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Prevalence of C*ryptosporidium* 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 Kinyioun 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.

Key words: *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 4^0C till 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 Kinyoun and Trichrome 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

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stained with Kenyon's carbol fuchsine for 5 minutes. After de-staining with 1% aqueous sulfuric acid (2-3 minutes) the slides were thoroughly washed with water. These slides were now treated with Loffler'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 C*ryptosporidium.*

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. Slides were placed in the first two jars which contain 70% alcohol + iodine for 1 min. each. These slides, then dipped four times in a jar containing 70% alcohol. Now slides were left in warm trichrome stain 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 (χ^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].

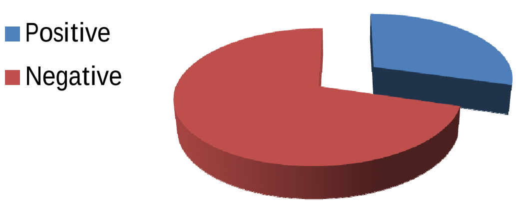

Figure 1: Presence of *Cryptosporidium* in water from schools.

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 (Fig. 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).

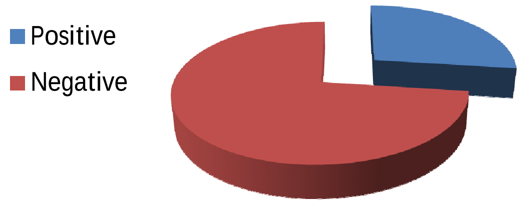

Figure 2: Presence of *Cryptosporidium* in water from houses.

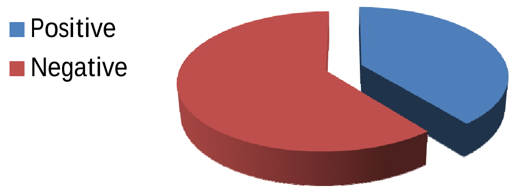

Figure 3: Presence of *Cryptosporidium* in water from mosques.

Parasite	Stain Used	Jeddah	Makkah	Total	P Value
Cryptosporidium	Kinyoun	56/161	21/84	77/245	0.07
Giardia	Trichrome	1/161	2/84	3/25	0.27

Table 1: Prevalence of *Cryptosoridium* and *Giardia* in Jeddah and Makkah cities.

Parasite	Stain used	Tap Water	Drinking Water	Total	P Value
Cryptosporidium	Kinyoun	63/124	14/121	77/245	0.001
Giardia	Trichrome	3/124()	0/121	3/245	0.24

Table 2: Prevalence of *Cryptosporidium* and *Giardia* in tap water and drinking water in Jeddah.

Parasite	Stain Used	Tap Water	Drinking Water	Total	P Value
Cryptosporidium	Kinyoun	21/84	0/84	21/84	0.07
Giardia	Trichrome	2/84	0/84	2/84	0.27

Table 3: Prevalence of *Cryptosporidium* and *Giardia* in tap water and drinking water in Makkah.

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).

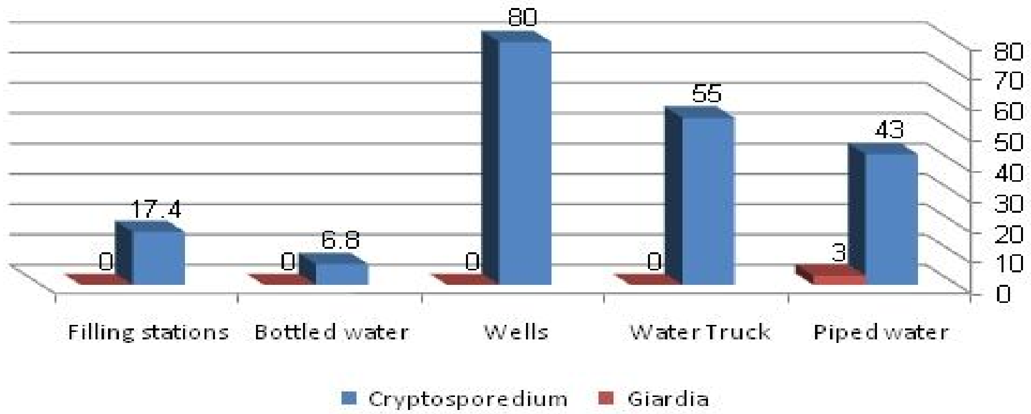

Figure 4: Contamination of water sources by *Cryptosporidium* and *Giardia.*

Parasite	Stain used	Pipe Water	Tap Water	Truck	Wells Company Drinking. Water	Filling Station Drinking Water	Total	P value
Cryptosporidium	**Kinyoun**	**52/121**	**12/22**	**4/5**	**5/74**	**4/23**	**77/245**	**0.001**
Giardia	**Trichrome**	**3/121**	**0/22**	**0/5**	**0/74**	**0/23**	**3/245**	**0.11**

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

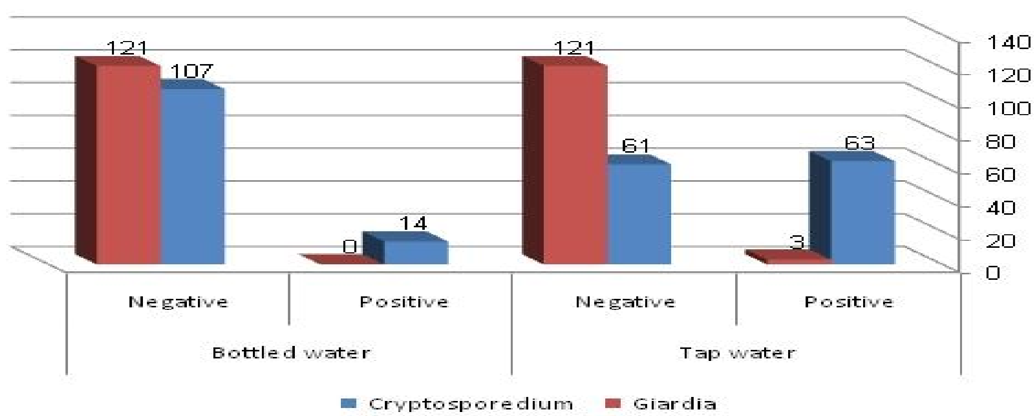

Figure 5: Presence of *Cryptosporidium* and *Giardia* in tap water and bottled water.

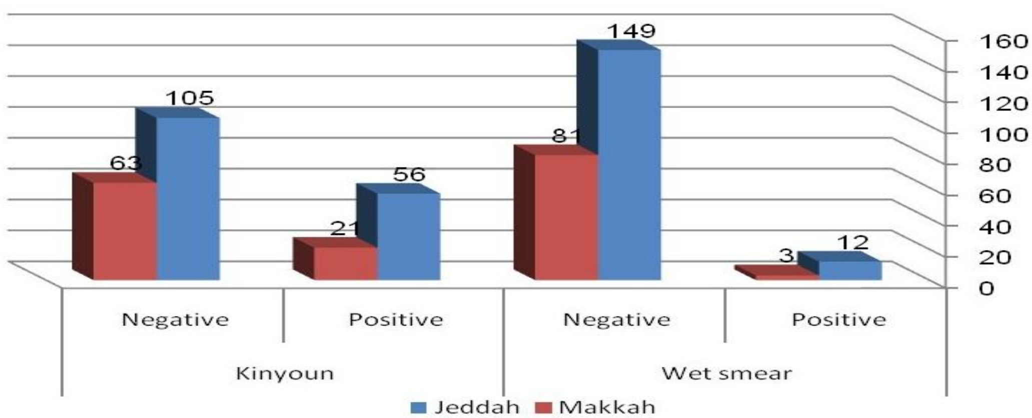

Figure 6: Presence of *Cryptosporidium* in water samples collected from Jeddah and Makkah by wet smear and Kinyoun technique.

Parasite	Stain Used	Filtered Water	Unfiltered Water	Total	P Value
Cryptosporidium	Kinyoun	5/13	27/232	32/245	0.38
Giardia	Trichrome	0/13	3/232	3/245	0.84

Table 5: Prevalence of *Cryptosporidium* and *Giardia* in filtered and unfiltered water.

Parasite	Stain Used	Rich	Medium	Poor	Total	P Value
Cryptosporidium	Kinyoun	2/4	23/46	5/11	30/61	0.81
Giardia	Trichrome	0/4	0/46	0/11	00/61	

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

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%.

Parasite	Stain used	Washing/Cleaning/ Drinking	Washing / Cleaning	Ablution	Total	P value
Cryptosporidium	Kinyoun	12/24	20/39	20/40	52/103	0.98
Giardia	Trichrome	0/24	0/39	1/40	1/103	0.27

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

The effect of water filters was also taken into the consideration for the presence or absence of *Cryptosporidium* and/or *Giardia* (Table 5). *Cryptosporidium* oocytes 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).

Parasite	Stain Used	Houses	School	Mosques	Total	P value
Cryptosporidium	Kinyoun	33/122	13/44	31/79	77/245	0.07
Giardia	Trichrome	0/122	2/44	1/79	3/245	0.06

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

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 are 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 immune-compromised 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 mostly 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.

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 water borne diarrhoea, which sometimes lead to fatality especially in young children could be prevented.

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