Molecular Detection of Cryptosporidium parvum in Different Water Sources of District Peshawar, Pakistan


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Abstract: Background cryptosporidium parvum is a zoonotic parasite which is a clinically important protozoan that causes Cryptosporidiosis infection, having typical symptoms include watery diarrhea, nausea, vomiting, fever and abdominal cramps etc. Material and Methods this study was designed for molecular detection of C. parvum in different water sources. A total of 300 water samples were collected from May, 2011 to April, 2012, from different water sources of district Peshawar and brought to the Department of Zoology KUST Khyber Pakhtunkhwa for further processing. The residue obtained after water filtration thorough whattman filter paper subjected to DNA extraction and PCR was conducted for detection of C. parvum. Results out of 300 water samples analyzed that the prevalence rate of C. parvum was 14.66% (44/300) in different water sources followed by 3.57% (256) in Tube well water, 2.74% (273) in Bore well water, 19.82% (22111) in Tap water and 30% (1860) in Drain water samples. While overall prevalence of C. parvum in different sites were unlike, in Saddar was 10% (6/60), in Shaheen Town, 17.5% (7/40), in University Town, 6.66% (4/60), in Hayatabad, 5% (3/60), in Khwaja Town, 12.5% (5/40) and in Faisal Colony, the prevalence rate was 47.5% (19/40). Conclusion from the current study, it was revealed that C. parvum is present in water sources of few areas in district Peshawar, which may be due to improper management of water scheme and flood. It is also revealed from the study that water should be properly treated for human consumption.

Key words: Cryptosporidium parvum · PCR · Zoonotic Parasite · Diarrhea · Flood

INTRODUCTION

Cryptosporidium parvum is a clinically important zoonotic parasitic protozan causes Cryptosporidiosis infection, transmitted by contaminated water having typical symptoms include watery diarrhea, vomiting, nausea, abdominal cramps and fever etc. [1]. Zoonotic infections have serious threat to the financial system and global health [2]. About 3.5 million people, including three million children die per year world wise and 98% deaths occur in the emerging republics because of extensive waterborne outbreaks. Each year 1.5 million deaths occur because of diarrheal diseases [3]. The association of Cryptosporidium with the host cell inhabiting out of the cells mostly in the epithelium of the mucosa [4]. Lakes, canal, ground water, rainwater, seawater and atmospheric water are the chief sources of drinking water, which depend on the pollutant sources and their pollution, are also different [5]. Waterborne transmission is the main pathway of disease concerned with a number of great outbreaks [6]. About 20% of childhood diarrheal cases occurred by Cryptosporidium in the developing countries [7]. A main reason of Cryptosporidiosis in children is diarrhea without or with human immunodeficiency (HIV) virus in emerging republics [8]. Water was contaminated at high level in the
tap water sources, especially in Faisal Colony in district Peshawar [3]. The contamination of different water sources in Khyber Pakhtunkhwa revealed that Cryptosporidium species were detected 19.5% microscopically [9] in district Peshawar 9% children were infected through C. parvum including 77.8% well water and 22.2% municipal water consumers [10].

C. parvum poses a significant risk to public health [11] spread by contaminated municipal water system and insufficiently treated water supplies and found throughout the world [12] which is main frequent enteric parasites of domestic animals [13]. The detection of C. parvum is different from the environmental and clinical samples. The techniques include microscopy, Polymerase Chain Reaction (PCR) and enzyme immunoassay (EIA) techniques [14]. We are facing a water crisis due to increasing world population and growing contamination of the usual resources [9]. Moreover, better off avoiding is to promote the practices of hand washing and hygienic standards in the mass [1]

Keeping the importance of the Cryptosporidium parasite in the water sources and its ill effect, the current study was designed to find out C. parvum in different water sources in district Peshawar using molecular techniques such as PCR.

MATERIALS AND METHODS

Study Area: The current study was carried out in District Peshawar, Khyber Pakhtunkhwa, having 1,257 square km area, lies between 33° 44' and 34° 15' north latitude and 71° 22' and 71° 42' east longitude. In district, Peshawar the total population is 2.019 million in which 1.061 million are Male population and 0.958 million are female population. Most the people have an occupation of agriculture, but few people are professional like doctors, engineers some are business persons and merchants etc. [3]. The samples were collected from Saddar, Shaheen Town, University Town, Hayatabad, Khwaja Town and Faisal Colony sites from 1st May, 2011 to 30th April 2012.

Samples Collection: Water samples (n=300) were collected in labeled (Date of collection, name of the area and the type of water) and sterilized bottles from six different areas for the detection of C.parvum including 56, tube well water, 73 bore well water, 111 tap water and 45 samples of drain water. The size of each sample was one liter and the experimental work was done in the Molecular Parasitology and Virology Laboratory, Department of Zoology KUST, Kohat. A well reported Performa was also filled during sample collection to obtain some information like socioeconomic condition of the people living at the collection sites for which the Ethical Committee of Kohat University of Science and Technology approved the current work [3].

Samples Processing: Whatman filter paper was used to filter the water samples in the Water Filtration Assembly. The filtered residue was centrifuged for 10 minutes at 6000 rpm, then the supernatant was discarded and the residue was obtained in eppendorf tubes for further centrifugation at 10000 rpm for 8 min. About 10il residues were placed on the slides and made a thin film on it through wooden stick and stained with Ziehl-Neelsen [10] and were observed under Tri ocular microscope at 10X, 40X and 100X magnification. The microscopically confirmed positive samples, identified [15] and were used for positive control [3].

DNA Extraction and DNA Amplification (PCR): DNA was extracted from 200il filtered residue of each sample through GF-1 Nucleic Acid Extraction Kits (Vivantis) with prescribed protocol [3, 12, 15]. Then extracted DNA was subjected to thermal cycler (NyxTechnix, USA) for PCR reaction along with Taq DNA polymerase (Fermentas, USA). The PCR mixture was composed of 5µL of extracted DNA with 10X PCR Buffer 2.2 µL, MgCl 2.4 µL, dNTPs, 1.0 µL, 1.0 µL of each 10 Pico moles of forward and reverse primers, dH O (Medicated), 7.1 µL and 0.3 µL Taq DNA polymerase enzyme [3, 15] with some modifications. Forward primer was AWA72F (3'-AGTGCTTAAAGCAGGCAACTG-5') and Reversed primer was AWA1235R (5'-CGTTAACGGAATTAACCAGAC-3') making 256-bp of the amplicon size targeting of 18S rRNA [12, 15]. The PCR program was set at 35 cycles, including initial denaturation was 96°C for 5 minutes while each cycle was composed of 3 steps denaturation at 96°C for 30 seconds, annealing at 65°C for 30 seconds and elongation at 72°C for 40 seconds [15].

Gel Electrophoresis: In 2% agarose gel 12µL sample containing 10µL of the PCR product mixture and 2µL loading dye was loaded along with 12µl of DNA Ladder (50bp). The gel was run at 500 mA current and 120 volts of voltage for 25 min. The gel was then examined by UV transiluminator. The specific DNA amplified product of each sample was determined by identifying 256-bp bands for C. parvum comparing with Fermentas GeneRuler 50 base pairs, DNA ladder (#SM0373) [12, 15].
Prevalence Rate: The prevalence rate was determined by the following formula \[3, 9, 12, 15\].

\[
\text{Prevalence Rate} = \frac{\text{No. of parasite detected in water sample}}{\text{Total no. of water samples examined}} \times 100
\]

Data Analysis: Statistical analysis was performed by using “STATISTIX”, version 9.0, Korean made software. Variables included for evaluation were tube well, bore well, tap and drain water and it values were considered significant [3].

RESULTS

A total of 300 water samples were collected from 6 different areas for the detection of \textit{C. parvum} including 56 samples of tube well water, 73 bore well water, 111 tap water and 60 samples were collected from drain water as shown in (Table 1).

Area Wise Prevalence: By means of molecular detection the present study showed that \textit{C. parvum} was absent in all samples collected from different sources, except tap water and drain water which showed 15\% (3/20) and 25\% (3/12) respectively in Saddar while 4.76\% (1/21) in tap and 21.43\% (3/14) in drain water samples collected from University Town. Similarly, in Hayatabad the tap water samples showed 4.53\% (1/23) and drain water samples showed 16.67\% (2/12) positive results for \textit{C. parvum} while tap water was 28.57\% (2/7) and drain water was 50\% (3/6) in Khwaja Town. Shaheen Town showed 14.29\% and 33.33\% positive results for \textit{C. parvum} in out of 7 and 3 water samples of tube well and bore well respectively. The tap and drain water samples showed 10\% (2/20) and 30\% (3/10) respectively positive results for \textit{C. parvum}. While \textit{C. parvum} was detected, 50\% (1/2) in tube well water, 8.33\% (1/12) in bore well water, 65\% (13/20) in tap water and 66.67\% (4/6) in drain water samples, collected from Faisal Colony, (Table 2).

Source Wise Prevalence of \textit{C. Parvum}: In the present study \textit{C. parvum} was absent in tube well water samples, collected from Saddar, University Town, Hayatabad and Khwaja Town in out of 15, 13, 17 and 2 samples respectively. While \textit{C. parvum} was present in those well water samples which were collected from Faisal Colony and Shaheen Town is showing 50\% (1/2) and 14.29\% (1/7) positive results. Similarly, in \textit{C. parvum} was also absent in bore well water samples, collected from saddar, University Town, Hayatabad and Khwaja Town in out of 13, 12, 8 and 25 water samples respectively. While 33.33 \% (1/3) and 8.33 \% (1/12) detected positively in bore water samples, collected from Shaheen Town and Faisal Colony respectively.

Table 1: Water samples collected from different areas of District Peshawar

<table>
<thead>
<tr>
<th>Location</th>
<th>Tube Well</th>
<th>Bore Water</th>
<th>Tap Water</th>
<th>Drain Water</th>
<th>Total Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saddar</td>
<td>15</td>
<td>13</td>
<td>20</td>
<td>12</td>
<td>60</td>
</tr>
<tr>
<td>Shaheen Town</td>
<td>7</td>
<td>3</td>
<td>20</td>
<td>10</td>
<td>40</td>
</tr>
<tr>
<td>University Town</td>
<td>13</td>
<td>12</td>
<td>21</td>
<td>14</td>
<td>60</td>
</tr>
<tr>
<td>Hayatabad</td>
<td>17</td>
<td>8</td>
<td>23</td>
<td>12</td>
<td>60</td>
</tr>
<tr>
<td>Khwaja Town</td>
<td>2</td>
<td>25</td>
<td>7</td>
<td>6</td>
<td>40</td>
</tr>
<tr>
<td>Faisal Colony</td>
<td>2</td>
<td>12</td>
<td>20</td>
<td>6</td>
<td>40</td>
</tr>
</tbody>
</table>

\(P=0.0000 <0.05\), Significant

Table 2: Prevalence of \textit{C. parvum} in different areas of District Peshawar

<table>
<thead>
<tr>
<th>Area</th>
<th>Tube well water</th>
<th>Bore water</th>
<th>Tap Water</th>
<th>Drain Water</th>
<th>Overall Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive/total (%)</td>
<td>Positive/total (%)</td>
<td>Positive/total (%)</td>
<td>Positive/total (%)</td>
<td>Positive/total (%)</td>
</tr>
<tr>
<td>Saddar</td>
<td>0/15 (0%)</td>
<td>0/13 (0%)</td>
<td>3/20 (15%)</td>
<td>3/12 (25%)</td>
<td>6/60 (10%)</td>
</tr>
<tr>
<td>Shaheen Town</td>
<td>1/7 (14.29%)</td>
<td>1/3 (33.33%)</td>
<td>2/20 (10%)</td>
<td>3/10 (30%)</td>
<td>7/40 (17.5%)</td>
</tr>
<tr>
<td>University Town</td>
<td>0/13 (0%)</td>
<td>0/12 (0%)</td>
<td>1/21 (4.76%)</td>
<td>3/14 (21.43%)</td>
<td>4/60 (6.66%)</td>
</tr>
<tr>
<td>Hayatabad</td>
<td>0/17 (0%)</td>
<td>0/8 (0%)</td>
<td>1/23 (4.35%)</td>
<td>2/12 (16.67%)</td>
<td>3/60 (5%)</td>
</tr>
<tr>
<td>Khwaja Town</td>
<td>0/2 (0%)</td>
<td>0/25 (0%)</td>
<td>2/7 (28.57%)</td>
<td>3/6 (50%)</td>
<td>5/40 (12.5%)</td>
</tr>
<tr>
<td>Faisal Colony</td>
<td>1/2 (50%)</td>
<td>1/12 (8.33%)</td>
<td>13/20 (65%)</td>
<td>4/6 (66.67%)</td>
<td>19/40 (47.5%)</td>
</tr>
<tr>
<td>Total</td>
<td>2/56 (3.57%)</td>
<td>2/73 (2.74%)</td>
<td>22/111 (19.82%)</td>
<td>18/60 (30%)</td>
<td>46/300 (14.66%)</td>
</tr>
</tbody>
</table>

\(\%\) Percentage, \(P=0.0007 <0.05\), Significant
C. parvum was also detected positively in tap water samples collected from Saddar 15% (3/20), Shaheen Town, 10% (2/20), University Town 4.76% (1/21), Hayatabad 4.35% (1/23), Khwaja Town 28.57% (2/7) and Faisal Colony 65% (13/20). While the drain water samples of Saddar showed 25% (3/12), Shaheen Town, 30% (3/10), University Town 21.43% (3/14), Hayatabad 16.67% (2/12), Khwaja Town, 50% (3/6) and Faisal Colony 66.67% (4/6) significant positive results for C. parvum (Table 2).

Overall Prevalence of C. Parvum: Overall prevalence of C. parvum was 14.66% (44/300) recorded; in different water samples were detected significantly positive for C. parvum.

Overall area-wise prevalence of C. parvum in all sources collected from different areas in district Peshawar was 10%. (6/60) in Saddar, 17.5% (7/40) in Shaheen Town, 6.66% (4/60) in University Town, 5% (3/60) in Hayatabad, 12.5% (5/40) in Khwaja Town and 47.5% (19/40) in Faisal Colony. While overall source-wise prevalence of C. parvum was 3.57% (2/56) in Tube well water, 2.74% (2/73) in Bore well water, 19.82% (22/111) in Tap water and 30% (18/60) in Drain water samples (Table 2).

In the current study the drain water was more contaminated with C. parvum than other sources. The negative result may be due to problem in handling or it was also possible that these samples may not contain C. parvum.

DISCUSSION

The present study was Co related to different studies, conducted by different people in District Peshawar or in Pakistan as well as throughout the world. A similar study was also conducted in district Kohat according to which out of 150 water samples collected from three different sources like tap water (effluent), open well water and stream water (influent) were examined through PCR too containing overall prevalence 12% (18/150), in which Cryptosporidium spp. was 6.66% (10/150) in tap, well and spring water [15] sources. From region to region the type and frequency of various parasites are different [16] which may be due to the change of environment and geography [15].

Similarly, the present study was correlated with the similar work carried out microscopically in three districtts of the Khyber Pakhtunkhwa Province, Pakistan in 2011, where all of the three sources of water were contaminated with protozoa. The results indicate overall prevalence of 65.5% (295/450) of protozoa, including 19.5% (88/450) Cryptosporidium spp [9]. In another study conducted in District Bannu contains 36% (25/75) overall prevalence of parasites was in which C. parvum was 7.84% (4/51) detected in tap water 62.5% (5/8) in pond water, 16.66% (2/12) in bore well water and 25% (1/4) in Hand pump water samples [12].

According to another study, conducted in the same district, different water sources were also contaminated with other zoonotic parasite, E. histolytica showing overall prevalence 11.33% (34/300) followed by 3.57% (2/56) in tube well, 2.74% (2/73) in bore well, 14.41% (16/111) in tap water and 23.33% (14/60) in drain water [3] and the “May be due to flooding and improper management of water scheme” [3].

Another study was also conducted in Russia and Bulgaria by different people for the detection of C. parvum in drinking water samples of different origin (Surface, tap, bottled, well, spring and waste water) were collected from Rostov (Southern Russia), Sofia and Varna (Bulgaria) contaminated (18.1%) with C. parvum in tap, river, waste and well water [17]. In the present study the rate of contamination was 14.66% (44/300) which was lower than in the above study.

In various countries of the world the incidence of cryptosporidiosis has been reported 8.9% in Bangladesh [18] 8.8% in Iraq [19, 20], 5.3% in Nigeria [21] 7.3% in India [20] which were lower than our results and 13.5% in Bethlehem, Palestine [20] which was slightly similar to our results.

In patients with AIDS the C. parvum is an important cause of chronic diarrheal disease [22] which can cause severe symptoms in immunocompromised person [23].

A study was recorded in the district Peshawar area in which, out of 200 children suffering from diarrhea having Cryptosporidium oocysts 9.0% infection commonly in children between 1 - 24 months of age and 83.3% children were infected, who contact with animals directly or Indirectly. Most of them, who infected by C. parvum were (77.8%) well water consumers, the diagnosis was performed through microscopy [10] while we have diagnosed it through PCR and our result was very high because the PCR is more sensitive than Microscopy.

The study was also done in Lahore (Pakistan) in different animals, cow and buffalo, which showed 25.6% overall prevalence of C. parvum [24].

In the current study, most of the positive samples belonged to rural areas having low socioeconomic conditions. “Where there no awareness regarding cleanliness, sterilization and disinfection” [3].
There are many factors affecting the prevalence of *C. parvum* consisting of location of sampling, the diversity of animals in the areas, season, climatic condition, volume of sample, the population of the animals in the areas and rainy seasons etc. So the transmission of *C. parvum* is different in emerging countries than that of developed countries [25].

Overall pH value was 8.21 (±0.06) including, tube well water 8.16 (±0.20), Bore well water 8.30 (±0.32) tap water 8.26 (±0.24) and drain water 8.11 (±0.48) collected from different areas of district Peshawar [3] previously. This showed no effect on the prevalence of *C. parvum* too. That may be due to low basicity of the water samples.

The present results may help the people for their health in prevention and supervision for amebiasis especially in children. It may also help the people in molecular detection of the parasites in the local laboratories.

Microscopic examination was not enough to detect low level of infection, but amplification of DNA can reveal it. So this study is entirely based on the detection of *C. parvum* through the PCR diagnosis. Clinician and laboratories should be encouraged to include *C. parvum* diagnostic techniques while dealing with diarrheal stool samples of young children. The study also revealed that a proper treatment of water for human consumption is required in district Peshawar.

**CONCLUSION**

It was concluded from the current study that high level of contamination of water was found in different water sources, which needs of a suitable source of drinking water to identify the threshold of water source contamination that requires treatment. Preventing waterborne disease and the health effects of water contamination is vital to our nation’s public health due to the fact that access to harmless drinking water is required cornerstone of public health. It was also concluded from the current study that zoonotic parasites could easily spread by means of water, which cause different diseases like cryptosporidiosis in human beings as well as in animals.

It was recommended that PCR is more sensitive and accurate for the detection of *C. parvum*. Pure and filtered water should be used to minimize the health hazards. Furthermore, it is recommended that water sources may have proper sanitary measures to avoid the spread of zoonotic parasites.

**Competing Interests:** The authors declare that they have no competing interests.

**REFERENCES**


