Journal of Population Therapeutics & Clinical Pharmacology

Molecular Detection of Rotavirus and Coronavirus in Potable Water Samples of District Faisalabad

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Abstract

Background: Rotavirus causes gastroenteritis in infants and young children, and it has been distributed worldwide. Coronavirus is common all around the world. It infects the respiratory tract of both humans and animals.

Methods: To explore the presence of rotavirus and corona virus in water, 50 potable water samples were collected from different areas of Faisalabad, Pakistan. Sewage water, ground water and filtered water samples were collected in sterilized falcon tubes and transported to the lab under refrigerated conditions. RNA was extracted from the water samples using the kit. Extracted viral RNA was used as a template for the synthesis of cDNA using oligoprimers against conserved regions of both Rotavirus and Coronavirus. RT-PCR was done, and gel electrophoresis was performed. **Results:** PCR results of rotavirus gave product size of 981 bps. Rotavirus was detected in 42 % (21) samples and in the case of coronavirus all the samples were negative. Results indicated that only rotavirus is prevalent in Faisalabad.

Conclusion: This study indicated that rotavirus was prevalent in the potable water of Faisalabad; its presence was higher in sewage water and lower in ground water. Coronavirus was not present in the water samples of Faisalabad.

Key Words: Potable water, Prevalence; PCR; Sewage; Ground water, Filtered; Virus.

Introduction

Rotavirus is a non-enveloped 70 nm wheel like particle belonging to the family *Reoviridae* and genus *Rotavirus*. The genome of Rotavirus consists of 11 segments of double stranded RNA, it encodes six Structural (VPs) and six nonstructural proteins (NSPs). The virus has protein capsid of icosahedral

symmetry and 100 nm in diameter having three protein layers. The outer layer is composed of two proteins VP4 and VP7 while middle layer consists of VP6 and the inner layer is composed of VP2 which encloses protein VP3, a viral capping enzyme and VP1 which is RNA dependent RNA polymerase. The two outer capsid proteins induce protection and contain neutralizing epitopes (Sadiq *et al*., 2018).

Rotavirus is classified into seven different groups (RVA-RVG) based on VP6 structural protein antigenicity. The most widespread is group A Rotavirus which has four subgroups. In the majority of cases Rotavirus, A causes severe diarrhea in infants and children. Rotavirus is classified into P and G genotypes on the bases of VP4 and VP7 respectively. Reassortment occurs because of the combination of GP genotypes. The most common genotype combinations in the world are G1P8, G2P4, G3P8, G4P8 and G9P8 (Moresco *et al*., 2016).

Rota virus is contagious and highly resistant to environmental conditions. (Sadiq *et al*., 2018). Due to poor health care facilities and poor diet in developing countries, rotavirus causes 800,000 deaths of children. In India, the most infections of diarrhea were seen within the first two years of life, having a peak at 9-11 months. In the year 2004, 527000 children of age less than five died because of rotavirus, 85 among these deaths were in Sub Saharan Africa and South East Asia (Salim *et al.*, 2014).

Coronavirus is positive sense, single stranded RNA virus it causes diseases in humans, birds, and mammals. It belongs to the family *Coronaviridae*. Coronavirus is enveloped, pleomorphic or spherical having diameter of 80-120 nm. The surface spikes of the virus are 17-20 nm and appear as club shaped. The genome size of the virus is 30kb, encoding nonstructural proteins such as RNA dependent RNA polymerase and helicase and three structural (S, M, E and N) proteins. Spike (S) glycoprotein which helps in attachment and fusion with the host cell membrane, Membrane (M) glycoprotein gives shape to the envelop, envelop (E) protein is present in small amount and nucleocapsid (N) protein binds the RNA like beads on a string (Masters, 2006. The subfamily Coronavirus has four major genera alpha CoV, beta CoV, delta CoV and gamma CoV (Al-hazmi, 2016). Alpha and beta coronaviruses are found in mammals. Every subtype has different serotypes. Coronavirus NL63 and 229E belong to alpha genus while OC43, HKU1, SARS CoV, MARS CoV and 2019-nCoV belong to beta coronavirus which was discovered in China in 2019. Gamma and delta coronaviruses infect birds (Narayanan *et al.*, 2015). In 2003 outbreak of SARS CoV (Severe Acute Respiratory Syndrome Virus) occurred worldwide causing infection in 8896 people and killing 9.5% of the infected population (Drexler *et al.*, 2014). World Health Organization declared that novel coronavirus is PHEIC (public health emergency of international concern). On February 11, 2020 the number of confirmed cases was 44672 in China, among these cases, 8225 were the severe cases and the number of deaths was 395 (Jiehao *et al.*, 2019).

Coronavirus is transmitted via air droplets from animals to human and can also be transmitted from human to human. Virus replicates in ciliated epithelium it causes cellular damage and inflammation at the infection site. Coronavirus also infects bats, pigs, camels, dogs, cats, mice, and birds (Alosail and Alwazzah., 2017). Different diagnostic techniques are used to detect rotavirus and coronavirus in water samples. Mainly used methods for the detection of rotavirus and coronavirus include electron microscopy (EM), Poly acrylamide gel electrophoresis (PAGE), Latex Agglutination Test (LAT), enzyme linked immunosorbent assay (ELISA), hybridization assays, lateral flow immunochromatography and polymerase chain reaction (PCR) based assays. These methods can detect viruses from water efficiently (Izzo *et al*., 2012). Objectives of this study include Detection of Rotavirus and Coronavirus from potable water samples and determination of the prevalence of Rotavirus and Coronavirus in district Faisalabad.

Materials and Methods

Study Area: Present study was conducted in District Faisalabad Pakistan. Faisalabad is located between 31° 25′ 0″ N-73° 5′ 28″ E. it is the second largest district of Punjab. A geographical map of District Faisalabad is shown in figure 1.

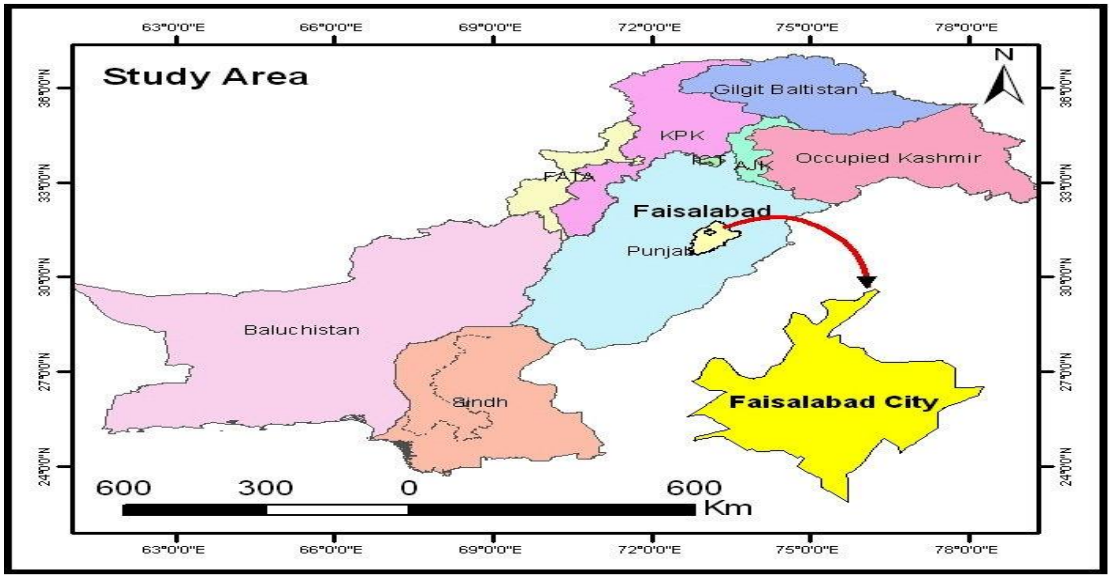

Figure1: showed the Geographical map of District Faisalabad, Punjab Pakistan

Study Design

Study was designed to evaluate the presence of Rotavirus and Coronavirus in water samples of district Faisalabad, Punjab Pakistan. The quality of drinking water was determined to estimate the risks to human health due to contaminated water. The study was conducted in the months of November and December and presence of Viruses was detected by using PCR.

Sample Collection

A total of 50 potable water samples were collected from various sources to determine the presence of rotavirus and coronavirus in the water samples. Samples were collected in 15ml sterilized falcon tubes for the isolation of both viruses. Sewage water samples were collected from different hospitals, including Allied hospital and District Head Quarter (DHQ) hospital to detect the presence of viruses in sewage samples. Filtered and ground water samples were also collected from different areas of Faisalabad. The samples were transported immediately at controlled temperature to the Microbiological Water Testing Laboratory, Institute of Microbiology University of Agriculture Faisalabad. Samples were stored at 4°C in refrigerator till further processing (Hassine *et al*., 2015).

RNA Extraction

RNA was extracted by using (thermo scientific) genejet RNA purification kit. The material used in RNA Extraction and the kit was taken to the laminar air flow to perform RNA extraction.

cDNA Synthesis

After purification of RNA from water samples cDNA synthesis was done. cDNA was prepared by reverse transcription reaction. Extracted viral RNA was used as a template for the synthesis of cDNA using oligoprimers against conserved regions of both Rotavirus and Coronavirus. Commercial cDNA

synthesis kit (Easy Script) was used according to manufacturer's protocol. The synthesized cDNA was stored at -20°C for future use.

Results

PCR Amplification of Rotavirua

The PCR products were separated using Gel Electrophoresis and the presence of virus was confirmed by analyzing the fragments on the gel. Positive water samples gave product size of 981 bps as shown in the figure: 2.

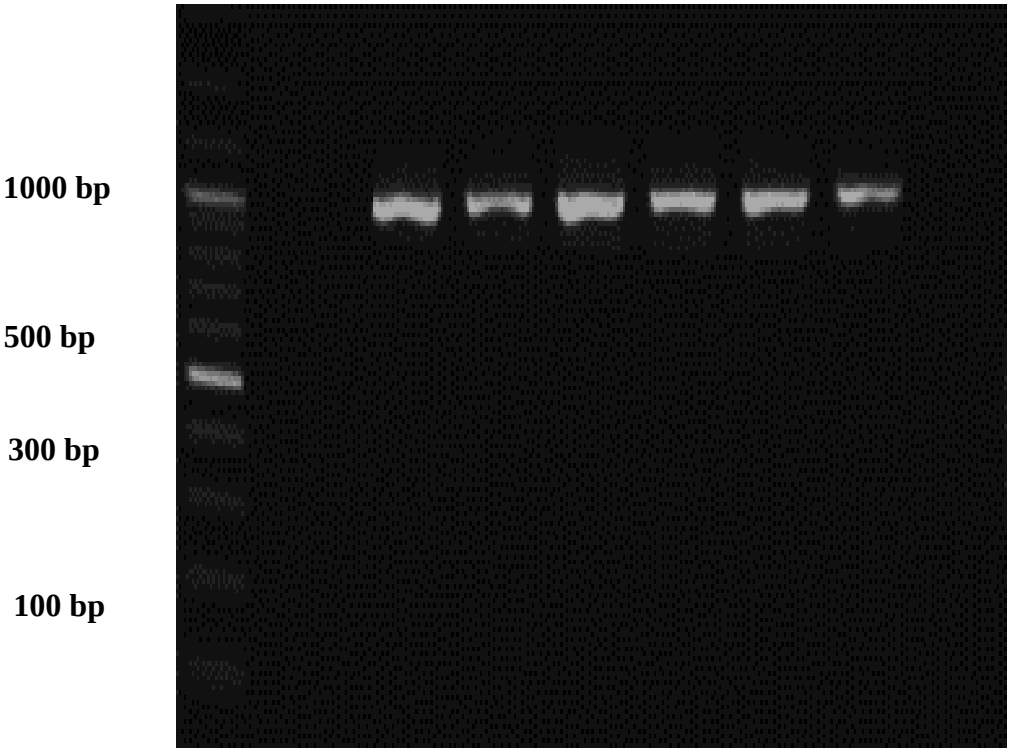

Figure 2 Represents PCR Amplification of Rotavirus. Lane M shows a molecular marker of 1000bp. Lane 1 represents negative samples, while Lane 2- 8 show PCR product of 981bp.

Amplification of rotavirus was done using polymerase chain reaction. Genome segments were amplified using forward and reverse primers. To amplify DNA all, the reagents were added in the PCR tube and set in thermal cycler. Out of 50 samples 21 samples were tested positive for rotavirus. Out of 20 sewage samples collected from Allied hospital (AL 1- AL 20) 9 sewage samples were tested positive and 11 samples were negative for the presence of rotavirus. Forty-five % of sewage samples collected from Allied hospital were positive. Out of 10 sewage samples collected from DHQ hospital (DHQ 1 – DHQ 10) 6 samples were positive for the presence of rotavirus and 4 samples were negative. The percentage of positive samples collected from DHQ was 60. All the filtered water samples were negative. Out of 10 ground water samples 6 were tested positive and 4 were negative. Polymerase chain reaction results of rotavirus detection are given in table:1.

Table 1: Polymerase Chain Reaction Results of Rotavirus Detection from the Water Samples.

Sr. No.	Location	Total No. of samples	Type of samples	Positive samples	Percentage (+ve)

1	Allied hospital	n = 20	Sewage water	AL 2, AL 5, AL 6, AL 9, AL 11, AL 12, AL 15, AL 19, AL 20	45%
2	DHQ	n = 10	Sewage water	DHQ 1, DHQ2, DHQ 5, DHQ 7, GHQ 8, DHQ 9	60%
3	Rehmanpura	n = 2	Filtered Water	-	50%
			Ground Water	Positive	
4	ABC Road	n = 2	Filtered Water	-	-
			Ground Water	-	
5	Gulshan Hyat	n = 2	Filtered Water	-	-
			Ground Water	-	
6	Shahbaz Garden	n = 2	Filtered Water	-	50%
			Ground Water	Positive	
7	Bharwal Colony	n = 2	Filtered Water	-	-
			Ground Water	-	
8	Muslim Town	n = 2	Filtered Water	-	50%

			Ground Water	Positive	
9	Shahi Chowk	n = 2	Filtered Water	-	50%
			Ground Water	Positive	
10	Sialvi Colony	n = 2	Filtered Water	-	-
			Ground Water	-	
11	Peoples Colony	n = 2	Filtered Water	-	50%
			Ground Water	Positive	
12	Madhan Pura	n = 2	Filtered Water	-	50%
			Ground Water	Positive	

PCR Amplification of Coronavirus

Amplification of coronavirus was done using polymerase chain reaction. All the 20 sewage samples collected from Allied Hospital (AL 1- AL 20) were negative for the presence of coronavirus. Ten sewage samples collected from DHQ hospitals (DHQ 1 – DHQ 10) were tested negative for the presence of coronavirus.

Discussion

Water is an essential requirement for all the human beings. Access to safe drinking water is a basic right of every human being. The unavailability of clean water has a great impact on developing countries. Contaminated water and poor hygienic conditions lead to the cause of water borne diseases such as diarrhea gastroenteritis and hepatitis. Present study showed the presence of rotavirus in 42% of samples.

In previous studies the presence of rotavirus was different in various areas (Mukaratirwa *et al.*, 2018) (Rayamajhi *et al.*, 2018) (Ahmad *et al.*, 2018) (Mursalova *et al.*, 2018) (Mast *et al.*, 2010) (Salim *et al.*, 2014) and (Hassine *et al*., 2015)**.** Out of 50 samples, 21 samples were positive for rotavirus and in 29 samples there was no detection of rotavirus, which indicated that 42 percent of samples were positive for rotavirus. Positive samples of rotavirus included ground water and sewage water, whereas filtered water did not show the presence of rotavirus. Five ground samples were positive for rotavirus and 21 sewage samples were positive and all the filtered water samples were negative. There was no detection of coronavirus in any of the 50 samples.

Conclusion

This study indicated that rotavirus was prevalent in the potable water of Faisalabad; its presence was higher in sewage water and lower in ground water. Coronavirus was not present in the water samples of Faisalabad. Rotavirus infections need medical attention. Preventive measures should be adopted to avoid the spread of rotavirus. There should also be proper water purification and water treatment systems to stop the spread of rotavirus. There was no detection of coronavirus in all the samples.

Conflict of Interest*:* The Authors declare that there are no conflicts of interest.

Acknowledgement

The authors are thankful to the Institute of Microbiology University of Agriculture Faisalabad for providing the technical support.

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