Microbiological quality of groundwater sources used by rural communities in Limpopo Province, South Africa

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Abstract A survey of the microbiological quality of water from 194 boreholes (97 privately owned and 97 communal boreholes) in the rural Thitale-Hlanganani area of the Limpopo Province, South Africa was carried out between August 2002 and August 2003. Very little information on the microbiological quality of privately-owned boreholes in rural communities is available raising concerns about the safety of these groundwater supplies. In this study, levels of total coliforms, thermotolerant (faecal) coliforms, faecal enterococci, Clostridium perfringens (vegetative cells and spores) and somatic coliphages were determined for community and privately-owned borehole water. The average counts for total coliforms, faecal coliforms, faecal enterococci and Clostridium perfringens exceeded the South African recommended guideline limits of 0–10 counts 100 ml−1 for total coliforms and 0 counts 100 ml−1 for faecal coliforms, faecal enterococci and Clostridium perfringens respectively. Comparisons between the levels of indicator bacteria present in private and communal boreholes during dry seasons indicated a statistical difference for faecal enterococci bacteria (p = 0.005) and Clostridium perfringens (p = 0.08). Comparisons between the levels of indicator bacteria present in private and communal boreholes during rainy seasons indicated statistical differences between total coliforms (p = 0.05), faecal coliforms (p = 0.03) and Clostridium perfringens (p = 0.009) bacteria. No significant differences in the presence of somatic coliphages in both private and communal borehole water were seen. The results indicated the need for environmental impact assessment studies to monitor the microbiological quality of groundwater sources in rural communities.

Keywords Boreholes; groundwater; microbiological quality; rural region; South Africa

Introduction

The scarcity of water in rural communities of South Africa has influenced the utilization of groundwater sources and through various water supply projects, the Department of Water Affairs and Forestry (DWAF) is drilling boreholes on a continuous basis (Xu and Braune, 1995; Wright, 1995). However, due to non-compliance by the communities to these water projects, the lack of borehole maintenance and the financial burden on the communities to maintain the boreholes themselves, most of these rural villages have serious problems regarding the availability of water for basic domestic purposes (Basselin, 1992; Dreyer, 1998). In addition, the rural communities use the borehole water untreated because it is believed that underground water is a microbiologically safer source of water compared to surface water (DWAF, 2002). However, the transmission of pathogenic microorganisms through contaminated water remains a serious health issue globally because of sporadic waterborne outbreaks and reported causes of mortality in developing countries mostly affecting young children, the elderly and immune-compromised individuals (Kempster and Smith, 1985; Pieterse, 1989; Grabow, 1996; Jagals et al., 1997).
South Africa is experiencing a high number of children under the age of five years suffering from diarrhea every year and outbreaks of cholera and typhoid fever has been reported in Limpopo, Maphumalanga and Kwazulu Natal Provinces (DWAF, 2001; DOH, 2005). About 11.8% of the South African population lives in the Limpopo Province which has a very dry climate and cannot meet the requirements of providing the population with potable water with the present available water resources (Dreyer, 1998; Census, 2001). Some rural households in the Province have their own private boreholes which are used as an alternative means of potable water supply during times of water shortages in the communal boreholes (Dunker, 2000; Hazelnot, 2000).

Whenever communal boreholes are drilled in rural areas, the responsibility for monitoring the water quality is given to Environmental Health Practitioners (EHPs) in local authorities to ensure safety for the consumers (DWAF, 2002). According to the National Health Act (2000) and the White Paper on Basic Household Sanitation Policy (2001), the EHPs are given responsibility for promoting health and hygiene awareness practices to the community, through monitoring compliance with health legislation, regulation and norms and standards. However, no responsibility with regard to private boreholes in rural communities is directed as the EHPs and therefore the microbiological water quality of private boreholes is not known. In addition, the policy-makers in the District Municipalities usually do not have by-laws that regulate privately-owned boreholes used for domestic purposes. It is only mentioned in the National Water Act of 1998 that licenses are not required for a borehole used for domestic purposes. The act further gives an obligation to the Municipalities to regulate private borehole water for domestic purposes only if they find it is necessary.

The objectives of this study was to compare the microbiological quality of water from privately-owned and communal boreholes in rural villages using standard bacterial and viral indicators to indicate faecal pollution. A second aim was to determine the seasonal variation of the microbiological quality of water during dry and rainy seasons.

Materials and methods
Sample collection
In total, 97 privately owned and 97 communal boreholes in the Thitale-Hlanganani area of Limpopo Province, South Africa were included in this study. During the study period 1180 water samples were taken from both private and communal boreholes during dry (590 samples) and rainy (590 samples) seasons and tested for the presence of total coliforms, faecal coliforms, faecal enterococci, *Clostridium perfringens* and somatic coliphages. Water samples taken between August and September 2002 and between May and August 2003 represented the dry seasons whereas water samples taken between October and November 2002 and between January and March 2003 represented the rainy seasons. Water samples were taken directly from the boreholes in locations where hand water pumps were used. Where the borehole water was connected to a tap or distributed via storage tanks or reservoirs, water samples were collected from the nearest tap available from the borehole as guided by South African Bureau of Standard (SABS) (1984) and DWAF (1996). Water samples (2 L) were taken in sterile collection bottles using standard collection technique as specified by SABS (1984), and transported on ice to the microbiology laboratory and analyzed within eight hours. During water collection, a form was used for capturing the data for both communal and private borehole water. Data captured included date of collection, time of collection, borehole location, name of village where samples were taken, and the reasons for failing to collect a water sample where relevant.
Microbiological analysis

All tests were performed in duplicate. Total coliforms, faecal coliforms, faecal enterococci and Clostridium perfringens counts were determined by passing 100 ml volumes of each sample through 0.45 μm pore size, 47 mm diameter Gelman filter membranes using the standard membrane filtration technique (Standard Methods, 1995). Selective media were used for the enumeration of total coliforms (mEndo agar, Difco Laboratories, Detroit, MI, USA), faecal coliforms (mFC agar, Difco Laboratories, Detroit, MI, USA), faecal enterococci (mEnterococcus, Difco Laboratories, Detroit, MI, USA) and Clostridium perfringens (supplemented OPSP agar-Oxoid). The mEndo agar plates and mEnterococcus plates were incubated aerobically at 37EC for 24 hours and 48 hours respectively. Faecal coliform plates were incubated for 24 hours at 44.5EC. Clostridium perfringens agar plates were incubated in microaerophillic conditions at 37EC for 48 hours using anaerogens sachets (Oxoid). Results were recorded as colony forming units per 100 milliliters (cfu.100 ml$^{-1}$).

Qualitative presence–absence spot tests on 500 ml water samples for the detection of low numbers of coliphages in water samples were performed as previously reported (Grabow et al., 1993; Uys, 1999). Briefly, 5 g trypicase peptone (Difco Laboratories, Detroit, MI, USA), 0.5 g yeast extract (Difco Laboratories, Detroit, MI, USA), 4 g sodium chloride (Merck, Darmstadt, Germany) and 5 ml calcium–glucose solution was added to 500 ml of each water sample together with 1 ml of the Escherichia coli strain WG5 (ISO, 2000) specific host culture. The calcium–glucose solution contained 3 g CaCl$_2$·2H$_2$O (Merck, Darmstadt, Germany) and 10 g Glucose (Merck, Darmstadt, Germany) dissolved in 100 ml distilled water and decontaminated with 0.22 μm membrane filtration (Merck, Darmstadt, Germany) (ISO, 1995). The presence–absence water sample was incubated at 37EC for 24 hours under aerobic conditions and the presence of somatic coliphages was determined by standard direct plaque assay (Grabow, 2001).

Statistical analysis

Data was analyzed using non-parametric tests which included the Fisher exact, chi-square and Mann–Whitney $U$ test. The significant level used was $p = 0.05$.

Results and discussion

Microbiological quality of private borehole water (Table 1)

The total coliform counts during the rainy season and dry season varied between a minimum of 0 counts.100 ml$^{-1}$ and a maximum of 1000 counts.100 ml$^{-1}$. The average counts for dry and rainy seasons were 29.1 counts.100 ml$^{-1}$ and 85.6 counts.100 ml$^{-1}$, respectively. The contamination of the private borehole water during the rainy season was higher than in the dry season ($p < 0.0001$). Counts of faecal coliform bacteria were higher than the South African recommended guideline value of 0 cfu.100 ml$^{-1}$ (DWAF, 1996).

Table 1 Bacterial indicator counts (counts.100 ml$^{-1}$) detected in private boreholes during the dry and rainy seasons indicating statistical differences in indicator counts. Results are presented as mean ± standard deviation (range)

<table>
<thead>
<tr>
<th></th>
<th>Total coliform</th>
<th>Faecal coliform</th>
<th>Faecal enterococci</th>
<th>Clostridium perfringens</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dry</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Count</td>
<td>$29.1 \pm 114.3$</td>
<td>$7.8 \pm 48.2$</td>
<td>$5.2 \pm 19.2$</td>
<td>$11.5 \pm 83.9$</td>
</tr>
<tr>
<td>Range</td>
<td>(0–1000)</td>
<td>(0–800)</td>
<td>(0–200)</td>
<td>(0–1000)</td>
</tr>
<tr>
<td><strong>Rainy</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Count</td>
<td>$85.6 \pm 215.6$</td>
<td>$19.0 \pm 84.8$</td>
<td>$9.6 \pm 29.7$</td>
<td>$18.2 \pm 101.6$</td>
</tr>
<tr>
<td>Range</td>
<td>(0–1000)</td>
<td>(0–900)</td>
<td>(0–800)</td>
<td>(0–800)</td>
</tr>
</tbody>
</table>

*Wilcoxon rank-sum (Mann–Whitney) test
The high counts in the two water sources could increase the risk to consumers of being infected by potential waterborne pathogens such as *Salmonella*, *Shigella*, *Vibrio cholerae*, *Campylobacter jejuni*, *Campylobacter coli*, *Yersinia enterocolitica* and pathogenic *Escherichia coli* which can cause diseases such as gastroenteritis, dysentery, cholera, typhoid fever and salmonellosis (DWAF, 1996).

The faecal coliform counts varied between a minimum of 0 counts.100 ml⁻¹ and a maximum of 900 counts.100 ml⁻¹. The average counts for dry and rainy seasons were 7.8 counts.100 ml⁻¹ and 19.0 counts.100 ml⁻¹. There was a significant difference (\( p = 0.011 \)) between the dry and rainy seasons. The South African recommended guidelines for water intended for domestic purposes state that 0 counts.100 ml⁻¹ of faecal coliform bacteria should be detected in the water sample (DWAF, 1996).

The faecal enterococci counts during the dry season varied between a minimum of 0 counts.100 ml⁻¹ and a maximum of 200 counts.100 ml⁻¹ and for the rainy seasons between 0 counts.100 ml⁻¹ and a maximum of 800 counts.100 ml⁻¹. The average counts during the dry seasons were 5.2 counts.100 ml⁻¹ and 9.6 counts.100 ml⁻¹ for the rainy seasons. These results indicated no significant difference between the dry and rainy seasons (\( p = 0.0555 \)). The counts of faecal streptococci in the two water sources indicated a potential risk for waterborne diseases to the consumers during exposure and consumption (DWAF, 1996).

The *Clostridium perfringens* during the dry season varied between a minimum of 0 counts.100 ml⁻¹ and a maximum of 1000 counts.100 ml⁻¹ with an average of 11.5 counts.100 ml⁻¹. During the rainy season, *Clostridium perfringens* counts varied between a minimum of 0 counts.100 ml⁻¹ and a maximum of 800 counts.100 ml⁻¹ with an average of 18.2 counts.100 ml⁻¹. A significant difference in the contamination of private borehole water by *Clostridium perfringens* were seen during the dry and rainy season (\( p = 0.0004 \)).

**Microbiological quality of communal borehole water (Table 2)**

The total coliform counts during the rainy season and dry season varied between a minimum of 0 counts.100 ml⁻¹ and a maximum of 1000 counts.100 ml⁻¹. The average counts for the dry and rainy seasons were 19.3 counts.100 ml⁻¹ and 55.4 counts.100 ml⁻¹, respectively. The contamination of the private borehole water during the rainy season was higher than in the dry season (\( p = 0.0025 \)). Counts of faecal coliform bacteria were higher than the South African recommended guideline value of 0 cfu.100 ml⁻¹ (DWAF, 1996). The high counts in the two water sources could increase the risk to consumers of being infected by potential waterborne pathogens such as *Salmonella*, *Shigella*, *Vibrio cholerae*, *Campylobacter jejuni*, *Campylobacter coli*, *Yersinia enterocolitica* and pathogenic *Escherichia coli* which can cause diseases such as gastroenteritis, dysentery, cholera, typhoid fever and salmonellosis (DWAF, 1996).

**Table 2** Bacterial indicator counts (counts.100 ml⁻¹) detected in communal boreholes during the dry and rainy seasons indicating statistical differences in indicator counts. Results are presented as mean ± standard deviation (range)

<table>
<thead>
<tr>
<th></th>
<th><em>p-value</em></th>
<th><em>p-value</em></th>
<th><em>p-value</em></th>
<th><em>p-value</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Total coliform</td>
<td>0.0025</td>
<td>0.5815</td>
<td>0.0021</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Faecal coliform</td>
<td>4.4 ± 19.9</td>
<td>1.7 ± 8.4</td>
<td>2.7 ± 10.3</td>
<td></td>
</tr>
<tr>
<td>Faecal enterococci</td>
<td>0.0021</td>
<td>1.9 ± 11.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clostridium perfringens</td>
<td>0.0001</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Wilcoxon rank-sum (Mann–Whitney) test*
The faecal coliform counts varied between a minimum of 0 counts.100 ml\(^{-1}\) and a maximum of 236 counts.100 ml\(^{-1}\) for the dry seasons and 250 counts.100 ml\(^{-1}\) for the rainy season. The average counts for the dry and rainy seasons were 4.4 counts.100 ml\(^{-1}\) and 7.5 counts.100 ml\(^{-1}\), respectively. There was no significant differences (\(p = 0.581\)) between the dry and rainy seasons. The South African recommended guidelines for water intended for domestic purposes state that 0 counts.100 ml\(^{-1}\) of faecal coliform bacteria should be detected in the water sample (DWAF, 1996).

The faecal enterococci counts during the dry season varied between a minimum of 0 counts.100 ml\(^{-1}\) and a maximum of 116 counts.100 ml\(^{-1}\) and for the rainy season between 0 counts.100 ml\(^{-1}\) and a maximum of 188 counts.100 ml\(^{-1}\). The average counts during the dry season were 1.7 counts.100 ml\(^{-1}\) and 11.2 counts.100 ml\(^{-1}\) for the rainy season. These results indicated a significant difference between the dry and rainy seasons (\(p = 0.0021\)). The counts of faecal streptococci in the two water sources indicated a potential risk for waterborne diseases to the consumers during exposure and consumption (DWAF, 1996).

*Clostridium perfringens* during the dry and rainy seasons varied between a minimum of 0 counts.100 ml\(^{-1}\) and a maximum of 100 counts.100 ml\(^{-1}\) with an average of 2.7 counts.100 ml\(^{-1}\) during the dry season and an average of 1.9 counts.100 ml\(^{-1}\) during the rainy season. No significant difference in the contamination of private borehole water by *Clostridium perfringens* were seen during the dry and rainy seasons (\(p = 0.0001\)).

Comparison of microbiological quality of ground water between private and communal boreholes during the dry and rainy seasons (Tables 3 and 4)

Total coliform and faecal coliform bacteria levels present in the private and communal borehole water samples during the dry season, indicated no significant differences (\(p = 0.2379\) for total coliforms and \(p = 0.2777\) for faecal coliforms). However, significant differences were seen with levels of faecal enterococci (\(p = 0.0055\)) and *Clostridium perfringens* (\(p = 0.0898\)) bacteria during the dry season. Private boreholes seemed more contaminated by these two indicator microorganisms than communal borehole sources.

Faecal enterococci bacteria levels present in the private and communal borehole water samples during the rainy season, indicated no significant differences (\(p = 0.6324\)). However, significant differences were seen with levels of total coliforms (\(p = 0.05\), faecal coliforms (\(p = 0.0333\)) and *Clostridium perfringens* (\(p = 0.0086\)) bacteria during the rainy season. Observations indicated that the private borehole water were unprotected because many of the boreholes had unprotected casings around the opening of the boreholes and some of the casings were corroded.

Prevalence of somatic coliphages (Figure 1)

During the dry seasons about 97% of private and 95% communal borehole water samples had no somatic coliphages present; while between 3% of the private and 5% of the

<table>
<thead>
<tr>
<th></th>
<th>Total coliform</th>
<th>p-value</th>
<th>Faecal coliform</th>
<th>p-value</th>
<th>Faecal enterococci</th>
<th>p-value</th>
<th>Clostridium perfringens</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Private</td>
<td>29.1 ± 114.3</td>
<td>0.2379</td>
<td>7.8 ± 48.2</td>
<td>0.2777</td>
<td>5.2 ± 19.2</td>
<td>0.0055</td>
<td>11.5 ± 83.9</td>
<td>0.0898</td>
</tr>
<tr>
<td></td>
<td>(0–1000)</td>
<td></td>
<td>(0–800)</td>
<td></td>
<td>(0–200)</td>
<td></td>
<td>(0–1000)</td>
<td></td>
</tr>
<tr>
<td>Communal</td>
<td>19.3 ± 79.5</td>
<td></td>
<td>4.4 ± 19.9</td>
<td></td>
<td>1.7 ± 8.4</td>
<td></td>
<td>2.8 ± 10.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(0–1000)</td>
<td></td>
<td>(0–236)</td>
<td></td>
<td>(0–116)</td>
<td></td>
<td>(0–100)</td>
<td></td>
</tr>
</tbody>
</table>

*Wilcoxon rank-sum (Mann–Whitney) test*
communal borehole water samples tested positive for the presence of somatic coliphages. No significant difference in the presence of somatic coliphages in both private and communal borehole water was found.

Conclusions

Despite the Government’s effort to provide potable safe water and proper sanitation systems to rural communities, the shortage of water and the sanitation backlog is still very prevalent in the Tshitale-Hlanganani area and the Vhembe district in Limpopo Province as a whole. Private boreholes drilled by individual community members are also increasing in these villages mainly because the individual households want to be prepared in times of water shortages. Unfortunately, many of these boreholes are not protected, and are open to microbial contamination from humans, animals and the environment. Observations made during this study indicated that private borehole water becomes contaminated because of unprotected casings around the opening of the boreholes. The surface water run-off gains access to the boreholes during the rainy season. Corrosion of borehole casings also causes microorganisms to get access to the borehole water. Most of communal boreholes were protected with a cement slab around the casing and a small house around it. Regrowth of bacteria in storage tanks and reservoirs as well as hygienic handling of such storage facilities was recorded as factors causing contamination of borehole water. It was also observed that some of the tanks and reservoirs had not been cleaned or disinfected since they were installed. In addition, it was found that some of the boreholes were drilled next to sanitary facilities, i.e. pit latrines and septic tanks which poses a health risk because of increased contamination by human faeces. The distance between the sanitary facilities and borehole water sites were also questionable and raises the question if proper Environmental Impact Assessment (EIA) is carried out when sites for drilling boreholes is selected.

Table 4 Comparison between bacterial indicator counts detected in private and communal boreholes during the rainy season. Results are presented as mean ± standard deviation (range)

<table>
<thead>
<tr>
<th></th>
<th>Total coliform</th>
<th>p-value</th>
<th>Faecal coliform</th>
<th>p-value</th>
<th>Faecal enterococci</th>
<th>p-value</th>
<th>Clostridium perfringens</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Private</td>
<td>85.6 ± 215.6</td>
<td>0.0500</td>
<td>19.0 ± 84.8</td>
<td>0.0333</td>
<td>9.6 ± 29.7</td>
<td>0.6324</td>
<td>18.2 ± 101.6</td>
<td>0.0086</td>
</tr>
<tr>
<td>(0–1000)</td>
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<td></td>
<td>(0–800)</td>
<td></td>
<td>(0–800)</td>
<td></td>
</tr>
<tr>
<td>Communal</td>
<td>55.4 ± 145.9</td>
<td></td>
<td>7.5 ± 27.0</td>
<td></td>
<td>11.2 ± 29.7</td>
<td></td>
<td>1.9 ± 11.1</td>
<td></td>
</tr>
<tr>
<td>(0–1000)</td>
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<td>(0–250)</td>
<td></td>
<td>(0–188)</td>
<td></td>
<td>(0–100)</td>
<td></td>
</tr>
</tbody>
</table>

*Wilcoxon rank-sum (Mann–Whitney) test

Figure 1 Presence–absence of somatic phages in private and communal boreholes
Very limited data is available regarding the microbiological quality of water from private boreholes drilled for domestic purposes in South Africa. This study addressed the issue in an area of Limpopo Province. However, more studies are needed in South Africa to get a broad picture of the microbiological quality of private borehole water. The Municipal by-law regarding the drilling and utilization of borehole water in private households should be reviewed in order to have proper control measures and improve the health of the community.

Acknowledgements
The assistance of undergraduate students Mr. P. Ramudingana and Mr. L. Nemarude is appreciated.

References


