Incidence of Entamoeba Histolytica in Well Water in Samaru-Zaria, Nigeria

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Abstract. The use of untreated or inadequately treated water can cause gastroenteritis and other waterborne diseases like amoebic dysentery, and presents immediate effects on a large number of population. Wells serve as the most affordable source of water in the rural areas but they are prone to surface runoff and seepages from septic systems/pit latrines. This research was aimed at assessing the incidence of Entamoeba histolytica in well water used for human consumption and other domestic activities in Samaru-Zaria, Nigeria. Associated risk factors of well water contamination were studied. Membrane filtration technique was employed in filtering 70 well water samples (of 20 liters each) at the flow rate of 3 liters/min through Millipore filter paper of nominal porosity of 0.45µm. Retained particulates were eluted in distilled water and concentrated by centrifugation. Wet mounts of the sediments were examined under 10x and 40x objectives of the light microscope. The incidence of Entamoeba histolytica was 38.6%. There was 72.9% level of parasitic contamination (including other parasites). Other medically important parasites were found in the well water samples, which included Enterobius vermicularis (2.9%), larvae of Strongyloides stercoralis (7.1%). The ANOVA and Chi Square (χ²) were used in the analysis of risk factors of well contamination (p ≤ 0.05).

Keywords: Entamoeba histolytica, well water, parasitic contamination, cysts, occurrence, Samaru-Zaria, Nigeria

1. INTRODUCTION

Potable and safe drinking water is indispensable for the health and economic development of a nation. Waterborne transmission is a highly effective means for spreading infectious agents to a large portion of a population. In selecting a site for a house or village; the first consideration should be the presence, within a reasonable distance of a good supply of potable water. It is necessary that the people using the wells should be shown how to protect them from surface runoffs and direct seepages from septic tanks, so that they may be kept clean and may not become a source of danger (Hurst et al., 2002, CDC, 2014). Aside from bacteria, other organisms like algae, fungi, protozoa and viruses can be present in water (Odeyemi and Olanipekum, 2007). Approximately, 40 million people worldwide suffer from amoebiasis per year, with 40,000 deaths due to amoebic dysentery, intestinal diseases and liver abscess (Munazza, 2011). As such, supply of safe drinking water has been emphasized by WHO in order to reduce the prevalence of parasitic diseases (Ghodratollah, 1999).

Wells that are either open and/or are at the same level with the ground are exposed to surface runoffs or contamination by septic system failures (Centers for Disease Control and Prevention (CDC), 1977; Moore et al., 1993; Barwick et al., 2000; Karanis, 2007). Disease outbreaks can be caused by water contamination and through drinking of contaminated water (CDC, 2014). Three ways to contract amoebiasis are through contaminated water, contaminated food and direct faecal-oral route (Robert, 2002; Jonathan et al., 2012). Contaminants in water can lead to health issues such as gastrointestinal illness. Microbial and parasitic contamination of a private well can impact not only the household served by the well, but also nearby households using the same aquifer (CDC, 2014).

An estimate of 4 billion cases of diarrhea and 1.9 million deaths exist in developing countries mostly among young children due to inadequate access to safe drinking water and improper sanitation (Daniele and Williams, 2008). Since the establishment of the Safe Drinking Water Act (SDWA) of 1974, with amendments in 1986 and 1996 for the protection of water sources like ground water wells, the private wells serving less than 25 individuals were not included. Therefore, safeguarding these wells is the responsibility of the owners (US EPA, 2004).

Surveillance of drinking water sources is imperative to minimize contaminations by protozoa...
and ensure continuous supplies of healthy water world-wide (Bouzid et al., 2008). This study was aimed at finding the incidence of *Entamoeba histolytica* in well water used for human consumption and other domestic activities in Samaru-Zaria, Nigeria, and to assess possible risk factors in well water parasitic contamination.

2. MATERIALS AND METHODS

2.1. Study Area

Samaru-Zaria is located in Kaduna State in Northern Nigeria on latitude 11.1667°, longitude 7.6333° and 2398 feet above the sea level (http://www.fallingrain.com/world/NI/00/Samaru.htm).

The study area was sub-divided into four sampling locations, which included: (a) Quarter ‘3’; (b) Market Area; (c) Hayin Dogo and; (d) Daranka, all in Samaru-Zaria, Nigeria.

2.2. Sample Collection and Procedure

A total of 70 well water samples were collected from randomly selected wells in Samaru-Zaria, Nigeria. The samples were obtained by fetching water into graduated galloons (20L) and then conveying them to the laboratory in Department of Microbiology, Faculty of Science, Ahmadu Bello University Zaria, Nigeria.

2.3. Laboratory Procedure

Preliminary preparation of the well water samples was done by diluting them with normal physiological saline (0.85%). Membrane filtration of water samples using the analytical procedure of membrane filtration technique (Millipore filter paper of nominal porosity of 0.45µm) at a flow rate of 3 liters/min was carried out immediately on arrival at the laboratory (Kulkarni et al., 1993; Aldom and Chagla, 1995; Medema, 1999). Retained particulates containing protozoan cysts were eluted from the filter in distilled water and concentrated by centrifugation (Musial, 1987; US EPA, 2001, US EPA, 2005; Bouzid et al., 2008; Azman et al., 2009). Lugol’s iodine was used for wet mount preparations of recovered sediments after the centrifugation and microscopy was done using x10 and x40 objectives of the light microscope (Parija and Prabhakar, 1995). Colour atlases were used in the identification of the parasites (Sullivan, 2000; Chiodini et al., 2001; Kayser, 2005). Data collected regarding the wells were subjected to statistical analysis by Analysis of Variance (ANOVA), Chi Square (\(\chi^2\)) and simple arithmetic percentages (Giuliano and Polanowicz, 2008).

3. RESULTS

The incidence values for *E. histolytica*, and other medically important parasites (*Enterobius vermicularis, Strongyloides stercoralis*) were evaluated (table1). Other parasites encountered in some of the wells were not of medical importance. Of all the 70 sampled wells, 51(72.9%) were found to contain one or more of the listed parasites in the table, while 19 (27.1%) of the wells were found to be free of those parasites (fig 1). The mean distribution of parasites based on the sampled areas compared by ANOVA showed statistical significance between the levels of contamination of the well water samples and the various sampling areas. The highest mean of contamination recorded for *Entamoeba histolytica* was in Quarter ‘3’ (1.53±0.40) while the least was 0.11±0.11 in Danraka (table 2).

The frequency of *Entamoeba histolytica* occurrence in the four sampling locations evaluated by Chi Square (\(\chi^2\)) analysis (table 3) indicated that the cysts of *E. histolytica* occurred most in Hayin Dogo (61.5%). Quarter ‘3’ had the highest parasitic contamination of 94.1%, but the Market Area had the least parasitic contamination.

The effectiveness of the physical protection of wells by covering/casing was evaluated using categorized variables: “Uncovered” and “Covered” for open wells and properly cased wells respectively. There was more occurrence of *E. histolytica* in the uncovered wells (43.3%) than in the covered wells (35.0%). The parasitic contamination was also higher in the uncovered wells with an occurrence of 80.0%, while the covered wells had 65.0% (table 4).

The index of surrounding hygiene of the sampled wells was analyzed in a Chi Square test, categorized as “Clean” and “Unclean”. Significant differences occurred. *Entamoeba histolytica* cysts occurred more in wells with unclean surroundings (48.9%), while those wells with relatively clean surroundings had 20.0%. The parasitic contamination was also higher in wells with unclean surroundings than in those wells with relatively clean surroundings (table 5).

Water clarity level was related to presence of parasites in a Chi Square test with the variable categorized as “Clear” and “Turbid”. Wells with turbid water had higher contamination with *Entamoeba histolytica* (51.9%) than those with relatively clear water (30.2%). Also, the parasitic contamination was lesser in clear water than in the turbid well water samples (table 6).

The risk factor of grazing or roaming animals around the wells revealed significant differences in
the occurrences of parasites between wells frequented by grazing animals and those not. Wells that were frequented by roaming animals had higher contamination of 53.1% for *E. histolytica*. Parasitic contamination was also high in the wells frequented by animals (81.3%) than in those not (63.2%) as illustrated in table 7.

**Table 1**: Occurrence of *Entamoeba histolytica* cysts and other Parasites in well water in Samaru-Zaria, Nigeria

<table>
<thead>
<tr>
<th>Parasite</th>
<th>*Number of positive samples out of 70</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Entamoeba histolytica</em> cysts</td>
<td>27</td>
<td>38.6</td>
</tr>
<tr>
<td><em>Enterobius vermicularis</em> ova</td>
<td>2</td>
<td>2.9</td>
</tr>
<tr>
<td><em>Strongyloides stercoralis</em> larvae</td>
<td>5</td>
<td>7.1</td>
</tr>
<tr>
<td>Total</td>
<td>34</td>
<td>48.6</td>
</tr>
</tbody>
</table>

*Co-contamination of two parasites occurred in (four) wells. Other parasites are not of medical importance.

**Table 2**: Mean distribution of parasites according to sampling areas

<table>
<thead>
<tr>
<th>Sampling Location</th>
<th>Number of Samples out of 70</th>
<th>Means ± SEM Entamoeba*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quarter 3</td>
<td>17</td>
<td>1.53 ± 0.40</td>
</tr>
<tr>
<td>Market Area</td>
<td>31</td>
<td>0.42 ± 0.15</td>
</tr>
<tr>
<td>Hayin Dogo</td>
<td>13</td>
<td>1.38 ± 0.39</td>
</tr>
<tr>
<td>Daranka</td>
<td>9</td>
<td>0.11 ± 0.11</td>
</tr>
</tbody>
</table>

*F = 5.440, df = 3, p = 0.002. Mean values with different superscripts are significantly different. Mean values were separated using Duncan’s multiple range tests (DMRT). F = ANOVA value; a, b = means that were significantly different; SEMs standard error mean.

**Table 3**: Frequency of parasite occurrence according to the sampling area

<table>
<thead>
<tr>
<th>Location</th>
<th>Number of Samples out of 70</th>
<th><em>Entamoeba</em></th>
<th>Parasitic Contamination**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quarter 3</td>
<td>17</td>
<td>10 (58.8)</td>
<td>16 (94.1)</td>
</tr>
<tr>
<td>Market Area</td>
<td>31</td>
<td>8 (25.8)</td>
<td>19 (61.3)</td>
</tr>
<tr>
<td>Hayin Dogo</td>
<td>13</td>
<td>8 (61.5)</td>
<td>9 (69.2)</td>
</tr>
<tr>
<td>Daranka</td>
<td>9</td>
<td>1 (11.1)</td>
<td>6 (66.7)</td>
</tr>
</tbody>
</table>

*x² = 10.833, p = 0.013;  **x² = 5.980, p = 0.113

**Fig. 1**: Parasitic contamination profile of wells in Samaru-Zaria, Nigeria
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Table 4: Effects of Well Casing on the Level of Parasitic Contamination

<table>
<thead>
<tr>
<th>Protection</th>
<th>Number of Samples out of 70</th>
<th>Entamoeba* Count and % Positive</th>
<th>Parasitic Contamination** Count and % Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uncovered</td>
<td>30</td>
<td>13 (43.3)</td>
<td>24 (80.0)</td>
</tr>
<tr>
<td>Covered</td>
<td>40</td>
<td>14 (35.0)</td>
<td>26 (65.0)</td>
</tr>
</tbody>
</table>

\[ \chi^2 = 0.502, \ p = 0.478; \ \chi^2 = 1.890, \ p = 0.169 \]

Table 5: Nature of Surrounding Hygiene and Effects on the Level of Well Contamination

<table>
<thead>
<tr>
<th>Surrounding Hygiene</th>
<th>Number of Samples out of 70</th>
<th>Entamoeba* Count and % Positive</th>
<th>Parasitic Contamination** Count and % Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clean</td>
<td>25</td>
<td>5 (20.0)</td>
<td>14 (56.0)</td>
</tr>
<tr>
<td>Unclean</td>
<td>45</td>
<td>22 (48.9)</td>
<td>36 (80.0)</td>
</tr>
</tbody>
</table>

\[ \chi^2 = 5.661, \ p = 0.017; \ \chi^2 = 4.536, \ p = 0.033 \]

Table 6: Clarity of Water and Contamination Levels

<table>
<thead>
<tr>
<th>Clarity</th>
<th>Number of Samples out of 70</th>
<th>Entamoeba* Count and % Positive</th>
<th>Parasitic Contamination** Count and % Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clear</td>
<td>43</td>
<td>13 (30.2)</td>
<td>29 (67.4)</td>
</tr>
<tr>
<td>Turbid</td>
<td>27</td>
<td>14 (51.9)</td>
<td>21 (77.8)</td>
</tr>
</tbody>
</table>

\[ \chi^2 = 3.272, \ p = 0.070; \ \chi^2 = 0.868, \ p = 0.351 \]

Table 7: Effect of Grazing (Roaming) Animals around the Wells on the Level of Well Contamination

<table>
<thead>
<tr>
<th>Grazing Animals</th>
<th>Number of Samples Out of 70</th>
<th>Entamoeba* Count and % Positive</th>
<th>Parasitic Contamination** Count and % Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absent</td>
<td>38</td>
<td>10 (26.3)</td>
<td>24 (63.2)</td>
</tr>
<tr>
<td>Present</td>
<td>32</td>
<td>17 (53.1)</td>
<td>26 (81.3)</td>
</tr>
</tbody>
</table>

\[ \chi^2 = 5.269, \ p = 0.022; \ \chi^2 = 2.786, \ p = 0.095 \]

4. DISCUSSION

Most rural and urban areas of Nigeria depend on ground water (Jokotagba et al. 2012). This is the situation in Samaru-Zaria. Parasitic contamination of well water is a threat to water safety and human health. The highest mean occurrence of *E. histolytica* cysts was 1.53 ± 0.40 at Quarter “3”. Most of the wells in Quarter “3” are public wells and are poorly handled, always uncovered with stray animals always coming around to drink stagnant water around the wet surroundings. Animals’ fecal wastes can be accidentally transferred into the wells through the fetching ropes that lash the ground. The high level of parasitic contamination of 51(72.9%) out of 70 well water samples was far higher than that in the study of Yousefi et al. (2009) were only 19.9% of samples were parasitic. *Entamoeba histolytica* was the most prevalent of all the parasites found in Samaru wells because they form cysts that are resistant to chlorination (Yousef et al., 2009; Sam and Bob, 2013). Wells that were without casings (uncovered) had higher chances of being contaminated with protozoan cysts. There was 43.3% occurrence of *E. histolytica* cysts in uncovered wells but lesser in wells that were properly covered. It is particularly important that private wells should be kept under favorable surrounding hygiene. Wells with unhygienic surroundings presented higher occurrence of *E. histolytica* cysts (48.9%). The presence of parasites like *Entamoeba histolytica* can result in disease outbreaks (CDC, 2114; Barwick et al., 2000). It has resulted in nine (2.8%) of the total 30% water-associated outbreaks reported in North American and European countries (Karanis et al., 2007).

In the tropics amebiasis is transmitted via fecal-contaminated water due to poor environmental and institutional sanitation (Jonathan et al., 2012). This could be due to possible cross contaminations by external factors like rain splashes and/or air currents containing fecal-borne debris. It was also found out that wells with turbid water largely presented more parasitic contamination but those with clearer water had less protozoan cysts. Turbidity can interfere with the action of chlorine. The suspended particles provide attachment surfaces for parasites. It is important to exclude the activities of (domesticated, roaming, grazing) animals from near the wells. This will be in an effort to prevent fecal cross contamination of the wells. Wells that were always
frequented by animals showed higher occurrence of *E. histolytica* cysts (53.1%) than those not. However, parasites were found in covered wells with clean areas and in the absence of grazing because ground water are prone to fecal contamination from a variety of sources (Morteza A (2001) like closeness to a septic tank (Zamzaka et al. 2004; Louis and Egbuna, 2012). Contamination of a single well is not limited to that well alone but also wells using the same aquifer (CDC, 2014). As such, even wells that were covered with clean surroundings and excluded from animals had parasites.

5. CONCLUSION

Generally, the wells in Samaru-Zaria, Nigeria had high level of parasitic contamination, where 51 out of 70 wells were examined with parasites, but there was total occurrence of 48.8% of medically important parasites. The incidence of *Entamoeba histolytica* was 38.6%. The local people have little to virtually no essential awareness of the possible presence and danger of pathogenic organisms in well water and so go about using inadequately protected wells. Cleaning of surrounding area, covering of wells and absence of grazing animals are important factors that can reduce incidence of *E. histolytica* in well water because wells with opposite conditions had higher incidence of parasites. The contamination of one well can affect surrounding wells served by the same aquifer. Although, most rural areas and parts of urban areas lack good water supply, water obtained from hand-dug wells should be treated by boiling, filtration and/or chlorination before use. Therefore, unless a well is adequately protected or its water is treated, should not be used for domestic consumption.

REFERENCES


Mr. Henry Gabriel Bishop was born on 1st January, 1989 in Bitaro-Kwoi, Jaba Local government Area of Kaduna State, Nigeria. He obtained his first degree (BSc. Microbiology, FIRST CLASS) from Ahmadu Bello University Zaria (A.B.U.), Nigeria in 2012. He was a recipient of Certificate of Recognition and A.B.U. Student of Excellence Award by the Vice Chancellor, Student Affairs Division, A.B.U. Zaria in May, 2013. He is presently undergoing his Masters Degree programme in Medical Microbiology (Parasitology) at the Department of Microbiology, A.B.U Zaria, where he is also a staff (Graduate Assistant). He has interest in immunology and immunobiology of Parasites (*Schistosoma haematobium* in co-infection with bacteria in children). Mr. Bishop is a writer (of novels and inspirational books). He has one conference paper and three journal papers in press.

Associate Professor Dr. Helen I. Inabo obtained her first degree in 1984 from Ahmadu Bello University (A.B.U.), Zaria, Nigeria. She later pursued her Masters degree in Medical Microbiology (Parasitology) in A.B.U. Zaria and graduated in 1991. Dr. H.I. Inabo obtained her doctorate from A.B.U. Zaria in 1996. She is currently an Associate Professor in the Department of Microbiology, A.B.U. Zaria. She has 65 peer reviewed journal publications (international and internal) and several conference papers. She had graduated 25 post graduates (MSc. and ph.D). Her area of research is parasitology.