Research Article

Relation between Water Source and Rotaviral infection: An Observational Study

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Abstract

Fecal pollution of water is a major concern in river based countries as it is associated with transmission of the pathogens. Rotavirus is one of them which is the major cause of acute severe dehydrating diarrhoea in children below five years.
This cross-sectional observational study was designed to determine the association of contaminated water source
with Rotaviral infection among under five children admitted in the Department of Pediatrics, Sylhet MAG Osmani
Medical College Hospital, Sylhet and was carried out in the Department of Microbiology during the period from 1st
January to 31st December, 2018. Total 184 under five children with acute watery diarrhoea were enrolled in this
study by convenient sampling. Stool samples were obtained and assayed for rotavirus antigens by enzyme linked
immunosorbent assay (ELISA).

Rotaviral antigen was found positive in 86 cases. The Rotavirus infection was found highest in age group of 7 to 12
months (50.56%) and in male (59.30%) children. It was found significantly higher in patients from lower socio-
economic condition (64.00%), in rural area (48.75%) and those who consumed unboiled pond water (94.44%). It
was found higher among those children who were not exclusively breastfed (83.87%). Bottle feeding, lower
educational level of mother and improper hand washing may serve as predisposing factors of rotavirus disease in
these children.

Using of contaminated drinking and household water has an important relation with Rotaviral infection. Ignorance
of using boiled water can play a significant role for increased prevalence of Rotaviral diarrhoea.

Keywords: Rota virus; Drinking water source; watery diarrhoea; ELISA

Abbreviation: Enzyme Linked Immunosorbent Assay (ELISA)

1. Introduction

Several enteric viruses causing water related diseases with great impact on public health are present in fecally
contaminated water. Rotavirus is one of them which is the major cause of acute severe dehydrating diarrhoea in
children below five years [1]. This virus was first described by electron microscopic examination of duodenal
biopsies from children with acute gastro-enteritis [2]. Rotavirus is transmitted through the faeco-oral route. High
viral shedding of infected person with low infective dose and resistence to adverse environmental conditions
contribute this virus as major environmental pollutants [3, 4]. Surface water can be polluted by discharges entering
from community sewage treatment plant, untreated sewage and livestock facilities. Other sources can be direct fecal
voiding of both human and livestock which is a common practice of rural area. The virus may be found in private
water sources like well, tube-wells, ponds that have been contaminated with the feces from infected humans. Waste
can pollute water by many ways, including sewage overflows, community sewage system plants, slaughterhouse
discharge, untreated sewage and polluted storm water overflow, all leading to very high viral load. Surface water
also can be polluted by urban surface water runoff which can be resulting from roof and road surfaces contaminated
with droppings of avian, pet and stray animals [5, 6, 7].

Several predisposing factors are responsible for Rotaviral infection in children. The most common factors are low
birth weight, male gender, 6-24 months age group (due to more exposure to contaminated materials in this age
group), children attending daycare, poor food hygiene, playing with toys, bottle-feeding, low literacy status of mother, improper hand washing, contaminated drink water [8,9] Hospital acquired infection due to Rotavirus also occurs [3].

Clinical presentation of watery diarrhoea resembles the same with different etiology. But Rotaviral diarrhea is responsible for significant number of morbidity and mortality. Many countries reported increased hospital admissions, emergency department visits and clinic visits among infants with Rotaviral diarrhoea [6]. In Bangladesh, Rotavirus is the major cause of under-five diarrhoea and diarrhoeal deaths. A study conducted by icddr, b in Matlab, Bangladesh from 2006 to 2012 revealed that prevalence of Rotaviral diarrhoea was 20.3% among under 5 children [10]. According to WHO, Rotaviral diarrhoea causes 1000-2700 deaths each year in children <5 years of age in Bangladesh [11].

Sensitive detection assays should be introduced for virus detection. Early detection can help in treatment and the whole prognosis of the disease. Immunological assays like ELISA and ICT can detect the causative agent within short time [6].

Good hygiene reduces the transmission of virus. But even in the most hygienic societies, virtually all children experience Rotaviral diarrhoea as a result of high infectivity of the virus. Exclusive breast feeding, proper hand washing and safe drinking water can help to control disease spread.

There is no report regarding contaminated water use and occurrence of Rotaviral diarrhoea in our country. So, this study was designed to assess the relation between water source and Rotaviral diarrhoea among children less than 5 years.

2. Materials and Methods

This cross-sectional observational study was carried out in the department of Microbiology in collaboration with the department of Paediatrics, Sylhet MAG Osmani Medical College Hospital from 1st January 2018 to 31st December 2018. All admitted children under 5 years of age with acute watery diarrhoea were included in this study. Detail history was taken from the parents of the children. Those who met the selection criteria were enrolled as study population. Children suffering from chronic diarrhea (diarrhoea for ≥ 14 days) and bloody diarrhea were excluded. After explaining the purpose of the study, informed written consent was taken from the patient’s legal guardian. Data collection and potential risk factors were determined through personal interview and a pre-designed data collection sheet. Prior to the beginning of this study, approval of the research protocol was obtained from the Ethical Review Committee of Sylhet MAG Osmani Medical College, Sylhet.

The patients residing within Sylhet city were classified as urban people and those residing outside Sylhet city were classified as rural people.
2.1 Specimen collection
Fresh stool samples were collected from the admitted patients in properly labeled clean, dry, wide-mouthed, leak-proof plastic containers. Stool samples were carried to the laboratory within 1 to 2 hours of collection.

2.3 Laboratory procedure
Macroscopic examination was done for colour, consistency, presence of mucus or blood in stool samples.

2.4 Rotaviral antigen detection by enzyme linked immunosorbant assay (ELISA)
Monoclonal antibodies against the product of the sixth viral protein (VP6) used in a sandwich type method. The assay was carried out according to the manufacturer’s instructions.

2.5 Statistical methods
For data analysis, $\chi^2$ (Chi square) test was done and $p<0.05$ was considered significant.

3. Result
3.1 Demographic profile of study participants
In our study, 184 under 5 years old children participated who were suffering from acute watery diarrhoea. Majority of these participants was male (66.30%) and from rural background (86.96%). More than half (54.35%) of the participants belonged to lower socio-economic status in our study, 66.30% of the study participants were breastfed exclusively, however, nearly two-third of them were bottle-fed (63.04%). Every participants had diarrhoea and other symptoms were vomiting, abdominal pain, fever and dehydration. Most of the guardians gave the history of hand washing after toilet use (86.96%). Nearly half of our respondents consumed tube-well water (48.91%) and just above a quarter of them consumed boiled water (26.09%), though, 9.78% of the respondents used un-boiled pond water for drinking. Among the children who took part in our study, almost equal proportion had mothers who completed primary (33.70%) and secondary (34.78%) level education respectively.

Table I: Demographic profile of study participants (N=184)

<table>
<thead>
<tr>
<th>Variables</th>
<th>n</th>
<th>(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>122</td>
<td>66.30</td>
</tr>
<tr>
<td>Female</td>
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</tr>
<tr>
<td>Residence</td>
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<td></td>
</tr>
<tr>
<td>Rural</td>
<td>160</td>
<td>86.96</td>
</tr>
<tr>
<td>Urban</td>
<td>24</td>
<td>13.04</td>
</tr>
<tr>
<td>Socioeconomic status</td>
<td></td>
<td></td>
</tr>
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<td>Middle</td>
<td>84</td>
<td>45.65</td>
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<td>Lower</td>
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<tr>
<td>Breast feeding</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### 3.2 Distribution of study population according to stool antigen test by ELISA
Following table shows the stool antigen positive in 86 (46.74%) and negative in 98 (53.26%) patients.

**Table II:** Total number of positive and negative cases observed by ELISA

<table>
<thead>
<tr>
<th>ELISA</th>
<th>Positive</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>86</td>
<td>46.74</td>
</tr>
<tr>
<td>Negative</td>
<td>98</td>
<td>53.26</td>
</tr>
<tr>
<td>Total</td>
<td>184</td>
<td>100.0</td>
</tr>
</tbody>
</table>

### 3.3 Prevalence and distribution of ELISA positive Rotavirus diarrhoea
It was revealed from our study that, prevalence of Rotavirus induced acute watery diarrhoea (ELISA positive) was highest among 7-12 months age group children (55.56%) followed by 13-24 months age group children (48.28%). This prevalence was also higher among male children (59.30%) and children from rural residence (48.75%).
compared to their female and urban counterparts respectively. This prevalence was also higher among children
belonged to low socio-economic status (64.00%) than the children belonged to middle socio-economic status
(26.19%). Expectedly, our study found that, prevalence of Rotavirus diarrhoea was higher among children who were
not exclusively breastfed (80.65%, $p<0.0001$) and were bottle-fed (73.53%, $p<0.0001$) and these associations were
statistically significant. Almost all of our study participants who consumed un-boiled water were found to suffer
from Rotavirus induced diarrhoea (pond water-94.44%, tap water-82.14%), however, only 4.17% of children who
consumed boiled water suffered from this condition. In our study, acute watery diarrhoea was found highly
prevalent among the children of less educated mothers (primary complete-83.87%, secondary incomplete-43.48%)
than their counterparts whose mother had better education (secondary complete-15.62%, higher secondary-33.33%)
(Table III)

Table III: Prevalence of Rotavirus diarrhoea (ELISA +ve) among under 5 years old children (N=184)

<table>
<thead>
<tr>
<th>Variables</th>
<th>ELISA (+ve)</th>
<th>ELISA (-ve)</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>(%)</td>
<td>n</td>
</tr>
<tr>
<td>Age (months)</td>
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<td></td>
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<tr>
<td>0-6</td>
<td>2</td>
<td>11.11</td>
<td>16</td>
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<tr>
<td>7-12</td>
<td>50</td>
<td>55.56</td>
<td>40</td>
</tr>
<tr>
<td>13-24</td>
<td>28</td>
<td>48.28</td>
<td>30</td>
</tr>
<tr>
<td>25-59</td>
<td>12</td>
<td>33.33</td>
<td>12</td>
</tr>
<tr>
<td>Gender</td>
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<tr>
<td>Male</td>
<td>51</td>
<td>59.30</td>
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<tr>
<td>Female</td>
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<td>27</td>
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<tr>
<td>Residence</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urban</td>
<td>8</td>
<td>33.33</td>
<td>16</td>
</tr>
<tr>
<td>Rural</td>
<td>78</td>
<td>48.75</td>
<td>82</td>
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<td>Socioeconomic status</td>
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</tr>
<tr>
<td>Middle</td>
<td>22</td>
<td>26.19</td>
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<tr>
<td>Lower</td>
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<td>64.00</td>
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<tr>
<td>EBF</td>
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<td>86</td>
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<tr>
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<td>80.65</td>
<td>12</td>
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<td>Bottle feeding</td>
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<tr>
<td>Yes</td>
<td>50</td>
<td>73.53</td>
<td>18</td>
</tr>
<tr>
<td>No</td>
<td>36</td>
<td>31.03</td>
<td>80</td>
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<tr>
<td>Source of drinking water</td>
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<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>------------------------</td>
<td>-------</td>
<td>-----</td>
<td>-----</td>
</tr>
<tr>
<td>Boiled</td>
<td>2</td>
<td>4.17</td>
<td>46</td>
</tr>
<tr>
<td>Not boiled (pond water)</td>
<td>17</td>
<td>94.44</td>
<td>1</td>
</tr>
<tr>
<td>Not boiled (tap water)</td>
<td>23</td>
<td>82.14</td>
<td>5</td>
</tr>
<tr>
<td>Not boiled (tubewell)</td>
<td>44</td>
<td>48.89</td>
<td>46</td>
</tr>
</tbody>
</table>

**Mother’s education**

<p>| | | | | | |</p>
<table>
<thead>
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</thead>
<tbody>
<tr>
<td>Primary complete</td>
<td>52</td>
<td>83.87</td>
<td>10</td>
<td>16.13</td>
<td>0.000*</td>
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<tr>
<td>Secondary incomplete</td>
<td>20</td>
<td>43.48</td>
<td>26</td>
<td>56.52</td>
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<tr>
<td>Secondary complete</td>
<td>10</td>
<td>15.62</td>
<td>54</td>
<td>84.38</td>
<td></td>
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<tr>
<td>Higher secondary</td>
<td>4</td>
<td>33.33</td>
<td>8</td>
<td>66.67</td>
<td></td>
</tr>
</tbody>
</table>

*P value <0.05 statistically significant

χ² test was employed to analyze the data

### 4. Discussion

In the present study, highest prevalence of Rotaviral diarrhoea was found in children of 7-12 months of age group. This is in agreement with the results of a study done in Nigeria where most of the infected children were found between 7 to 12 months of age group [12]. It appeared that infants below 6 months of age are initially protected to some extent against Rotavirus diarrhoea due to presence of maternal antibodies. In this age group (7 – 12 months), children start crawling and develop tendency to put almost everything into mouth which can increase the chance of infection [13]. Another reason can be that the weaning is started at this age. So, there is chance of contamination of food during preparation using unboiled water and if hand washing after using toilet or changing diaper of the baby and food hygiene is not maintained properly. Frequency of Rotaviral infection was less in higher age group due to acquisition of antibody by natural infection [14].

This prevalence was found higher among children from rural residence compared to their urban counterparts. This prevalence was also higher among children belonged to low socio-economic status (64.00%). This finding is in resemblance with the finding of an Indian and an Ethiopian study [15, 16]. These findings can be explained by unhygienic behavior, lack of health education, not having or using sanitary latrine and less ability to avail standard health care facility. As Rotaviral infection is highly communicable, overcrowding living condition can also explain this finding [15].

Expectedly, our study found prevalence of Rotavirus diarrhoea was significantly higher among children who were not exclusively breastfed and were bottle-fed. Dhiman showed that bottle feeding increases chance of Rotaviral infection (52.38%) [16]. John, Devgan and Mitra [17] from India and Azeez and Alsakee [18] from Iraq reported the same. Feeding bottles can easily be contaminated and use of unboiled water for washing feeding bottles or to prepare artificial feeds may be the reason behind the higher rate of infection among bottle-fed children.

In our study, acute Rotaviral diarrhoea was found highly prevalent among the children of less educated mothers. This correlates with a previous study done by Sisey et al. who found that majority of children of illiterate mother had
Rotavirus infection [15]. This can be explained by lack of maintenance of hygiene. Ignorance of using boiled water during food preparation and in other household works can also play a significant role for increased prevalence of Rotavirus diarrhoea in the children of less educated mother.

Almost all of our study participants who consumed un-boiled water were found to suffer from Rotavirus induced diarrhoea, however, a small number of children who consumed boiled water suffered from this condition. Contaminated drinking water may be an important source of infection. In rural area, river water get contaminated with the feces from infected human due to unhygienic behavior. Shallow Wells may be more vulnerable to such contamination after flooding as it has chance to be immersed by floodwater. The waterborne transmission may dominate during dry seasons and also during increased rainfall [5, 15]. Another study done in Northwest Argentina showed that Arias-Arenales river water was tested positive for Rotavirus genotypes which were also found common circulating genotypes isolated from from local people. Researchers suggested that these rotaviruses could be originated from storm water discharge and excreted by the infected people causing contamination of water. Thus the river water could be the potential source of rotavirus infection of local people [19]. According to CDC, rotavirus may be found in different water sources such as private wells that have been contaminated with the feces from infected humans. Waste can enter the water through different other ways like sewage overflows, sewage systems that are not working properly, and polluted storm water runoff. Wells may be more vulnerable to such contamination after flood particularly if the wells are shallow, have been dug or bored, or have been submerged by floodwater [7]. An epidemiological study was conducted in Philippines to investigate a rotavirus outbreak in 2010 due to drinking water contamination. They reported 2936 suspected cases with 22 deaths. Most people of Zamboanga City rely on water refilling stations as their major source of drinking water. The majority of active case patients reported obtaining their drinking water from water refilling stations. This was the first rotavirus outbreak in the Philippines with isolation of rotavirus from water refilling stations. Among them, five stations were investigated and researchers found that some refilling stations had no sanitary permit. Water handlers were mostly children. Hand pumps were attached to the water distribution pipes to add pressure to the faucet. Pipes were also submerged in the sewer system. These sources also did not meet the water-quality standards. Researchers suggested that rotavirus was the cause of this outbreak. The reason could be water handlers were the active cases. As Rotavirus is highly transmissible person-to-person contamination might play a role in the increase of number of the cases [6].

5. Conclusion
Bangladesh is a river based heavily populated country. It is also a flood prone area. Water source contamination during flood is very common. Again, unhygienic toilet practice in rural area play a great deal in contamination of drinking water. We found contaminated water has an important relation with Rotaviral infection. Rolling boiling of household drinking water, regular disinfection of water reserves and water testing once in a year after disinfection can be play very important role in preventing Rotaviral infection.

6. Limitations
1. The study was conducted in a single center which may not represent the overall disease burden in different other
hospitals and geographical locations of the country.
2. Sample size was small.
3. Pollution source identification was not done.
4. Water examination for pathogens was not done.

**7. Recommendations**
1. Further studies with large sample size involving multicenter should be carried out to determine the actual disease burden.
2. Water samples from different areas should be examined.
3. A technical working group focusing on water safety can be created.
4. A multidisciplinary city water safety plan should be employed.
5. Rotavirus vaccines should be included in the national immunization programme.

**8. Conflict of interest:** Nothing to declare

**9. Funding:** Self-funding.

**10 References**
7. Center for Disease Control and Prevention (CDC). Rotavirus and Drinking water from Private Wells.


