) assumed for computing C_{so} was 70-84 days, i.e. the same as in the farmers scenario. Longer sludge drying time (t_1 = 150-200 days) was tested as a way to mitigate risks for consumers.

2.7. Dose-response assessment

Dose-response curves were used to determine the relationship between ingested dose and probability of infection. Navarro et al. (2009) formulated a dose-response model for Ascaris based on epidemiological data collected in Mexico (Blumenthal et al., 1996; Cifuentes et al., 1993), which has been used by most QMRA studies since 2009 and also in this study.

Dose-response models (Crockett et al., 1996; Haas et al., 1999; Strachan et al., 2001) have continuously been tested and re-fitted against outbreak data for EHEC where doses are known (Strachan et al., 2005; Teunis et al., 2004, 2009). The most recent assessment and fitting of models to the global *E. coli* O157:H7 outbreak (Teunis et al., 2008) concluded that infectivity may vary widely between strains and that the best-fitting model is a beta-Poisson model using a beta-binomial likelihood. Both the Ascaris and EHEC models in this study used the simplified Beta-Poisson equation:

$$P_i(D) = 1 - \left[1 + \frac{D}{\beta}\right]^{-\alpha} \quad (7)$$

with median infection as:

$$N_{50} = \beta\left(2^{\frac{1}{\alpha}} - 1\right) \quad (8)$$

where P_i represents the probability of infection for an ingested dose (D) in each event.

The probability of illness was then calculated using an infection to illness rate (λ):

$$P_{ill}(D) = P_i(D) \times \lambda \quad (9)$$

In the case of EHEC, the rate for *E. coli* O157:H7 (λ = 0.35) was used (Machdar et al., 2013). Considering the heterogeneity of illness for Ascaris (Walker et al., 2013), λ was modelled as a PERT distribution (0.15, 0.27, 0.39) (Dold and Holland, 2011; Mara and Bos, 2009). The annual probability of illness (P_{ill_yr}) was then calculated as:

$$P_{ill\_yr\_a} = 1 - (1 - P_{ill})^n \quad (10a)$$

or

$$P_{ill\_yr\_b} = 1 - \prod_1^n (1 - P_{ill}) \quad (10b)$$

where n is number of events (days of exposure).

Equation 10a was used with a constant infection to illness rate (λ) across events and equation 10b with a variable probability of illness. Note that the model considers probability of infection at any event as independent from other events, with no immunity developed after infection. In the consumer scenario, a single portion (30 g, 52 events) was taken as the minimum consumption unit of vegetables and assumed to occur on a weekly basis.

2.8. Risk characterisation

The disease burden was calculated and expressed in DALYs per 10,000 persons and year (Lopez et al., 2006; Mara and Bos, 2009; Murray et al., 2012). The WHO threshold of 10^{-6} DALY would correspond to 0.01 years lost per 10,000 persons a year, and a 10^{-4} DALY pppy to 1 year lost per 10,000 persons a year. Outcome of illness in terms of proportion with mild (*mi*), moderate (mo), severe (*s*) and fatal (*f*) severity weighting and duration of each outcome used for calculating DALYs (eq. 11) were based on previous studies (Katukiza, 2013; Pullan et al., 2014). For each scenario, the probability of illness from equation 10 was multiplied by the frequency (*F*), severity (*S*) and duration (*d*) of each outcome of illness as:

$$DALY_{mi,mo,s,f} = P_{ill\_yr} \times \sum \left(F_{mi,mo,s,f} \times S_{mi,mo,s,f} \times d_{mi,mo,s,f}\right) \quad (11)$$

The mean value of DALY for each scenario (expressed as $\log_{10}$) was then calculated as:

$$DALY_{mean} = mean\left(log_{10}\left(DALY_{mi,mo,s,f}\right)\right) \quad (12)$$

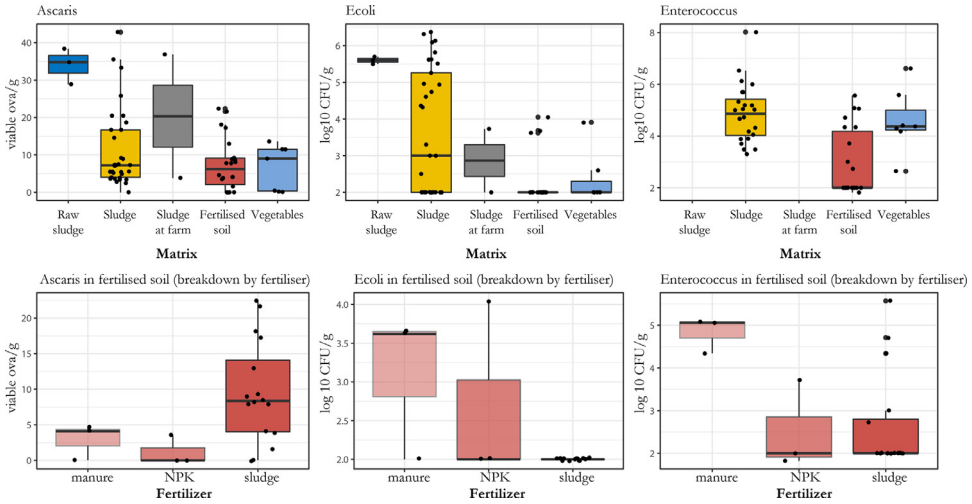

Fig. 2. Concentrations of a) viable *Ascaris lumbricoides* eggs, b) *Escherichia coli* and c) *Enterococcus* spp. in (top row) five matrices sampled along the faecal sludge management and reuse chain and (bottom row) field soil fertilised with three different fertilisers. Concentrations are shown as inter-quartile range boxes, with whiskers showing the 1.5 inter-quartile range and individual samples (dots).

2.9. Quality assurance and data analysis

To verify the accuracy of the *E. coli* enumeration, an additional sample was inoculated with *E. coli* ATCC-25922. Concentration of pathogens in the inoculum was determined by turbidity using a McFarland Standard. A solution of 0.5% was used, indicating a concentration of 1.5×10^8 cfu mL^{-1}. Analysis of the inoculated sample detected a concentration of 1.8×10^7 cfu mL^{-1}. In the case of Ascaris, data obtained from the field analysis were concentration-adjusted, assuming that full recovery of eggs was not achieved. Distribution fitting was done using R software (R Foundation for Statistical Computing, 2013) and the package fitdistrplus (Delignette-Muller and Dutang, 2015). For both organisms, a normal distribution on log-transformed data was used (see Table 1). Environmental samples with no detected microorganism concentrations were set to the detection limit (100 cfu g^{-1}) for distribution fitting. In the QMRA model, a Monte Carlo simulation was used in each step, by randomly sampling 10,000 times a value within the range or distribution used for a given parameter.

Sensitivity analysis on the decay model (TM) for the consumer and farmer scenarios was run using the R package multisensi (Bidot et al., 2018) and with parameter values in the same range as the QMRA model to simulate additional uncertainty (see supplementary material for details).

3. Results

3.1. Environmental sampling

3.1.1. Indicator bacteria

Salmonella spp. was not detected (detection limit 1 in 25 g) in any of the dried faecal sludge samples analysed (n=15) and was not investigated in other sludge, soil and vegetable samples. The raw faecal sludge (n=3) contained *E. coli* concentrations of 5.6 $\log_{10}$ cfu g^{-1}, while the dried sludge, which was sampled over 2 years (n=33), showed high variability, with *E. coli* ranging from below detection limit to 6.4 $\log_{10}$ cfu g^{-1} faecal sludge (Table 2). *Enterococcus* spp. was detected in all dried faecal sludge samples, at an average concentration of 4.9 $\log_{10}$ cfu g^{-1}. In soil samples, the highest concentrations of *E. coli* and *Enterococcus* were found in fields fertilised with chicken manure (Fig. 2). No consistent difference in bacterial concentration was observed between the two soil depths. *Enterococcus* spp. was found on all vegetables (mean 5.2 $\log_{10}$ cfu g^{-1}), while *E. coli* was detected in five out of seven vegetable samples (mean 2.3 $\log_{10}$ cfu g^{-1}). *E. coli* was detected in crops even when not detected in the faecal sludge-fertilised soil in which these crops were growing.

3.1.2. Ascaris

Viable Ascaris eggs were detected in all samples. The highest concentration was detected in raw sludge (mean 66 eggs g^{-1}, 52% egg viability). As expected, mean concentration was lower in dried sludge (21 eggs g^{-1}, 53% viability) and sludge-fertilised soil (15 eggs g^{-1}, 63% viability). The dried faecal sludge stored on two farms (n=2) had concentrations of viable eggs of 11 and 43 eggs g^{-1}, which is within the range measured in dry sludge at the plant (Fig. 2). However, Ascaris was also detected in fields fertilised with chicken manure (7 eggs g^{-1}, 43% viability) and NPK (5 eggs g^{-1}, 23% viability). Vegetable samples were found to be contaminated, with cabbages having an average of 172 viable eggs per item (0.4 egg g^{-1}, 67% viability), while onion leaves and amaranth had an average of 17 eggs g^{-1} (65% viability).

3.2. Worker scenario

The workers' schedule, with different duties, resulted in a daily risk of infection that was higher for EHEC (3.8-24% depending on duty) than for Ascaris (0.03-4.4%). However, the pattern was similar for both pathogens, with generic duties giving the least risk, followed by working at drying beds, working at sedimentation beds and working at the inlet. Thus, raw sludge gave the highest risk of infection (Table 4). The probability of illness over different time frames (24, 30 and 90 days) of the workers' rolling schedule in the base (b) and mitigation (m) scenario showed that workers reached 99% probability of illness from EHEC within one month of working, whereas for Ascaris one year of working was required to reach 99% probability of illness (Table 4). Mitigation measures had most effect for Ascaris, decreasing the probability of infection over a 3-month period from 85% to 36%.

3.3. Farmer scenario

The QMRA model resulted in yearly ingestion of 5.9 g soil, considering the first 10 days after the two fertilisation events as high-intensity work activity (average 60 mg day^{-1}) and the remaining days as low-intensity work (average 12 mg day^{-1}).

Table 4
Estimated average risk of infection with *Ascaris lumbricoides* and enterohaemorrhagic *Escherichia coli* (EHEC) for workers over five periods (1, 14, 30, 90 and 365 days) and the health burden as disability-adjusted life years per 10,000 persons and year (DALYs) for the base scenario (b) and with mitigation measures (m).

Organism	Duty	Daily		14 d		30 d		90 d		Year		DALYs (years lost per 10,000 persons & yr)	
		b	m	b	m	b	m	b	m	b	m	b	m
EHEC	Generic	3.8%	3.1%	88%	61%	99%	85%	100%	100%	100%	100%	30.1	30.1
	Drying beds	7.6%	5.6%										
	Inlet	24%	17%										
	Grit	22%	14%										
Ascaris	Generic	0.03%	0.14%	22%	5.8%	40%	11.3%	85%	36%	99%	83%	15.3	9.0
	Drying beds	0.27%	0.57%										
	Inlet	4.4%	0.46%										
	Grit	3.3%	0.26%										

For farmers, the field data model (FM) estimated yearly risk of illness from Ascaris at 70% and from EHEC at 26%. Mitigation by using a face mask during the first 10 days from fertilisation reduced the risk of illness to 12% for Ascaris and 21% for *E. coli*. The decay model (TM) predicted a yearly risk of illness for farmers from EHEC of 1.2% for the base scenario and 0.8% for the mitigated scenario. Yearly risk of illness from Ascaris in the decay model (TM) was estimated at 1.4% in the base scenario and 1.0% in the mitigated scenario for the decay model (TM). Field data from the same farm gave a yearly risk odds-ratio (OR) of 2.8 for soil fertilised with sludge (mean 3.9 viable eggs g^{-1}, 60-80 days from fertilisation) and without faecal sludge but NPK (1.2 viable eggs g^{-1}, 60-80 days from fertilisation).

3.4. Consumer scenario

The field model based on concentrations detected in samples of vegetables predicted a 100% yearly probability of illness caused by EHEC and Ascaris infection for leafy vegetables (n=7). Probability of illness for a single event was estimated at 19.7% for EHEC and 11% for Ascaris. Using concentrations found in cabbage (n=3), the annual and weekly risks were much lower: 0% in both time frames for EHEC and 26% and 0.06%, respectively, for Ascaris. The theoretical model predicted a risk for EHEC of almost 0%, while for Ascaris the yearly probability of illness from consuming leafy vegetables was estimated at 64% and 19% for a shorter (70-84 days) and longer period (150-200 days) of sludge drying, respectively, while for cabbage the probability of illness was 15% and 2% for the same shorter and longer drying periods, respectively.

4. Discussion

4.1. Environmental sampling of the faecal sludge management and reuse chain

4.1.1. Sludge and soil

The *E. coli* concentration in raw faecal sludge (mean 5.6 $\log_{10}$ g^{-1}) was slightly lower than found in pit latrines in Kampala (6-7 $\log_{10}$ g^{-1}), whereas the Ascaris egg concentration (mean 66 ±18 eggs g^{-1}) was higher than reported for pit latrines (19-25 eggs g^{-1}) (Kabenge et al., 2017). Drying of the sludge lowered the concentration of Ascaris eggs (to 21±23 eggs g^{-1}), although with rather high variation in concentration (Fig. 2), which may reflect variations in raw sludge or indicate that the sludge had been subjected to different storage times. Navarro et al. (2009) found that even with different treatments, helminth eggs are likely to remain in faecal sludge. Since Ascaris eggs can persist at moisture content down to 5% (Senecal et al., 2020), drying may not be efficient for Ascaris sanitisation. A study by Kone et al. (2007) investigating faecal sludge drying in a similar set-up observed a reduction from 60 to 22-38 eggs g^{-1} total solids when drying from 3% to 20% total solids, with viability around 50%, which is in agreement with others (Navarro et al., 2009; Seidu et al., 2008) and the present study. To reach WHO standards (WHO, 2006b) or Class B sludge in South Africa (Department Water and Agriculture of South Africa, 2006) (1 egg g^{-1}), and based on decay of -0.0095 $\log_{10}$ viable eggs day^{-1} (Table 1), the raw faecal sludge would need to be dried for 190 days. Field data points with known sludge age (n=12) were used to estimate a decay rate. The result (-0.0093 day^{-1} with R^2=0.36) was compatible with the log-decay rate used in the TM (-0.0095 day^{-1}). A curve fit on predicted (y) versus measured log-values (x) was also produced (y = 0.38x+0.80 R^2=0.37).

Concentration of Ascaris eggs in soil fertilised with sludge on the farms sampled had a mean of 9.5 viable eggs g^{-1}. For samples obtained from the same farm, sludge-, manure- and NPK-fertilised fields had a mean concentration of 3.9, 2.9 and 1.2 eggs g^{-1}, respectively, and differences were not significant (p = 0.47).The detection of ascaris eggs in soils that were not fertilised with sewage sludge may be explained by residual eggs from previous sludge applications or other potential sources of pathogens (e.g. irrigation, free-ranging pigs). The bacterial sampling also indicated sources other than sludge application (Table 2). However, for bacterial contamination there are more likely sources among wild and domestic fauna.

Even based on few data, the difference in soil concentration indicates that the last sludge application could contribute up to 70% of the Ascaris egg concentration in soil. However, when considering the overall/true risk to farmers and consumers, the background levels need to be included.

In most cases (19/22), *E. coli* was not detected (<100 cfu g^{-1}) in soil fertilised with faecal sludge, but was found in soil fertilised with manure or NPK. A study on farmer exposure in Ghana (Antwi-agyei et al., 2016) reported an *E. coli* concentration in soil of 2.3 $\log_{10}$ cfu g^{-1}, which is similar to that found in this study.

4.1.2. Crops

Escherichia coli was detected on leafy vegetables, in an average concentration of 1.6 $\log_{10}$ cfu g^{-1}, which is similar to that reported for leafy vegetables in Ghana (10^2 faecal coliform bacteria g^{-1}) (Amoah et al., 2007a). It also agrees with the estimated survival time of coliforms on crops of 15-30 days (WHO, 2006b, p. 46). *E. coli* was detectable on vegetables despite not being present in the soils where the vegetables were grown, indicating other potential sources of contamination, as the survival in soil is expected to be higher than on vegetables (Oliveira et al., 2012).

Ascaris concentration on vegetables (11 ±1.9 viable eggs g^{-1} on leafy vegetables and 0.30 ±0.18 viable eggs g^{-1} cabbage)

Table 5
Risk as probability of illness (%) for different scenarios and disability-adjusted life years (DALYs) per 10,000 persons and year assuming a DALY per infection for EHEC of 0.012 and for Ascaris of 0.0062

Scenario	Pathogen	Probability of illness (%)		DALYs (yrs per 10,000 persons & yr)	
		FM base/mitigated	TM base/mitigated	FM base/mitigated	TM base/mitigated
Workers	E.coli O157:H7	100/100	na	120/120	na
	Ascaris	99/83	na	62/51	na
Farmers	E.coli O157:H7	26/21	1.2/0.8	31/25	1/1
	Ascaris	70/12	1.4/1	43/7	1/1
Consumers	E.coli O157:H7 leafy veg.	100	0	120	0
	E.coli O157:H7 cabbage	0	0	0	0
	Ascaris leafy veg.	100	64/19	62	39/12
	Ascaris cabbage	26	15/2	16	9/1

was of the same magnitude as reported previously in a similar context (2.7 eggs g^{-1} spring onions and 0.4 eggs g^{-1} cabbage) (Amoah et al., 2006). The concentration on leafy vegetables was very close to that in the dried sludge (Table 2). This would represent rather high soil contamination, despite harvesting being performed in very controlled ways to avoid soil contamination, which may not be the case during large-scale routine harvest. Since concentrations on cabbage are counts per unit weight, considering only the outer leaves or the whole cabbage (as done in this study) would change estimates significantly.

4.1.3. Sampling strategy

Unlike *E. coli*, *Enterococcus* spp. was present in concentrations above the detection limit in all matrices analysed. This may indicate slower decay and/or other sources of contamination, and suggests that *Enterococcus* spp. may be a better indicator of soil and crop contamination by persistent pathogens. However, *Enterococcus* spp. may also be of environmental origin, so distinguishing *Enterococcus* spp. of faecal origin (*E. faecalis* and *E. faecium*) is required for accurate detection of faecal contamination (Bartz et al., 2017). *E. coli* may serve as a model for Gram-negative bacterial pathogens, e.g. *Salmonella* and *Shigella*.

4.2. Risk characterisation

In all scenarios, the burden of disease expressed as years of life lost per 10,000 people (Table 5) was at least two orders of magnitude higher than the WHO tolerable additional disease burden of DALYs lost (0.01 per 10,000 or 10^{-6} pppy) (Table 4 and 5). The burden of disease was below the WHO value only for EHEC when consuming cabbage, based on field data, and for both crops in the TM, the latter due to assumed decay in the environment. A target of 1% of local prevalence (4.5% for Ascaris, 25% for *E. coli* risk to farmers), as previously proposed (Mara, 2011; Mara and Sleigh, 2010), may be a more meaningful level for acceptable risk. Figs. 5 and 6 show the risk of illness across the faecal sludge treatment-reuse chain.

The contrasting risk estimates from the FM and TM depend on the different concentrations of pathogens measured versus assumed at each step of the treatment-reuse chain (Fig. 3). These differences are caused by considering in the TM only the last sludge application, not including possible additional sources of pathogens and/or residual eggs from previous fertilisation occurring in the field. The TM also suffers from uncertainties in the application rate of sludge and migration rate from soil to crop. Fitting the TM to field data was tested, where possible (section 4.2.4).

4.2.1. Worker scenario

Dividing the scenario into daily duties showed that risk varied significantly depending on work tasks at the treatment plant. Unsurprisingly, working with raw faecal sludge posed the highest risk and should be given priority in mitigation efforts. Evaluating the risk over time showed that, while the yearly risk of illness remained almost 100% for both pathogens studied, mitigation effects were detectable in a shorter time frame and more prominent for Ascaris (Table 3).

In Uganda, the prevalence of Ascaris in workers managing drainage channels at another WWTP (Bugolobi) is reported to be 2.3% and that in workers managing faecal sludge 0% (Fuhrimann et al., 2016a). Overall prevalence of Ascaris in Uganda is estimated to be 4.5% (Karagiannis-Voules et al., 2015). The QMRA for 30 and 90 days in the present study estimated a risk of infection of 40% and 85%, respectively. Considering that workers at Lubigi WWFSTP are dewormed every 90 days, this could be considered the highest risk of infection. The results support the deworming interval (Table 4). Even considering the shorter time frame, the estimated risk of illness was over 10-fold higher than reported for Bugolobi WWTP. It was not stated whether the Bugolobi workers received deworming similar to workers at the Lubigi plant (Fuhrimann et al., 2016a).

In a survey on faecal sludge workers, the self-reported prevalence of diarrhoea over a 14-day recall period was 32.8% (Fuhrimann et al. 2016b), while a review by Thorn and Karekes (2001) reported prevalence of gastrointestinal problems in WWTP workers of 8% (Germany in 1954) and 13% (Sweden in 1978). Using a time frame of two weeks, which would cover the duration of illness in 99% of cases for EHEC (Table 1), a risk of illness of 88% for the base scenario and 61% for the mitigated scenario was estimated from the models, i.e. much higher than the self-reported value. For both pathogens, the higher probability of illness calculated via QMRA compared with prevalence reported by others could be caused by overestimation of involuntary ingestion. Host characteristics such as age and immunity could also play a role (Walker et al., 2013, p. 165; Holland, 2009), and may not be accounted for in the dose-response curve. In the studies cited, use of protection measures was not mentioned, but could explain the lower prevalence of infection.

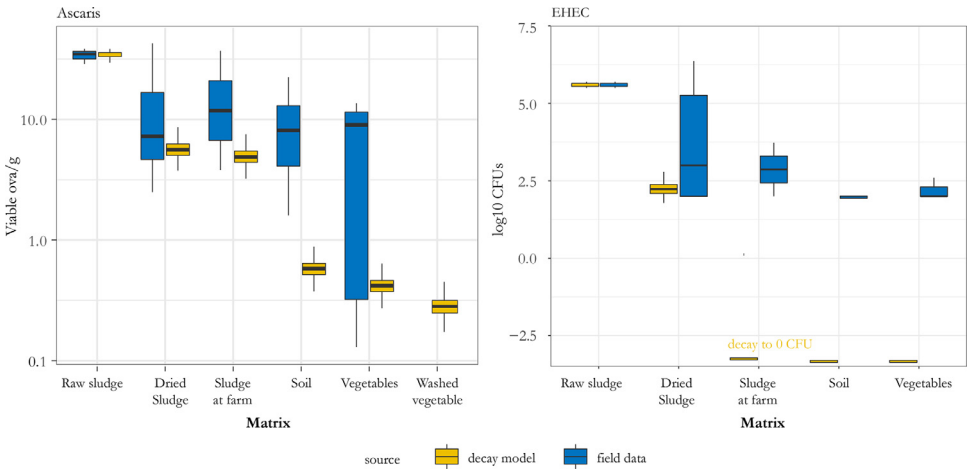

Fig. 3. Comparison of concentrations obtained from field sampling (blue) and estimates obtained from the theoretical model (yellow) for different matrices. For Ascaris, concentrations on vegetables and washed vegetables refer to leafy vegetables.

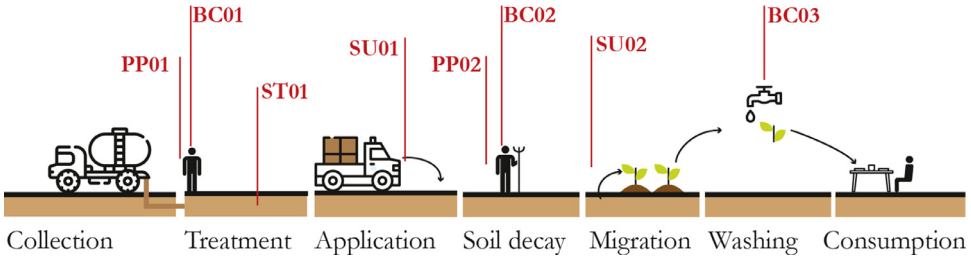

Fig. 4. Mitigation measures that could be adopted along the faecal sludge treatment-reuse chain. PP01 = personal protection equipment for workers (face shield and gloves); BC01 = behavioural change by workers (avoid eating near beds, improve personal hygiene before eating); ST01 = sludge treatment (extend drying time); SU01 = sludge use (control application rate); PP02 = personal protection for farmers (gloves, shoes especially in the first days after fertilisation); BC02 = behavioural change by farmers (personal hygiene); SU02 = sludge use (apply crop restriction); BC03 = behavioural change by consumers (improve washing of produce).

4.2.2. Farmer scenario

In a study by Fuhrimann et al. (2016a), 25% of farmers self-reported cases of diarrhoea over a period of two weeks. In comparison, the QMRA, over the same period, predicted a probability of illness from EHEC of 4% (FM) and below 0.01% (TM). The lower estimate (FM) may be partly explained by EHEC being only one of many possible causes of diarrhoea and partly by underestimation of involuntary ingestion for farmers. That the general concentration of *E. coli* was analysed and assumed to represent a fraction of EHEC is a matter of uncertainty for both models (FM and TM).

For Ascaris, risk estimates diverged significantly between the TM and FM, due to the different egg concentration in soil used in the two models: 9.5 viable eggs g^{-1} (FM) and 0.24 viable eggs g^{-1} (TM). Several scenarios were explored in order to reconcile the two models. Using the considered parameters within a reasonable range did not lead to a convincing result. The difference between the two models could be caused by uncertainties connected to the parameters used in the TM (decay dynamics in sludge and soil, sludge application rate, analytical recovery, time passed between fertilisation), but also by assuming only one source of pathogens. The existence of other sources was indicated by the concentration of *E. coli* and *Enterococcus* found in NPK-fertilised fields, but also by the limited difference between concentrations found in dried sludge (11 viable eggs g^{-1}) and soil (mean of 9.5 viable eggs g^{-1} across all fields). Despite these considerations, given the limited amount of sampled farms, further conclusions cannot be drawn. Additional studies, perhaps in the form of controlled experiments, are required to better understand the application and fate of Ascaris in farm soil.

The yearly risk odds-ratio (OR) of 2.8 was higher than the value of 1.7 reported by Tran-Thi et al (2017) but lower than the 3.5-5.4 reported in other studies (Amoah et al., 2016; Blumenthal and Peasey, 2002; Pham-Duc et al., 2013).

4.2.3. Consumer scenario

For EHEC, the FM and TM produced contradictory yearly risk estimates, e.g. risk was negligible in TM, due to the fast decay assumed (Bartz et al., 2017), and 100% in FM, due to the detection of *E. coli* on vegetables (mean 2.6 $\log_{10}$ CFU g^{-1}). These differences could be reconciled considering that water used for irrigation can also be a source of pollution (Amoah et al., 2007a) and that contamination may occur between farm and market: 80% of vegetables sampled at a market in Ghana were contaminated with *E. coli* (0.6-3.8 $\log_{10}$ g^{-1}) (Antwi-Agyei et al., 2015). The estimated 100% probability of illness from EHEC in the FM was significantly higher than that estimated previously for Côte d'Ivoire, which has a yearly infection risk of 12-23% (Kouamé et al., 2017). In conclusion, for EHEC the QMRA model based on extrapolation from *E. coli* field data and weekly consumption indicated an almost certain risk of illness on a yearly basis, but the source of this pathogen is unlikely to be the sludge used on fields.

For Ascaris, the slower decay in soil would theoretically allow this pathogen to survive and be present on the crop at harvest. The divergence between TM and FM in soil concentration estimates (Fig. 3) also affected crop concentration and risk estimate. The FM predicted a yearly probability of illness of 100% (based on concentrations found on leafy vegetables) and 26% (based on concentrations found on cabbage). The TM prediction was 66% for leafy vegetables and 19% for cabbages. The results from both models indicated that leafy vegetables pose a several-fold higher risk than cabbage. This is linked to the higher Ascaris egg concentration found on leafy vegetables (11.4 viable eggs g^{-1}) compared with cabbage

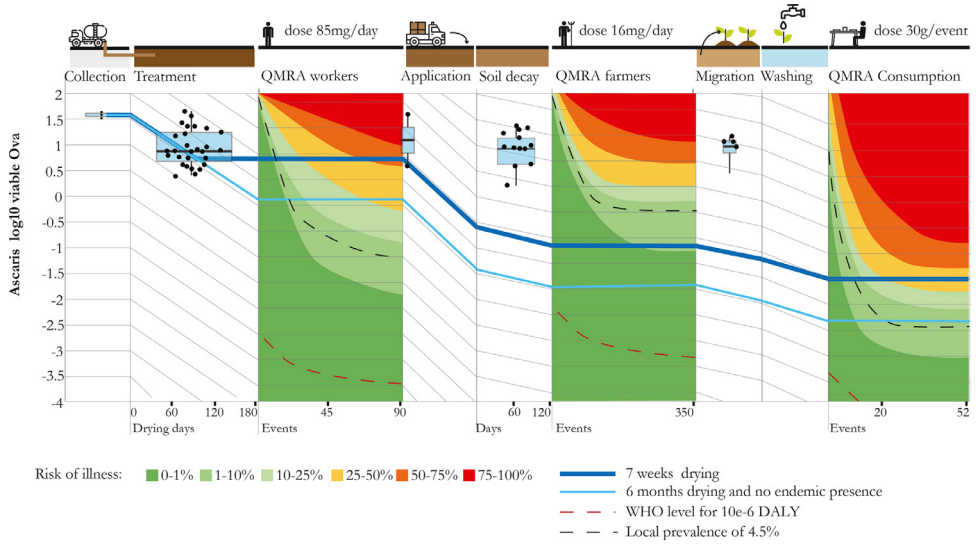

Fig. 5. Heat map showing the probability of illness from *Ascaris lumbricoides* (colour codes indicate percentages) for workers and farmers as a function of days and for consumers as a function of eating events (x-axis), depending on Ascaris egg concentration (y-axis). Box plots show concentrations based on field data (field model, FM) and the blue line concentrations based on theoretical estimates (theoretical model, TM). Boxplot width and position (not the single dots) indicate assumed age of the sample. The red dashed line shows Ascaris egg concentration required to reach the WHO threshold tolerable added burden of disease of 10^{-6} disability-adjusted life years (DALYs), the black dashed line shows infection rate at the local Ascaris egg prevalence of 4.5%. Note that the QMRA heatmap for workers is simplified by assuming one activity and one ingestion, and therefore not comparable to other results shown in the manuscript.

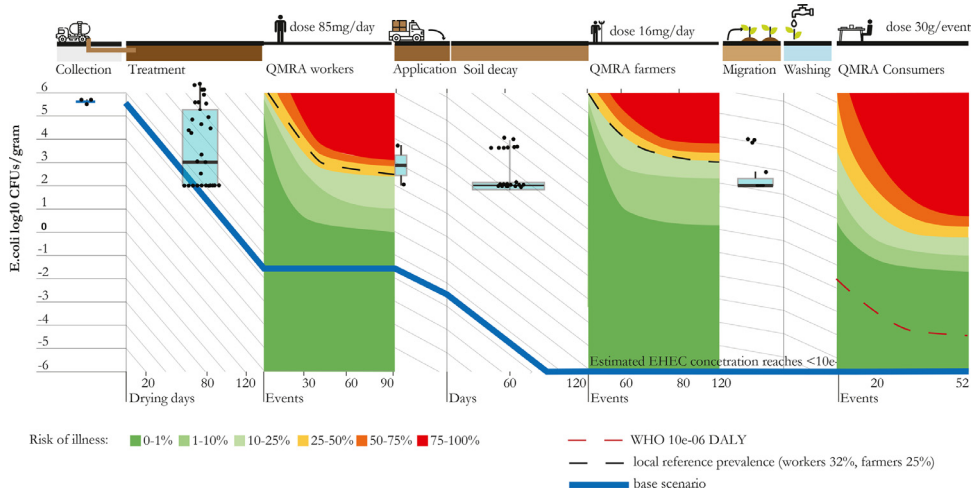

Fig. 6. Heat map showing the risk of illness from enterohaemorrhagic *Escherichia coli* (EHEC) for each target group along the faecal sludge management chain as a function of days/events of exposure (x-axis) and pathogen concentration (y-axis). Boxplots show field data, with boxplot width and position (not the single dots) indicating assumed age of the sample and the blue line concentrations based on theoretical estimates (theoretical model, TM). The black dashed line indicates level of local prevalence. The red dashed line shows the EHEC concentration required to reach the WHO threshold for tolerable added burden of disease of 10^{-6} disability-adjusted life years (DALYs). Red grid lines in the background show mean decay rate at each stage of the treatment and reuse process.

(0.30 viable eggs g^{-1}). Both models showed that the crop chosen for the QMRA may also be very influential for the outcome: leafy vegetables showed higher concentration and risks. Regarding cabbages, it should also be considered that while the pathogen concentrations on external leaves may be similar, the greater weight of the inner cabbage head results in much lower counts per unit weight.

4.2.4. Fitting field data

Despite the significant differences in outcome between the two models, an effort was made to adjust parameters to fit the TM to field data. For Ascaris, the calibrated model was considered over a time frame of one month, while for EHEC the time frame was two weeks. These periods were chosen based on the assumed duration of illness (Table 1). For the workers scenario, matching the national Ascaris prevalence of 4.5% would require daily ingestion just lower than that calculated in the mitigated scenario: for activities with raw sludge 20 mg and for dried sludge 10 mg. For EHEC, a 33% risk would be matched by ingestion of 6 mg of raw sludge, assuming the same concentration in dried sludge as in the base scenario.

For the farmers and consumers scenarios, fitting the TM proved more difficult. In particular, fitting the concentration found in soil with reasonable sludge application rate was not possible. Ascaris decay rate in soil and residual eggs from previous fertilisation were areas of great uncertainty. Assuming Ascaris levels found in NPK-fertilised fields as a background concentration, field data fitted with a decay rate of 90% (T_{90}) at 234 days. For the TM, the background concentration was not included but a more conservative approach was taken on decay (T_{90} between 120-500 days). This decay should produce a residual load after several fertilisations of around 0.45 viable egg g^{-1}. Migration rate from soil to crop was estimated based on limited data points obtained from field data,

but it would be beneficial to do further research on the topic. A model reflecting the full risks would probably require inclusion of additional sources of pathogens, e.g. via irrigation, and would benefit from a larger sample and closer monitoring to determine key parameters (R_{app}, R_m, C_{res}). The development of such a combined fertilisation-irrigation model could be the objective of further studies.

4.2.5. Uncertainty in QMRA

For involuntary ingestion, estimates were based on published literature and may be considered reasonable, but it is not known how accurately they describe the specific context. Acquired immunity and differences in age or gender were not considered in the model, unlike in other studies (Karavarsamis and Hamilton, 2010). The infection to illness rate was modelled using a single value (0.35) for EHEC and PERT(0.15, 0.27, 0.39) for Ascaris. In future QMRA, validation with health data from workers could reduce these uncertainties. Statistical methods used may also have a significant impact (Poma et al., 2019), as may dose-response parameters (Kundu et al., 2014). QMRA is mostly used for estimating single exposure events, but it would be worth exploring its use for continuous exposure. The data collected during interviews and site visits were not sufficient to accurately estimate fertiliser application rate (R_{app}) and pathogen migration from soil to crop (R_m). The TM was not able to explain the majority of the Ascaris concentration found in soil. Improvement work on the model should include other possible sources of pathogens apart from sludge and residual eggs from previous applications. Ascaris survival in soil was modelled using literature data but, given the wide range of available decay rates, further context-specific studies would be beneficial. Running two models based on different inputs led to different risk estimates, indicating the importance of contextualising QMRA with local data in combination with published data.

4.3. Risk mitigation

Four types of mitigation measures were identified: improved treatment (extension of drying), limitation in the use of sludge (application control, limitation for certain crops), use of personal protection equipment (mask, face shields, shoes, gloves) and behavioural change for workers along the faecal sludge treatment-reuse chain (Dumba et al., 2013; Strunz et al., 2014) (Fig. 4).

4.3.1. Worker scenario

Reducing involuntary ingestion could be done at two points: during work activities and during eating at the workplace. Protective equipment should be used, especially at the inlet and sedimentation beds. The estimated daily risk of illness would then drop from 24% to 17% (EHEC, inlet) and from 22% to 13% (EHEC, sedimentation beds). Since a face mask is difficult to wear for hours in a warm climate, a face shield may be better option. It could also be cleaned and reused. Risks from eating could be reduced by revising hygiene practices and lunch location, and banning food consumption near the beds. However, the impact of these measures is difficult to predict, given the numerous uncertainties involved. Ascaris risk of illness during the 90 days between deworming could be decreased from 58% to 18%. Reduction in the risk from EHEC may only be detected over a 14-day period (reduction from 88% to 60%).

4.3.2. Farmer scenario

Sensitivity analysis showed that sludge drying time (range 70-200 days) was the main factor in controlling EHEC infection (Global Sensitivity Index 0.27) and Ascaris (GSI 0.34). A first mitigation approach would consist of extending the drying period of the sludge at the WWTP or at the farm after purchase. Another set of mitigation strategies could act at farmer behaviour level, particularly during the days with intense work in contact with soil. Protective equipment to reduce involuntary ingestion by 90% would decrease Ascaris infection (based on field data) from 62% to 22%, but would have no major impact on EHEC infection (22% to 21%). Previous studies have shown that shoes and improved personal hygiene may reduce the risk of illness (Strunz et al., 2014).

4.3.3. Consumer scenario

Sensitivity analysis showed that several factors influenced the final risk estimates for consumers. For Ascaris, controllable factors (drying time (t), application rate (Rapp), washing (Rw), ingestion (I)) were responsible for 92% of the results (GSI: A=0.321, t=0.29, I=0.05, Rw=0.26). Thus mitigation efforts should concentrate on extending drying time (illness probability reduction from 58% to 30%), promoting appropriate sludge application rates in agriculture and good produce washing practices by consumers. Restriction on crop type was not tested numerically, but the high variation in pathogen concentrations reported for different crops suggests that it could have a significant impact on the final risk of illness. These mitigations would be aligned with guidelines for South Africa Class B sludge with the following additional measures: sludge not used in growing vegetables to be eaten raw, crop that touches the ground should not be harvested within 14 months after last sludge application. It is also recommended not to exceed an application rate of 10 ton per ha and year.

For EHEC, the mitigation focus should be on the final part of the treatment-reuse chain (from harvest to point of sale), by avoiding additional pollution most likely coming from water.

Conclusions

- The concentrations of faecal indicator organisms and pathogens detected in sludge, soil and vegetables suggested that the latest sludge application plays an important role, but is not the sole source of pathogens along the reuse chain.
- QMRA estimates based on field sampling and theoretical decay calculations differed, indicating the importance of contextualising risk assessment. QMRA models based on field data showed that yearly risk of infection was high for all groups considered (workers, farmers, consumers).
- QMRA based on a decay model showed high yearly risk for Ascaris, but not for EHEC.
- EHEC from faecal sludge is unlikely to be present on harvested crops.
- Possible mitigation strategies for workers and farmers showed a reduction in monthly risk of 30-70%.
- Sludge drying time was found to be an important factor in the farmer and consumer scenarios (impact of 25-30% on risk estimates), and is therefore a potential mitigation tool.

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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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.watres.2021.117068.

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