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Full Length Research Paper

Rotavirus infection and its monitoring in waste water using RT- PCR in Jeddah, Saudi Arabia

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Rotaviruses are the most common etiological agent of severe diarrhea in infants and young children Sewage systems are important nodes to monitor enteric pathogens transmitted via water. The aim of this study was to assess the presence of rotaviruses in wastewater receiving streams in Jeddah, Saudi Arabia, to provide viral fate and transport data for further epidemiological studies. In this study, one hundred of waste water samples were collected between 2009 and 2010 from the wastewater receiving outlet of AL-Misk Lake in Jeddah city. Samples were screened for the presence of rotavirus by reverse transcriptase-polymerase chain reaction (RT-PCR) technique. A total of 65 (65%) samples were found to be positive for rotavirus using this technique. The seasonal distribution of rotavirus diarrhea showed a winter peak, with an unusual peak from June to September.

Keywords: Rotavirus infection, waste water, polymerase chain reaction, seasonality, Jeddah.

INTRODUCTION

Rotaviruses (RVs) are enteric pathogens which cause severe gastroenteritis primarily in young children and are major causes of infant hospitalization and mortality worldwide (Tate et al., 2012).

After replicating in the gastrointestinal tract, these viruses are excreted and may be dispersed in environmental waters (Bosch et al., 1988 and Gajardo et al., 1995). Rotaviruses have been implicated in water-borne gastroenteritis outbreaks in many countries (Ansari et al., 1991).

The stability of human rotaviruses in environmental water and their resistance to physicochemical treatment processes in sewage treatment plants may facilitate their transmission (Rao et al., 1988; Sobsey, 1989).

The advent of the polymerase chain reaction (PCR) has greatly enhanced the ability to detect human enteric viral pathogens in the environment, including water, municipal wastes, sewage, food and air (Soule et al., 2000; Abbaszadegan et al., 2003; Gajardo Sano et al., 2003). Since RVs are known to be present in stool samples, it was of interest to determine the relative abundance of these viruses in the waste water using sensitive, specific, and reproducible RT-PCR assay. The aim of this study was to assess the in Jeddah city, Saudi Arabia, to provide viral fate for further epidemiological studies.

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MATERIALS AND METHODS

Sewage samples

A total of 100 waste water samples (5 Liter each) were collected from the receiving outlet of the waste water lake in east of Jeddah, Saudi Arabia (Figure 1). Two samples a week were collected from 28 January 2009 to 25 February 2010. The samples were transported to the King Abdul aziz University virology laboratory in Jeddah.

Concentration and reconstruction of viruses from wastewater

The sample was filtered through a filter paper (Whatman® Schleicher and Schuell, England) and through a positive charged nitrocellulose membrane (0.45µm pore size RANKEM) followed by an elution with

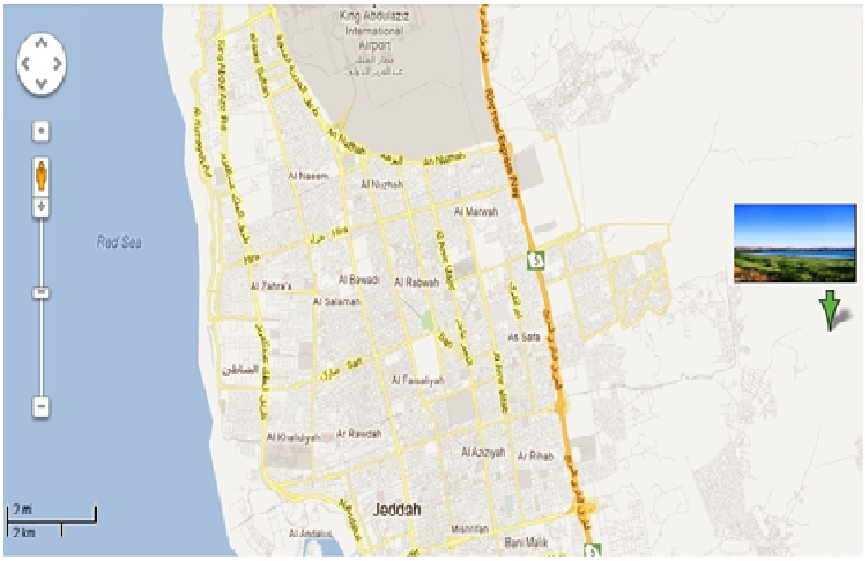

Figure 1. Geographic location of the wastewater receiving site at AL-Misk Lake

3.0% beef extract, pH 9.0. The pH of the filtrate was adjusted to pH 9.5 and centrifuged at 3,000 x g for 15 min. The sediment was dissolved in 0.15 M Na_2HPO_4 (pH 9.0).

Viral RNA Extraction

The QIAamp viral RNA mini kit (Qiagen, Hilden, Germany) was used to extract viral RNA from the waste water sample according to the manufacturer's instructions. Extracted viral RNA was stored at -20˚C until used.

Rotavirus detection

For generic detection of rotaviruses, a reverse transcription (RT)-PCR method based on amplification of a VP6 fragment was performed using the (QIAGEN® One step RT-PCR Kit). Primers VP6-3 ('5-GCTTTAAAACGAAGTCTTCAAC-3'); positions 2 to 23 of human strain Wa (accession number K02086) and VP6-4 ('5_-GGTAAA TTACCAATTCCTCCAG-3'); positions 187 to 166 of human strain Wa (accession number K02086). Mixture supplemented with each primer at a concentration of 0.6 µM, were used in an RT reaction in a 50 µl (final volume) mixture, each deoxynucleoside triphosphate at a concentration of 400 µM , and 16 µl of a denatured (5 min at 99℃) double-stranded RNA sample. The reaction mixture was incubated for 60 min at 50℃. The PCR program included a 9-min denaturation step at 95℃ and 40 cycles of amplification for 1 min at 94℃, for 1 min at 50℃, and for 1 min at 72℃, followed by a final elongation step of 7 min at 72℃.

The PCR products were then electrophoresed on 2% agarose gel. The amplified products 189 bp. fragment of the VP6 gene were visualized under ultra violet light and fragment sizes were compared with commercially available size standards (100 bp. DNA ladder, Promega).

RESULTS

Rotavirus detection

A total of 100 waste water samples were screened by RT-PCR by using specific primers for HRV. Overall, 65 (65%) of waste water samples were positive for RV (Figure 2a and 2b).

Figure 2a and 2b: 2% Agarose gel showing the positive samples of the nRT-PCR products with 189 bp. fragment length on the gel electrophoresis. Ladder 1Kbp ladder, PC (Positive control, Live attenuated human G1 monovalent, RIX4414 strain of rotavirus vaccine, Glaxosmith Kline as positive control, which is proceeded through extraction using QIA gene kit and in parallel with the DNA ladder included in each RT-PCR). Some of 65 Positive samples (2, 10, 14, 15, 21, 23, 24, 26, 27, 28, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, and 49).

Human rotavirus infection through year 2007-2009

The statistical analysis of data collected from 5 hospitals in Jeddah city showed that there was a decreasing of the

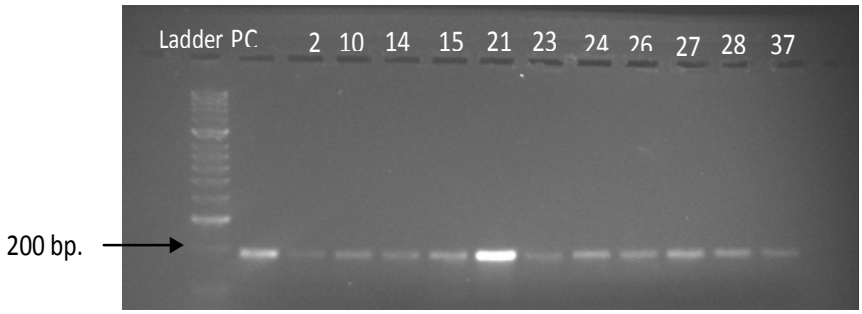

Figure 2a. Positive samples

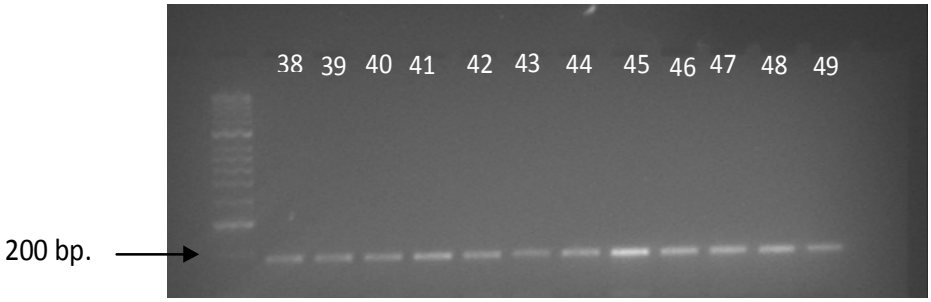

Figure 2b. Positive samples

Table 1. Number of infected people with HRV collected from 5 major hospitals in Jeddah city through the years 2007 - 2009

Hospitals		Year			Total	*p* value
		2007	2008	2009		
King Abdulaziz University	Count	8	7	3	18	<0.001
	Total (%)	44	39	17	100	
Bugshan	Count	59	50	6	115	<0.001
	Total (%)	51.3	43.5	5.2	100	
King Khaled National Guard	Count	52	85	29	166	<0.001
	Total (%)	31.3	51.2	17.5	100	
Ghassan Najib Pharaon	Count	76	39	17	132	<0.001
	Total (%)	57.6	29.5	12.9	100	
International Medical Center	Count	23	33	60	116	<0.001
	Total (%)	19.8	28.4	51.7	100	
Total	Count	218	214	115	547	<0.001
	Total (%)	39.9	39.1	21.0	100%	

number of RV infection throughout the years 2007 - 2009. The RV infections were 40%, 39.1% and 21% of total number of patients 547 of the five hospitals (Table 1). Analysis of data showed that there was significant difference in the rate of RV infection through out the years 2007 - 2009 with *p* value <0.001 (Table 1).

Human rotavirus infection according to gender

The prevalence of RV infection in the entire collected data was performed. Although the number of males infected with HRV 294 (54%) was more than the number of females 253 (46.3%) through the years 20007 - 2009

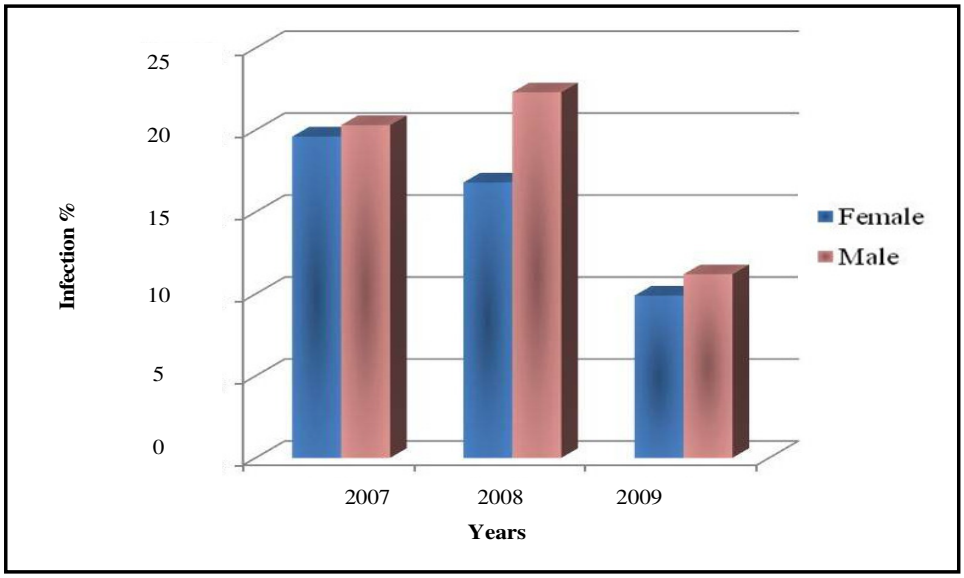

Figure 3. Distribution of HRV infection among genders

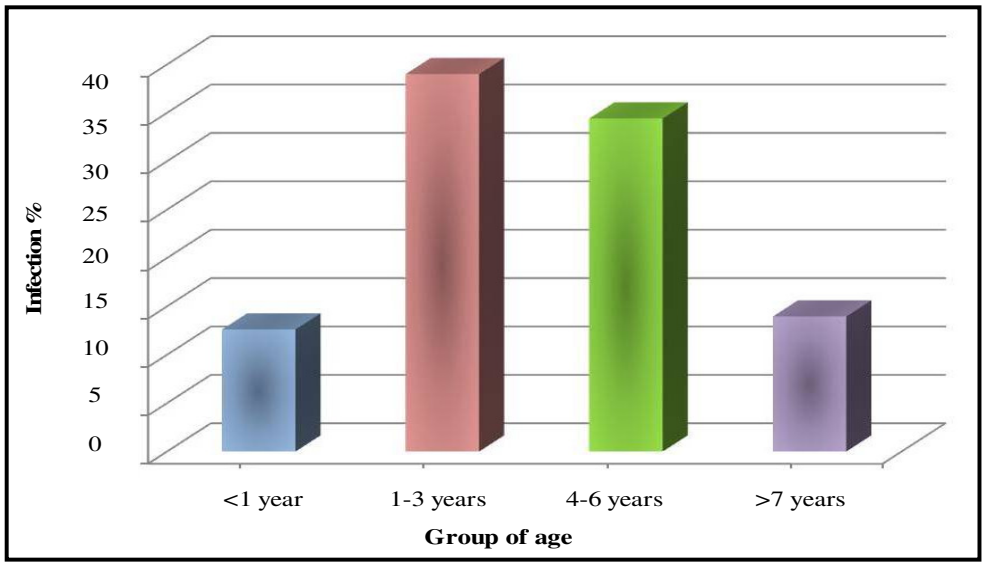

Figure 4. Percentage of HRV infections in different age groups

(Figure 3), there is no significant difference in the rate between the males and females ($p > 0.05$).

Human rotavirus infection according to age group

Statistical analysis of data showed that there was a significant difference ($p < 0.001$) in the rate of HRV infection between all age groups. The rate of HRV infection was higher in 1-3 years age group (39%)] than < 1 year (13%). The rate of HRV infection was higher in 4-6 years age group (34.4%) than children at 7 year age group (14%) Figure 4.

In addition, there was a clear increasing in the number of HRV infection in children at 1-3 year group of age throughout 2007 – 2009 with significant value of $p < 0.001$. What did you use as statistical program?

Seasonality of infection

Figure 5 shows the infection cases fro each month of the

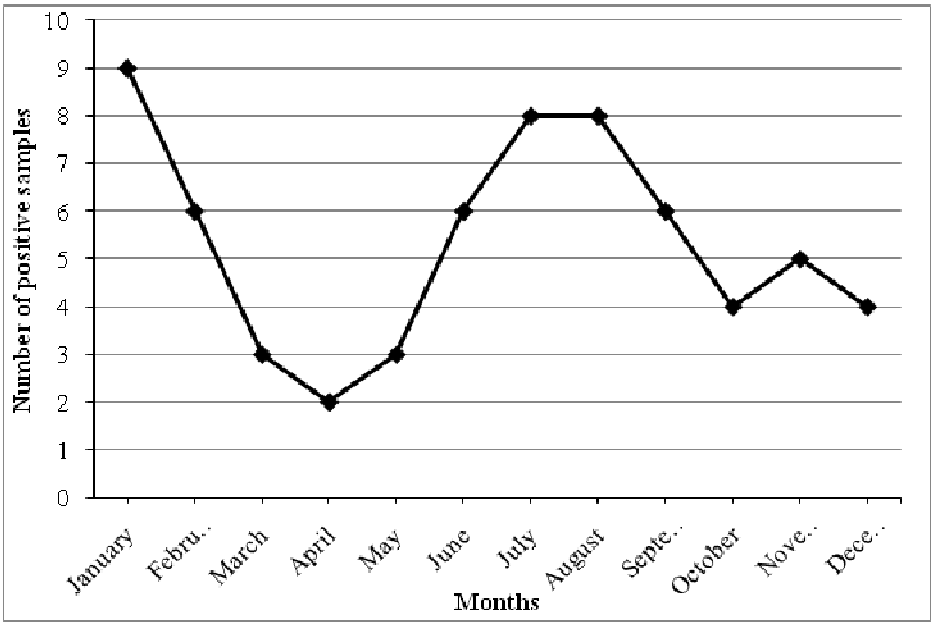

Figure 5. Monthly distribution of rotavirus in wastewater from 28th of January 2009 – 31st of January 2010

study period. Distribution of these cases showed a pattern of higher cases in the cooler months (January, February) and the warmer months (June –September) in Jeddah.

DISCUSSION

RVs were detected from waste water samples throughout the study years 2009 - 2010, suggesting that the virus is circulating around the year with a peaking incidence in the cooler and warmer months.To what do you associate these peaks as you are in the discussion part

Molecular techniques, such as RT-PCR, nested PCR, rPCR and multiplex PCR are used to detect RVs from water. In This study, RV was screened in waste water collected from the outlet of waste water lake (AL-Misk Lake) of Jeddah city. HRV was detected in waste water samples by using RT-PCR.

According to Pina et al. (1998) and Park et al. (2010), PCR has led to higher rates of detection of enteric viruses in environmental samples. In addition, Borchardt et al. (2003) detected 8% of enteric viruses (enteroviruses, RV, NVs and HAVs) from 50 household wells by PCR, while no virus was detected by cell culture. Conventional RT-PCR considers as a simple, easy, efficient and high sensitive technique, and this is a suitable technique to detect RV from waste water sample. Moreover, Villena et al. (2003), found, that 85.7% of 35 waste water samples from Greater Cairo were RV positive by RT-PCR. In Barcelona (Spain), 239 (66.9%) of 357 waste water samples were positive. Moreover, Abbaszadegan et al. (1999) have used RT-PCR to detect RV from 130 groundwater samples and they succeeded in detecting 14% positive samples for RV. In addition, Gratacap-cavallier et al. (2000) has found 7% positive samples of RV from 56 water samples taken at homes of infected children using RT-PCR. Buesa et al. (1996) approved that RT-PCR are more sensitive than ELISA, PAGE and EM. Nishimura et al. (1993), Ushijima et al. (1994), Husain et al. (1995), Gladstone et al. (2008), Kamel et al. (2009) and Brassard et al. (2011**)** stated that RT-PCR is sensitive and suitable technique in detecting RV from other samples such as serum, stool, central nervous system (CNS) and food samples**.**

Borchardt et al. (2003), used the same technique in detecting 4 (8%) RV in 1 contaminated well out of total 50 Wisconsin household wells. After two years Brassard et al.. (2005), approved that the analytical sensitivity of the RT-PCR is at least a hundred fold more sensitive for RVs (10^{-3} $TCID_{50\%}$/ml) compared to the multiplex. Rodríguez-Díaz et al. (2009) used the nested or seminested PCR and they found that HRVs were the waterborne gastroenteritis viruses most frequently detected both in waste water and in the highly polluted Guaire River. Twenty three (77%) samples that were positive for HRVs of total 30 samples processed and included both waste water and waste water-polluted river waters. Recently, Barril et al.. (2010), found that all 52 waste water samples tested for RV-A detection by RT-PCR followed by seminested PCR were positive for RV-A.

In Jeddah city, which has high temperatures and high humidity, RV was detected throughout the whole study period, and showed higher cases in the cooler and the

warmer months of the study. In Florida Custodio et al. (2010) has found that there is no definite season. Also, Dutta et al. (1990) has referred that RV could be detected throughout the year from diarrhoea cases in Bahrain with no seasonal trend and it did not show any correlation with mean monthly temperature and humidity. Despite, Kheyami et al.. (2006) has found the peak between November and February. Milaat and EL-Assouli (1995) has agreed that RV frequency rate is higher in cooler months, while Bahl et al. (2005) referred in Delhi the same observation.

This study, for the first time, revealed the whole year prevalence of rotaviruses in wastewater in Jeddah, and demonstrated the impact of wastewater discharge on the potential spreading of infectious rotaviruses and public health.

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