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Detection of *Escherichia coli*, rotavirus, and *Cryptosporidium* spp. from drinking water, kitchenware, and flies in a periurban community of Lusaka, Zambia

Yasuhiro Asada[a], Min Li Chua[b], Mayu Tsurumi[b], Taro Yamauchi[c,d], Imasiku Nyambe[e] and Hidenori Harada[d,f,*]

[a] Department of Environmental Health, National Institute of Public Health, Saitama, Japan
[b] Graduate School of Global Environmental Studies, Kyoto University, Kyoto, Japan
[c] Graduate School of Health Sciences, Hokkaido University, Sapporo, Japan
[d] Research Institute for Humanity and Nature, Kyoto, Japan
[e] Integrated Water Resources Management Centre, c/o School of Mines, University of Zambia, Lusaka, Zambia
[f] Graduate School of Asian and African Area Studies, Kyoto University, Kyoto, Japan
*Corresponding author. E-mail: harada.hidenori.8v@kyoto-u.ac.jp

HH, 0000-0002-7685-7751

ABSTRACT

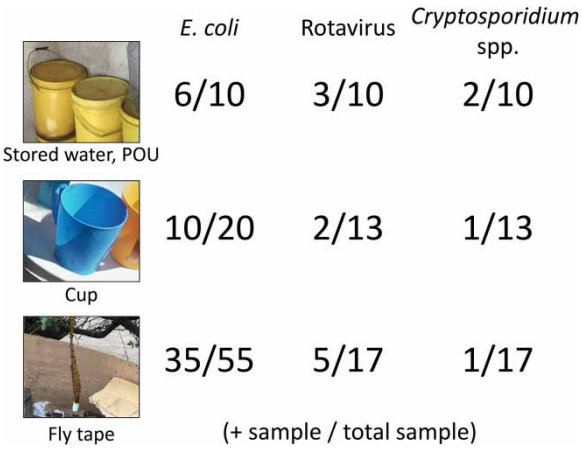
Fecal contamination with a poor water, sanitation and hygiene environment in urban informal settlements poses diarrhea risks. Little information is available on the contamination of environmental media with enteric pathogens in such settlements. We investigated the contamination of *Escherichia coli*, rotavirus, and *Cryptosporidium* spp. in water, on kitchenware, and on flies in urban informal settlements of Chawama and Kanyama, Lusaka, Zambia. These environmental media were examined by XM-G agar cultivation for *E. coli* and specific real-time RT-PCR assays to detect rotavirus and *Cryptosporidium* spp. *E. coli*; rotavirus, and *Cryptosporidium* spp. were detected in samples of household stored drinking water (6 of 10 samples, 3 of 10 samples, and 2 of 10 samples, respectively), cups (10 of 20 samples, 2 of 13 samples, 1 of 13 samples, respectively), and flies (35 of 55 samples, 5 of 17 samples, 1 of 17 samples, respectively). The ranges of rotavirus concentrations in household stored drinking water, on cups, and flies were 2.9×10^2–2.2×10^5 copies/L, 1.2×10^2–4.3×10^2 copies/cup, and 5.0×10^1–2.0×10^2 copies/fly, respectively. These results indicate the contribution of drinking water and kitchenware to enteric pathogen exposure and potential role of flies in microbial transmission.

Key words: *Cryptosporidium* spp., *E. coli*, Lusaka, rotavirus, urban informal settlements, WASH

HIGHLIGHTS

- The study was conducted in a periurban informal settlement of sub-Saharan Africa.
- Rotavirus and *Cryptosporidium* spp. were detected from point-of-use drinking water.
- These two pathogens were also detected from drinking cups and flies.
- People are exposed to these enteric pathogens from drinking water as well as cups.
- The results imply the role of flies as a vector of these enteric pathogens.

GRAPHICAL ABSTRACT



INTRODUCTION

Water, sanitation, and hygiene (WASH) are the basic needs for healthy living. However, many people are still living under inadequate WASH conditions, despite the global efforts under the Millennium Development Goals and Sustainable Development Goals. Poor WASH conditions are the major cause of diarrhea. The mortality rate due to diarrhea has been the highest in low- and middle-income countries, particularly in sub-Saharan Africa (UNICEF & WHO 2009). More than 100 million people in sub-Saharan Africa are estimated to be using unimproved sanitation and drinking water contaminated with feces (Bain *et al.* 2014; UNICEF & WHO 2019). These statistics have largely been contributed by the slow progress in WASH aspects in countries with high poverty and a dense population in urban informal settlements (UN-HABITAT 2016). The reduction of WASH-related health risks should be addressed, particularly in the urban informal settlements in sub-Saharan Africa.

By 2015, 60% of the informal settlements in sub-Saharan Africa only had access to unimproved sanitation, and were subjected to poorly managed environments (UN 2017). Urban informal settlements have been struggling with access to adequate WASH facilities. For example, in urban Uganda (Katukiza *et al.* 2014), 87.8 and 11.8% of the population at the study site used tap water and unprotected spring water for daily living, respectively. However, *Escherichia coli*, a major indicator bacterium of fecal contamination, was detected from tap water and unprotected spring water at the point-of-source (mean: 0.5 and 2.5 $\log_{10}$ CFU/100 mL, respectively). In another case study in urban Kenya (Bauza *et al.* 2019), *E. coli* was detected in 20% of stored water and in 65% of kitchenware used for dietary purposes.

Enteric pathogens are detected from stool samples owing to poor WASH conditions in sub-Saharan Africa. In sub-Saharan Africa, diarrheal deaths in children aged <5 years were mostly attributable to rotavirus and *Cryptosporidium* infection (Troeger *et al.* 2017). Rotavirus is the main cause of diarrhea among young children in sub-Saharan Africa. Investigations of rotavirus diarrhea associated with children aged <5 years are frequently conducted. Forty percent of stool samples from hospitalized patients with acute gastroenteritis among children aged <5 years in eight sub-Saharan countries were positive for rotavirus (Mwenda *et al.* 2010). Rotavirus infection in sub-Saharan Africa was responsible for a high rate (range: 38.9–45.5%) of diarrheal deaths in 2000–2013 (Tate *et al.* 2016). Thirteen percent of stool samples from children aged <5 years presenting to outpatient departments with diarrhea or a history of diarrhea were positive for *Cryptosporidium* species (Krumkamp *et al.* 2021). Detection rates of *Cryptosporidium* oocysts in stool samples from 19,033 patients at all ages and 2,762 patients in the 1- to 4-year age group in Zambia were 1.46 and 3.8%, respectively (Mulunda *et al.* 2020). Cryptosporidiosis is one of the AIDS-defining illnesses and people living with HIV infections in sub-Saharan Africa, responsible for 70% of the global burden of HIV infections (Kharsany & Karim 2016), are especially vulnerable to diarrhea due to cryptosporidiosis. Although the presence of rotavirus and *Cryptosporidium* spp. in stool samples has been investigated in many studies, little is known about the contamination of important exposure media, such as drinking water and kitchenware, in the sub-Saharan African context, although the quantitative data through transmission-exposure pathways of such enteric pathogens need to be essentially examined.

In addition to the contamination of the exposure media, flies may act as vectors in the transmission of fecal pathogens, as described in the F-diagram of Wagner & Lanoix (1958). After contacting feces, flies may carry pathogens to the surfaces of exposure media, such as food, and then the pathogens are ingested by a person (Wagner & Lanoix 1958). Previous studies on urban informal settlements have shown the effect of flies in transmitting fecal microorganisms.

For example, in urban Nigeria, Oyeyemi *et al.* (2016) revealed that flies were commonly found in indoor and outdoor environments, and were suggested to be important agents of pathogen transmission to humans at the study site. In urban Bangladesh, Lindeberg *et al.* (2018) reported that the risk of rice contamination by *E. coli* was significantly higher when there were flies landing on the rice (odds ratio (OR): 5.4; 95% confidence interval (CI): 2.5–11.7; $p < 0.001$), and diarrheagenic *E. coli* and *Shigella* species were detected on the surface of the contaminated rice. Thus, flies are potential vectors that transmit enteric pathogens to exposure media in the kitchen. Although enteric pathogen transmission from feces to flies has been studied in laboratory-scale experiments (Tan *et al.* 1997; Graczyk *et al.* 1999, 2003), little is known about the contamination of wild flies with enteric pathogens in the field.

In the present study, we aimed to investigate enteric pathogen contamination in the living environment, with a focus on exposure media in kitchens, in a periurban informal settlement of sub-Saharan Africa. Together with *E. coli*, the contamination levels of rotavirus and *Cryptosporidium* spp. in household stored drinking water and cups in kitchens, as exposure media, were investigated in a periurban community (Chawama and Kanyama) of Lusaka, the capital city of Zambia, with large informal settlements in periurban areas having limited access to WASH. As a potential vector of rotavirus and *Cryptosporidium* spp., we also investigated the contamination of these enteric pathogens from wild flies caught in the community. Rotavirus was quantified and *Cryptosporidium* spp., which are assumed to have low contamination in the living environment, were detected by RT-qPCR. To the best of our knowledge, this is the first study to quantify rotavirus in flies and on kitchenware and to detect *Cryptosporidium* spp. contamination on the kitchenware.

MATERIALS AND METHODS

Study area

The study was conducted in Lusaka City, Zambia, with a population of approximately 2.5 million people in 2018. The focus of the study was on two densely populated urban slums called the Chawama (440 ha; 188,000 inhabitants; 40,000 households) and Kanyama compounds (3,000 ha; 360,000 inhabitants; 76,000 households) (Central Statistics Office 2012).

According to a study conducted on the same compounds (Nyambe *et al.* 2020), the community had limited access to improved water sources and sanitation facilities. Among the residents, 84.9% had access to improved drinking water sources, such as public taps and boreholes with hand pumps, whereas 15.1% used an unimproved water source, open dug wells with or without covers, linings and slabs as an alternative. The majority (80.5%) used toilets shared with neighbors where pit latrines with or without solid wall/roofing were commonly found. However, signs of open defecation were still observable, as human feces were scattered on open grounds. Stored water collected from public stand taps shared with several households and located outside was kept in plastic pails in kitchens for drinking and cooking. An open dug well is an alternative water source used for flushing toilets and cleaning. After washing and drying, kitchenware, such as cups and plates ready for use, were kept in kitchens located inside houses. At the study site, stagnant water was commonly found near water sources, such as taps, open dug wells, and outdoor bathing sites, where water could accumulate in potholes on the ground. Flies were also observed moving freely in kitchens, outside houses, near toilets and near the waste bag of each household where they dispose of daily waste outside the house.

This study was approved by the Research Institute for Humanity and Nature in Japan (ref: 2018-8) and the Excellence in Research Ethics and Science (ERES) Converge in Zambia (ref: 2019-Feb-009). All households were notified of the purpose and the survey process, and written consent was obtained prior to conducting the survey in each household.

Samples

All the samples were collected during 16 October–14 November 2019. Nine types of environmental media were targeted for the analysis of *E. coli* as a fecal indicator, rotavirus, and *Cryptosporidium* spp. as enteric pathogens. Water samples were obtained using sterilized sampling bags. Stored water was sampled from 20 L plastic pails in the kitchens of 10 households; they were originally drawn from taps by local people. Open dug well water was sampled from four wells using buckets that were regularly used by the residents. Some of the open dug well water was sampled repeatedly, and in total, seven samples

were collected from four wells. Stagnant water was sampled at 10 locations: near taps (6), open dug wells (3), and a bathing site (1). All water samples were used for the analyses of both *E. coli* and enteric pathogens.

From 16 kitchens, 33 cups ready for use were collected: 20 for *E. coli* analysis and 13 for enteric pathogen analysis. The inner surfaces of the cups were carefully swabbed on the site using swab test kits (Pro-media ST25 PBS, ELMEX, Japan); swabs were placed inside a plastic tube containing phosphate buffer solution (PBS) for the following laboratory analysis.

Fly tapes were hung at five locations for each of the seven households to catch flies on the surface of the sticky tape: inside the toilet (7), outside toilet (7), inside kitchen (7), outside house entrance (7), and around waste bag (7). After 1 h of hanging, the tapes were kept in sterilized plastic bags for the following laboratory analysis. After collection, all samples were transported on ice to the laboratory within 4 h. In the laboratory, a maximum of five flies were collected from each tape using sterilized forceps; each fly was transferred into a 50-mL Falcon tube and vortexed to rinse the surface of flies with 40 mL of phosphate-buffered saline. These solutions were used as fly samples for subsequent analyses.

Analysis of *E. coli*

Each of the samples was agitated rigorously. Of stored tap water samples, 100 mL was filtrated to test by the culture method for detection of *E. coli* following the WHO guideline for drinking-water quality (World Health Organization 2017). Other types of water samples were filtered in the following different volumes: 10 mL of 100 mL open dug well water and 1 mL of 100 mL stagnant water. For cup swab samples, 4 mL of 10 mL PBS solution in the swab was filtered. For fly samples, 10 of 40 mL of PBS solution was filtered. The tested volume of water and PBS solution was decided based on a preliminary survey to have appropriate concentration for the following test. The test for *E. coli* was conducted via membrane filtration (0.45 μm pore size, EMD Millipore Microfil V Filtration Device, Fisher Scientific, USA) and agar cultivation (XM-G agar, Nissui, Japan) with incubation at 37 °C for 22 h. Colonies of *E. coli* appeared blue on the filter. As the maximum reliable count of *E. coli* on the filter was 300, the upper detection limits for water samples were 300 CFU/100 mL for stored tap water, 3,000 CFU/100 mL (equivalent to 300 CFU/10 mL) for open dug well water, and 30,000 CFU/100 mL for stagnant water (300 CFU/mL). For the cup swab samples, the upper detection limit was 750 CFU/cup (300 CFU/4 ml for 10 mL). For the fly samples, the upper detection limit was 1,200 CFU/fly (300 CFU/10 mL for 40 mL).

Analysis of rotavirus and *Cryptosporidium* spp.

Pretreatment to concentrate rotavirus was conducted as described by Katayama *et al.* (2002). For water samples, $MgCl_2$ was added and mixed with 100 mL of water at a concentration of 25 mM. For fly samples, each individual fly was collected from fly tapes using sterilized forceps, mixed with 40 mL of PBS in a 50-mL Falcon tube, and $MgCl_2$ was added at a concentration of 25 mM. These solutions were filtered through a mixed cellulose ester membrane (Millipore, HA mixed cellulose, pore size of 0.45 μm and diameter of 47 mm). The membranes were frozen until the analysis. For swab samples, the tubes containing swab and solution were kept frozen and used for the following analysis.

Viral and protozoan RNA were extracted from the membranes and swab samples using the NucliSENS kit (bioMérieux, Lyon, France) according to Miura *et al.* (2019). A murine norovirus (MNV) was used as a process control virus to analyze rotaviruses. Briefly, MNV was added to the membranes and swab samples in a 50-mL tube, and the tube was incubated for 5 min at −80 °C and for 2 min at 56 °C, four times. After the incubation, 15 mL of lysis buffer was added to the tube. The tube was vortexed for 15 s and incubated at 56 °C for 30 min. The tube was centrifuged at 2,300 × *g* for 5 min. The supernatant was collected in a 15-mL tube, and 140 μL of magnetic silica particles was added to the supernatant. All washing steps for the supernatant were performed using the NucliSENS miniMAG (bioMérieux, Lyon, France) according to the manufacturer's instructions, and the extracted RNA was collected in 100 μL of elution buffer.

For rotavirus, a LightCycler® 480 System II (Roche) was used. The viral RNA was amplified using an RNA Ultrasense™ One-Step Quantitative RT-PCR System (Thermo Fisher Scientific, Japan) with previously reported primers, probes, and PCR conditions for MNV and rotavirus A (Miura *et al.* 2019). For *Cryptosporidium* spp., RT-PCR was performed on a Veriti Thermal Cycler (Applied Biosystems), and real-time PCR was performed on a LightCycler® 480 System II. Protozoan RNA was amplified using a Cycleave RT-PCR *Cryptosporidium* (18S rRNA) (Takara, Japan) according to the manufacturer's instructions. Mulunda *et al.* (2020) identified four species (*C. hominis*, *C. parvum*, *C. felis*, and *C. meleagride*) of *Cryptosporidium* from stool samples of patients in Lusaka, Zambia. This kit can detect 10 species of *Cryptosporidium* spp., including these four species.

Undiluted and 10-fold diluted RNA extracts were tested to verify inhibition in real-time RT-PCR with TaqMan probe detection in each sample. The number of genome copies in each sample was calculated from the cycle threshold (C_T) values less than 40 and a standard curve generated with serial dilutions of plasmid DNA containing each target region (Miura *et al.* 2019). Standard curves with amplification efficiencies of 85–110% were used for quantification (Miura *et al.* 2019). When the MNV recovery rate of the sample was above 1%, the virus concentration in the sample was calculated based on the volume of RNA extracts and samples (Miura *et al.* 2019). Non-specific amplification was checked by 1.5% agarose gel electrophoresis.

RESULTS

Occurrence of *E. coli* in the living environment

A summary of *E. coli* concentrations on nine types of samples is presented in Tables 1–3. *E. coli* was detected in 68 of 102 samples. Among the water samples (Table 1), *E. coli* was detected in all 10 samples of stagnant water on the ground at high levels (>30,000 CFU/100 mL). Although the samples of open dug well water were directly collected from the wells, *E. coli* was detected in all seven samples, among which the concentration in five was >3,000 CFU/100 mL. The results for *E. coli* concentration in stagnant water indicated heavy fecal contamination of the living environment, potentially causing fecal contamination of open dug well water.

For stored tap water samples, which were used for direct drinking, *E. coli* was detected in six of 10 samples. In addition, the concentration in three samples was >300 CFU/100 mL, indicating fecal contamination of drinking water tested. In addition to stored tap water, *E. coli* was detected on half of the 20 cups (Table 2); these cups were used for drinking or collecting stored tap water from plastic pails. Out of 20 cups, the concentration on seven cups was >750 CFU/cup.

For fly samples (Table 3), the number of flies caught and the *E.* coli concentrations in them varied among the five locations. The average number of flies caught per tape for an hour was 0.9 fly inside the toilet space, 3.1 flies outside the toilet space, 4.3 flies in the kitchens, 4.9 flies at the house entrance, 9.7 flies near waste bags. The proportion of *E. coli*-positive flies inside the kitchen was the lowest (40.0%; 6 of 15 flies), although the average number of flies in the kitchen was higher than that of flies inside and outside the toilet space. Nevertheless, two flies from the kitchen had *E. coli* concentrations of >1,200 CFU/fly. All

Table 1 | Summary of *Escherichia coli* concentration in water samples (CFU/100 mL), from Chawama and Kanyama, Lusaka, Zambia with the numbers showing the sample number (percentage) under each category

				***E. coli* concentration (CFU/100 mL)**					
	Sample number (*n*)	**Positive sample number**	**N.D.**	**$0 < x \leq 10$**	**$10 < x \leq 100$**	**$100 < x \leq 300$**	**$300 < x \leq 3{,}000$**	**$3{,}000 < x \leq 30{,}000$**	**$30{,}000 < x$**
Stored water, POU	10	6 (60.0)	4 (40.0)	2 (20.0)	1 (10.0)	0 (0.0)	3[a] (30.0)	–	–
Open dug well, POS	7	7 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (14.3)	1 (14.3)	5[a] (71.4)	–
Stagnant water	10	10 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	10[a] (100.0)

POU, point-of-use; POS, point-of-source; N.D., not detected.

[a]The lower end of the range is the maximum detection limit of the sample, and the number indicates the sample number with concentration above the higher detection limit.

Table 2 | Summary of *Escherichia coli* concentration on cup samples (CFU/cup), from Chawama and Kanyama, Lusaka, Zambia with the numbers showing the sample number (percentage) under each category

				***E. coli* concentration (CFU/cup)**			
	Sample number (*n*)	**Positive sample number**	**N.D.**	**$0 < x \leq 10$**	**$10 < x \leq 100$**	**$100 < x \leq 750$**	**$750 < x$**
Cups	20	10 (50.0)	10 (50.0)	2 (10.0)	1 (5.0)	0 (0.0)	7[a] (35.0)

N.D., not detected.

[a]The lower end of the range is the maximum detection limit of the sample, and the number indicates the sample number with concentration above the higher detection limit.

Table 3 | Summary of *Escherichia coli* concentration in fly samples (CFU/fly), from Chawama and Kanyama, Lusaka, Zambia with the numbers showing the sample number (percentage) under each category

					***E. coli* concentration (CFU/fly)**				
	Average fly number caught	**Fly number tested**	**Positive fly number**	**N.D.**	**$0 < x \leq 10$**	**$10 < x \leq 100$**	**$100 < x \leq 1{,}000$**	**$1{,}000 < x \leq 1{,}200$**	**$1{,}200 < x$**
Fly inside toilet space	0.9	4	4 (100.0)	0 (0.0)	0 (0.0)	1 (25.0)	0 (0.0)	0 (0.0)	3[a] (75.0)
Fly outside toilet space	3.1	4	3 (75.0)	1 (25.0)	1 (25.0)	1 (25.0)	1 (25.0)	0 (0.0)	0 (0.0)
Fly in kitchen	4.3	15	6 (40.0)	9 (60.0)	3 (20.0)	1 (6.7)	0 (0.0)	0 (0.0)	2[a] (13.3)
Fly at house entrance	4.9	15	11 (73.3)	4 (26.7)	0 (0.0)	1 (6.7)	5 (33.3)	0 (0.0)	5[a] (33.3)
Fly near waste bag	9.7	17	11 (64.7)	6 (35.3)	2 (11.8)	1 (5.9)	1 (5.9)	0 (0.0)	7[a] (41.1)

N.D., not detected.

[a]The lower end of the range is the maximum detection limit of the sample, and the number indicates the sample number with concentration above the higher detection limit.

the four flies inside the toilet were positive for *E. coli*, with three having concentrations of >1,200 CFU/fly, although the average number of flies caught on a tape was the lowest (0.9 flies per tape). The average number of flies caught per tape near waste bags located outside was the highest in all five locations, with 11 of 17 flies being positive for *E.* coli, of which the concentration in seven flies was >1,200 CFU/fly.

Occurrence of rotavirus and *Cryptosporidium* spp. in the living environment

Amplification efficiencies of standard curves by real-time RT-PCR with TaqMan probe detection of rotavirus, MNV and *Cryptosporidium* spp. were 97, 94, and 86%, respectively (Supplementary Figure S1). Rotavirus and *Cryptosporidium* spp. were detected by real-time RT-PCR in eight and three of the nine sample types, respectively (Table 4). The genotypes of rotavirus and the species of *Cryptosporidium* spp. were not identified from amplification products by real-time RT-PCR. All detection samples gave C_T values less than 40 and recovery rates above 1% estimated from C_T values of RNA extracts of MNV. But inhibition was detected in three water samples and one cup sample, comparing C_T values of undiluted RNA extracts to those of 10-fold diluted RNA extracts. The concentrations of these samples were calculated from C_T values of 10-fold diluted RNA extracts. Also, specific amplification was detected from all detection samples (Supplementary Figure S2). A summary of the concentrations of rotavirus and *Cryptosporidium* spp. in water, flies, and cups is presented in Table 4. Of the 27 water samples, 7 and 2 were positive for rotavirus and *Cryptosporidium* spp., respectively, with a maximum concentration of 2.2×10^5 copies/L (stored water) and 3.6×10^4 copies/L (stored water), respectively. Rotavirus was detected from all of the three types of water: stagnant water near water sources, water from open dug wells, which were unimproved drinking water sources, and stored water from public taps and boreholes, which were improved water sources for drinking and cooking. In contrast, *Cryptosporidium* spp. was detected only from the stored water. Of the 13 cup samples, 2 and 1 were positive for rotavirus and *Cryptosporidium* spp., respectively. These exposure media were contaminated with rotavirus and *Cryptosporidium* spp.

Of the 17 fly samples, 5 and 1 were positive for rotavirus and *Cryptosporidium* spp., respectively, with the concentrations ranging from 5.0×10^1 to 2.0×10^2 copies/fly at four locations (inside toilet, outside toilet, in the kitchen, and on waste bag)

Table 4 | Contamination levels of rotavirus and *Cryptosporidium* spp. in water, on cups, and in flies in Chawama and Kanyama, Lusaka, Zambia

	Rotavirus		***Cryptosporidium* spp.**	
	Positive samples/total samples (positive rate: %)	**Concentration**	**Positive samples/total samples (positive rate: %)**	**Concentration**
Water				
Stored water, POU	3/10 (30.0)	2.9×10^2–2.2×10^5 (copies/L)	2/10 (20.0)	2.2×10^3–3.6×10^4 (copies/L)
Open dug well, POS	2/7 (28.6)	3.5×10^4–1.9×10^5 (copies/L)	0/7 (0.0)	–
Stagnant water	2/10 (20.0)	4.0×10^1–4.1×10^3 (copies/L)	0/10 (0.0)	–
Cup	2/13 (15.4)	1.2×10^2–4.3×10^2 (copies/cup)	1/13 (7.7)	1.3×10^2 (copies/cup)
Fly				
Fly inside toilet space	1/1 (100.0)	8.0×10^1 (copies/fly)	0/1 (0.0)	–
Fly outside toilet space	1/2 (50.0)	5.1×10^1 (copies/fly)	0/2 (0.0)	–
Fly in kitchen	1/5 (20.0)	5.0×10^1 (copies/fly)	0/5 (0.0)	–
Fly at house entrance	0/4 (0.0)	–	0/4 (0.0)	–
Fly near waste bag	2/5 (40.0)	5.2×10^1–2.0×10^2 (copies/fly)	1/5 (20.0)	8.5×10^2 (copies/fly)

POU, point-of-use; POS, point-of-source.

and a concentration of 8.5×10^2 copies/fly on the waste bag. Many flies were observed in the living environment of the targeted area. The flies might be contaminated with rotavirus and *Cryptosporidium* spp. in various parts of the living environment, where people had limited access to improved sanitation facilities.

DISCUSSION

Transmission routes of enteric pathogens include contaminated hands, drinking water, utensils, and flies (Brown *et al.* 2013). In this survey, we focused on stored drinking water and cups, as important exposure media in kitchens, and flies, as potential vectors of enteric pathogens.

E. coli was detected in most of the water samples, and the *E. coli* concentrations in some water samples were remarkably high. The presence of *E. coli* in various types of water, including stored tap water for drinking, well water and stagnant water, indicated that water might have been contaminated with human and/or animal feces. The contamination of uncovered open well water and stagnant water may be due to their exposure to the surrounding ground surface that was potentially contaminated with feces. As a common practice at the study site, plastic buckets and cups were used to collect open dug well water and stored tap water, respectively. Although we did not sample the surface of the plastic buckets used for collecting open dug well water, the cups for the stored tap water were contaminated (Table 2). This suggests potential cross-contamination between the point-of-source and point-of-use water sources, as revealed in some areas such as Bolivia (Rufener *et al.* 2010) and Bangladesh (Harada *et al.* 2018).

The number of flies varied at different locations and in kitchens, and they carried high *E. coli* concentrations that could potentially contaminate the living environment. Flies may carry feces from the source of feces in toilets and transmit them to the kitchen environment, where food and water are prepared, as observed in the flies in kitchens (Table 3). Similarly, previous studies showed that *E. coli* concentrations in stored food in the kitchen were positively associated with the presence of flies in urban Bangladesh (Lindeberg *et al.* 2018) and with *E. coli* concentrations in flies in urban Bangladesh (Doza *et al.* 2018). As *Cryptosporidium* spp. and rotavirus were detected in flies, our results suggest that flies may act as potential vectors of enteric pathogens in the kitchen. Furthermore, a previous study at the same study site found that certain households disposed of feces with solid wastes (Nyambe *et al.* 2018). This WASH practice may attract or breed more flies with contamination of enteric pathogens. WASH management at dumpsites could be important not only in toilets and kitchens, but also in controlling the transmission of enteric pathogens in the living environment.

Recent studies have investigated rotavirus in drinking water sources in rural areas of sub-Saharan Africa (Verheyen *et al.* 2009; Kiulia *et al.* 2010). Although the data are outdated, Kelly *et al.* (1997) investigated the presence of *C. parvum* in water sources using 100 L of water samples in Zambia. Oocysts of *C. parvum* were identified in five (Chipata supply, Chawama borehole, Misisi quarry, George zone (LWSC), and George JICA borehole) of six water sources in Zambia. However, there are no studies on the detection of these pathogens with regard to the use of drinking water. In the present study, rotavirus was detected in all three water types including stored drinking (tap) water in the kitchens (point-of-use); *Cryptosporidium* spp. was in stored drinking (tap) water in the kitchen (point-of-use). This point-of-use contamination indicates the exposure of rotavirus and *Cryptosporidium* spp. through contaminated drinking water.

Rotavirus and *Cryptosporidium* spp. were also detected on the surfaces of cups. These pathogens may be transferred from contaminated sources, such as human hands, water, or flies. For example, hands and flies contaminated with these pathogens frequently contact kitchenware surfaces and kitchenware is washed with contaminated water. Drying of cups with cloth is also one of the factors associated with *E. coli* contamination (Benwic *et al.* 2018). However, no information is available on the detection of rotavirus and *Cryptosporidium* spp. on cups. We showed the contamination of kitchenware with rotavirus and *Cryptosporidium* spp. The concentrations of rotavirus on the kitchenware are also shown. These findings suggest that people living in the target area are also exposed to enteric pathogens from the kitchenware.

Rotaviruses (Rzezutka & Cook 2004) and *Cryptosporidium* oocysts (Robertson *et al.* 1992) can survive for months in some types of water, such as tap water and river water. Rotaviruses can also survive and remain infectious on nonporous materials (glass, stainless steel, a smooth or rough plastic) (Sattar *et al.* 1986). In a previous study, *Cryptosporidium* oocysts were found to be viable in dry soils at 32 °C for 10 days (Fayer *et al.* 1998). *Cryptosporidium* oocysts have the potential to survive under high temperatures and dry conditions. Therefore, these enteric pathogens may maintain infectivity in stored drinking water and on kitchenware.

The 50% infection doses (ID_{50}s) for rotavirus and *Cryptosporidium* spp. are 6.17 focus-forming units (FFU) (approximately 9.6×10^4 particles) (Haas *et al.* 1999) and 12.1; 132; 2,066 oocysts (UCP, IOWA, TAMU strain) (Messner *et al.* 2001),

respectively. Detection of rotavirus and *Cryptosporidium* spp. by real-time RT-PCR in the present study do not necessarily indicate the existence of infectious viral particles of rotavirus and oocysts of *Cryptosporidium* spp. Considering the detection of rotavirus and *Cryptosporidium* spp. in stored drinking water and on kitchenware, we suggest that these exposure media may promote infection with these pathogens. The detection rate of rotavirus in stool samples from children in Zambia was the highest, being 67.6% (Chisenga *et al.* 2018). In addition, *Cryptosporidium* spp. was detected in 1.46% of 19,033 stool samples from patients, collected between 2017 and 2019, in Lusaka, Zambia (Mulunda *et al.* 2020). The findings of this study are supported by the detection rates 67.6% (rotavirus) (Chisenga *et al.* 2018), 1.46% (*Cryptosporidium* spp.) (Mulunda *et al.* 2020) of these enteric pathogens in stool samples from diarrheal patients in the same region.

Enteric pathogens are detected in flies during field and laboratory experiments. In addition, these pathogens can survive in flies for some days (Motarjemi *et al.* 1993). The role of flies as vectors of infectious diseases in humans has previously been suggested (Tan *et al.* 1997; Graczyk *et al.* 1999, 2003). There is no field study on the detection of rotavirus and *Cryptosporidium* spp. in wild-caught flies at various locations of the living environment, although rotavirus was detected in a field study at households in or close to food preparation areas in India (Collinet-Adler *et al.* 2015). Many flies were observed in the living environment of the targeted area. Detection of rotavirus and *Cryptosporidium* spp. on flies caught from various locations of the living environment indicated that flies play a potential role in the contamination of enteric pathogens.

LIMITATIONS OF THE STUDY

There are some limitations to the present study. Because the RT-qPCR assay was applied to achieve greater sensitivity, no quantitative information on *Cryptosporidium* spp. was collected. Considering the small sample size, it was not possible to compare sufficient quantitative data among the exposure media and vectors of enteric pathogens. The present study investigated the stored drinking water at the point-of-use since the contamination of this water has direct impact on humans, who use the water; water contamination at the source was out of the scope of this study. It is necessary to track the sources of enteric pathogens to evaluate transmission routes. Nevertheless, our study in a periurban context contributes to society by providing essential information on enteric pathogen contamination in living environments, especially in poor WASH environments. Our future studies will focus on the evaluation of transmission and exposure routes for these pathogens by analysis of more samples determined from the findings of the present study and employing genome sequence analysis.

CONCLUSIONS

E. coli, rotavirus, and *Cryptosporidium* spp. in water and on cups, as exposure media, and on flies, as potential vectors of enteric pathogens, in the living environment were investigated in a periurban community of Lusaka. Widespread contamination of *E. coli*, rotavirus, and *Cryptosporidium* spp. has been observed from the detection of these microorganisms in stored tap water for drinking, dug well water for domestic use, stagnant water on the ground, cups, and flies. To our knowledge, the present study provides the first quantitative information on rotavirus in the kitchenware and flies and the first information on *Cryptosporidium* spp. contamination in the kitchenware. These data indicate that drinking water and kitchenware contributed to enteric pathogen exposure. Detection of flies in a living environment implies the potential role of flies as vectors of rotavirus and *Cryptosporidium* spp.

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AUTHOR CONTRIBUTIONS

Y.A. designed the experiment of enteric pathogens, analyzed the data of enteric pathogens. M.L.C. and M.T. conducted the field work, sampling, and *E. coli* analysis. M.L.C. analyzed the data of *E. coli*. Y.A. and M.L.C. wrote the first draft of the manuscript. T.Y. and I.N. supported and supervised the field work. H.H. designed the overall study and edited the manuscript. All authors reviewed and approved the final manuscript.

DATA AVAILABILITY STATEMENT

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

CONFLICT OF INTEREST STATEMENT

The authors declare there is no conflict.

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