Original Article

Prevalence of Cryptosporidium and Giardia lamblia in Water Samples from Jeddah and Makkah Cities

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Abstract: Water contamination by Giardia lamblia and Cryptosporidium is one of the causes of diarrhoea throughout the world. A total of 161 and 84 samples were collected from Jeddah and Makkah cities, respectively. Each sample was concentrated by double centrifugation and the sediment was examined as a wet smear and after staining with Trichrome and Kinyoun stains. The results showed that 56 (35%) and 1 (0.62 %) samples of Jeddah were positive for the oocyst of Cryptosporidium and cyst of Giardia, whereas only 21 (25%) and 2 (2.4 %) samples of Makkah showed positivity for oocysts and cyst of these parasites. Overall Cryptosporidium contamination in bottled water and water from filling stations was 6.8% and 17.4%, respectively. Maximum contamination for Cryptosporidium was recorded in tap water which was 51% and 25% in Jeddah and Makkah, respectively.

Keywords: Cryptosporidium, Giardia, Water contamination, Jeddah, Makkah.

1. Introduction

Cryptosporidiosis and Giardiasis are waterborne diseases prevalent all over the world. Both of them are closely linked to urban slum conditions such as overcrowding and high level of faecal pollution by animal and human excreta in developing countries [1, 2]. Organisms of the genus Cryptosporidium are small parasites that infect microvilli of epithelial cells, lining the digestive and respiratory organs of vertebrates [3-6]. Cryptosporidium is a major cause of acute diarrhoea in children and chronic persistent diarrhoea in HIV-infected individuals with low CD4 counts. The infection is self-limiting in immunocompetent hosts but can be severe, persistent and life threatening in the immune compromised and malnourished [7-11]. Giardia lamblia infections usually clear up within a few weeks. It is marked by abdominal cramps, bloating, nausea and bouts of watery diarrhoea. Infection is more common in children than in adults [12-14]. Giardia lamblia can cause asymptomatic colonization or acute or chronic diarrhoeal illness. The organism has been found in raw water supplies. It is a common cause of chronic diarrhoea and growth retardation in children in developing countries, including Saudi Arabia [15]. Since the source of infection for both cryptosporidiosis and giardiasis is contaminated water; the present study was undertaken to see the contamination rate of different sources of water in Jeddah and Makkah cities and its probable role in transmission of these diseases.

2. Materials and Methods

Water samples were collected in sterile containers from randomly selected houses, mosques and schools in Jeddah and Makkah cities. Samples were placed in the refrigerator at 4°C until the day of examination. Each sample was concentrated by double centrifugation (at 5300 rpm for 10 min.) and the sediment was examined as a wet smear as well as after staining with Trichrome and Kinyoun stains to see the presence of oocyst and cyst of Cryptosporidium and Giardia lamblia as described earlier [16,17].

Oocysts in the specimens are usually difficult to detect without special stains. Modified Acid-Fast Stain (Kinyoun's stain) was used to detect oocysts of Cryptosporidium. Slides were prepared from the sediment obtained after centrifugation and fixed in absolute methanol for 30 Seconds. These slides were stained with Kinyoun's carbol fuchsin for 5 minutes. After destaining with 1% aqueous sulfuric acid (2-3 minutes), the slides were thoroughly washed with...
water. These slides were now treated with Loeffler’s alkaline methylene blue and then washed and air dried. Now slides were examined under 100x objective lens of a research microscope for the presence of cyst of *Cryptosporidium*.

Trichrome Stain is rapid and simple which gives excellent differentiation of the morphological details of intestinal parasites. It consists of six jars. The jar that contains the trichrome stain has a band around it that heat the stain to 53°C. The heated stain results in more efficient penetration into organisms. The modified decolorizer aides in enhancing the trichrome stain. Stains and Trichrome stains showed much better results in 90% alcohol for 20 seconds and then dipped four times in 90% alcohol for dehydration. After the staining and dehydration process, these slides were treated with a clearing agent (Xylene) and finally mounted in a suitable mounting medium and examined under 100x objective lens of the microscope for the presence of *Giardia* cyst.

The chi-square ($\chi^2$) test was applied to assess the trends of these contaminations. A $-value of <0.05 was considered significant.

### 3. Results

Kinyoun and Trichrome stains showed much better results in detecting *Cryptosporidium* and *Giardia* as compared to wet smear (p < 0.001) and therefore considered as a method of choice for this study. Earlier workers had also made a comparative study of using aforesaid techniques for detection of *Cryptosporidium* and *Giardia* [18-21].

Results are summarized in Tables 1-8 and Fig. 1-6. Oocysts and cysts of both *Cryptosporidium* and *Giardia lamblia* were recovered from water samples collected from houses, mosques and schools from Jeddah and Makkah (Figs. 1-3). 35% and 25% samples were contaminated with *Cryptosporidium* oocyst in Jeddah and Makkah, respectively (Table 1). 11.6% of drinking water and 51% of tap water samples were positive for *Cryptosporidium* in Jeddah. In Makkah, there was no contamination of *Cryptosporidium* oocyst in drinking water, but it was present in 25% of tap water samples. 0.62% and 2.4% of Jeddah and Makkah tap water samples were found positive for *Giardia lamblia* cyst, whereas drinking water was devoid of this contamination (Table 2, 3).

### Table 1. Prevalence of *Cryptosporidium* and *Giardia* in Jeddah and Makkah cities.

<table>
<thead>
<tr>
<th>Parasite</th>
<th>Stain used</th>
<th>Tap Water</th>
<th>Drinking Water</th>
<th>Total</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Cryptosporidium</em></td>
<td>Kinyoun</td>
<td>63/124</td>
<td>14/121</td>
<td>77/245</td>
<td>0.001</td>
</tr>
<tr>
<td><em>Giardia</em></td>
<td>Trichrome</td>
<td>3/124</td>
<td>0/121</td>
<td>3/245</td>
<td>0.24</td>
</tr>
</tbody>
</table>

Drinking refilled bottled water samples showed less contamination and only 11.6% contained *Cryptosporidium* oocysts (p<0.001) and no cyst for *Giardia*. But *Giardia* cysts were found in 2.4% of tap water samples. Samples which were collected from tap water that was transported by pipes showed a *Cryptosporidium* contamination rate of 43% and those transported by truck showed a still higher rate of contamination of 54.5% (p <0.001). There were only 5 tap water samples supplied by wells, 80% of them showed positive results for *Cryptosporidium* (Fig. 4). Bottled water and water from filling stations had a contamination rate of 6.8 and 17.4%, respectively. *Giardia* contamination was found only in 2.5% of samples transported by pipes and was not seen in water samples from wells nor those transported by trucks (Table 4).
Prevalence of Cryptosporidium and Giardia lamblia in Water Samples

Table 4. Distribution of *Cryptosporidium* and *Giardia* in different sources of water.

<table>
<thead>
<tr>
<th>Parasite</th>
<th>Stain used</th>
<th>Pipe Water</th>
<th>Tap Water</th>
<th>Truck</th>
<th>Well Company</th>
<th>Filling Station</th>
<th>Total</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cryptosporidium</td>
<td>Kinyoun</td>
<td>52/121</td>
<td>12/22</td>
<td>4/5</td>
<td>5/74</td>
<td>4/23</td>
<td>77/245</td>
<td>0.001</td>
</tr>
<tr>
<td>Giardia</td>
<td>Trichrome</td>
<td>3/121</td>
<td>0/22</td>
<td>0/5</td>
<td>0/74</td>
<td>0/23</td>
<td>3/245</td>
<td>0.11</td>
</tr>
</tbody>
</table>

Fig. 4. Contamination of water sources by *Cryptosporidium* and *Giardia*.

Fig. 5. Presence of *Cryptosporidium* and *Giardia* in tap water and bottled water.

Fig. 6. Presence of *Cryptosporidium* in water samples collected from Jeddah and Makkah by wet smear and Kinyoun technique.
Table 5. Prevalence of Cryptosporidium and Giardia in filtered and unfiltered water.

<table>
<thead>
<tr>
<th>Parasite</th>
<th>Stain Used</th>
<th>Filtered Water</th>
<th>Unfiltered Water</th>
<th>Total</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cryptosporidium</td>
<td>Kinyoun</td>
<td>5/13</td>
<td>27/232</td>
<td>32/245</td>
<td>0.38</td>
</tr>
<tr>
<td>Giardia</td>
<td>Trichrome</td>
<td>0/13</td>
<td>3/232</td>
<td>3/245</td>
<td>0.84</td>
</tr>
</tbody>
</table>

Table 6. Prevalence of Cryptosporidium and Giardia in people of different economic status.

<table>
<thead>
<tr>
<th>Parasite</th>
<th>Stain Used</th>
<th>Rich</th>
<th>Medium</th>
<th>Poor</th>
<th>Total</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cryptosporidium</td>
<td>Kinyoun</td>
<td>2/4</td>
<td>23/46</td>
<td>5/11</td>
<td>30/61</td>
<td>0.81</td>
</tr>
<tr>
<td>Giardia</td>
<td>Trichrome</td>
<td>0/4</td>
<td>0/46</td>
<td>0/11</td>
<td>0/61</td>
<td>0.64</td>
</tr>
</tbody>
</table>

Tap water was found to be used by people for the purpose of washing and cleaning. However, some people still use tap water for drinking. In this study, 14.3% of tap water samples obtained from the houses of people who used it for cleaning and washing only. Furthermore, 12.5% of samples collected from houses where people use tap water for washing, cleaning and drinking, were positive for Cryptosporidium (Table 7). On the other hand, none of these samples showed Giardia cysts. Half of the Ablution water samples that were obtained from Jeddah’s mosques were positive for Cryptosporidium and it gives a total of 50%.

Table 7. Prevalence of Cryptosporidium and Giardia in water used for different purposes.

<table>
<thead>
<tr>
<th>Parasite</th>
<th>Stain Used</th>
<th>Washing/Cleaning/Drinking</th>
<th>Washing / Cleaning</th>
<th>Ablution</th>
<th>Total</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cryptosporidium</td>
<td>Kinyoun</td>
<td>12/24</td>
<td>20/39</td>
<td>20/40</td>
<td>52/103</td>
<td>0.98</td>
</tr>
<tr>
<td>Giardia</td>
<td>Trichrome</td>
<td>0/24</td>
<td>0/39</td>
<td>1/40</td>
<td>1/103</td>
<td>0.27</td>
</tr>
</tbody>
</table>

The effect of water filters was also taken into the consideration for the presence or absence of Cryptosporidium and/or Giardia (Table 5). Cryptosporidium oocysts were found in 27 (11.6%, n = 232) of unfiltered water compared to 5 (38.5%, n = 13) of filtered water samples (p <0.38). Giardia cysts were found in 1.3% of unfiltered water, but not in filtered water. Only 13 houses out 232 included in this study were using water filters indicating that 94.7% of the samples were unfiltered and only 5.3% were filtered. 38.2% of water samples were collected as desalinated water that was transported through pipes showed 38% positivity for Cryptosporidium and 2.3% for Giardia. Refilled drinking bottled water too had contamination. About 26.2% of such bottled water was contaminated by Cryptosporidium but negative for Giardia.

This study also looked at the economic state and its relation to the prevalence of Cryptosporidium and/or Giardia. Two third of water samples were collected from houses of medium income individuals which contained Cryptosporidium in 50% of cases (p <0.81). Similar contamination rates were recorded from the houses of poor and rich people. As for different sites like houses, mosques and schools are concerned, all of them showed positivity for Cryptosporidium oocyst. 33 (27%, n = 122) were positive from houses, 31 (39.2%, n = 89) from mosques and 13 (29.5%, n = 44) from schools. As for contamination by the Giardia cyst is concerned, it was 2 (4.5%, n = 44) and 1 (1.3%, n = 79) in schools and mosques, respectively (Table 8).

Table 8. Prevalence of Cryptosporidium and Giardia in samples collected from houses, schools and mosques.

<table>
<thead>
<tr>
<th>Parasite</th>
<th>Stain Used</th>
<th>Houses</th>
<th>School</th>
<th>Mosques</th>
<th>Total</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cryptosporidium</td>
<td>Kinyoun</td>
<td>33/122</td>
<td>13/44</td>
<td>31/79</td>
<td>77/245</td>
<td>0.07</td>
</tr>
<tr>
<td>Giardia</td>
<td>Trichrome</td>
<td>0/122</td>
<td>2/44</td>
<td>1/79</td>
<td>3/245</td>
<td>0.06</td>
</tr>
</tbody>
</table>

4. Discussion

Most of the references about Cryptosporidiosis and Giardiasis are related with their positivity in diarrhoeal patients, particularly in children. There are only a few references regarding water contamination by these parasites from few countries. During present study, it was found that Cryptosporidium contamination was more in Jeddah than Makkah (i.e. 51% and 25%). This raises a big question about the cleanliness of water supplied to the people. Higher contamination rates (23-26%) of Cryptosporidium oocyst were recorded from different sources of drinking water in Brazil, Canada and Spain as well [13, 22, 23]. It seems that the contamination in Jeddah and Makkah would have occurred during transportation through damaged pipes by sewage. The majority of such tap water is usually transported to houses, schools and mosques using pipes and then stored in water reservoirs underground till used. Many pipes are broken and their water content might be contaminated by seepage since there is no efficient sewage system available in both the cities. Reason for less contamination rate by Cryptosporidium oocyst in Makkah could not be ascertained, as mode of transport of water is almost same in both the cities. Small sample size and better maintenance of pipeline might be the reason. Earlier clinical studies showed that in Jeddah, 32% of the symptomatic and 4.7% of asymptomatic children were found to be excreting Cryptosporidium oocysts [24]. Still higher rate of Cryptosporidiosis (60-70%) was recorded in immunocompromised patients in Riyadh [9]. The prevalence of human Cryptosporidium infection in

Saudi Arabia and neighboring countries ranged from 1% to 37% with a median of 4% [25]. In Kuwait out of 3.4-4% cases of cryptosporidial diarrhoea, the majority (41.4%) were children in the 4–8 years of age [26]. These findings clearly indicate that this higher prevalence rate of cryptosporidiosis is due to drinking water contaminations with Cryptosporidium oocyst. As for giardiasis is concerned, we observed Giardia cyst in 0.62% and 2.4% tap water samples of Jeddah and Makkah, respectively. Slightly higher rate of around 6% contamination by Giardia cyst was recorded in Brazil [13]. Still higher rate of Giardia contamination in treated water (18-19%) was recorded in Canada and Spain [22, 23]. Earlier workers recorded 4-10% prevalence rate of Giardiasis in diarrhoeic patients of northern, southern, eastern, western and central regions of the Kingdom. These findings were based on stool examinations in the above mentioned regions of the kingdom where most children of 5-13 years of age were infected [27, 28]. All these findings strongly suggest that it is contaminated water, which contributes to giardiasis in the kingdom as well as other countries, and supports present findings in which we reported 2.4% tap water contaminated with Giardia cyst in Jeddah tap water. Cryptosporidium cyst was observed in unfiltered as well as filtered water, which reflects the maintenance problem. However, the filter quality and maintenance record were never examined. Cryptosporidium contamination rate was almost same in the samples collected from the houses of poor, medium and rich persons. This clearly suggests that the economic status of the individual has no impact on the prevalence of these two parasites (Table 6).

In the present study both bottled as well as tap water in Jeddah and Makkah which cater the need of people for drinking and washing purposes showed contamination with Cryptosporidium oocyst, along with few Giardia cyst in tap water, might be infecting the people in these and other cities as well. It may be taken as a serious warning by Saudi authorities who may ask the person concerned responsible for examination of water samples for parasitic contamination to analyse the drinking water samples properly and find out the lapses so that remedial measure must be applied to remove contaminations of these parasites which are the causative agents of diarrhoea, particularly in children who are most susceptible and may succumb to it.

5. Recommendation

On the basis of the findings of the present study, it is concluded that the tap and drinking water samples from Jeddah and Makkah showed a considerable amount of contamination by Cryptosporidium and Giardia parasites. Since, both these parasites are the causative agents for diarrhoea in a fair percentage of the population, need immediate attention. Corrective measures must be taken to get rid of these infections from both drinking and tap water so that waterborne diarrhoea, which sometimes leads to fatality especially in young children, could be prevented.

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References

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