Biosand water filter evaluation: pilot study of field use indicators in Cyegera, Rwanda
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ABSTRACT
Diarrheal diseases are a global public health burden, killing 1.8 million people annually. Diarrhea disproportionately affects children and those in poverty. Most diarrheal cases can be prevented through safe drinking water, basic hygiene and/or sanitation measures, with drinking water interventions having the most impact on reducing diarrheal disease. There is no generally agreed-upon field method for determining biosand water filter effectiveness that is usable in low-resource communities. A pilot study was conducted of potential field use indicators, including the Colilert coliform presence/absence (P/A) test, hydrogen sulfide, alkalinity, hardness, pH, and fluorescently labeled latex microspheres. The study included both laboratory and field testing. The Colilert P/A test had the highest correlation to the United States Environmental Protection Agency standard method (IDEXX Quanti-trays), but more data are needed before making a recommendation. This study adds to understanding about evaluation of biosand water filters and provides preliminary data to address the need for a field use indicator for biosand water filters.

Key words | biosand filters, diarrhea, household water treatment, WASH

INTRODUCTION
Description of the problem
Diarrhea and other water-related diseases are a global public health burden, killing 1.8 million people annually (Prüss-Ustün et al. 2014). Diarrhea disproportionately affects children and those in poverty. Globally, diarrhea is the second leading cause of death of children age five and under, with approximately 760,000 children dying annually (WHO 2013). Ninety percent of diarrreal deaths occur in developing nations (Gill et al. 2012); it is the primary cause of childhood morbidity and mortality in sub-Saharan Africa (O’Reilly et al. 2012).

Biosand water filters
One point-of-use method to improve drinking water and reduce disease is the biosand water filter (BSF), created by Dr. David Manz in the 1990s at the University of Calgary and promoted by his organization, Centre for Affordable Water and Sanitation Technology (CAWST) (CAWST 2009). An adaptation of the traditional slow sand filter, it was made smaller and modified for intermittent use, for utilization in households globally (CAWST 2009).

BSFs (structure displayed in Figure 1) are composed of a concrete or plastic container and layers of sand and rock prepared using CAWST methods (CAWST 2013). Ninety percent of diarrreal deaths occur in developing nations (Gill et al. 2012); it is the primary cause of childhood morbidity and mortality in sub-Saharan Africa (O’Reilly et al. 2012).

Studies have shown 27–74% reduction in incidence of diarrheal disease with BSF use (O’Connell et al. 2017a). BSFs have been shown to remove significant amounts of
Escherichia coli in both laboratory and real-world settings ranging from 48% to 100%, with fecal coliform removal of 33.7–96.1% (O’Connell et al. 2017a). Further, BSFs have also been shown to remove echovirus type 12 (93%) and bacteriophages (82%) (Elliot et al. 2008). Studies indicate a high long-term user acceptance in communities ranging from 77% to 94% (Earwaker 2006; Fiore et al. 2010; Aiken et al. 2011; Stuber et al. 2011; Mangoua-Allali et al. 2012). BSF life span is shown to vary widely, ranging from under a year to over 12 years (Sisson et al. 2013). Therefore, it is necessary to test the filters over time to determine if they are working effectively. However, in rural developing communities where BSFs are often used, it is often not feasible to use standard laboratory testing due to limitations in electricity, transportation times, and funding. Currently, studies do not point to an agreed-upon field method for testing (O’Connell et al. 2017a).

Study community

Cyegera is a small community in Southern Province, Rwanda. Diarrhea causes 17% of total deaths in Rwanda (Prüss-Ustün et al. 2014). Demographic and Health Surveys 2010 data show that 13% of children under 5 years old had diarrhea in the 2 weeks preceding the survey (DHS Program 2015). The health workers at the clinic in Cyegera reported that diarrhea and intestinal parasites are of major concern in the community, especially in children (personal communication, July 2014). Data from the local Compassion International branch show that 2.13% of the children, aged 6–14 years, enrolled in the program had diarrhea from July 2014 to June 2015 (personal communication, June 2015). Diarrhea was second to malaria in causing illness in children enrolled in the program and it continues to be a problem for older children, but the greatest risk is
Study aim

The specific aim of this study was to carry out a pilot laboratory trial and a field trial in Cyegera of selected potential field use indicators of biosand water filters and to obtain data for further study.

METHODS

Laboratory testing of field use indicators

To address the gap in the literature of evidence-based methods usable in a remote field setting to determine whether a BSF is functioning, a pilot study was conducted of six existing potential field use indicators (FUIs): Colilert’s presence/absence (Colilert P/A), Hach’s hydrogen sulfide, alkalinity and hardness kits, a Mettler Toledo EL-2 battery-powered pH meter, and Sigma Aldrich fluorescently labeled latex microspheres. Laboratory procedures were approved by the East Tennessee State University (ETSU) Biosafety Committee.

Description of FUIs and standard

IDEXX Colilert Quanti-trays were used as the standard for comparison of the FUIs. The system provides most probable number (MPN) counts for coliforms (IDEXX 2015a). The number of trays that change color to yellow are counted and the MPN table used to determine results (IDEXX 2015a). IDEXX recommends incubating (IDEXX 2015b), but for this study, incubation was not done so that results would be replicable in the field. Thus, results (color change) were read at 12-hour increments for 48 hours, rather than only at the recommended 24-hour interval.

The Hach alkalinity test kit determines the total alkalinity of the water sample (Hach 2015). The Hach hardness procedure was used to determine hardness of the sample.

A Mettler Toledo EL-2 battery-powered digital pH meter was used to test pH of each sample.

Two types of Sigma Aldrich latex microspheres were used: 2.0 μm yellow-green and 1.0 μm red. At first use, 1 mL of each size was mixed in the influent to be put in the four filters in treatment group A. The microspheres were used only in treatment group A because microspheres or microbeads are sometimes used in filter media as a filtration aid and could, therefore, potentially affect the filtration process (Balsimo & Mary 1994).

Study design

Laboratory evaluation of the FUIs occurred using nine BSFs constructed during August and September 2014 at ETSU. The filters were segregated into three groups: four filters receiving microspheres in the first influent (group A), four filters not receiving microspheres (group B), and one control (C). Group A and group B were established to delineate whether the microspheres affected filter operation or parameter measurement because they were being poured directly into the BSFs. The control BSF was used to detect any environmental fluctuations influencing the testing. Unamended tap water was used for the control. Escherichia coli was added to the influent so that there was a known contaminant for testing.

The E. coli was isolated from an environmental sample and tests were done to ensure that it was not of the O157:H7 serotype. The sample was initially cultured on Eosin Methylene Blue (EMB) agar plates, and then transferred to slants of Tryptic Soy agar. To prepare E. coli for addition to the influent, 300 mL of Tryptic soy broth was inoculated with a single loop of E. coli and incubated at 35 °C for 18 hours. This broth was divided into 100 mL increments and centrifuged for 10 minutes at 800 RCF (relative centrifugal force), producing an E. coli ‘pellet’ at the base of each centrifuge container, above which the broth was removed. Two mL of phosphate-buffered saline (PBS) was then added, the mixture vortexed, and this solution was transposed for influent seeding.

Subsequently, 2,000 mL samples were collected from each filter, and 200 mLs of each sample was taken for the Colilert Quanti-tray test and for the microspheres. The Quanti-tray was conducted according to the manufacturer’s procedures.
Field trial of field use indicators

Researchers traveled to Cyegera, Rwanda, during 4–30 June 2015 to test the FUIs under real-world conditions. It was a cross-sectional study intended for future scale-up and repetition to become a longitudinal study. ETSU and University of Rwanda Internal Review Boards and the Rwanda National Ethics Committee did not require the FUI data collection procedures to be reviewed because it was not considered human subjects’ research.

BSF installation

For the field trial, eight new BSFs constructed by the Rwandese Health and Environment Project Initiative (RHEPI) were installed in homes throughout the village near water sources, and two existing BSFs in the children’s home were re-installed. The project was financed through an ETSU Research Development Committee Major Grant. The process of BSF installation was described using IBM-WASH psychosocial and technological factors at all levels (Dreibelbis et al. 2013). The BSFs were intended for community access due to budget constraints, but were installed in individual homes so that there was a sense of individual responsibility for maintenance and reporting of any problems.

Sample collection and FUI procedures

Water samples were collected from each filter at installation and on 27 June 2015. It was intended that the second set of samples would be taken 2 weeks after installation to allow the biolayer to form; however, samples were tested slightly earlier. This was due to schedule delays in installing new BSFs and time troubleshooting the older BSFs. Further, the FUIs needed 48 hours to process. Samples were collected and tested from the four community water sources on 22 June 2015. FUI procedures from laboratory testing were followed in the field with one exception – the Colilert P/A test was read every 6 hours instead of 12 due to possible ambient air temperature differences.

In July 2017, 2 years after installation, the BSFs were visited again. One had been destroyed in an accident in which it was tipped over. Another had been discontinued due to the owner moving away. New residents of the house where the filter was not in use were educated and said they were eager to utilize it. Samples from these filters were taken on 24 and 26 July 2017, and tested with Colilert P/A.

In May 2018, follow-up revealed that filters installed in 2015 were all in use. One had a rusty diffuser plate, which was replaced. Water from existing filters and sources was sampled on 1 June 2018. Samples were tested with Colilert P/A.

Data analysis

Laboratory data and field data were entered into Microsoft Excel spreadsheets. The method detection limit as provided by the manufacturers was used when results were below the detection limit (IDEXX Colilert Quanti-tray – one organism per 100 mL, Colilert P/A test – two heterotrophs per 100 mL, Hach hydrogen sulfide kit – 0–5 mg/L, Hach hardness – 1–20 mg/L, and Hach alkalinity – 5–100 mg/L). All data were imported into SPSS version 23.0.

Transformations were performed on the results of each test to make the data as normally distributed as possible to meet requirements for use of statistical testing. Data for pH and Colilert P/A were used without transformation. Logarithmic transformation produced the most normal data for Quanti-tray MPN and Hach hardness tests. Square root transformation produced the most normal data for Hach alkalinity and hydrogen sulfide tests. Pearson’s R statistic was performed for each FUI compared to Quanti-trays to determine correlation. Field trial data were reported for the one FUI that had correlation to log Quanti-tray MPN.

RESULTS

Laboratory evaluation

The microbeads that were put into the first influent were never seen in effluent samples collected. Because no difference was indicated in the filter group with microbeads, results from filter groups A and B were combined for analysis. Negative values for the Colilert P/A at 12 or 24 hours prevented correlation analysis for these time periods.
Pearson’s R correlation is provided in Table 1 for each test compared to Quanti-tray results.

Quanti-tray MPN data were categorized per the WHO recommended acceptable drinking water concentration for *E. coli* in drinking water, which is <10 MPN per 100 mL (WHO 2014). This corresponds to <1 log MPN. Categorical MPN results were compared to the categorical Colilert P/A results using chi-square. The null hypothesis for the chi-square tests was that there was no significant difference between results of the risk-categorized MPN and Colilert P/A results. Chi-square results for the categorical log Quanti-tray MPN and Colilert P/A are displayed in Tables 2 and 3.

The $\chi^2$ for Table 2 was 22.143 ($p \leq 0.001$). Because one cell had a value less than five, it was necessary to use Fisher’s exact test, which was also significant ($p \leq 0.001$). Type II error was calculated at 0.068 and type I error at 0.423. The $\chi^2$ for Table 3 was 13.767 ($p \leq 0.001$). Again, Fisher’s exact test was significant ($p \leq 0.001$). Type II error was calculated at 0.033 and type I error at 0.575.

### Field trial results

In the field trial, none of the samples collected changed color for the Hach alkalinity test. Further, results for Colilert P/A readings under 36 hours could not be used because correlation to Quanti-trays could not be established from the laboratory data and results. Because correlation to Quanti-trays was low for other tests, field trial results were analyzed for Colilert P/A readings. Colilert P/A test was the recommended field use indicator based on the laboratory testing.

In 2017, samples from the seven BSFs in use were tested using Colilert P/A and results were negative at 36- and 48-hour readings. A sample from the BSF not in use was also taken and tested. Results indicated the presence of fecal coliforms at 36 hours, probably because the biolayer was not formed yet. A water source was tested for comparison and positive for fecal coliforms at only 24 hours.

In 2018, samples from the existing BSFs and source waters were tested using Colilert P/A. All water before filtration resulted in positive readings at 24 hours. Two samples after filtration also tested positive at 24 hours. One of these was from the filter that had a rusted diffuser plate which was replaced. The other positive sample was from a filter, which was resampled, yielding a negative result through 48 hours of incubation. The other six samples from BSFs were negative at 24 hours but positive at 36 hours. An important difference between sampling events in 2017 and in 2018 is season. In May 2018, the rainy season was still underway, likely impacting contamination levels of source waters. Table 4 displays test results of samples taken from drinking water sources, from BSFs at installation, and from BSFs on 27 June 2015, July 2017, and May 2018.

| Table 1 | Pearson’s R statistic results – categorical log MPN for each FUI
<table>
<thead>
<tr>
<th>Field use indicator</th>
<th>Pearson’s R compared to categorical log Quanti-tray MPN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colilert P/A at 36 hours</td>
<td>0.642</td>
</tr>
<tr>
<td>Colilert P/A at 48 hours</td>
<td>0.503</td>
</tr>
<tr>
<td>pH meter</td>
<td>–0.037</td>
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<tr>
<td>Hach hardness (log)</td>
<td>–0.014</td>
</tr>
<tr>
<td>Hach alkalinity (square root)</td>
<td>–0.075</td>
</tr>
<tr>
<td>Hach hydrogen sulfide (square root)</td>
<td>0.151</td>
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| Table 2 | Chi-square Colilert P/A 36 hour
<table>
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<th>Log Quanti-tray MPN categorical</th>
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<th>P</th>
<th>Total</th>
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<tbody>
<tr>
<td>$\leq$1 log MPN/100 mL</td>
<td>41</td>
<td>11</td>
<td>52</td>
</tr>
<tr>
<td>$&gt;$1 log MPN/100 mL</td>
<td>5</td>
<td>15</td>
<td>18</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>44</td>
<td>26</td>
<td>70</td>
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</tbody>
</table>

| Table 3 | Chi-square Colilert P/A 48 hour
<table>
<thead>
<tr>
<th>Log Quanti-tray MPN categorical</th>
<th>A</th>
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<th>Total</th>
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<td>$\leq$1 log MPN/100 mL</td>
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<tr>
<td>$&gt;$1 log MPN/100 mL</td>
<td>1</td>
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<tr>
<td><strong>Total</strong></td>
<td>30</td>
<td>40</td>
<td>70</td>
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DISCUSSION

Laboratory testing of FUIs

Interpretation of laboratory evaluation of the FUIs should include consideration of the wide range of corresponding Quanti-tray MPN to the Colilert P/A results. The positive results for 36-hour readings of the P/A test corresponded to log MPN values of <0 to 3.38. The negative results for 36-hour readings correlated to log MPN values ranging from <0 to 2.21. Three of the negative results for Colilert P/A, out of 70 testing dates, corresponded to log MPN values that are above the WHO value, and six of the positive results for Colilert P/A corresponded to log MPN values within the WHO recommendation.

Further testing and more data would potentially enhance the predictive value and reduce the type I and II errors. Reduced correlation between Quanti-tray MPN and 48-hour readings of Colilert P/A was likely because positive results for Colilert P/A at 48 hours corresponded to a broader range of Quanti-tray MPN. While only one negative P/A result corresponded to an MPN value above one, positive P/A results corresponded about half of the time to MPNs within acceptable risk. While risk of a type II error, which would result in recommending use of unsafe water, is reduced with the 48-hour reading of Colilert P/A, the risk of a type I error is increased. A type II error is more dangerous in terms of preventing diarrhea, but a type I error, which would result in not recommending use of safe drinking water, could be detrimental in communities with limited water quantity.

Field trial of FUIs

Several factors are important to note when interpreting the results of the field trial. The 27 June 2015 results were obtained 12 days after installation of eight of the BSFs, 9 days after the re-installation of another, and only 7 days after re-installation of the final BSF. Therefore, the final testing date did not allow full formation of the biolayer. Further, because the Colilert P/A test was conducted without incubation or other temperature controls, the laboratory results and field trial results likely differ because of ambient temperature. However, the results from July 2017 indicate that seven BSFs were improving water quality compared to unfiltered water after 2 years of use. Data from May 2018 indicate that filters were less effective in removing contaminants than in 2017, but were reducing contaminants from source water. These results are likely influenced by collection during the rainy season, which is shown in studies to impact water quality overall (Prüss-Ustün et al. 2014; O’Connell et al. 2017b).

LIMITATIONS

Important limitations included failure to collect and test multiple samples per filter on testing days for purposes of

<table>
<thead>
<tr>
<th>Date</th>
<th>Source</th>
<th>Incubation time and number of samples</th>
<th>24 hours</th>
<th>36 hours</th>
<th>48 hours</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Negative</td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>June 15, 2015</td>
<td>Water sources</td>
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<td>0</td>
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<tr>
<td>June 15, 2015</td>
<td>Filters at installation</td>
<td>8</td>
<td>0</td>
<td>2</td>
<td>6</td>
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<tr>
<td>June 27, 2015</td>
<td>Filters</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>July 26, 2017</td>
<td>Water source</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>July 26, 2017</td>
<td>Filters</td>
<td>8</td>
<td>0</td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td>May 31/June 12, 2018</td>
<td>Water sources</td>
<td>0</td>
<td>7</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>May 31, 2018</td>
<td>Filters</td>
<td>8</td>
<td>1</td>
<td>3</td>
<td>6</td>
</tr>
</tbody>
</table>

Table 4 | Field trial Colilert P/A results
reliability analysis, and small sample sizes for both laboratory testing and the field trial. Additionally, although source waters were tested for comparison to filtered samples in the field trial, results would be strengthened by testing samples directly before and after filtering. Further, summaries of field testing were based on the significant assumption that Colilert P/A tests could be used to estimate risk in the same way that Quanti-trays could.

RECOMMENDATIONS

More study is needed both in laboratory and field settings. This should include testing multiple samples of effluent per filter per day to determine reliability of FUIs. Samples should be collected for each effluent to avoid missing data such as breakthrough of microbeads. Having longer time periods between testing dates would be aided by scheduling a longer time at the study location. Use of Quanti-trays with and without incubation would allow for more information to examine when comparing to non-incubated FUI results. Future studies may benefit from reading the Colilert test at more narrow time intervals between 36 and 48 hours to provide more information on the best balance of error probability and to identify a more optimal predictor of risk. Finally, the Colilert P/A test should be further studied with the BSF along with other indicator tests such as the water canary (Water Canary 2015) and the filter clogging assay (Hammond 2015). Follow-up on the BSFs from the field trial is recommended.

CONCLUSION

In conclusion, BSFs are a viable solution to reduce burden of disease by increasing drinking water quality, and Colilert P/A is recommended for further study as a field use indicator for biosand water filters. The Colilert P/A test deserves further investigation as a FUI. Future testing of Colilert P/A and other FUIs can also be improved, most significantly through larger samples sizes, adequate length of testing time, and inclusion of other promising tests. Much can be done to improve future evaluation of BSFs, with focus on length of follow-up and controlling for variables external to drinking water, including season.

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