Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol. 25(2): 735 – 749 (2021)
www.ejabf.journals.ekb.eg

Molecular Quantification of Human Bocavirus in Environmental Water Samples in Giza, Egypt

Neveen Magdy Rizk and Ibrahim Ahmed Hamza*
Environmental Virology Laboratory, Water Pollution Research Department,
National Research Centre, Cairo, Egypt.
***Corresponding Author: ibrahimnrc@gmail.com**

ARTICLE INFO
Article History:
Received: Dec. 6, 2020
Accepted: April 19, 2021
Online: April 30, 2021

Keywords:
Human bocavirus,
Nile water,
Drinking water,
Wastewater,
qPCR.

ABSTRACT

There are extremely limited data on the prevalence and quantification of human bocavirus (HBoV) genotypes in different environments, particularly for rivers and drinking water. Here we performed quantitative real-time PCR (qPCR) to investigate the presence and load of HBoV genotypes in Nile water, drinking water, and effluent of the activated sludge wastewater treatment facility in Giza, Egypt. The results revealed that the detection rate of HBoV was 95.8% (23/24) and 29.2% (14/48) in effluents of wastewater treatment facility and Nile water samples, respectively. HBoV was not found in drinking water samples from the Giza governorate. The mean concentration values of human bocavirus genotypes HBoV-1, HBoV-2/4 and HBoV-3 were 0.52 $\log_{10}$ GC/L, 2.2 $\log_{10}$ GC/L, 0.3 $\log_{10}$ GC/L, respectively in HBoV-positive Nile water samples (n = 14), while in HBoV-positive effluent of wastewater samples (n = 23) were 0.77 $\log_{10}$ GC/L, 2.3 $\log_{10}$ GC/L, and 1.6 $\log_{10}$ GC/L, respectively. The viral type had a significant impact on the prevalence of the virus in surface water as well as effluent samples. A seasonal pattern of HBoV subtypes was observed in surface water (P = 0.0002), as the highest detection rate was found during winter. Furthermore, the discharge of the effluent with higher viral concentration into the water sources leads to viral contamination of these sources, which may be a probable source for human infections. This study is executed to fill a gap of knowledge concerning the quantification and genotyping of bocavirus in Nile water, and enrich the scientific literature body by providing data about HBoV in environmental samples.

INTRODUCTION

Contamination of water supplies with enteric viruses is a significant concern for public health. The water plays a meaningful role as a transmission route of many enteric viruses. Furthermore, the main source of viral contamination is through discharging of treated or non-treated sewage in surface waters. Environmental epidemiological studies are crucial because many infectious diarrheal diseases remain with unknown etiological agents **(Fong and Lipp, 2005; Bridge *et al.*, 2010; Ong *et al.*, 2016).** Diarrheal illnessess cause about 88% of deaths worldwide (Liu *et al*., 2012), and WHO estimated that about

1.7 million deaths from ~ 4 billion cases of diarrhea annually, primarily related to unsafe drinking water and poor hygiene **(Bosch, 2007).**

Human bocavirus was first reported in Swedish children's respiratory tract samples in 2005 **(Allander *et al.*, 2005)**. Bocavirus belongs to the Parvoviridae family, which causes infection only in vertebrates **(Jartti *et al.*, 2012; Cotmore *et al.*, 2014)**. HBoVs are single-stranded icosahedral, non-enveloped DNA viruses with a length of 5.3 kb. HBoVs consist of three open reading frames (ORFs), two non-structural proteins (NS1 and NP1) are encoded by ORFs (1, 3), and the viral capsid proteins 1 and 2 (VP1 and VP2) are encoded by ORF2 **(Allander *et al.*, 2005; Gurda *et al.*, 2010; Schildgen *et al.*, 2012; Zhao *et al.*, 2012)**.

Four genotypes have recently been identified, including HBoV-1, HBoV-2, HBoV-3, and HBoV-4 **(Allander *et al.*, 2005; Arthur *et al.*, 2009; Kapoor *et al.*, 2010, 2011)**, Acute respiratory infections are usually associated with the first genotype (HBoV-1), while the remaining genotypes are found initially in stool samples of patients suffering from gastroenteritis **(Allander *et al.*, 2005; Arthur *et al.*, 2009; Kapoor *et al.*, 2011)**. However, the pathogenesis of HBoV is not clear because of the lack of animal models and specific cell lines **(Song *et al.*, 2010; Koseki *et al.*, 2012)**. The evolution of HBoV subtypes has been causally associated with a high frequency degree of recombination at VP1 and NP1 genes within/and between bocavirus species **(Kapoor et al., 2010)**. Different qPCR and conventional PCR (single, nested) targeting different genes (NS1, NP1, VP1, VP2) for detection of HBoV were well established **(Allander *et al.*, 2005; Zhao *et al.*, 2012; Misigo *et al.*, 2014; Hamza et al., 2017; Lin *et al.*, 2020)**. Globally, HBoV genotypes have been identified without any geographical or boundary restrictions. The infection rate with HBoV is more likely to be higher in developing countries (from 29.2% to 63.0%) than in developed countries (from 1.3% to 6.3 %) **(Guido *et al.*, 2016)**.

Several environmental studies on various enteric viruses including adenovirus, rotavirus, norovirus and astrovirus in Egypt have been found **(Ali *et al.*, 2004; Kamel *et al.*, 2009; Rizk and Allayeh, 2018; Rizk *et al.*, 2019; Gad *et al.*, 2019)**. Globally, other reports recorded the prevalence of HBoV in wastewater (60–93%) and surface water (37–40%) **(Blinkova *et al.*, 2009; Hamza *et al.*, 2009; Bibby and Peccia, 2013; Myrmel *et al.*, 2015; Iaconelli *et al.*, 2016; La Rosa *et al.*, 2017; Hamza *et al.*, 2017)**. However, environmental epidemiological studies of HBoV in Egypt are limited **(Hamza *et al.*, 2017; Shaheen *et al.*, 2019)**, and collectively did not consider quantitation and genotyping of HBoV in Nile river water, and did not test HBoV in drinking water. The presence of HBoV in water used for drinking or recreation purposes, may increase the potential risk of infection as has been proposed for other enteric viruses **(Lodder and Husman, 2005; Hamza *et al.*, 2009; Wang *et al.*, 2010)**. To the best of our knowledge, no studies on the presence of HBoV in drinking water were found, and so far, one study about the quantification and genotypes of HBoV in environmental samples in Egypt is

present. Therefore, we aim to assess the prevalence, viral loads and genotypes of HBoV in Nile water, drinking water and effluents of wastewater treatment facility.

MATERIALS AND METHODS

A total of 120 samples were collected from Nile water (n=48), drinking water (n=48), and from effluents (n=24) of Zenin wastewater treatment facility (ZWWTF) with a designed capacity of 330,000 m^3/day. The surface water samples were collected from Nile river beside Gazirat Al Dahab drinking water treatment facility (GDWTF). The drinking water samples were collected from two sites, the first site near the drinking water station (GDWTF) at Al Munib district, and the second site far from the drinking water station at Faisal district. Both districts were supplied by drinking water from GDWTF. The sample volumes were 5 liters, 10 liters and 20 liters for the effluent of ZWWTF, Nile water and drinking water, respectively.

Samples concentration and processing

Water samples were separately concentrated by filtration through negatively charged nitrocellulose membranes (0.45µm pore size) after addition of $MgCl_2$ to a final concentration of 0.5 mM and acidification to pH 3.5. The adsorbed viruses to the membrane were eluted with 75ml of 3% beef extract-0.05M glycine buffer, pH 9.5 (Lab-Limco powder, OXOID, UK). All samples were reconcentrated using an organic flocculation **(APHA, 2012)**. The eluate was acidified to pH 3.5 using HCl , centrifuged at 3000 rpm for 15 min, the supernatant was discarded, and the pellet was dissolved in 1 ml of Na_2HPO_4 (0.14 N, pH 9).

Nucleic Acid Extraction

Viral nucleic acids were extracted from 200µl of the concentrated sample using DNeasy PowerLyzer PowerSoil Kit (QIAGEN-USA) according to the manufacturer's instructions. The obtained nucleic acid was dissolved in 80µl of elution buffer and kept at -70^oC until used.

Detection and Quantification of HBoV by qPCR

All primers used in the current study are listed in Table 1. For HBoV-1, the quantification protocol specific to NP1 gene was used **(Hamza *et al.*, 2009)**. A single sense primer was shared in HBoV -2, 3 and -4 quantification while the same antisense primer was used in the qPCR of HBoV-2 and -4 (Kantola et al., 2010). SYBR green qPCR assay was conductnded for HBoVs quantification using a Maxima SYBR Green qPCR Master Mix Kit (Thermo Scientifc). The real-time PCR amplification thermal conditions were as follow; 30 s initial denaturation step at 95^oC, 40 cycles of denaturation at 95^oC for 15 s and annealing-extension at 55^oC for 30 s. The specificity of the reactions was determined by melting curve analysis of the amplicons. The genome copy number of Bocavirus genotypes (HBoV-1, HBoV-2/4 and HBoV-3) was determined by comparison with a standard curve generated with serial dilutions of positive control of the PCR product from each genotype. The PCR product was purified using Wizard® SV Gel and

the PCR Clean-Up System (Promega, USA). Nucleic acid concentrations of the purified PCR products were determined by NanoDrop Fluorospectrometer (Thermo-Scientific, USA). The number of DNA copies was determined by multiplying the DNA concentration by Avogadro's constant and dividing by the product size and average weight of a base pair. The standard curves of each examined organism were separately prepared by tenfold serial dilution of the nucleic acid standard ranging from 5×10^1 to 5×10^7 copies/ reaction. Virus concentration per liter GC/L was calculated according to the following equation: $\mathbf{GC/L} = \frac{\mathbf{GC\ x\ DF}}{\mathbf{V}}$

Where GC is genome copy number per reaction, DF is the dilution factor for the volume reductions that occur during the concentration, DNA extraction and qPCR steps, and V is volume of original water sample assayed in liters **(Hamza *et al.*, 2017)**. Moreover, no PCR inhibitors were found in the examined samples.

Table (1): Primer sequences of Bocavirus genotypes

Virus	Target gene	Primer name	Sequence (5`–3`)	Fragment length (bp)	Reference
HBoV-1		NP1-F2421	TGGCAGACAACTCATCACAG		**(Hamza *et al.*, 2009)**
	NP1	NP1- R2544	TCTTCGAAGCAGTGCAAGAC	123	
HBoV-2/4		HBoV234F	GCACTTCCGCATYTCGTCAG		
	NS1	HBoV24R	AGCAGAAAAGGCCATAGTGTCA	100	**(Kantola *et al.*, 2010)**
HBoV-3		HBoV234F	GCACTTCCGCATYTCGTCAG		
	NS1	HBoV3R	GTGGATTGAAAGCCATAATTTGA	100	

Statistical analyses

Statistical analyses were performed using GraphPad Prism version 8.3.0 software (USA). The critical *P*-value for the test was set at <0.05. One-way ANOVA was used to test the correlation between the relative distribution of different bocavirus genotypes in surface or effluent samples. Two-way ANOVA was used to teste the significance effect of the seasons and the viral types on the prevalence of the virus.

RESULTS

Prevalence of bocavirus genotypes in water samples

In the present study, the prevalence rate of HBoV was 95.8% and 29.2% in effluent and Nile water samples, respectively. HBoV could not be detected in drinking water samples collected from Giza governorate. HBoV-1, HBoV-2/4 and HBoV-3 were found in the effluent as well as Nile water samples. The highest prevalence rate (20.8%) was

recorded for HBoV-2/4, followed by 6.3% for HBoV-1 and 2.1% for HBoV-3 in Nile water samples. Concerning the effluents, the mixed contamination of HBoV-2/4&HBoV-3, and single contamination by HBoV-2/4 recorded the highest rates (25% for each), followed by co-detection of HBoV-1&HBoV-2/4&HBoV-3, which accounted for 16.7%. Then the co-detection of HBoV-1&HBoV-3 and HBoV-1&HBoV-2/4 were recorded in 12.5% and 8.3% of effluent samples, respectively. Furthermore, single detection of HBoV-3 was detected in 8.3% of effluents (Table 2).

Table (2): Prevalence of Bocavirus genotypes in water samples

Water Type	No. of Samples	No. of ositive amples		HBoV-1		HBoV-2/4		HBoV-3		HBoV-1&HBoV-2/4		HBoV-1&HBoV-3		HBoV-2/4 &HBoV-3		HBoV-1&HBoV-2/4&HBoV-3	
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Effluent	24	23	95.8	0	0	6	25	2	8.3	2	8.3	3	12.5	6	25	4	16.7
Nile	48	14	29.2	3	6.3	10	20.8	1	2.1	ND	ND	ND	ND	ND	ND	ND	ND
Drinking	48	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

HBoV-1&HBoV-2: The presence of HBoV-1 and HBoV-2/4 subtypes in the same sample (dual contamination)

HBoV-1&HBoV-3: The presence of HBoV-1 and HBoV-3 subtypes in the same sample (dual contamination)

HBoV-2/4 &HBoV-3: The presence of HBoV-2/4 and HBoV-3 subtypes in the same sample (dual contamination)

HBoV-1&HBoV-2/4&HBoV-3: The presence of HBoV-1, HBoV-2/4 and HBoV-3 subtypes in the same sample (triple contamination)

*** No. of positive samples was calculated based on the presence of one or more HBoV subtypes in the sample**

ND: not detected

Quantification of HBoV in surface water and effluent samples

The HBoV-1 concentration ranged from 2.29 to 2.59 $\log_{10}$ GC/L in Nile river water, with mean value of 0.52 $\log_{10}$ GC/L. While HBoV-2/4 concentration ranged from 2.44 to 3.57 $\log_{10}$ GC/L, with a mean value of 2.2 $\log_{10}$ GC/L. Moreover, HBoV-3 was detected in one Nile water sample with a viral load of 1.8 $\log_{10}$ GC/L. Statistically, the type of the virus had a significant impact ($P < 0.0001$; $R^2 = 0.4258$) on the distribution of the virus in surface water, as the detection rate of HBoV-2/4 was significantly higher than HBoV-1 and HBoV-3 (Figure 1). In effluent samples, the viral concentrations ranged from 1.14 to 3.07 $\log_{10}$ GC/L for HBoV-1, 1.57 to 4.09 $\log_{10}$ GC/L for HBoV-2/4, 1.17 to 5.12 $\log_{10}$ GC/L for HBoV-3. The mean values for HBoV-1, HBoV-2/4 and HBoV-3 were 0.77 $\log_{10}$ GC/L, 2.3 $\log_{10}$ GC/L and 1.6 $\log_{10}$ GC/L in effluent samples, respectively. There

was a significant correlation (P = 0.001; R^2 = 0.18) between HBoV subtypes and the viral distribution, indicating a significant difference between the prevalence of HBoV1 versus HBoV-2/4 in effluent samples using Tukey's multiple comparisons test, however, no significant difference was observed between HBoV1 versus HBoV-3 and HBoV-2/4 versus HBoV-3 (Figure 2).

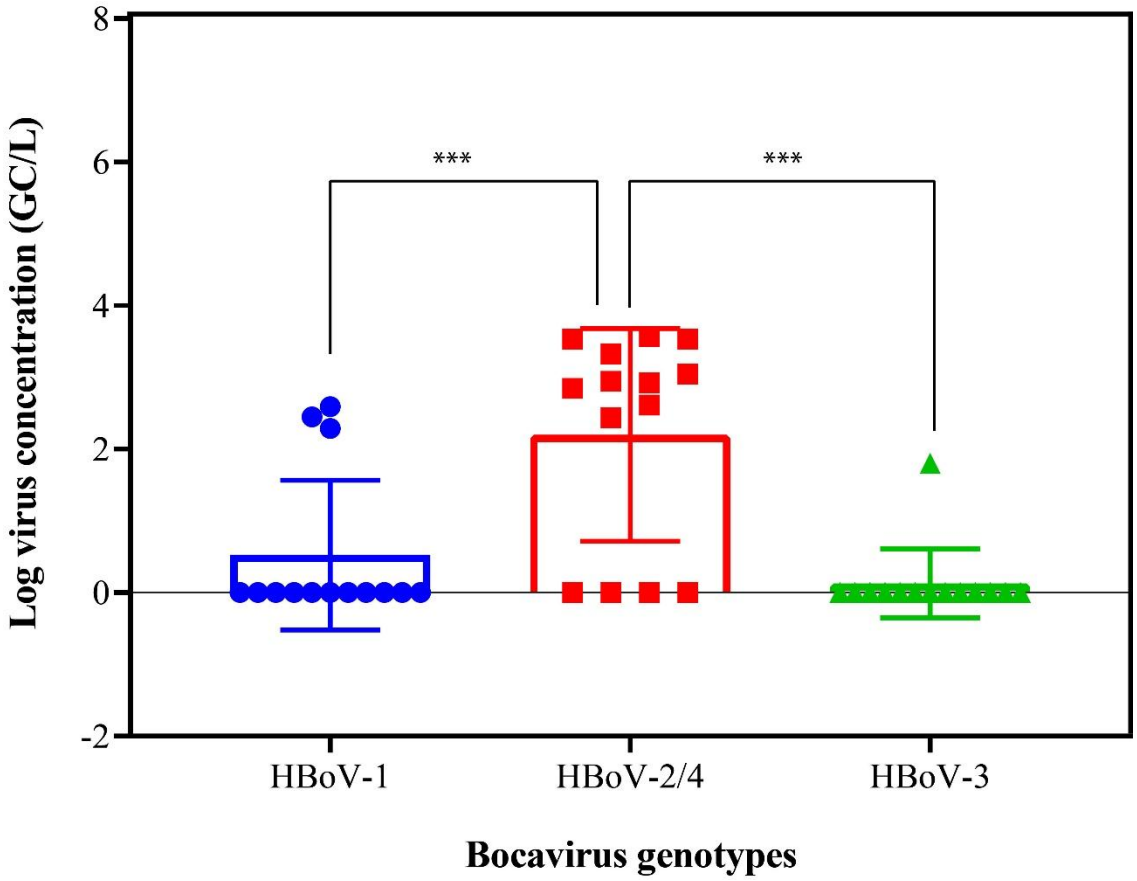

Figure (1): Box and Whiskers showing the abundance of bocavirus genotypes in the total number of HBoV in Nile river water (n = 14).

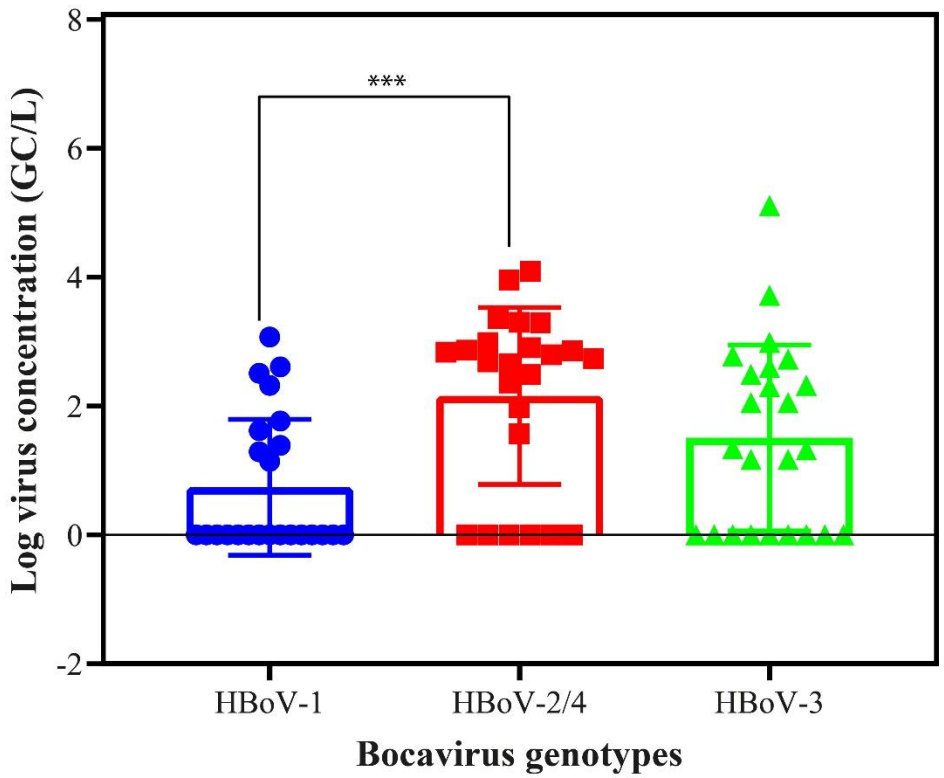

Figure (2): Box and Whiskers plot showing the abundance of bocavirus genotypes in effluents of WWTF.

Variation of HBoV genotypes in source water

HBoV can be detected during the year in river water except in the summer months. However, in effluent samples, no seasonal variations were found. The prevalence of HBoV-2/4 in surface water samples (positive for HBoV; n=14) was 50%, 14.3%, and 7.1% in winter, autumn, and spring, respectively. Concerning HBoV-1 and HBoV-3, both were detected only in winter season (Figure 3). Statistically, there was a strong significant impact (P = 0.0002) of the seasonal variation on the viral distribution in surface water samples. In effluents, the prevalence rates of HBoV-1 were 17.4% in winter, 8.7% in summer, 8.7% in autumn, and 4.3% in spring. However, the ratios of HBoV-2/4 were 26.7% in spring, 21.7% in summer, 21.7% in winter and 8.7% in autumn. Furthermore, the prevalence of HBoV-3 was 21.7%, 17.4%, 13% and 13% in winter, spring, summer and autumn, respectively (Figure 4).

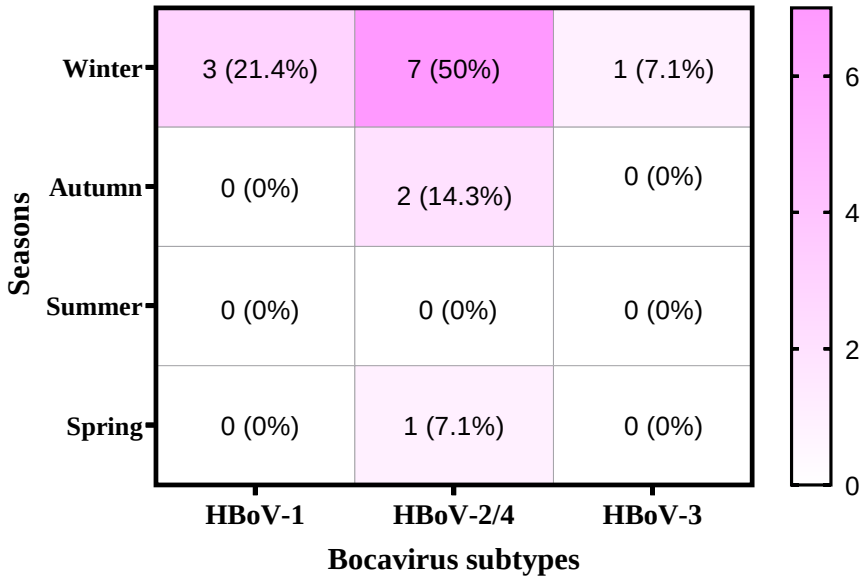

Figure (3): Heatmap showing the temporal variation of HBoV genotypes in Nile river. The values in the cells indicating different HBoV subtypes positive samples in different seasons and their percentages from HBoV-positive samples (n=14).

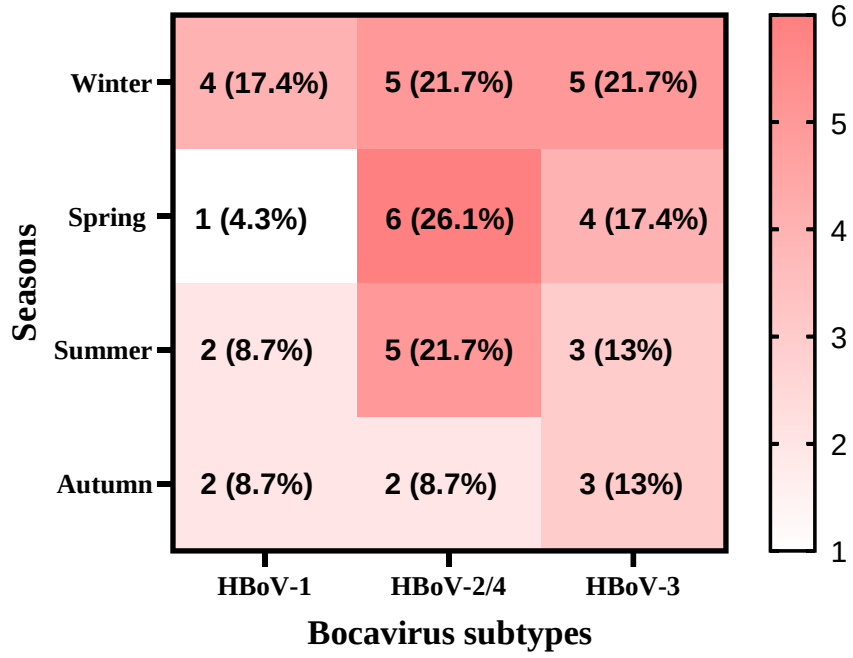

Figure (4): Heatmap showing the temporal distribution of HBoV subtypes in the effluent. The values in the heatmap cells indicate the number of positive samples of HBoV subtypes and their percentages from HBoV-positive effluent samples (n=23).

DISCUSSION

Limited data are available about the circulation of human bocaviruses in environmental samples in Egypt **(Hamza *et al.*, 2017; Shaheen *et al.*, 2019)**, especially in Nile water and drinking water. This is the first study on the quantification of HBoV in Nile water, and so far the studies on HBoV in rivers are limited worldwide **(Hamza *et al.*, 2009; La Rosa *et al.*, 2017; Salvo *et al.*, 2018; Shaheen *et al.*, 2019)**. Nevertheless, the study of Shaheen and colleagues determined only the prevalence of HBoV in Nile water, without addressing neither viral loads nor viral subtypes. Our study provides a comprehensive data about the subtypes of HBoV and their load in the Nile river. Different HBoV subtypes were found in river water, HBoV-1(4.2%), HBoV-2/4 (18.8%) and HBoV-3 (2.1%). Globally, two previous studies considering HBoV subtypes in the rivers were found. The first one was in Italy where HBoV-2 and HBoV-3 were detected in Tiber river **(La Rosa *et al.*,2017)**, and the second study determined HBoV-3 in surface water from rivers in Uruguay **(Salvo *et al.*, 2018)**. The difference of the detected HBoV genotypes in surface water of different countries depends on the type of infection in the population. In the present study, the HBoV at the genotype level loads ranged from 1.8 to 3.57 $\log_{10}$ GC/L in Nile water, which was nearly similar to that detected from Ruhr and Rhine rivers (1.5 to 3.5 $\log_{10}$ GC/L) in Germany **(Hamza *et al.*, 2009)**. It is worth noting that the HBoV prevalence in Nile water was 29.2% in this study. However, higher detection rate of HBoV in Tiper river (37.5%) in Italy **(La Rosa *et al.*, 2017)**, and in Ruhr & Rhine rivers (40.8%) in Germany was found **(Hamza *et al.*, 2009)**, and lower rates were detected in rivers (3%) of Uruguay **(Salvo *et al.*, 2018)** and Nile river (12.5%) in Egypt **(Shaheen *et al.*, 2019)**. These differences in the viral prevalence rates in different rivers might be due to the difference in contamination loads discharging in the rivers. Moreover, the absence of HBoV in the Al Munib district (the site near Gazirat Al Dahab DWTF) indicated the ability of the DWTF to remove the viral loads detected in the nearby source water. Furthermore, the absence of HBoV in Faysal district, the far site from Gazirat Al Dahab DWTF, indicated that the distribution system was not subjected to contamination between the two sites.

The study results indicated a high contamination level (95.8%) of HBoV in the effluent, and HBoV-1, HBoV-2/4, and HBoV-3 were observed in the effluent as well as in surface water. The high frequency of HBoV in effluent indicates a high percentage of infected persons in the given community and inappropriate wastewater treatment technology. As such, HBoV could be excreted in asymptomatic patients or in undiagnosed respiratory or gastroenteritis disease, and released in the environment in higher concentrations **(Kapoor *et al.*, 2011; Proenca-Modena *et al.*, 2013)**. Previous studies found that the prevalence rates of HBoV ranged from 25% to 100% in the effluents samples collected from wastewater treatment facilities **(Myrmel *et al.*, 2015; Hamza *et al.*, 2017; Salvo *et al.*, 2018; Shaheen *et al.*, 2019)**. They proposed HBoV as a viral indicator of fecal contamination due to its high frequency **(La Rosa *et al.*, 2017;**

Salvo *et al.*, 2018). Notably, higher prevalence rates of HBoV were found in the effluents of the activated sludge WWTF, while lower rates were found in WWTF using other different technologies (e.g. stabilization ponds) **(Salvo *et al.*, 2018)**. In general, the stabilization pond treatment technology is more effective than the activated sludge technology to remove enteric viruses **(Verbyla and Mihelcic, 2015)**. For example, Salvo and colleagues detected HBoV with frequencies ranging from 67 to 90%, and HBoV was detected in all cities in Uruguay except in one city, which presented a meaningful lower frequency (33%). They explained that reduction in frequency to the difference in methods of treatment where their examined effluent samples were obtained from a stabilization pond treatment facility **(Salvo *et al.*, 2018)**. The stabilization pond is able to remove 2 to > 6 $\log_{10}$ viral units, depending on hydraulic retention time **(Verbyla and Mihelcic, 2015)**. However, activated sludge treatment technology can remove about 2 $\log_{10}$ enteric viruses **(Naughton and Rousselot, 2017)**. In our study, since no raw sewage samples were collected, we could not determine or evaluate the performance of the activated sludge WWTF.

The concentration of HBoV in effluents of wastewater treatment plants is available **(Iaconelli *et al.*, 2016; Hamza *et al.*, 2017)**. Quantitatively, the mean values for HBoV-1, HBoV-2/4 and HBoV-3 were 0.77 $\log_{10}$ GC/L, 2.3 $\log_{10}$ GC/L and 1.6 $\log_{10}$ GC/L, respectively (Figure 2). The virus loads observed in the current study were quite similar to the values obtained in previous studies **(Iaconelli *et al.*, 2016; Hamza *et al.*, 2017)**. In this study, the abundance of HBoV-2/4 and HBoV-3 was observed in the effluent samples, and this pattern was similar to the previous studies **(Myrmel *et al.*, 2015; Iaconelli *et al.*, 2016; Hamza *et al.*, 2017; Salvo *et al.*, 2018)**. In other words, the widespread of HBoV-2/4 and HBoV-3 might be due to differences in pathogenesis, transmission and persistence of HBoV subtypes. As such, HBoV-1 is principally associated with respiratory diseases, while HBoV-2/4 and HBoV-3 are mainly frequent in gastroenteritis patients **(Arthur *et al.*, 2009; Zhao *et al.*, 2012)**. The positive samples for HBoV were mainly found in winter for surface water samples, and found all over the year for effluent samples, indicating a clear seasonal pattern of HBoV in surface water samples only (Figure 3 and 4). More strikingly, temporal patterns of HBoV were observed in winter months **(Chow *et al.*, 2008; Ong, Schuurman, and Heikens, 2016)** and spring/summer months **(Choi *et al.*, 2006; Arthur *et al.*, 2009; Nawaz *et al.*, 2012)**. On the contrary, no clear temporal pattern for the virus was reported in other studies **(Hamza *et al.*, 2009; Iaconelli *et al.*, 2016; Hamza *et al.*, 2017)**.

CONCLUSION

This is the first report to evaluate the different HBoV genotypes and their concentrations in the Nile river, Egypt and provides an evidence about the absence of the HBoV in drinking water. In addition, the data obtained in this study raise a concern about the contamination of Nile water by HBoV, which has been causally associated with

discharging treated/untreated wastewater. Although HBoV was not determined in drinking water, a potential role of river water in the transmission of the virus through other means (e.g. recreational activities) should not be ignored, and the protection of the river from pollution is the whole community`s responsibility. Further studies are needed to determine the actual risk of infection via contaminated surface water and to answer open questions about HBoV infectivity, persistence, and disinfection.

Acknowledgments

This work has been funded by the National Research Centre, Egypt, in-house project number 12030103.

REFERENCES

Ali, M. A.; Al-Herrawy, A. Z. and El-Hawaary, S. E. (2004). Detection of enteric viruses, *Giardia* and *Cryptosporidium* in two different types of drinking water treatment facilities. Water Res., 38(18):3931-3939.

Allander, T.; Tammi, M. T.; Eriksson, M.; Bjerkner, A.; Tiveljung-Lindell, A. and Andersson, B. (2005). Cloning of a human parvovirus by molecular screening of respiratory tract samples. Proceedings of the National Academy of Sciences of the United States of America, 102: 12891–12896.

APHA (2012). Standard Methods for the Examination of Water and Wastewater. Stand. Methods 541. Edition 22th. https://doi.org/ISBN 9780875532356

Arthur, J. L.; Higgins, G. D.; Davidson, G. P., Givney, R. C. and Ratcliff, R. M. (2009). A novel bocavirus associated with acute gastroenteritis in Australian children. PLoS Patho., 5: e1000391.

Bibby, K. and Peccia, J. (2013). Identification of viral pathogen diversity in sewage sludge by metagenome analysis. Environ. Sci. Technol., 47(4): 1945–1951.

Blinkova, O.; Rosario, K.; Li, L.; Kapoor, A.; Slikas, B.; Bernardin, F., Breitbart, M. and Delwart, E. (2009). Frequent detection of highly diverse variants of Cardiovirus, Cosavirus, Bocavirus, and Circovirus in sewage samples collected in the United States. J Clin Microbiol., 47(11): 3507-3513.

Bosch, A. (2007). Human viruses in water: Perspectives in medical virology. 1st Edition. Elsevier Science.Department of Microbiology, School of Biology, Barcelona, Spain.eBook ISBN: 9780080553276

Bridge, J. W.; Oliver, D. M.; Chadwick, D.; Godfray, H. C. J.; Heathwaite, L.; Kay, D.; Maheswaram, R.; Mcgonigle, D. F.; Nichols, G.; Pickup, R.; Porter, J.; Wastlingl, J. and Banwart, S. A. (2010). Engaging with the water sector for public health benefits: Waterborne pathogens and diseases in developed countries. Bull. World Health Organ., 88 (11): 873 – 875.

Choi, E. H.; Lee, H. J.; Kim, S. J.; Eun, B. W.; Kim, N. H.; Lee, J. A.; Lee, J. H.; Song, E. K.; Kim, S. H.; Park, J. Y. and Sung, J. Y. (2006). The Association of

Newly Identified Respiratory Viruses with Lower Respiratory Tract Infections in Korean Children, 2000–2005. Clin. Infect. Dis., 43(5): 585–592.

Chow, B. D. W.; Huang, Y. T. and Esper, F. P. (2008). Evidence of human bocavirus circulating in children and adults, Cleveland, Ohio. J Clin Virol., 43(3): 302-306.

Cotmore, S. F.; Agbandje-McKenna, M.; Chiorini, J. A.; Mukha, D. V.; Pintel, D. J.; Qiu, J.; Soderlund-Venermo, M.; Tattersall, P.; Tijssen, P.; Gatherer, D. and Davison, A. J. (2014). The family Parvoviridae. Arch Virol., 159(5): 1239-1247.

Fong, T. T. and Lipp, E. K. (2005). Enteric Viruses of Humans and Animals in Aquatic Environments: Health Risks, Detection, and Potential Water Quality Assessment Tools. Microbiol Mol Biol Rev., 69(2): 357-371.

Gad, M. A.; Allayeh, A. K.; Elmahdy, E. M.; Shaheen, M. N. F.; Rizk, N. M.; Al-Herrawy, A. Z.; Saleh, F. E. Z. R. and Marouf, M. A. (2019). Genotyping and interaction-reality of *A*canthamoeba, enteric adenovirus and rotavirus in drinking water, Egypt. Egypt. J. Aquat. Biol., 23(2): 65–79.

Guido, M.; Tumolo, M. R.; Verri, T.; Romano, A.; Serio, F.; De Giorgi, M.; De Donno, A.; Bagordo, F. and Zizza, A. (2016). Human bocavirus: Current knowledge and future challenges. World J Gastroenterol., 22(39): 8684–8697.

Gurda, B. L.; Parent, K. N.; Bladek, H.; Sinkovits, R. S.; DiMattia, M. A.; Rence, C.; Castro, A.; McKenna, R.; Olson, N.; Brown, K.; Baker, T. S. and Agbandje-McKenna, M. (2010). Human Bocavirus Capsid Structure: Insights into the Structural Repertoire of the Parvoviridae. J Virol., 84(12): 5880-5889.

Hamza, H.; Leifels, M.; Wilhelm, M. and Hamza, I. A. (2017). Relative Abundance of Human Bocaviruses in Urban Sewage in Greater Cairo, Egypt. Food Environ Virol., 9(3): 304-313.

Hamza, I. A.; Jurzik, L.; Wilhelm, M. and Uberla, K. (2009). Detection and quantification of human bocavirus in river water. J Gen Virol., 90(11): 2634–2637.

Iaconelli, M.; Divizia, M.; Della Libera, S.; Di Bonito, P.and La Rosa, G. (2016). Frequent Detection and Genetic Diversity of Human Bocavirus in Urban Sewage Samples. Food Environ Virol., 8(4): 289-295.

Jartti, T.; Hedman, K.;Jartti, L.; Ruuskanen, O.; Allander, T. and Söderlund-Venermo, M. (2012). Human bocavirus—the first 5 years. Rev Med Virol., 22(1): 46-64.

Kamel, A. H.; Ali, M. A.; El-Nady, H. G.; De Rougemont, A.; Pothier, P.and Belliot, G. (2009). Predominance and circulation of enteric viruses in the region of greater Cairo, Egypt. J Clin Microbiol., 47(4):1037-1045.

Kantola, K.; Sadeghi, M.; Antikainen, J.; Kirveskari, J.; Delwart, E.; Hedman, K.and Söderlund-Venermo, M. (2010). Real-time quantitative PCR detection of four human bocaviruses. J Clin Microbiol., 48(11): 4044–4050.

Kapoor, A.; Hornig, M.; Asokan, A.; Williams, B.; Henriquez, J. A.and Lipkin, W. I. (2011). Bocavirus episome in infected human tissue contains Non-Identical termini. PLoS ONE. 6(6): e21362.

Kapoor, A.; Simmonds, P.; Slikas, E.; Li, L.,; Bodhidatta, L.; Sethabutr, O.; Triki, H.; Bahri, O.; Oderinde, B. S.; Baba, M. M.; Bukbuk, D. N.;Besser, J.; Bartkus, J. and Delwart, E. (2010). Human Bocaviruses Are Highly Diverse, Dispersed, Recombination Prone, and Prevalent in Enteric Infections. J Infect Dis., 201(11): 1633–1643.

Katzenelson, E.; Fattal, B.and Hostovesky, T. (1976). Organic flocculation: an efficient second step concentration method for the detection of viruses in tap water. Appl Environ Microbiol., 32(4): 638–639.

Koseki, N.; Teramoto, S.; Kaiho, M.; Gomi-Endo, R.; Yoshioka, M.; Takahashi, Y.;Nakayama, T.; Sawada, H.; Konno, M.; Ushijima, H.; Kikuta, H.; Ariga, T.and Ishiguro, N. (2012). Detection of human bocaviruses 1 to 4 from nasopharyngeal swab samples collected from patients with respiratory tract infections. J Clin Microbiol., 50(6): 2118–2121.

La Rosa, G.; Sanseverino, I.; Della Libera, S.; Iaconelli, M.; Ferrero, V. E. V.; Barra Caracciolo, A. and Lettieri, T. (2017). The impact of anthropogenic pressure on the virological quality of water from the Tiber River, Italy. Letters in Applied Microbio., 65: 295-305.

Lin, M. Y. C.; Chan, H. C.; Chi, H.; Chiu, S. C.; Nora-Krukle, Z.; Rasa-Dzelzkaleja, S.; Vilmane, A.; Murovska, M.; Lin, J. H. and Liu, H. F. (2020). Genetic diversity and phylogenetic analysis of human bocavirus 2 in pediatric patients with acute gastroenteritis in Taiwan. Int J. Environ. Res. Public Health., 17(3): 1–9.

Liu, L.; Johnson, H. L.; Cousens, S.; Perin, J.; Scott, S.; Lawn, J. E.; Rudan, I.; Campbell, H.; Cibulskis, R.; Li, M.; Mathers, C. and Black, R. E. (2012). Global, regional, and national causes of child mortality: An updated systematic analysis for 2010 with time trends since 2000. The Lancet, 379 (9832): 2151-2161.

Lodder, W. J. and De Roda Husman, A. M. (2005). Presence of noroviruses and other enteric viruses in sewage and surface waters in The Netherlands. Appl Environ Microbiol.,71(3): 1453-1461.

Misigo, D.; Mwaengo, D. and Mburu, D. (2014). Molecular detection and phylogenetic analysis of Kenyan human bocavirus isolates. J Infect Dev Ctries., 8(2): 221-227

Myrmel, M.; Lange, H. and Rimstad, E. (2015). A 1-Year Quantitative Survey of Noro-, Adeno-, Human Boca-, and Hepatitis E Viruses in Raw and Secondarily Treated Sewage from Two Plants in Norway. Food Environ Virol., 7(3): 213-223.

Naughton, C. and Rousselot, O. (2017). Activated Sludge. Global Water Pathogen Pro ect. http://www. waterpathogens. org (. R. Mihelcic and ME Verb la (eds) Part 4 Management Of Risk from Excreta and Wastewater) http://www. waterpathogens. org/book/acti ated-sludge Michigan State Uni ersit, E. Lansing, MI, UNESC, 62.

Nawaz, S.; Allen, D. J.; Aladin, F.; Gallimore, C. and Iturriza-Gómara, M. (2012). Human bocaviruses are not significantly associated with gastroenteritis: Results of retesting archive DNA from a case control study in the UK. PLoS ONE, 7: e41346.

Ong, D. S. Y.; Schuurman, R. and Heikens, E. (2016). Human bocavirus in stool: A true pathogen or an innocent bystander? J Clin. Virol., 74: 45-49.

Proenca-Modena, J. L.; Martinez, M.; Amarilla, A. A.; Espínola, E. E.; Galeano, M. E.; Fariña, N.; Russomando, G.; Aquino, V. H.; Parra, G. I. and Arruda, E. (2013). Viral load of human bocavirus-1 in stools from children with viral diarrhoea in Paraguay. Epidemiol Infect., 141(12): 2576-2580.

Rizk, N. M. and Allayeh, A. K.(2018). Multiplex Semi-Nested RT-PCR for Genotyping of Rotaviruses Group A in Giza Tap Water, Egypt. Asian J. Water, Environ. Pollut., 15(2): 217-221.

Rizk, N.; Al-Herrawy, A.; Gad, M.; Shaheen, M. and Elmahdy, E. (2019). Existence and removal of rotaviruses group a and cryptosporidium species in a wastewater treatment plant. Pol. J. Environ. Stud., 28(6): 4331–4339.

Salvo, M.; Lizasoain, A.; Castells, M.; Bortagaray, V.; Castro, S.; Colina, R.; Tort, F. L. and Victoria, M. (2018). Human Bocavirus: Detection, Quantification and Molecular Characterization in Sewage and Surface Waters in Uruguay. Food Environ Virol. 10(2):193–200

Schildgen, O.; Qiu, J. and Sderlund-Venermo, M. (2012). Genomic features of the human bocaviruses. Future Virol., 7(1): 31–39.

Shaheen, M. N .; Abd El-Daim, S. E.; Ahmed, N. I. and Elmahdy, E. M. (2019). Environmental monitoring of Aichi virus and human bocavirus in samples from wastewater treatment plant, drain, and river Nile in Egypt. J Water Health,18(1): 30-37

Song, J. R.; Jin, Y.; Xie, Z. P.; Gao, H. C.; Xiao, N. G.; Chen, W. X.; Xu, Z. Q.; Yan, K. L.; Zhao, Y.; Hou, Y. De and Duan, Z. J. (2010). Novel human bocavirus in children with acute respiratory tract infection. Emerg Infect Dis., 16(2): 324-327.

Verbyla, M. E. and Mihelcic, J. R. (2015). A review of virus removal in wastewater treatment pond systems. Water Res., 71: 107-124

Wang, K.; Wang, W.; Yan, H.; Ren, P.; Zhang, J.; Shen, J. and Deubel, V. (2010). Correlation between bocavirus infection and humoral response, and co-infection with other respiratory viruses in children with acute respiratory infection. J Clin. Virol., 47(2):148-155.

Zhao, H.; Zhao, L.; Sun, Y.; Qian, Y.; Liu, L.; Jia, L.; Zhang, Y. and Dong, H. (2012). Detection of a Bocavirus Circular Genome in Fecal Specimens from Children with Acute Diarrhea in Beijing, China. PLoS One 7(11): e48980.

الملخص العربي

القياس الكمي الجزيئي لفيروس بوكا البشري في عينات المياه البيئية في الجيزة، مصر

نيفين مجدى رزق و ابراهيم احمد حمزة

قسم بحوث تلوث المياه، المركز القومي للبحوث، الدقي، الجيزة، مصر.

البيانات المتاحة عن انتشار الأنماط الجينية لفيروس بوكا البشري (HBoV) وتحديدها الكمي في العينات البيئية محدودة للغاية، وخاصة في الأنهار ومياه الشرب. في هذه الدراسة استخدمنا تفاعل البلمرة المتسلسل اللحظي للكشف عن الأنماط الجينية لفيروس البوكا وتركيزه في مياه الشرب والنيل ومخرج محطة صرف صحي في محافظة الجيزة، مصر. أظهرت النتائج أن معدل انتشار HBoV كان 95.8% (24/23) و29.2% (48/14) في عينات مياه الصرف الصحي ومياه النيل على التوالي. في حين ان جينات الفيروس لم تتواجد في عينات مياه الشرب التي تم جمعها من محافظة الجيزة.

الأنماط الجينية لفيروس البوكا المتحصل عليها، كانت HBoV-1 و HBoV-2/4 و HBoV-3 بتركيزات تراوحت من 1.14 إلى 5.11 $\log_{10}$(GC/L) ومن 1.8 إلى 3.57 $\log_{10}$ (GC/L) في عيناتخارج مياه الصرف الصحي ومياه النيل ، على التوالي. احصائيا،كان للنمط الجيني للفيروستأثير كبير على انتشار الفيروس في مياه النيل وكذلك عينات مياه الصرف الصحي.

في مياه النيل لوحظ أيضا ان لفيروس البوكا نمط موسمي، تبرز أهميته في فصل الشتاء ($P = 0.0002$) ، بينما لم يتم ملاحظة هذا النمط لفيروس البوكا في عينات مخرج مياه الصرف الصحي. وفي الختام، فإن تصريف مياه الصرف الصحي ذات التركيز الفيروسي العالي في مصادر المياه (مياه النيل) يؤدي إلى تلوث فيروسي لهذه المصادر، والذي قد يكون مصدرًا محتملاً للعدوى البشرية. تم تنفيذ هذه الدراسة لسد فجوة معرفية بشأن القياس الكمي والتعرف على الأنماط الجينية لفيروس البوكا في مياه النيل، وإثراء قاعدة البيانات الدولية والمحلية من خلال توفير بيانات حول HBoV في العينات البيئية.