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ROOF-HARVESTED RAINWATER IS A POTENTIAL SOURCE OF BACTERIAASSOCIATED DIARRHEA IN A PERI-URBAN SOUTHERN UGANDA SETTING: CROSS-SECTIONAL STUDY

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ABSTRACT

Background: In 2011, 41 countries experienced water stress with approximately 24% of them depleting their freshwater supply. They had to rely on alternative water sources such as roof harvested rain water (RHRW) with uncertainty about its bacterial safety for human consumption as well as domestic use.
Objectives: We aimed to study the presence of bacterial pathogens in roof-harvested rain water, their sero-typical identity as well as their antibiotic susceptibility profile.
Methods: A prospective cross-sectional study was carried out to assess the contamination of roof harvested rain water (RHRW) from Makondo Sub-parish, Lwengo district, Southern Uganda. RHRW in 47 households was purposively sampled between August, 2011 to February, 2012 and tested for salmonella and *E. coli* organisms using standard procedures.
Results: In August, *E. coli* contamination was 76.6% and Salmonella was 17.02%. There were decreasing trends for both *E. coli* and Salmonella overtime. *E. coli* contamination was generally significantly higher than that of Salmonella (P = 0.01). Among *E. coli* serotypes; serotype 09+ was the most predominant. Salmonella serotype 04+ was dominant throughout all the months. Most Salmonella isolates were resistant to Streptomycin and Ampicillin while *E. coli* were resistant to Ampicillin.
Conclusion: RHRW in Makondo sub-parish was contaminated with diarrheal agents of public health significance exhibiting varying antibiotic resistance. Households using this water should be sensitized about rendering this water safe againstmicrobial pathogens.

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INTRODUCTION

Climate change is threatening to contribute enormously to water scarcity. Alternative sources of water for drinking are encouraged globally such that everyone on earth should access safe and affordable drinking water (UNDP, 2015) .Roof harvested rain water (RHRW) is becoming a major source of household water in peri-urban and rural areas of developing countries.Despite Strategic Development Goal 6 to ensure safe water for drinking to the public, RHRW has been associated with microbiological contamination with diarrheal agentsin developed countries (Ahmed, 2012). Multiple studies have reported various pathogens including *Salmonella*, *Shigella*, *Vibrio*, *Legionella*, *Campylobacter*, *Cryptosporidium,* and *Giardia* spp. in samples taken from rainwater tanks (Fewtrell, 2007). Such microbes especially salmonella and *E. coli* O157:H7 are associated with serious diarrheal disease outbreaks with significant morbidity and mortalities in humans (Cheesbrough, 1984). The situation is worse among children less than 5 years old and immunocompromised adults (Stephanie, 2013). The significance of RHRW as a source of these diarrheal agents is not clearly documented in Uganda. We purposed to determine whether RHRW in Makondo parish of Lwengo district, Uganda, could be acting as the vehicle for diarrheal water borne disease agents.

MATERIALS AND METHODS

Study area

The study was conducted in Makondo sub-parish in Ndagwe Sub County, Lwengo district, located in Southern Uganda (Figure 1).

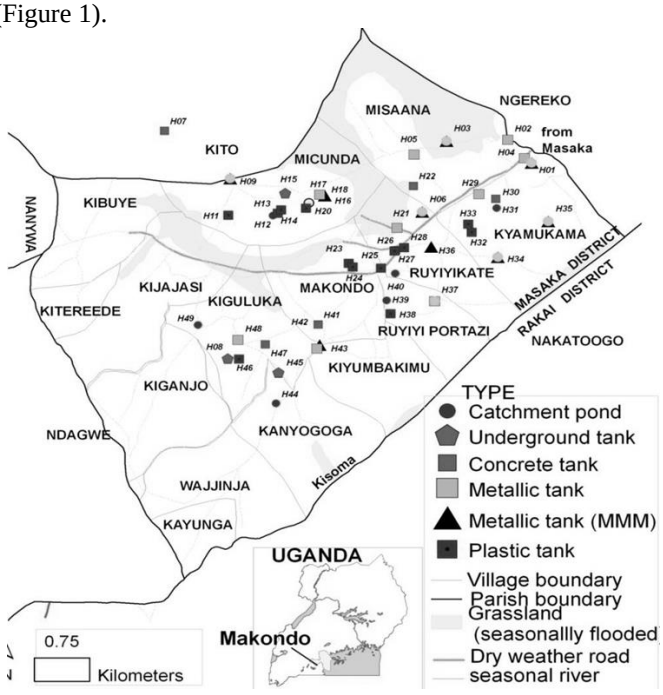


Figure 1: Map showing study area, letter 'H' represents House hold involved in the study- Makondo Parish Ndagwe Sub County, Lwengo district, Uganda

Makondo Sub-Parish is located between longitude 31° East to longitude 34° East and latitude 00 degrees Equator to 25° South of the Equator. Makondo is characterized by numerous hills and valleys and covers an area of 32.7 km^2.

The communities keep cows, goats and pigs as their main economic activity. Despite the major reliance on agriculture, Makondo has a difficult terrain and lies within the region receiving the least amount of rainfall in Uganda (750-1000 mm per year) thus the water sources on which both humans and their animals survive are constrained, leading the population to rely on harvesting rain water.

Study design and collection of samples

This was a prospective cross-sectional study in which RHRW from selected households were sampled and tested for bacterial contaminants. A total of 47 households practicing rainwater harvesting were selected purposively for the study based on accessibility. Some of the households were distributed along Kibuye – Masaka dry weather road, others were enclosed in seasonal grassland of Micunda, while the rest were scattered throughout the parish. The RHRW sources included catchment ponds, underground tanks, concrete tanks, metallic tanks and plastic tanks in different households and in any particular household, only one RHRW source was studied. All the 47 households were sampled for the month of August 2011; subsequently these households were sampled during the months of October and December 2011 and February 2012. However, due to lack of rains and hence drying of the tanks, only 18 households were sampled in the latter month. The water samples were collected from tank tap / source outlets into 250 ml sterile bottles and transported chilled to the Microbiology laboratory, College of Veterinary Medicine, Animal Resources and Biosecurity (COVAB), Makerere University, for bacteriological analysis within 24hours. The samples were analyzed for *E. coli* and *Salmonella* species.

Laboratory analysis for *Salmonella* spp

Isolation of S*almonella* was carried out according to standard procedure. Briefly, the roof-harvested rainwater sample in a 250 ml bottle was swirled gently to allow homogenization and then 45 mls was transferred into a 50 ml sterile falcon tube. The tube was centrifuged at 2.5 x g for 10 minutes and the supernatant decanted off leaving behind the residue of about 5 mls that was thoroughly vortexed and 2 mls of which was inoculated aseptically into 9 mls of peptone water. The inoculated peptone broth was incubated at 37ºC for 24 hours, after which it was thoroughly vortexed and 1.5 mls was inoculated into 9 mls of Rappaport as well as Selenite broth for enrichment. The organisms in Rappaport and Selenite broths were incubated at 42ºC for 24 hours. Following incubation, two loopfuls from either broth were inoculated onto XLD agar and thereafter incubated at 37ºC for 24 hours. Black colonies with pink surrounding on XLD were taken as *Salmonella* suspects. The latter were subjected to biochemical testing using Triple Sugar iron (TSI), Simmon's Citrate, Urea and Lysine Indole Motility (LIM). Subsequently one colony per plate was serotyped using polyvalent "O" antisera (Denka-Seinken Company Limited; Japan). The *Salmonella* polyvalent anti-sera panel included 01-035 and Vi antisera.

Laboratory analysis for*E. Coli*

Isolation of *E. coli* was carried out using standard procedure. Briefly, 250 mls water sample was swirled gently and 45 mls were transferred into a 50 ml sterile falcon tube. The tube was centrifuged at 2.5 x g for 10 minutes and the supernatant decanted off, the 5 mls residue was thoroughly vortexed and 2

mls was inoculated aseptically into 9 mls of peptone water and incubated at 37ºC for 24 hours. Following incubation, 3 loopfuls of the latter broth were aseptically picked and inoculated onto MacConkey agar and plates were incubated overnight at 37°C. Characteristic medium pink colonies of *E. coli* were selected for subculture to obtain pure cultures for further identification(5). Pure *E. coli* colonies were Gram stained and biochemical identification of isolates was performed using sugar fermentation tests (Dextrose, Sucrose, Lactose Maltose and Mannitol), indole test, methyl- red, VogesProskauer and Simmon's Citrate tests according to standard procedures (Cheesbrough, 1984). One colony per plate was serotyped using *E. coli* polyvalent "O" antisera ranging from 01-09+ according to the supplier of the antisera (Denka-Seinken Company Limited; Japan). Visible agglutination was taken as positive whereas no agglutination / flocculation was taken as a negative test.

Antibiogram of isolates

The antimicrobial sensitivity testing of *Salmonella* and *E. coli* isolates was done on Muller-Hinton agar, following the commercial disc diffusion method of Kirby-Bauer. Isolates were tested against Streptomycin (10 µg), Gentamicin (10 µg), Kanamycin (30 µg), Chloramphenicol (30 µg), Piperacillin (100 µg), Ampicillin (10 µg), Cefmetazole (30 µg), Fosfomycin (50 µg), Tetracycline (30 µg), Sulfamethoxazole and Trimethoprim (Septrin) (19 µg), Ofloxacin (5 µg) and Nalidixic acid (30 µg) (Japan, Mast Diagnostics, and Merseyside, U.K). One to five colonies from a purity plate were picked with a wire loop and emulsified in 5 ml of sterile saline. Then the turbidity of the saline was adjusted to match 0.5 Mc Farland turbidity standards (Beckton and Dickson, UK). A sterile cotton swab was dipped into the saline suspension and cultures were inoculated on sterile Mueller-Hinton agar by surface spreading. Antibiotic discs were placed over the seeded agar plates (six discs per plate) which were subsequently incubated aerobically at 37°C for 24 hours (Carter, 1979). After incubation the diameter of inhibition zones were measured. The results were interpreted as sensitive, intermediate or resistant based on the interpretive chart of Kirby-Bauer.

Data analysis

Data were entered in Microsoft Excel program and descriptive statistics were performed. Data presentation was performed using line graphs, and tables. Comparison of the prevalence of *E. coli* and *Salmonella* species was carried out using the Chi-square for trend. Statistical significance was considered at p value ≤ 0.05.

Ethical consideration

Approval to conduct this research was obtained from Makerere University, College of Veterinary Medicine Research Ethics Committee. Communities of Lwengo were penetrated after written permission by the local authorities. House hold heads were requested for written permission to obtain samples from their rain water harvesting tanks.

RESULTS

Prevalence and trend of RHRW bacterial contamination

Fig 2: Results showed that 76.6% of the RHRW sources were contaminated with *E. coli* bacteria while 17.02% had

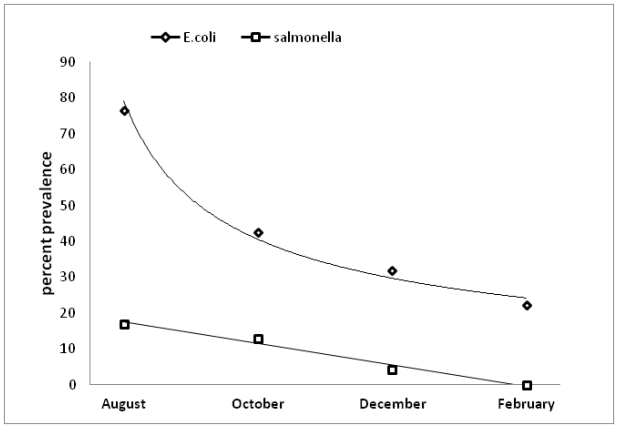
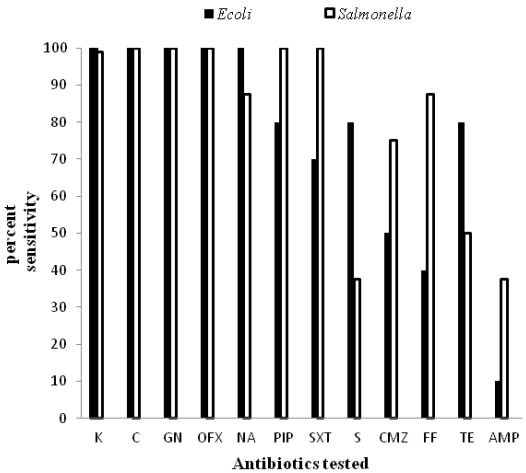


Figure 2. Prevalence of *E. coli* and *Salmonella* in roof-harvested rainwater from Makondo Parish Ndagwe Sub County, Lwengo district, Uganda

Salmonella spp in the month of August 2011. Follow up samples showed that occurrence of both *E. coli* and *Salmonella* decreased from August 2011 to February 2012 following a decrease in rainfall pattern. The former organism showed an exponential parabolic decrease while the latter exhibited a linear decrease. The prevalence of *E. coli* was significantly higher than that of *Salmonella*spp (p value = 0.0116). The trend of occurrence of *E. coli* and *Salmonella* in the tanks was as shown in Apart from *E. coli* and Salmonella, other bacteria isolated were *Pseudomonas aeruginosa*, *Proteus species*, *Corynebacteria species*, and *Enterobacter* species.

Serotypes of *E. coli* and Salmonella isolates

Table1: The predominating *E. coli* and Salmonella serotypes by month were such that*E. coli* serotypes varied in their predominance with 07+, 08+, 09+, and 09+ predominating in August, October, December and February, respectively. Other *E. coli* serotypes were 01+ up to 06+ with differing proportions. Table2: *Salmonella* serotype 04+ was the most abundant in all the study months. Other *Salmonella* serotypes included 01+, 02+, 07+, 08+, 09+ and non-serotypable group. No *Salmonella* spp were recoveredin February 2012.



K=Kanamycin, C=Chloramphenicol, GN=Gentamicin, OFX=Ofloxacin, NA=Nalidixic acid, PIP=Piperacillin, SXT= Sulfamethoxazole and Trimethoprim (Septrin); S= Streptomycin, CMZ=Cefmetazole, FF=Fosfomycin, TE=Tetracycline, AMP=Ampicillin.

Figure 3:Antibiotic susceptibility of *E. coli* and *Salmonella* isolates from roof-harvestedfrom Makondo Parish Ndagwe Sub County, Lwengo district, Uganda. rainwater

Table 1.The different*E. coli* serotypes from roof-harvested rainwater from Makondo Parish Ndagwe Sub County, Lwengo district, Uganda

Month	Number of positive samples	(Serotype occurrence) percentage								
		01+	02+	03+	04+	05+	06+	07+	08+	09+
August	36/47	(0) 0.0%	(7) 9.33%	(0) 0.0%	(6) 6.47%	(0) 0.0%	(6) 6.47%	(10) 7.27%	(0) 0.0%	(7) 9.33%
October[a]	20/47	(2) 9.09%	(0) 0.0%	(4) 8.18%	(2) 9.09%	(1) 4.55%	(2) 9.09%	(3) 3.64%	(5) 22.73%	(3) 3.64%
December[b]	15/47	(2) 11.76%	(1) 5.88%	(1) 5.88%	(3) 7.65%	(2) 1.76%	(1) 5.88%	(1) 5.88%	(2) 1.76%	(4) 3.53%
February	4/18	(0) 0.0%	(0) 0.0%	(0) 0.0%	(0) 0.0%	(1) 25.0%	(1) 25.0%	(0) 0.0%	(0) 0.0%	(2) 50.0%

Table 1. The different *Salmonella* serotypes from roof-harvested waterfrom Makondo Parish Ndagwe Sub County, Lwengo district, Uganda

Month	Number of positive samples	(Serotype occurrence) percentage						
		01+	02+	04+	07+	08+	09+	Non-serotypable
August	8/47	(0) 0.0%	(1) 12.5%	(3) 37.5%	(2) 25.0%	(1) 12.5%	(1) 12.5%	(0) 0.0%
October	6/47	(1) 10.0%	(1) 10.0%	(4) 40.0%	(1) 10.0%	(1) 10.0%	(0) 0.0%	(2) 20.0%
December*	2/47	(0) 0.0%	(0) 0.0%	(2) 66.7%	(1) 33.30%	(0) 0.0%	(0) 0.0%	(0) 0.0%
February	0/18	(0) 0.0%	(0) 0.0%	(0) 0.0%	(0) 0.0%	(0) 0.0%	(0) 0.0%	(0) 0.0%

* Both colonies reacted with O4+ and one reacted with O7+ sera.

Antimicrobial sensitivity of the bacterial isolates

Figure 4 shows the antibiotic sensitivity of bacterial isolates. Most of the *Salmonella* isolates (>80%) were sensitive to Kanamycin, Chloramphenicol, Gentamicin, Ofloxacin, Nalidixic acid, Piperacillin, Septrin and Fosfomycin. There were high rates of *Salmonella* isolate resistance to Streptomycin and Ampicillin. On the other hand most of the *E. coli* isolates (>80%) were sensitive to Kanamycin, Chloramphenicol, Gentamicin, Ofloxacin and Nalidixic acid, however, most were resistant to Ampicillin.

DISCUSSION

Although the harvesting of rain water occurs countrywide, there was hardly any prior data nationally on the microbiological quality of roof harvested rain water that could be used to compare our findings. This is the first study reporting the bacteriological quality of RHRW from Makondo parish Lwengo district and draws attention to considering RHRW as one of the potential sources of pathogens. Our study found that the Roof-harvested rainwater in Makondo sub-parish was contaminated with *E. coli* and *Salmonella* organisms. In August 2011, *E. coli* prevalence was 76.6% while that of *Salmonella* was 17.02%. The prevalence of *E. coli* was almost similar to the 79% obtained in Denmark (Albrechtsen, 2002) and 72% found in South Korea (Lee, 2010). On the other hand the prevalence of *Salmonella* (17.02%) obtained in our study was higher than the 10.7% reported by (Ahmed, 2012) in Australia. Generally this information suggests that the rainwater catchment systems in the developed countries and that of the study area have similar common vulnerability to contamination. In addition higher level of microorganisms in August 2011 than the other succeeding months was probably due to the "first- flush effect" (Forster, 1996; Forster, 1998; Forster, 1999). In this month just prior to sampling, Makondo sub-parish had received the first rains that could have been responsible for washing off of the contaminated dirt and dust from the rooftops into the gutter and then tanks. However, our study showed that after August and onwards, *E. coli* and *Salmonella* registered decreasing trends over months following the rainfall trend. The observed prevalence of *E. coli* ranging from 22.22% - 76.60% is similar to the prevalence range of the organisms that were obtained in developed countries(Albrechtsen, 2002; Lye, 1987).

Similarly the prevalence of *Salmonella* ranging from 0-17.02% was also falling in the ranges obtained in developed countries Ahmed *et al*. 2010) suggesting contamination levels keep fluctuating. It is probably true that these organisms have a common source, that is, the organisms arise from dust, animal, birds and reptile excreta that are washed off down the roof into the water tanks at the onset of rains as suggested earlier (Lee, 2010). During the study there were steady rains from August onwards to December, it is thus possible that these rains caused a dilution factor of the roof containment and the environment leading to the decreasing prevalence of the organisms. It became increasingly dry from January- February 2012 suggesting that dry conditions adversely affect the survival of the micro-organisms especially *Salmonella* (Fig. 2). *Escherichia coli* are believed to be more persistent (several weeks to months) in the environment owing to its adaptive characteristics to harsh environment and this may account in part to the parabolic asymptotic nature of the *E. coli* recovery curve (Forster, 1996), (Ahmed, 2012) Arguably the negative gradient possessed by *Salmonella* linear curve could indicate that these organisms are not as tolerant to dry conditions as *E. coli* are. No studies to isolate and serotype *Salmonella* and *E. coli* from roof harvested water in Uganda had been done previously.

The variability of *E. coli* serotypes over the months, as observed in this study, was probably due to a wide number of hosts. The *E. coli* serotypes inhabit the gastrointestinal tract of varied wild and domestic animals, birds, reptiles, fish, amphibians and occasionally humans. Based on our data, special attention should be paid to serotype 09+ because it contains subtypes 0121, 0130, 0145 that are associated with gastroenteritis (Forster, 1998). The common *Salmonella* was serotype 04+ and according to Kauffmann Classification polyvalent serotype O4+ comprises of group B *Salmonellae* that includes *S.* paratyphi B var. *odense*, *S.* Typhimurium, *S.* abortus-equi, *S.* abortus-ovis, *S.* derby, *S.* saint-paule.t.c. The dominance of this serotype suggests occurrence of a common source of this Salmonella or a narrow range of hosts and perhaps it is relatively hardy as rains decrease and hence its survival advantage. It is worth noting that this serotype is also of public health importance due to certain sub groups such as *Salmonella* Typhimurium that is a significant cause of gastroenteritis(Franklin, 2008).

The varied sensitivity pattern of drugs as noted in this study underlies the fact that, various drugs have got different modes of action as well as the frequency of their use. Interestingly drug resistance was not a big problem in most of the isolates apart from Streptomycin and Ampicillin. The latter antibiotics have been widely used in human and veterinary practice in Uganda suggesting cross-species transfer of strains from humans/livestock to birds and other hosts that subsequently shed organisms that contaminate RHRW. Overall our findings suggest that most of the bacteria in the RHRW were environmental and were not much subjected to antimicrobial challenge.

Conclusion

Roof-harvested rainwater in Makondo sub-parish Lwengo district was contaminated with several serotypes of *Salmonella* and *E. coli*. Most of these pathogenic diarrheal organisms were environmental and exhibited little antimicrobial resistance. The contamination of the RHRW with these organisms was high at the onset of Rains but decreased with decreasing rains. Based on these data, the communities should be sensitized about contamination of the water with these waterborne pathogens and should be advised to chemically treat or boil their RHRW water prior to drinking so as to prevent disease outbreaks. Studies about the design of rain water harvesting systems especially ease of cleanliness of tanks, gutters etc. and socio-demographic factors of household members are warranted. Also Studies should be done to elucidate the virulence determinants of these organisms in order to ascertain their pathogenic significance to humans.

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