Supporting Information to:

A quantitative microbial risk assessment of pediatric infections attributable to ingestion of fecally contaminated domestic soils in low-income urban Maputo, Mozambique

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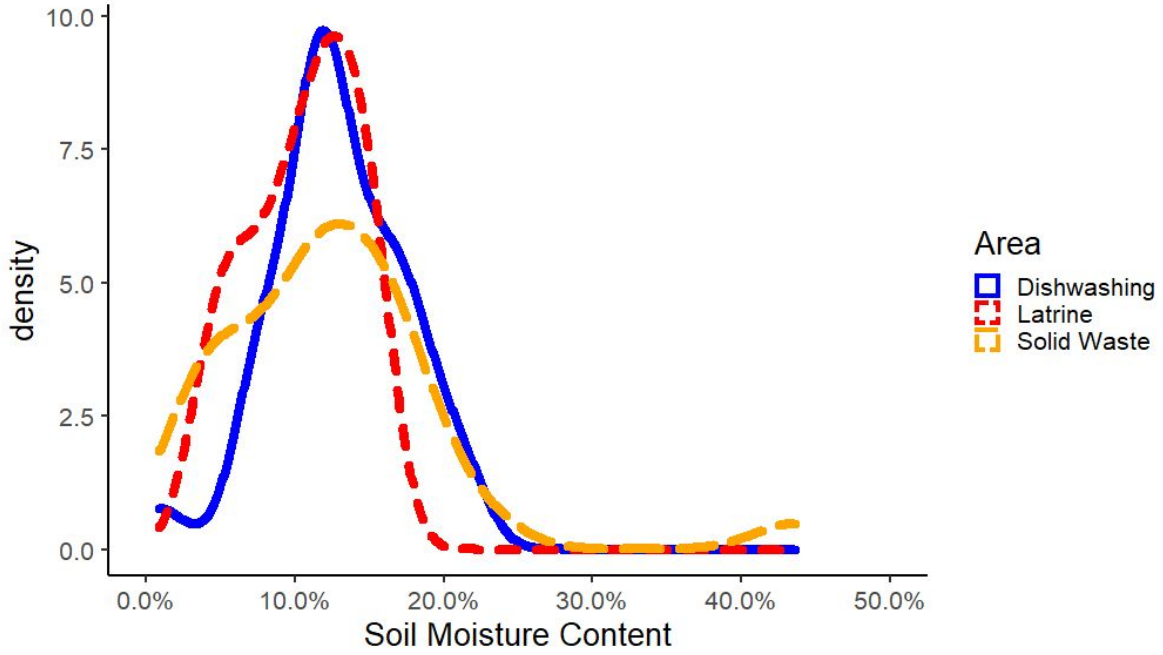
Supporting Information: 8 figures, 9 tables, 3 texts

Text S1. Soil sampling location selection

In February 2018 we met with an adult from eight households enrolled in the MapSan trial to evaluate the feasibility of various sampling locations. We asked participants to identify an area where the household's children most frequently play in the compound (cluster of households sharing common living space and sanitation). None of the respondents were able to identify an area where the children most frequently play. Instead, responses indicated that children typically play all over the yard or with their friends in a neighboring compound. Due to the inability to systematically identify an area where children frequently play, we chose standardized locations that existed at each compound in the study. The standardized locations chose were likely to have relatively higher fecal contamination than other compound locations to represent an upper bound of infection risk.

Figure S1. Soil moisture content



Kernel density plot of soil moisture content as a percent of overall mass by sampling location.

Table S1. Results from soil drying experiments

Sample	Number	Moisture Content at 1 hour	Moisture Content at 2 hours
A	1	0.28	0.28
A	2	0.27	0.27
A	3	0.26	0.26
B	4	0.11	0.11
B	5	0.13	0.13
B	6	0.13	0.13
C	7	0.33	0.33
C	8	0.35	0.35
C	9	0.34	0.33

We collected three soils in Atlanta using the methods described in this manuscript. One soil was high in sand content, one was high in clay content, and the third was high in organic matter. We dried each sample in triplicate for 1 hour, determined the moisture content, dried for an additional hour, and then determined the moisture content again. The data shows that drying for one hour vs. two hours made no substantial difference in the measured moisture content.

Text S2. Supplemental notes on ddPCR

Adapting qPCR assays to ddPCR

Our methods to adapt qPCR assays to ddPCR have been extensively described in Bivins *et al*. 2020.[1] Briefly, we first ran positive control materials (gBlock DNA) using a 10° Celsius annealing temperature gradient. Then, we used the results to run a second 4° Celsius annealing temperature gradient. From these results, we selected the annealing temperature that achieved the greatest difference between the positive and negative droplet fluorescence amplitudes. The thermocycling conditions used are listed in Table S1.

ddPCR Reactions

We singleplexed all assays and used a final primer concentration of 900 nM and probe concentration of 250 nM. Likewise, for the ybbW EvaGreen assay we used a primer concentration 250nM. Table S1 lists the reagents used in our ddPCR reactions, which included molecular-grade water, ddPCR Supermix for Probes or ddPCR EvaGreen Supermix (Bio-Rad, Hercules, CA), primers, probe, and extract. On each ddPCR plate we included at least one no-template control and at least one negative extraction control.

ddPCR Analysis

To differentiate between positive and negative partitions we used conservative manual thresholding (Figure S3). Due to the presence of 3 false positive partitions in our *beta-giardin* controls (Table S2) and based on best practices from the literature[2], we only called samples with 3 or more partitions above the manual threshold as positive for a gene target. In addition, we successfully detected the MS2 process control in each sample.

68 Table S2. Descriptions of molecular assays

Assay	Probe concentration (nM)	Forward / reverse primer concentration (nm)	Template volume (dilution)	Supermix or Mastermix (Volume)	Reaction Volume	Cycling conditions
MS2 (reverse transcription PCR, ABI 7500)	250	900	1 µL (none)	qScript™ XLT One-Step RT-qPCR ToughMix®, Low ROX™ (20 µL)	40 µL	1. 45°C 10 minutes 2. 94°C 10 minutes 3. 40× (94°C 0.5 minutes, 60°C 1 minute) 2°C/s ramp rate; sample volume 40 µL
ybbW (ddPCR, QX 200)	250	900	1 µL, 2uL (none, 1:100)*	QX200™ ddPCR™ EvaGreen Supermix (10.5 µL)	21 µL	1. 95°C 10 minutes 2. 40× (95°C 0.5 minutes, 59°C 2 minutes) 3. 4°C 5 minutes 4. 90°C 5 minutes 5. 4°C hold 1°C/s ramp rate, heated lid 105°C; sample volume 40 µL
ipaH (ddPCR, QX 200)	250	900	4 µL (none)	ddPCR™ Supermix for Probes (10.5 µL)	21 µL	1. 95°C 10 minutes 2. 40× (95°C 0.5 minutes, 58.7°C 1 minute) 3. 98°C 10 minutes 4. 4°C hold 2°C/s ramp rate, heated lid 105°C; sample volume 40 µL
beta-giardin (ddPCR, QX 200)	250	900	4 µL (none)	ddPCR™ Supermix for Probes	21 µL	1. 95°C 10 minutes 2. 40× (95°C 0.5 minutes, 58.7°C 1 minute) 3. 98°C 10 minutes

				(10.5 µL)		4. 4°C hold 2°C/s ramp rate, heated lid 105°C; sample volume 40 µL

*Different volumes and dilutions were run for the *ybbW* assay to obtain results within the quantifiable range. See Table S5 for additional details.

Table S3. Summary of molecular and culture-based assays from combined trial arms.

Log_{10} transformed density of *beta-giardin* gene copies per gram of dry soil				
Location	Prevalence	Mean (sd)	Median	Range
Latrine entrance	47% (14/30)	2.0 (0.70)	1.9	0.48, 3.3
Solid waste	30% (9/30)	1.8 (0.73)	1.8	0, 3.4
Dishwashing	47% (14/30)	2.3 (0.81)	2.0	0.85, 3.8
Total	41% (37/90)	2.0 (0.76)	1.9	0, 3.8
Log_{10} transformed density of *ipaH* gene copies per gram of dry soil				
Location	Prevalence	Mean (sd)	Median	Range
Latrine entrance	57% (17/30)	2.6 (0.74)	2.3	1.4, 4.9
Solid waste	30% (9/30)	2.3 (0.48)	2.4	1.1, 3.4
Dishwashing	47% (14/30)	2.4 (0.62)	2.3	1.3, 3.8
Total	44% (40/90)	2.4 (0.62)	2.3	1.1, 4.9
Log_{10} transformed density of *ybbW* gene copies per gram of dry soil				
Location	Prevalence	Mean (sd)	Median	Range
Latrine entrance	100% (30/30)	5.8 (0.33)	5.8	5.4, 6.8
Solid waste	100% (30/30)	5.7 (0.35)	5.7	4.9, 6.3
Dishwashing	100% (30/30)	6.1 (0.27)	6.1	5.5, 6.8
Total	100% (90/90)	5.9 (0.37)	5.9	4.9, 6.8
Log_{10} transformed count of CFU *E. coli* per gram of dry soil				
Location	Prevalence	Mean (sd)	Median	Range
Latrine entrance	87% (26/30)	3.0 (1.1)	3.2	0.90, 5.3
Solid waste	87% (26/30)	3.2 (1.1)	3.1	1.1, 5.2
Dishwashing	90% (27/30)	3.3 (1.2)	3.3	0, 5.3
Total	88% (79/90)	3.2 (1.1)	3.2	0, 5.3

Note: For reporting purposes all non-detects (ND) were imputed to random value from zero to the 95% LOD for molecular assays and from zero to the LOD for the culture-based assay. Data in this table is combined from both trial arms.

Table S4. Summary of molecular and culture-based assay from 15 MapSan intervention compounds

Log_{10} transformed density of *beta-giardin* gene copies per gram of dry soil				
Location	Prevalence	Mean (sd)	Median	Range
Latrine entrance	53% (8/15)	2.0 (0.79)	2.1	0.48, 3.2
Solid waste	60% (9/15)	1.9 (0.61)	1.9	0.90, 2.7
Dishwashing	47% (7/15)	2.5 (0.79)	2.5	0.85, 3.8
Total	53% (24/45)	2.2 (0.75)	2.2	0.48, 3.8
Log_{10} transformed density of *ipaH* gene copies per gram of dry soil				
Location	Prevalence	Mean (sd)	Median	Range
Latrine entrance	47% (7/15)	2.4 (0.35)	2.3	2.0, 3.1
Solid waste	60% (9/15)	2.3 (0.49)	2.4	1.1, 3.1
Dishwashing	33% (5/15)	2.5 (0.59)	2.4	1.5, 3.8
Total	47% (21/45)	2.4 (0.48)	2.3	1.1, 3.8
Log_{10} transformed density of *ybbW* gene copies per gram of dry soil				
Location	Prevalence	Mean (sd)	Median	Range
Latrine entrance	100% (15/15)	5.8 (0.37)	5.8	5.4, 6.8
Solid waste	100% (15/15)	5.6 (0.31)	5.6	4.9, 6.1
Dishwashing	100% (15/15)	6.2 (0.23)	6.2	5.9, 6.8
Total	100% (15/15)	5.9 (0.39)	5.9	4.9, 6.8
Log_{10} transformed count of CFU *E. coli* per gram of dry soil				
Location	Prevalence	Mean (sd)	Median	Range
Latrine entrance	80% (12/15)	3.4 (1.1)	3.4	1.4, 5.3
Solid waste	13% (2/15)	3.0 (1.2)	2.9	1.0, 5.2
Dishwashing	93% (14/15)	3.3 (0.99)	3.2	1.7, 5.3
Total	84% (38/45)	3.2 (1.1)	3.2	1.0, 5.3

Note: For reporting purposes all non-detects (ND) were imputed to random value from zero to the 95% LOD for molecular assays and from zero to the LOD for the culture-based assay

Table S5. Summary of molecular and culture-based assay from 15 MapSan control compounds

Log_{10} transformed density of *beta-giardin* gene copies per gram of dry soil				
Location	Prevalence	Mean (sd)	Median	Range
Latrine entrance	40% (6/15)	2.0 (0.62)	1.9	1.3, 3.3
Solid waste	13% (2/15)	1.6 (0.80)	1.5	0, 3.4
Dishwashing	33% (5/15)	2.0 (0.79)	1.7	1.1, 3.5
Total	29% (13/45)	1.9 (0.75)	1.7	0, 3.5
Log_{10} transformed density of *ipaH* gene copies per gram of dry soil				
Location	Prevalence	Mean (sd)	Median	Range
Latrine entrance	67% (10/15)	2.7 (0.96)	2.3	1.4, 4.9
Solid waste	27% (4/15)	2.3 (0.50)	2.3	1.5, 3.4
Dishwashing	33% (5/15)	2.2 (0.62)	2.3	1.3, 3.5
Total	42% (19/45)	2.4 (0.74)	2.3	1.3, 4.9
Log_{10} transformed density of *ybbW* gene copies per gram of dry soil				
Location	Prevalence	Mean (sd)	Median	Range
Latrine entrance	100% (15/15)	5.9 (0.30)	5.9	5.4, 6.4
Solid waste	100% (15/15)	5.8 (0.38)	5.9	5.0, 6.3
Dishwashing	100% (15/15)	6.1 (0.31)	6.1	5.5, 6.5
Total	100% (15/15)	5.9 (0.35)	5.9	5.0, 6.5
Log_{10} transformed count of CFU *E. coli* per gram of dry soil				
Location	Prevalence	Mean (sd)	Median	Range
Latrine entrance	67% (10/15)	2.7 (1.0)	2.9	0.90, 4.6
Solid waste	87% (13/15)	3.3 (1.0)	3.5	1.6, 5.2
Dishwashing	73% (11/15)	3.2 (1.5)	3.6	0, 5.1
Total	76% (34/45)	3.1 (1.2)	3.2	0, 5.2

Note: For reporting purposes all non-detects (ND) were imputed to random value from zero to the 95% LOD for molecular assays and from zero to the LOD for the culture-based assay

89 Table S6. Control experiment results

Molecular Assay		
	beta-giardin	
	Positive partitions	Negative partitions
Extraction controls	3	86243
No template controls	0	49747
	ipaH	
	Positive partitions	Negative partitions
Extraction controls	0	87230
No template controls	0	67520
	ybbW	
	Positive partitions	Negative partitions
Extraction controls	0	97015
No template controls	0	38749

91 Table S7. Droplet data

Compound	Area	Target	Dilution	Template Volume (µL)	Accepted Droplets	Positives	Negatives	Lambda*
1	Dishwashing	*beta-giardin*	none	4	17416	9	17407	5.17E-04
2	Dishwashing	*beta-giardin*	none	4	14919	0	14919	0.00E+00
3	Dishwashing	*beta-giardin*	none	4	16167	30	16137	1.86E-03
4	Dishwashing	*beta-giardin*	none	4	12411	3	12408	2.42E-04
5	Dishwashing	*beta-giardin*	none	4	12733	49	12684	3.86E-03
6	Dishwashing	*beta-giardin*	none	4	11080	52	11028	4.70E-03
7	Dishwashing	*beta-giardin*	none	4	14094	28	14066	1.99E-03
8	Dishwashing	*beta-giardin*	none	4	13337	0	13337	0.00E+00
9	Dishwashing	*beta-giardin*	none	4	11607	2	11605	1.72E-04
10	Dishwashing	*beta-giardin*	none	4	11875	13	11862	1.10E-03
11	Dishwashing	*beta-giardin*	none	4	10176	25	10151	2.46E-03
12	Dishwashing	*beta-giardin*	none	4	12971	0	12971	0.00E+00
13	Dishwashing	*beta-giardin*	none	4	17337	27	17310	1.56E-03
14	Dishwashing	*beta-giardin*	none	4	17428	0	17428	0.00E+00
15	Dishwashing	*beta-giardin*	none	4	12292	0	12292	0.00E+00
16	Dishwashing	*beta-giardin*	none	4	13922	0	13922	0.00E+00
17	Dishwashing	*beta-giardin*	none	4	10605	29	10576	2.74E-03
18	Dishwashing	*beta-giardin*	none	4	13288	0	13288	0.00E+00

19	Dishwashing	*beta-giardin*	none	4	13512	0	13512	0.00E+00
20	Dishwashing	*beta-giardin*	none	4	11655	0	11655	0.00E+00
21	Dishwashing	*beta-giardin*	none	4	16205	38	16167	2.35E-03
22	Dishwashing	*beta-giardin*	none	4	12861	0	12861	0.00E+00
23	Dishwashing	*beta-giardin*	none	4	12143	28	12115	2.31E-03
24	Dishwashing	*beta-giardin*	none	4	13072	5	13067	3.83E-04
25	Dishwashing	*beta-giardin*	none	4	10141	0	10141	0.00E+00
26	Dishwashing	*beta-giardin*	none	4	12350	18	12332	1.46E-03
27	Dishwashing	*beta-giardin*	none	4	14429	2	14427	1.39E-04
28	Dishwashing	*beta-giardin*	none	4	11882	0	11882	0.00E+00
29	Dishwashing	*beta-giardin*	none	4	13086	0	13086	0.00E+00
30	Dishwashing	*beta-giardin*	none	4	12163	0	12163	0.00E+00
1	Latrine	*beta-giardin*	none	4	12305	0	12305	0.00E+00
2	Latrine	*beta-giardin*	none	4	14131	19	14112	1.35E-03
3	Latrine	*beta-giardin*	none	4	12724	3	12721	2.36E-04
4	Latrine	*beta-giardin*	none	4	11267	0	11267	0.00E+00
5	Latrine	*beta-giardin*	none	4	11204	15	11189	1.34E-03
6	Latrine	*beta-giardin*	none	4	11593	8	11585	6.90E-04
7	Latrine	*beta-giardin*	none	4	15711	2	15709	1.27E-04
8	Latrine	*beta-giardin*	none	4	13504	0	13504	0.00E+00
9	Latrine	*beta-giardin*	none	4	11617	0	11617	0.00E+00

10	Latrine	*beta-giardin*	none	4	12410	5	12405	4.03E-04
11	Latrine	*beta-giardin*	none	4	11951	6	11945	5.02E-04
12	Latrine	*beta-giardin*	none	4	12395	0	12395	0.00E+00
13	Latrine	*beta-giardin*	none	4	11238	8	11230	7.12E-04
14	Latrine	*beta-giardin*	none	4	12709	32	12677	2.52E-03
15	Latrine	*beta-giardin*	none	4	11906	1	11905	8.40E-05
16	Latrine	*beta-giardin*	none	4	13375	0	13375	0.00E+00
17	Latrine	*beta-giardin*	none	4	13259	0	13259	0.00E+00
18	Latrine	*beta-giardin*	none	4	12289	2	12287	1.63E-04
19	Latrine	*beta-giardin*	none	4	10313	0	10313	0.00E+00
20	Latrine	*beta-giardin*	none	4	11595	0	11595	0.00E+00
21	Latrine	*beta-giardin*	none	4	13687	4	13683	2.92E-04
22	Latrine	*beta-giardin*	none	4	12618	4	12614	3.17E-04
23	Latrine	*beta-giardin*	none	4	9317	0	9317	0.00E+00
24	Latrine	*beta-giardin*	none	4	14103	0	14103	0.00E+00
25	Latrine	*beta-giardin*	none	4	12976	33	12943	2.55E-03
26	Latrine	*beta-giardin*	none	4	13623	3	13620	2.20E-04
27	Latrine	*beta-giardin*	none	4	14291	1	14290	7.00E-05
28	Latrine	*beta-giardin*	none	4	9654	1	9653	1.04E-04
29	Latrine	*beta-giardin*	none	4	11393	16	11377	1.41E-03
30	Latrine	*beta-giardin*	none	4	9548	3	9545	3.14E-04

1	Solid waste	*beta-giardin*	none	4	16651	1	16650	6.01E-05
2	Solid waste	*beta-giardin*	none	4	12627	8	12619	6.34E-04
3	Solid waste	*beta-giardin*	none	4	15515	0	15515	0.00E+00
4	Solid waste	*beta-giardin*	none	4	16374	0	16374	0.00E+00
5	Solid waste	*beta-giardin*	none	4	10867	0	10867	0.00E+00
6	Solid waste	*beta-giardin*	none	4	15988	0	15988	0.00E+00
7	Solid waste	*beta-giardin*	none	4	15880	2	15878	1.26E-04
8	Solid waste	*beta-giardin*	none	4	12425	6	12419	4.83E-04
9	Solid waste	*beta-giardin*	none	4	16851	0	16851	0.00E+00
10	Solid waste	*beta-giardin*	none	4	11960	1	11959	8.36E-05
11	Solid waste	*beta-giardin*	none	4	12602	12	12590	9.53E-04
12	Solid waste	*beta-giardin*	none	4	13966	11	13955	7.88E-04
13	Solid waste	*beta-giardin*	none	4	14523	10	14513	6.89E-04
14	Solid waste	*beta-giardin*	none	4	14396	2	14394	1.39E-04
15	Solid waste	*beta-giardin*	none	4	15559	14	15545	9.00E-04
16	Solid waste	*beta-giardin*	none	4	13587	1	13586	7.36E-05
17	Solid waste	*beta-giardin*	none	4	13485	0	13485	0.00E+00
18	Solid waste	*beta-giardin*	none	4	13312	54	13258	4.06E-03
19	Solid waste	*beta-giardin*	none	4	9024	0	9024	0.00E+00
20	Solid waste	*beta-giardin*	none	4	13121	1	13120	7.62E-05
21	Solid waste	*beta-giardin*	none	4	8818	0	8818	0.00E+00

22	Solid waste	*beta-giardin*	none	4	13890	0	13890	0.00E+00
23	Solid waste	*beta-giardin*	none	4	16237	0	16237	0.00E+00
24	Solid waste	*beta-giardin*	none	4	15245	0	15245	0.00E+00
25	Solid waste	*beta-giardin*	none	4	13517	0	13517	0.00E+00
26	Solid waste	*beta-giardin*	none	4	16424	0	16424	0.00E+00
27	Solid waste	*beta-giardin*	none	4	14232	13	14219	9.14E-04
28	Solid waste	*beta-giardin*	none	4	12493	0	12493	0.00E+00
29	Solid waste	*beta-giardin*	none	4	12582	29	12553	2.31E-03
30	Solid waste	*beta-giardin*	none	4	10231	0	10231	0.00E+00
1	Dishwashing	*ipaH*	none	4	10205	19	10186	1.86E-03
2	Dishwashing	*ipaH*	none	4	12649	19	12630	1.50E-03
3	Dishwashing	*ipaH*	none	4	15098	7	15091	4.64E-04
4	Dishwashing	*ipaH*	none	4	16470	0	16470	0.00E+00
5	Dishwashing	*ipaH*	none	4	17697	1	17696	5.65E-05
6	Dishwashing	*ipaH*	none	4	18042	39	18003	2.16E-03
7	Dishwashing	*ipaH*	none	4	14503	10	14493	6.90E-04
8	Dishwashing	*ipaH*	none	4	15766	3	15763	1.90E-04
9	Dishwashing	*ipaH*	none	4	14804	141	14663	9.57E-03
10	Dishwashing	*ipaH*	none	4	15758	2	15756	1.27E-04
11	Dishwashing	*ipaH*	none	4	15316	2	15314	1.31E-04
12	Dishwashing	*ipaH*	none	4	16136	1	16135	6.20E-05

13	Dishwashing	*ipaH*	none	4	13876	51	13825	3.68E-03
14	Dishwashing	*ipaH*	none	4	10317	0	10317	0.00E+00
15	Dishwashing	*ipaH*	none	4	13616	3	13613	2.20E-04
16	Dishwashing	*ipaH*	none	4	15605	5	15600	3.20E-04
17	Dishwashing	*ipaH*	none	4	16352	0	16352	0.00E+00
18	Dishwashing	*ipaH*	none	4	16051	89	15962	5.56E-03
19	Dishwashing	*ipaH*	none	4	9661	7	9654	7.25E-04
20	Dishwashing	*ipaH*	none	4	16580	1	16579	6.03E-05
21	Dishwashing	*ipaH*	none	4	15564	19	15545	1.22E-03
22	Dishwashing	*ipaH*	none	4	13099	0	13099	0.00E+00
23	Dishwashing	*ipaH*	none	4	11944	2	11942	1.67E-04
24	Dishwashing	*ipaH*	none	4	15370	1	15369	6.51E-05
25	Dishwashing	*ipaH*	none	4	14452	1	14451	6.92E-05
26	Dishwashing	*ipaH*	none	4	15727	0	15727	0.00E+00
27	Dishwashing	*ipaH*	none	4	15077	0	15077	0.00E+00
28	Dishwashing	*ipaH*	none	4	16857	0	16857	0.00E+00
29	Dishwashing	*ipaH*	none	4	10041	19	10022	1.89E-03
30	Dishwashing	*ipaH*	none	4	14182	0	14182	0.00E+00
1	Latrine	*ipaH*	none	4	12365	3	12362	2.43E-04
2	Latrine	*ipaH*	none	4	10049	0	10049	0.00E+00
3	Latrine	*ipaH*	none	4	10140	21	10119	2.07E-03

4	Latrine	*ipaH*	none	4	16987	0	16987	0.00E+00
5	Latrine	*ipaH*	none	4	12900	8	12892	6.20E-04
6	Latrine	*ipaH*	none	4	15469	0	15469	0.00E+00
7	Latrine	*ipaH*	none	4	10468	3	10465	2.87E-04
8	Latrine	*ipaH*	none	4	17184	2	17182	1.16E-04
9	Latrine	*ipaH*	none	4	17750	0	17750	0.00E+00
10	Latrine	*ipaH*	none	4	15320	2	15318	1.31E-04
11	Latrine	*ipaH*	none	4	3242	34	3208	1.05E-02
12	Latrine	*ipaH*	none	4	17409	2	17407	1.15E-04
13	Latrine	*ipaH*	none	4	11313	15	11298	1.33E-03
14	Latrine	*ipaH*	none	4	14775	1	14774	6.77E-05
15	Latrine	*ipaH*	none	4	18085	6	18079	3.32E-04
16	Latrine	*ipaH*	none	4	8962	0	8962	0.00E+00
17	Latrine	*ipaH*	none	4	16257	0	16257	0.00E+00
18	Latrine	*ipaH*	none	4	16527	3	16524	1.82E-04
19	Latrine	*ipaH*	none	4	14823	151	14672	1.02E-02
20	Latrine	*ipaH*	none	4	17267	2039	15228	1.26E-01
21	Latrine	*ipaH*	none	4	10459	1	10458	9.56E-05
22	Latrine	*ipaH*	none	4	10450	30	10420	2.87E-03
23	Latrine	*ipaH*	none	4	14492	39	14453	2.69E-03
24	Latrine	*ipaH*	none	4	14906	2	14904	1.34E-04

25	Latrine	*ipaH*	none	4	13861	14	13847	1.01E-03
26	Latrine	*ipaH*	none	4	13966	122	13844	8.77E-03
27	Latrine	*ipaH*	none	4	18856	0	18856	0.00E+00
28	Latrine	*ipaH*	none	4	7697	59	7638	7.69E-03
29	Latrine	*ipaH*	none	4	17512	5	17507	2.86E-04
30	Latrine	*ipaH*	none	4	17429	3	17426	1.72E-04
1	Solid waste	*ipaH*	none	4	11762	0	11762	0.00E+00
2	Solid waste	*ipaH*	none	4	13879	2	13877	1.44E-04
3	Solid waste	*ipaH*	none	4	16628	0	16628	0.00E+00
4	Solid waste	*ipaH*	none	4	16650	6	16644	3.60E-04
5	Solid waste	*ipaH*	none	4	15766	0	15766	0.00E+00
6	Solid waste	*ipaH*	none	4	15099	0	15099	0.00E+00
7	Solid waste	*ipaH*	none	4	12466	0	12466	0.00E+00
8	Solid waste	*ipaH*	none	4	11694	1	11693	8.55E-05
9	Solid waste	*ipaH*	none	4	15093	31	15062	2.06E-03
10	Solid waste	*ipaH*	none	4	12844	15	12829	1.17E-03
11	Solid waste	*ipaH*	none	4	15677	9	15668	5.74E-04
12	Solid waste	*ipaH*	none	4	17178	19	17159	1.11E-03
13	Solid waste	*ipaH*	none	4	10389	0	10389	0.00E+00
14	Solid waste	*ipaH*	none	4	16955	0	16955	0.00E+00
15	Solid waste	*ipaH*	none	4	16393	2	16391	1.22E-04

16	Solid waste	*ipaH*	none	4	9289	0	9289	0.00E+00
17	Solid waste	*ipaH*	none	4	17644	0	17644	0.00E+00
18	Solid waste	*ipaH*	none	4	15486	0	15486	0.00E+00
19	Solid waste	*ipaH*	none	4	15492	1	15491	6.46E-05
20	Solid waste	*ipaH*	none	4	6523	1	6522	1.53E-04
21	Solid waste	*ipaH*	none	4	17474	0	17474	0.00E+00
22	Solid waste	*ipaH*	none	4	16871	1	16870	5.93E-05
23	Solid waste	*ipaH*	none	4	15375	72	15303	4.69E-03
24	Solid waste	*ipaH*	none	4	18325	0	18325	0.00E+00
25	Solid waste	*ipaH*	none	4	15194	6	15188	3.95E-04
26	Solid waste	*ipaH*	none	4	19094	15	19079	7.86E-04
27	Solid waste	*ipaH*	none	4	13292	0	13292	0.00E+00
28	Solid waste	*ipaH*	none	4	17125	2	17123	1.17E-04
29	Solid waste	*ipaH*	none	4	10070	0	10070	0.00E+00
30	Solid waste	*ipaH*	none	4	16432	17	16415	1.04E-03
1	Dishwashing	*ybbW*	1:100	1	15536	88	15448	5.68E-03
2	Dishwashing	*ybbW*	1:100	1	16221	130	16091	8.05E-03
3	Dishwashing	*ybbW*	1:100	1	16270	99	16171	6.10E-03
4	Dishwashing	*ybbW*	1:100	1	17060	114	16946	6.70E-03
5	Dishwashing	*ybbW*	1:100	1	17640	481	17159	2.76E-02
6	Dishwashing	*ybbW*	1:100	1	15951	199	15752	1.26E-02

7	Dishwashing	*ybbW*	1:100	1	16918	116	16802	6.88E-03
8	Dishwashing	*ybbW*	1:100	1	15048	67	14981	4.46E-03
9	Dishwashing	*ybbW*	1:100	1	17798	169	17629	9.54E-03
10	Dishwashing	*ybbW*	1:100	1	17522	79	17443	4.52E-03
11	Dishwashing	*ybbW*	1:100	1	18942	86	18856	4.55E-03
12	Dishwashing	*ybbW*	1:100	1	18870	70	18800	3.72E-03
13	Dishwashing	*ybbW*	1:100	1	17086	67	17019	3.93E-03
14	Dishwashing	*ybbW*	1:100	1	15947	118	15829	7.43E-03
15	Dishwashing	*ybbW*	1:100	1	16103	68	16035	4.23E-03
16	Dishwashing	*ybbW*	1:100	1	17319	171	17148	9.92E-03
17	Dishwashing	*ybbW*	1:100	1	16124	134	15990	8.35E-03
18	Dishwashing	*ybbW*	1:100	1	17447	145	17302	8.35E-03
19	Dishwashing	*ybbW*	1:100	1	12442	18	12424	1.45E-03
20	Dishwashing	*ybbW*	1:100	1	15881	188	15693	1.19E-02
21	Dishwashing	*ybbW*	1:100	1	13833	42	13791	3.04E-03
22	Dishwashing	*ybbW*	1:100	1	16239	177	16062	1.10E-02
23	Dishwashing	*ybbW*	1:100	1	15386	74	15312	4.82E-03
24	Dishwashing	*ybbW*	1:100	1	16769	154	16615	9.23E-03
25	Dishwashing	*ybbW*	1:100	1	9864	88	9776	8.96E-03
26	Dishwashing	*ybbW*	1:100	1	17611	98	17513	5.58E-03
27	Dishwashing	*ybbW*	1:100	1	16073	54	16019	3.37E-03

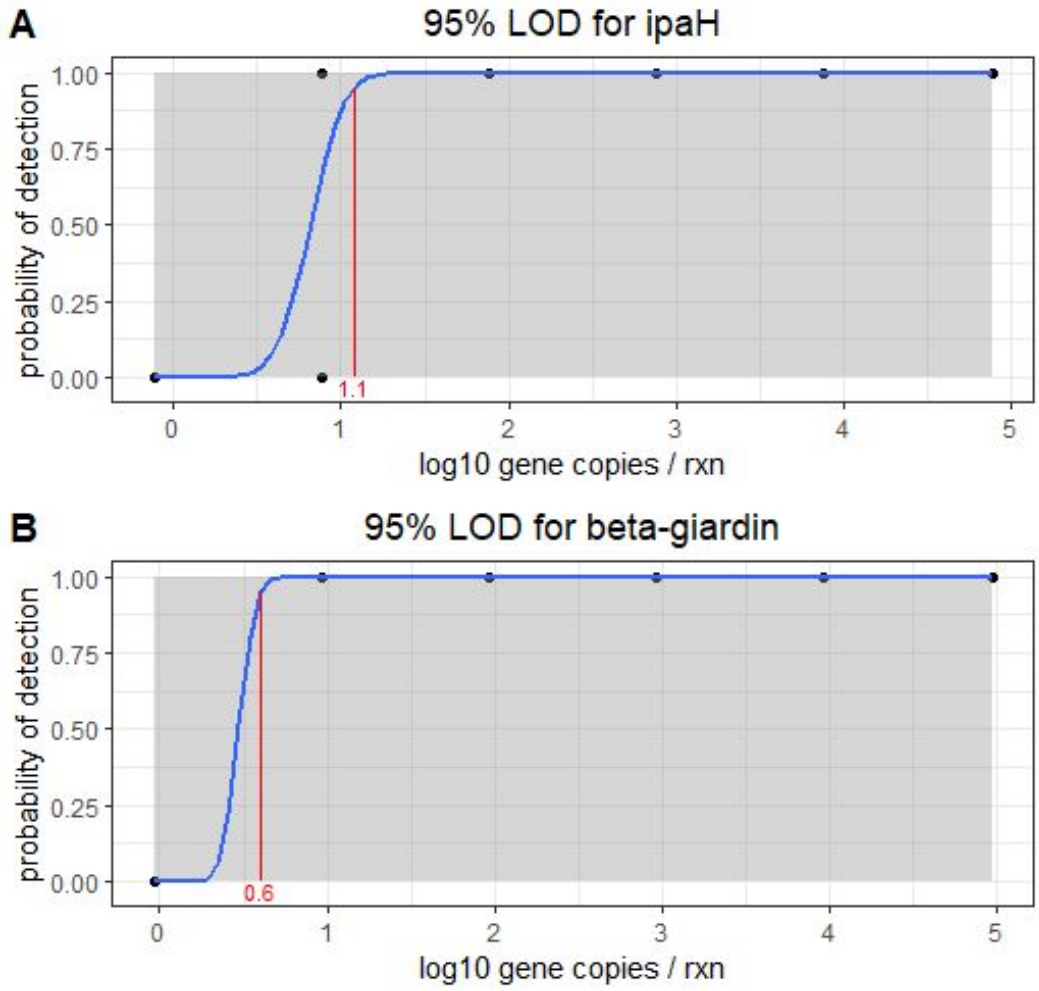
28	Dishwashing	*ybbW*	1:100	1	5526	7	5519	1.27E-03
29	Dishwashing	*ybbW*	1:100	1	16185	54	16131	3.34E-03
30	Dishwashing	*ybbW*	1:100	1	18489	83	18406	4.50E-03
1	Latrine	*ybbW*	None	1	14037	2692	11345	2.13E-01
2	Latrine	*ybbW*	1:100	2	13096	71	13025	5.44E-03
3	Latrine	*ybbW*	1:100	2	12363	112	12251	9.10E-03
4	Latrine	*ybbW*	None	1	15427	1437	13990	9.78E-02
5	Latrine	*ybbW*	None	1	16347	3097	13250	2.10E-01
6	Latrine	*ybbW*	1:100	2	11594	569	11025	5.03E-02
7	Latrine	*ybbW*	None	1	12699	2815	9884	2.51E-01
8	Latrine	*ybbW*	None	1	14116	3290	10826	2.65E-01
9	Latrine	*ybbW*	None	1	13419	1263	12156	9.88E-02
10	Latrine	*ybbW*	1:100	1	16851	59	16792	3.51E-03
11	Latrine	*ybbW*	1:100	1	18061	154	17907	8.56E-03
12	Latrine	*ybbW*	1:100	1	16509	49	16460	2.97E-03
13	Latrine	*ybbW*	1:100	2	12630	30	12600	2.38E-03
14	Latrine	*ybbW*	None	1	14486	1924	12562	1.43E-01
15	Latrine	*ybbW*	None	1	14480	2796	11684	2.15E-01
16	Latrine	*ybbW*	None	1	13850	3703	10147	3.11E-01
17	Latrine	*ybbW*	1:100	2	12062	138	11924	1.15E-02
18	Latrine	*ybbW*	None	1	14935	3993	10942	3.11E-01

19	Latrine	*ybbW*	1:100	1	14485	100	14385	6.93E-03
20	Latrine	*ybbW*	None	1	15263	4550	10713	3.54E-01
21	Latrine	*ybbW*	None	1	11732	3076	8656	3.04E-01
22	Latrine	*ybbW*	None	1	12712	3654	9058	3.39E-01
23	Latrine	*ybbW*	None	1	15530	3389	12141	2.46E-01
24	Latrine	*ybbW*	1:100	1	16405	194	16211	1.19E-02
25	Latrine	*ybbW*	1:100	2	12921	158	12763	1.23E-02
26	Latrine	*ybbW*	None	1	17215	2780	14435	1.76E-01
27	Latrine	*ybbW*	None	1	15052	1902	13150	1.35E-01
28	Latrine	*ybbW*	None	1	15245	3402	11843	2.53E-01
29	Latrine	*ybbW*	1:100	2	11530	23	11507	2.00E-03
30	Latrine	*ybbW*	None	1	13801	1662	12139	1.28E-01
1	Solid waste	*ybbW*	1:100	1	13466	43	13423	3.20E-03
2	Solid waste	*ybbW*	1:100	1	14498	47	14451	3.25E-03
3	Solid waste	*ybbW*	1:100	1	16257	13	16244	8.00E-04
4	Solid waste	*ybbW*	1:100	2	11285	35	11250	3.11E-03
5	Solid waste	*ybbW*	None	1	16297	576	15721	3.60E-02
6	Solid waste	*ybbW*	1:100	2	13463	88	13375	6.56E-03
7	Solid waste	*ybbW*	1:100	1	12201	28	12173	2.30E-03
8	Solid waste	*ybbW*	1:100	1	13746	79	13667	5.76E-03
9	Solid waste	*ybbW*	None	1	15955	1548	14407	1.02E-01

10	Solid waste	*ybbW*	1:100	2	12326	71	12255	5.78E-03
11	Solid waste	*ybbW*	1:100	2	13043	43	13000	3.30E-03
12	Solid waste	*ybbW*	1:100	2	14594	28	14566	1.92E-03
13	Solid waste	*ybbW*	1:100	1	14440	23	14417	1.59E-03
14	Solid waste	*ybbW*	1:100	1	15209	43	15166	2.83E-03
15	Solid waste	*ybbW*	None	1	18347	1882	16465	1.08E-01
16	Solid waste	*ybbW*	1:100	1	14662	128	14534	8.77E-03
17	Solid waste	*ybbW*	1:100	2	12336	100	12236	8.14E-03
18	Solid waste	*ybbW*	1:100	1	12289	38	12251	3.10E-03
19	Solid waste	*ybbW*	1:100	2	12039	69	11970	5.75E-03
20	Solid waste	*ybbW*	1:100	1	13776	88	13688	6.41E-03
21	Solid waste	*ybbW*	1:100	1	18726	71	18655	3.80E-03
22	Solid waste	*ybbW*	1:100	1	15772	145	15627	9.24E-03
23	Solid waste	*ybbW*	1:100	1	17458	119	17339	6.84E-03
24	Solid waste	*ybbW*	None	1	15208	599	14609	4.02E-02
25	Solid waste	*ybbW*	1:100	2	13891	44	13847	3.17E-03
26	Solid waste	*ybbW*	None	1	15531	1377	14154	9.28E-02
27	Solid waste	*ybbW*	1:100	2	13008	43	12965	3.31E-03
28	Solid waste	*ybbW*	1:100	2	12721	34	12687	2.68E-03
29	Solid waste	*ybbW*	1:100	1	13811	33	13778	2.39E-03
30	Solid waste	*ybbW*	None	1	11011	1515	9496	1.48E-01

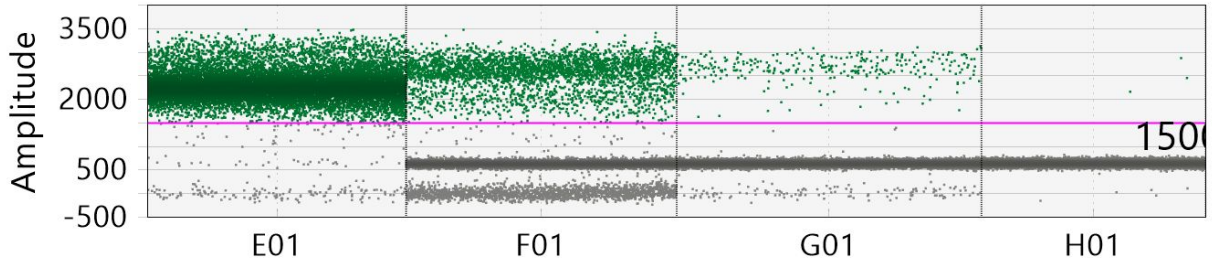
92 *Lambda = -ln(1-(k/n) where k is the positive number of droplets and n is the total number of droplets

Figure S2. 95% LOD determination



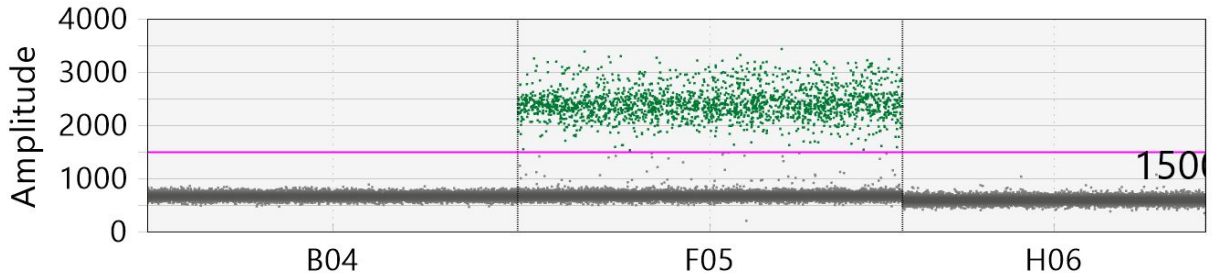
Determined following the method of Stokdyk *et al*. 2016

Figure S3. Example of manual thresholding



Example output of from manual thresholding in Quantasoft (Version 1.0.596; Bio-Rad, Hercules, CA) for the dilution series used to calculate the 95% LOD for the *ipaH* gene target. The amplitude on the y-axis represents the measured fluorescent signal of each dot, which represents each measured droplet in the ddPCR reaction. The vertical lines differentiate between the different wells. The pink horizontal line was draw by the user via manual thresholding. As such, the green dots above the pink line are classified as positive and the grey dots below are negative.

Figure S4. Example of ddPCR results



Well B04 is a no-template control. Well F05 is a sample that was positive for the *ipaH* gene target. Well H06 is a negative extraction control.

Figure S5. Photos of compounds in urban Maputo, Mozambique

A latrine entrance

A dishwashing area

A solid waste storage area

Text S3

Dose response harmonization and viability

Equation 1: For *Giardia duodenalis*

dose$_{Giardia}$ (cysts / day) = *beta-giardin* (gene copies/gram soil) × 1 cyst / 16 gene copies × (Viable$_{E.\ coli}$ / *ybbW*) × soil ingested (grams / day)

Where:
beta-giardin is the density of the *beta-giardin* gene (gene copies / gram soil) stochastic
Viable$_{E.\ coli}$ is the culturable *E. coli* count observed in soils (CFU / gram soil), stochastic
ybbW is the density of the *ybbW* gene in soils (gene copies / gram soil), stochastic
Soil ingested is the amount of soil ingested per day (grams / day), stochastic

Equation 2: For *Shigella spp.*

Dose$_{Shigella}$ (CFUs / day) = *ipaH* (gene copies/gram soil) × *ipaH*$_{\text{gene copies/genome}}$ × (Viable$_{E.\ coli}$ / *ybbW*) × soil ingested (grams / day)

Where:
ipaH is the density of the *ipaH* gene (gene copies / gram soil) stochastic
ipaH$_{\text{gene copies/genome}}$ is the *ipaH* gene copies per *Shigella* genome, stochastic
Viable$_{E.\ coli}$ is the culturable *E. coli* count observed in soils (CFU / gram soil), stochastic
ybbW is the density of the *ybbW* gene in soils (gene copies / gram soil), stochastic
Soil ingested is the amount of soil ingested per day (grams / day), stochastic

Dose response equations

Equation 3: For *Giardia duodenalis*

$$P_{inf} = 1 - \exp(-k * dose)$$

Where:

P_{inf} is the probability of infection
k is a parameter of the exponential model fit to dose response data (stochastic)
dose is the ingested dose of viable cysts (see equation 1), (stochastic)

Equation 4: For *Shigella spp.*

$$P_{inf} = 1 - [1 + dose \times (2^{1/\alpha} - 1)/N_{50}]^{-\alpha}$$

Where:

160 P_{inf} is the probability of infection

161 **alpha** is parameter of the approximate beta Poisson model, stochastic
162 $\mathbf{N_{50}}$ is the median infectious dose, stochastic
163 **dose** is the ingested dose of viable *Shigella* CFUs (see equation 2), stochastic

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167 Table S8. Estimated daily risk of infection

	Model output using soil ingestion estimates from US EPA Exposure Factors Handbook				
	Estimated daily risk of *Giardia duodenalis* infection (out of 10,000 people)				
	Percentile	**10th**	**50th**	**90th**	**MapSan point prevalence**
Age	<6 months	0.0016	0.087	4.4	13%
	6-11 months	0.0024	0.13	7.3	22%
	12-23 months	0.0038	0.21	10.5	59%
	24-71 months	0.0017	0.097	5.8	73%
	Estimated daily risk of *Shigella* spp. infection				
Age	<6 months	0.00075	0.037	2.0	5.0%
	6-11 months	0.0011	0.058	3.2	21%
	12-23 months	0.0019	0.090	4.9	36%
	24-71 months	0.00078	0.047	2.7	68%
	Model output using soil ingestion estimates from Kwong *et al*.				
	Estimated daily risk of *Giardia duodenalis* infection (out of 10,000 people)				
	Percentile	**10th**	**50th**	**90th**	**MapSan point prevalence**
Age	3-5 months	0.0078	0.44	23	13%
	6-11 months	0.011	0.60	32	22%
	12-23 months	0.012	0.63	33	59%
	24-35 month	0.0085	0.45	24	72%
	36-47 months	0.0082	0.46	24	75%
	Estimated daily risk of *Shigella* spp. infection				
Age	3-5 months	0.0036	0.19	10	5.0%
	6-11 months	0.0052	0.27	14	21%

	12-23 months	0.0054	0.28	15	36%
	24-35 month	0.0040	0.20	11	56%
	36-47 months	0.0041	0.21	11	73%
	Model output using geophagy estimates from Geissler *et al*.				
	Estimated daily risk of *Giardia duodenalis* infection (out of 10,000 people)				
	Percentile	10th	50th	90th	
	Geophagy	2.3	113	4500	
	Estimated daily risk of *Shigella* spp. infection				
	Geophagy	1.1	52	1600	

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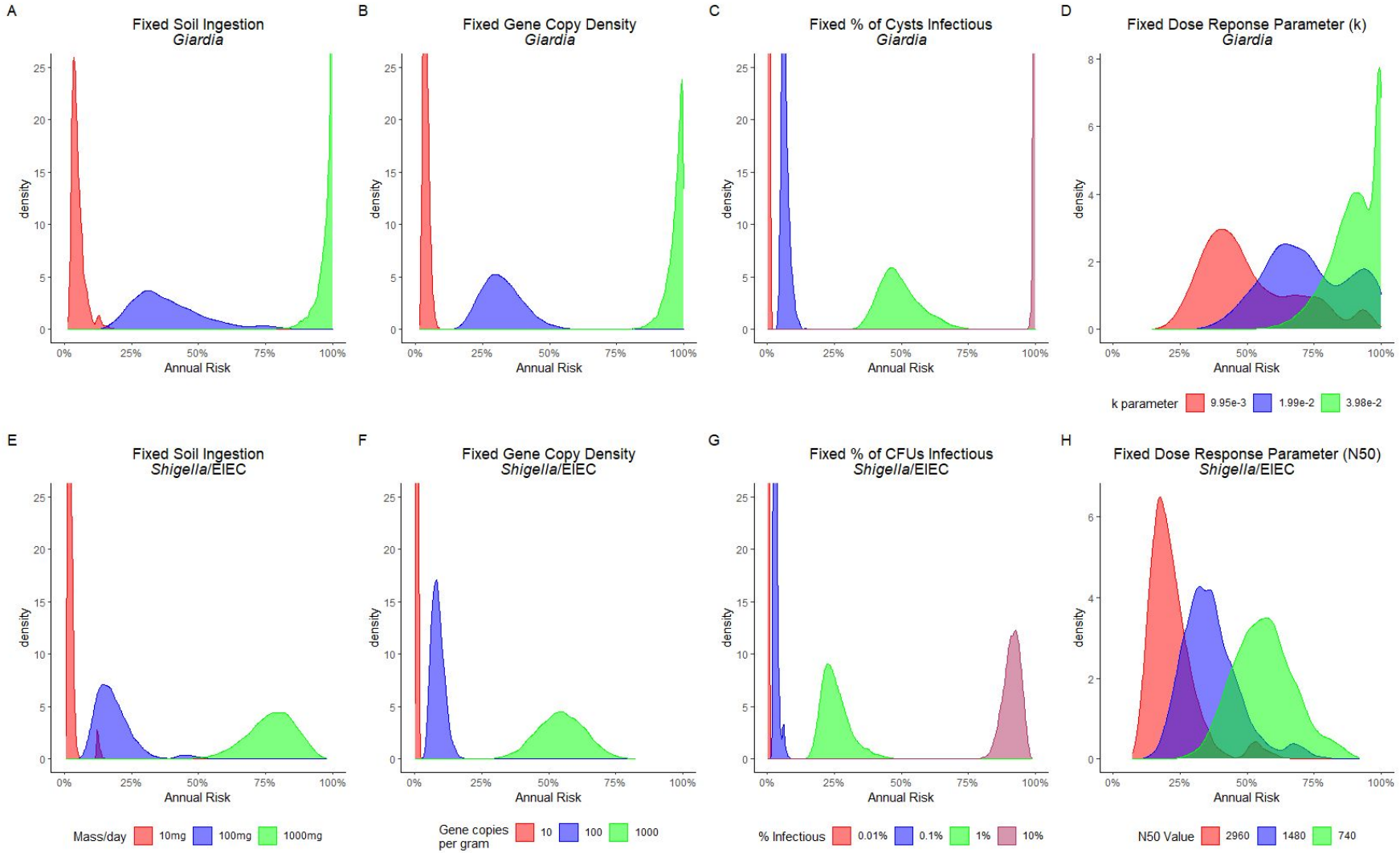
171 Figure S6. Sensitivity analysis graphs



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Results from sensitivity analysis that held individual parameters constant as part of the stochastic QMRA model. The stochastic parameters used are from Kwong *et al*. 2019 for children 12-23 months old

175 Table S9. Simulated dose reduction intervention

		Estimated annual risk out of 10,000		
	Dose reduction	10th percentile	50th percentile	90th percentile
Giardia duodenalis	None	5300	7100	9600
	1E-01	730	1200	2700
	1E-02	76	121	311
	1E-03	7.6	12	31
	1E-04	0.76	1.2	3.2
	1E-05	0.076	**0.12**	0.32
Shigella/EIEC	None	2800	4000	5560
	1E-01	330	540	1000
	1E-02	34	57	110
	1E-03	3.4	5.7	11
	1E-04	0.34	**0.57**	1.1
	1E-05	0.033	0.057	0.11

176 Note: bold indicates median risk ≤1 in 10,000 annual infection risk

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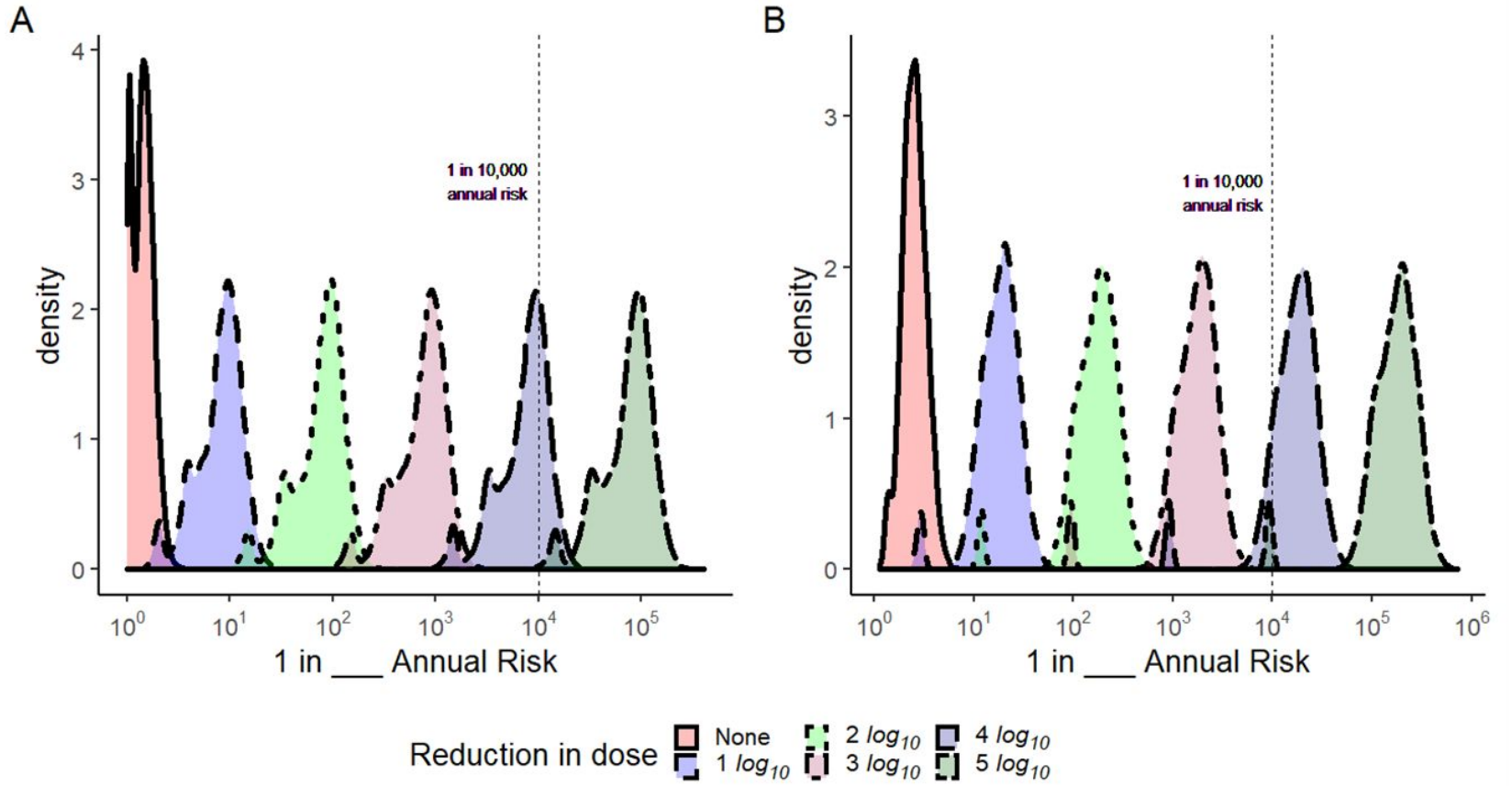
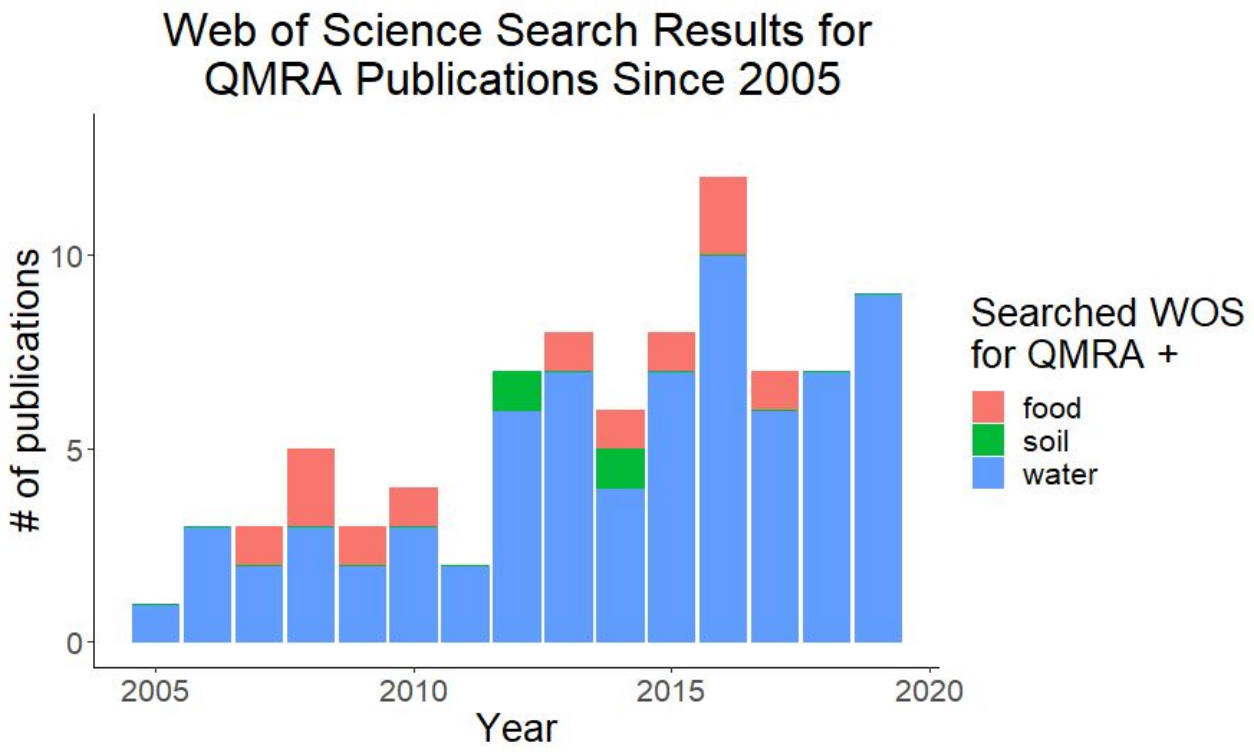
178 Figure S7. Simulated dose reduction intervention graphs



179

180 Simulated intervention by reducing the ingested dose parameter for Giardia duodenalis (7A) and *Shigella*/EIEC (7B). Stochastic
181 exposure parameters are from Kwong *et al*. 2019 for children 12-23 months old

Figure S8



Results from Web of Science search (https://www.webofknowledge.com/) for publications with titles containing the words “QMRA” OR “quantitative microbial risk assessment”, and either “water”, “soil”, or “food”

References

(1) Bivins, A.; Lowry, S.; Murphy, H. M.; Borchardt, M.; Coyte, R.; Labhasetwar, P.; Brown, J. Waterborne Pathogen Monitoring in Jaipur, India Reveals Potential Microbial Risks of Urban Groundwater Supply. *npj Clean Water* **2020**, *3* (1), 1–10.

(2) Basu, A. S. Digital Assays Part I: Partitioning Statistics and Digital PCR. *SLAS Technol. Transl. Life Sci. Innov.* **2017**, *22* (4), 369–386.