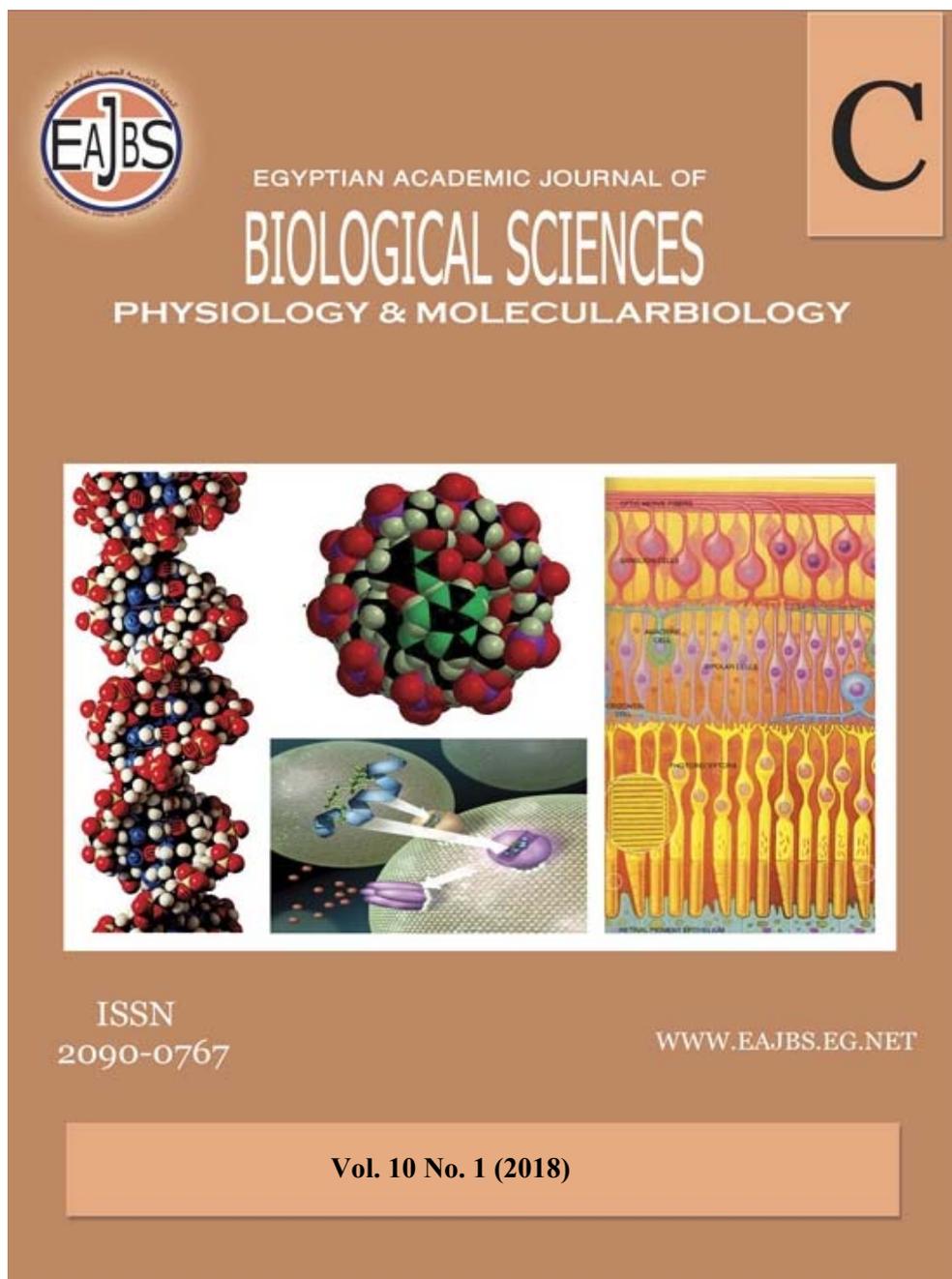


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## Molecular Detection of Hepatitis A Virus and Rotavirus in Water Samples Collected from Albaha, Saudi Arabia

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### ABSTRACT

Viral contamination of the water bodies is neglected area of research in Saudi Arabia. The study, first of its kind, using RT-PCR technique, reports Hepatitis A virus (HAV) and Rotavirus (RV) presence in almost 13% of the samples collected. This report may trigger the detection of accurate viral contamination status in Saudi Arabia's environmental waters.

### INTRODUCTION

Globally, waterborne disease is a huge burden causing approximately 2.2 million deaths, and more than the half of them are children under 5 years of age, annually from diarrhea, gastrointestinal diseases, and systematic illnesses (WHO, 2017). Approximately 9.1% of the global burden of disease and 6.3% of all deaths could be prevented by improving quality of water, sanitation, and general hygiene (Pruss-Ustun *et al.*, 2008).

Microbiological water quality is largely assessed through bacterial indicators like enterococci, fecal coliform, and total coliform bacteria. However, the major disadvantage using this approach is that important hazardous viruses like enteric viruses often go unnoticed (Schvoerer *et al.*, 2001).

Most of the human enteric viruses have a non-enveloped structure, and thus are resistant to common decontamination processes (Tuladhar *et al.*, 2012). This group represented by the astroviruses, rotaviruses, noroviruses, adenoviruses, and hepatitis A can survive for many months in diverse environments (Fong & Lipp, 2005; Pellegrinelli *et al.*, 2013) and have been found to be associated with many waterborne outbreaks (Fong & Lipp, 2005). Therefore, they may be highly recommended to be used as indicators of microbial water quality along with coliforms (Fong & Lipp, 2005; Griffin *et al.*, 2003; Noble *et al.*, 2003).

Although most of the enteric viral infections are asymptomatic in humans and primarily cause diarrhea and self-limiting gastroenteritis, but many of them cause

serious complications such as meningitis, hepatitis, acute flaccid paralysis, conjunctivitis, respiratory illness, and myocarditis among others (Palacios & Oberste, 2005).

Moreover, enteric viruses may further cause aseptic meningitis, encephalitis, and paralysis in immunocompromised individuals inflicting high mortality rates (Fong & Lipp, 2005).

Adenoviruses, ds-DNA viruses, are comparably more resistant to UV light than other enteroviruses and cause gastroenteritis along with conjunctivitis, cystitis and respiratory infections in humans (Doerfler, 1996).

The Hepatitis A and E virus outbreaks are primarily associated with contaminated water supplies (Khuroo & Khuroo, 2016). Rotavirus, ds-RNA virus, infection leads to severe diarrhea among infants and young children (Kapikian & Shope, 1996). Additionally, rotavirus also infects livestock affecting agriculture (Cho & Yoon, 2014).

In Albaha region, KSA, water reservoir dams supply the majority of water for the public and agricultural use. Few early studies analyzed the heavy metal content in the water bodies and the plantation found therein (Schvoerer *et al.*, 2001; Zabin SA, 2015). However, no studies have been conducted so far analyzing the presence of pathogenic microorganisms in these water bodies especially at a level of viruses.

Considering the significant risk to public health by human viral presence, this study, first report so far, attempts to detect the contamination of water bodies by Hepatitis A virus, Rotavirus, Enterovirus, and Human Adenoviruses from Albaha region of KSA.

## MATERIALS AND METHODS

### Sampling Sites :

The Al-Baha region is situated in the southwestern part of Saudi Arabia

with an area of 15,000 km<sup>2</sup> and with an estimated population of about 500,000 residents (The General Census Department, Saudi Arabia 2004). Water samples were collected from the dams located in Biljurashi (Bj) and Mikhwah (Mk) sites of the Albaha region during the period between February- April 2017.

### Water Sample Collection and Processing

30 samples (500 ml each) were collected from surface water in sterilized plastic bottles. Samples were stored at 4°C prior to processing. Adsorption elution method was used for samples concentration (Donaldson *et al.*, 2002; Karamoko *et al.*, 2005) Briefly, water samples were filtered through whatmann filter paper No. 10. before mixing with 0.3 g MgCl<sub>2</sub> and pH adjustment to 5.

Samples were passed through a 0.45 µm pore size and a 90 mm diameter negatively charged membrane filters (Millipore, USA) in a vacuum pump system to adsorb the viruses to the membrane.

After that, the membranes were rinsed with 150 ml of 0.5 mM H<sub>2</sub>SO<sub>4</sub> (pH: 3.0) to rinse out the cation. Viral particles adsorbed by the membrane were eluted using 5 ml of 1 mM NaOH (pH: 10.5) and the resulting filtrate was mixed with 25 µl of 50 mM H<sub>2</sub>SO<sub>4</sub> and 25 µl of 100x Tris-EDTA (TAE) buffer for neutralization. The eluate was stored at -80°C until processing.

### Nucleic Acid Isolation:

Viral RNA and DNA were isolated by using an RNA/DNA extraction kit (Invitrogen, USA) following the manufacturers guidelines. Viral RNA/DNA were kept at -80 °C until further use.

### Detection of Viruses Using PCR:

Rotavirus, Adenovirus, Hepatitis A virus, and Enterovirus contamination in the water samples were detected using specific primers according to the conditions as follows:

**Hepatitis A Virus:**

	Primer name	Sequence	Nucleotide position	Product size
HAV	HAV FP	GTTTTGCTCCTCTTTATCATGCTATG	2109–2135	246bp
	HAV RP	GGAAATGTCTCAGGTACTTCTTTG	2330–2355	HAV RP

cDNA synthesis: 5 µl of RNA Template was added to 4 µl of Moloney Murine leukemia virus (MMLV) buffer containing 2 µl of 10 mM dNTPs, 1.5 µl of HAV R- Primer, and 0.6 µl of MMLV Enzyme. Reaction tubes were placed in thermal cycler (Bio- Rad) at 42°C for 1 hour. PCR: 5 µl of cDNA template was added to 2.5 µl of 1x Taq reaction buffer containing 1.5 µl of 2 mM each dNTPs,

1.5 µl of forward and reverse primer, 0.5 µl of Taq DNA polymerase, and 7.5 µl of RNase free water. Reaction tubes were placed in a thermal cycler for an initial denaturation at 94°C for 3 min., followed by 35 cycles of denaturation at 94°C for 1 min., annealing at 55°C for 1 min., and extension at 72°C for 45 sec., completed by a final extension at 72°C for 7 min.

**Enterovirus:**

	Primer name	Sequence	Nucleotide position	Product size
EV	En-1F	CAAGCACTTCTGTTTCCCCGG	164–184	436bp
	En-1R	ATTGTCACCATAAGCAGCCA	599–580	
	En-2R*	CTTGCGCGTTACGAC	562–512	

cDNA synthesis: 10 µl of RNA Template was added to 4 µl of MMLV buffer containing 2 µl of 10 mM dNTPs, 1.5 µl of Enterovirus R- Primer, and 0.6 µl of MMLV Enzyme. Reaction tubes were placed in thermal cycler at 42°C for 1 hour. PCR: 7 µl of cDNA template was added to 2.5 µl of 1x Taq reaction buffer containing 2.5 µl of 2 mM each dNTPs, 1.5 µl of forward and reverse primers,

0.5 µl of Taq DNA polymerase, and 5.5 µl of RNase free water. Reaction tubes were placed in a thermal cycler for an initial denaturation at 94°C for 4 min., followed by 35 cycles of denaturation at 94°C for 1 min., annealing at 58°C for 1 min., and extension at 72°C for 1 min., completed by a final extension at 72°C for 7 min.

**Human Adenovirus:**

	Primer name	Sequence	Nucleotide position	Product size
HAdV	Ad-1F	CATTGCCAGGAATAAAGAA	32763–32782	429bp
	Ad-2F*	TCATATCATGGGTAACAGACAT	33000–33021	202bp
	Ad-2R*	CCCATGTAGGCGTGGACTTC	33182–33201	

PCR: 5 µl of DNA template was added to 2.5 µl of 1x Taq reaction buffer containing 2.5 µl of 2 mM each dNTPs, 2 µl of forward and reverse primers, 0.5 µl of Taq DNA polymerase, and 5.5 µl of RNase free water. Reaction tubes were placed in a thermal cycler for an initial denaturation at 94°C for 4 min., followed by 35 cycles of denaturation at 94°C for 1 min., annealing at 54°C for 30 sec., and extension at 72°C for 1 min., completed by a final extension at 72°C for 7 min.

**Gel Electrophoresis:**

PCR results were analyzed by gel electrophoresis. Briefly, 10 µl of PCR product mixed with 2 µl 6x loading dye (Invitrogen) was loaded into the wells of 2% 1X TAE agarose gel containing 2 µl of 10 mg/ml ethidium bromide ethidium-bromide, to which 90 Volts were applied for 25 to 35 minutes. A 3 kb DNA ladder (NEB, MA) was used for indication of PCR product fragment size. The Molecular Imager Gel Doc system (Bio-Rad Laboratories, Inc., CA) was used to visualize results under UV light.

## RESULTS AND DISCUSSION

Thirty water samples collected from different sites in Albaha region, KSA, were analyzed by conventional PCR for the prevalence of Hepatitis A virus, Rota virus, Enterovirus, and Human Adenovirus.

Of all the samples collected, Hepatitis A virus presence was detected

in 4 samples collected from Bj-1 and Mk-1 sampling sites with 13.33% prevalence. Rotavirus was detected in 4 samples collected from Bj-2 sampling sites with 13.33% prevalence. Enterovirus and Human adenovirus presence was not detected in any of the samples collected (Table 1).

**Table 1.** Detection pattern of Hepatitis A virus (HAV) and rotavirus (RV) in water samples collected from Albaha region, KSA.

Sampling site	Hepatitis A virus (HAV)	Rotavirus (RV)	Enterovirus (EV)	Human Adeno Virus (HAdV)
Bj-1	+ (2)	-	-	-
Bj-2	-	+ (4)	-	-
Bj-3	-	-	-	-
Bj-4	-	-	-	-
Mk-1	+ (2)	-	-	-
Mk-2	-	-	-	-

Numbers indicate samples; + indicates viral detection; – indicate absence of viral detection

Amplification of the target sequence of the pathogenic viruses using gene specific primers was visualized through

gel electrophoresis. Figure 1 clearly showed 246 bp amplification indicating the presence of Hepatitis A virus



**Fig. 1:** Agarose gel depicting the amplified PCR products of Hepatitis A virus (246 bps) and Rotavirus (380 bps) from eight water samples collected in and around Albaha region of KSA. Lanes MM: Molecular weight Marker (3000 – 100 bps); Lanes S1 – S4 shows water samples examined for presence of Hepatitis A virus; and lanes S5 - S8 shows water samples examined for presence of Rotavirus.

Figure 2 showed an amplification of 380 bp amplification demonstrating the presence of Rotavirus in the sample



**Fig. 2:** A representative gel picture. Lanes S1 & S2 show absence of any amplification in two water samples analyzed for Enteroviruses; lanes S3 & S4 show presence of Rotavirus (380bps); lanes S5 & S6 show absence of amplification for Human Adenovirus; and lanes S7 & S8 show presence of Hepatitis A virus (246bps).

The current study reports the efficient concentration and detection of different viruses and their prevalence in the water bodies in Albaha region. The study is the first from the region as well as Saudi Arabia reporting the presence of potentially problematic viral pathogens in the water sources. The early studies from the country so far have relied upon bacterial indicators alone for water quality assessment (Alqahtani *et al.*, 2015; Saatti AA, 2013).

A recent study was conducted in Makkah city to detect prevalence of pathogens in different drinking water resources using filtration method on solid and liquid selective media. Of all the water samples tested, 58% drinkable

wells water (DWW) samples, 42% non-drinkable wells water (NDWW) were found contaminated with *E. coli*. Whereas 92% DWW samples and 100% NDWW samples were found contaminated with *P. aeruginosa*. Approximately 8% DWW samples, 16% NDWW samples were found contaminated with *E. faecalis*. Additionally, 33% DWW samples, 8% NDWW sample were contaminated with *Aspergillus* spp. along with *Penicillium* spp found in 100% and 50% of SDW (Safe drinking water) and CDW (Circumpolar Deep Water) samples, respectively (E, 2013).

Another recent study reported the poor bacteriological quality of drinking

water samples collected from southwestern Saudi Arabia. Of all, 20% water samples collected from wells, 32% samples from water carrying tankers, 69% of samples from roof tanks were found positive for total coliforms. Furthermore, 25% and 10% of the water samples from in-house water storage tanks were found to be positive for *E. coli* and *Streptococcus faecalis*, respectively (Alqahtani *et al.*, 2015).

A pioneer study investigating the presence of potentially pathogenic non-cholera *Vibrio* species in environmental water (Non-Drinking Water Resource) of Eastern Region of Saudi Arabia, revealed the presence of five potentially pathogenic *Vibrio* species in the water samples.

Of all the samples, 38% were positive for *V. alginolyticus* species, 13.3 % for *V. parahaemolyticus*, 7.6% for *V. vulnificus*, 5.6% for *V. cholerae* non-O/non-O139 and 0.33% for *V. mimicus* (Saksela *et al.*, 1995).

Several other early studies from Saudi Arabia have reported the presence of pathogenic organisms primarily restricted to the bacterial contamination in drinking water that exceeds the guideline values recommended by national and international standards of drinking water (Alaa el-Din *et al.*, 1994; AlOtaibi, 2009; BM, 2010; Figueras & Borrego, 2010).

Approximately 1407 species of waterborne pathogens infect humans including 538 species of bacteria, 208 types of viruses, 57 species of parasitic protozoa, and many fungi and helminths species (Sobsey & Pillai, 2009).

More importantly, emerging waterborne pathogens like protozoa *Microsporidia*, as the bacteria *Mycobacterium avium intracellulare*, *Helicobacter pylori*, *Tsukamurella*, *Cystoisospora belli*, and viruses such as adenoviruses, parvoviruses, coronaviruses (SARS), and polyomavirus are showing resistance against common decontamination/

inactivation measures including chlorine, UV light and heat treatment (Nwachuku & Gerba, 2004).

The major challenge in the waterborne pathogen detection lies in the specificity, sensitivity, and the speed of the method (Kostic *et al.*, 2011; Zhao *et al.*, 2014).

So far, majority of studies conducted in Saudi Arabia have used culture dependent methods for pathogens detection in water. The limitations of these methods largely include low sensitivity and the large turnaround time along with the presence of viable but non-culturable (VBNC) state in many human pathogens like *Helicobacter pylori* giving rise to false negative results (Law *et al.*, 2014; Zhao *et al.*, 2014).

The current study, first known so far in Saudi Arabia, fills this lacuna by investigating the prevalence of Rotavirus, Human Adenovirus, Hepatitis A virus, and Enterovirus in water samples collected locally through a molecular method.

The choice of the viruses studied was dictated by their high impact on human health and long persistence in drinking water source.

In current study, environmental prevalence of Hepatitis A virus (HAV) in almost 13% samples collected was found lower than recently estimated anti-HAV prevalence rate in population of KSA i.e., 18.6% (Abdo *et al.*, 2012; Al Faleh *et al.*, 2008).

However, the current finding that HAV is present in the study area identifies a major risk factor of disease transmission, which may lead to the development of comprehensive prevention and detection programs.

More importantly reported here is the presence of Rotavirus, the most common cause of diarrhea in infants and young children, albeit in almost 13% of the samples collected from the water bodies (Tate *et al.*, 2012). Rotavirus presence in these bodies indicates

towards possible sewage contamination. An early study reported as high as 65% positivity in the waste water samples collected in Jeddah, KSA (Redwan, 2012). The water from these resources is used for the drinking water supply and agriculture irrigation purposes after the treatment. Stringent evaluation and monitoring of the treatment plants with respect to their efficacy in clearing the viral contamination cannot be emphasized more in the wake of the high stability of human rotaviruses in environmental water and their resistance to physicochemical treatment processes (Choi *et al.*, 2014; Rao *et al.*, 1986).

### CONCLUSION

Drinking water contamination with human pathogenic viruses may be a major source of widespread illness and mortality. Molecular technique used in the current study successfully identified the viral presence in two water bodies. The findings underline the urgent need of the strict microbiological monitoring of the water bodies used as the source of drinking water to accurately detect waterborne pathogens and protect public health.

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**Conflict of Interests:** The author declares no conflict of interests.

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## ARABIC SUMMARY

وجود فيروس التهاب الكبد A وفيروس العجلي في عينات مياه تم جمعها من منطقة الباحة  
بالمملكة العربية السعودية

شائع صالح ر. المالكي

قسم طب المختبرات ، كلية العلوم الطبية التطبيقية ، جامعة الباحة ، الباحة ، المملكة العربية السعودية  
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التلوث الفيروسي للمسطحات المائية مجال بحثي مهم في المملكة العربية السعودية. هذه الدراسة الأولى  
من نوعها، من خلال استخدام تقنية RT-PCR، تبين وجود فيروس الكبد الوبائي (HAV) وفيروس العجلي  
(RV) فيما يقرب من 13% من العينات التي تم جمعها. وقد يؤدي هذا التقرير إلى الكشف عن حالات التلوث  
الفيروسي الدقيقة في المياه البيئية في المملكة العربية السعودية.