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DETECTION AND FREQUENCY OF HEPATITIS A AND E VIRUSES IN MUSSELS COLLECTED FROM THE BOSPHORUS, IN ISTANBUL, TURKEY

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Summary

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The frequency of HAV and HEV in mussels has not previously been investigated in the Bosphorus, Istanbul, Turkey, and therefore the aim of this study was to investigate the frequency of HAV and HEV in mussels collected from the Bosphorus, Istanbul, Turkey. A total of 92 pooled samples representing 736 mussels originating from fish distributors located along the Bosphorus were collected. RNA was extracted using the RNeasy Plant Mini Kit and a Quantitative real-time RT-PCR performed by using primers specific for HAV and HEV. Among the 92 pooled samples tested, 3 (3.2 %) were found to be positive for HAV by Quantitative real-time RT-PCR. No HEV was detected in any of the mussels collected. A 174 bp product was observed on gel electrophoresis from HAV positive samples detected by real-time RT-PCR. This product was confirmed to be HAV by sequencing.

Key words: hepatitis A, hepatitis E, mussels, RT-PCR, Turkey, virus

INTRODUCTION

Hepatitis A and E are orally transmitted diseases which are endemic in Asia, Africa and the Middle East. Hepatitis A virus is recognised as a major cause of food and water-borne viral hepatitis worldwide and is frequently associated with the consumption of contaminated shellfish, sandwiches, fresh vegatables and fruits, and water (Koopmans & Duizer, 2004; Cook & Rzezutka, 2006; Rodríguez-Lázaro *et al.*, 2012). Foodborne transmission of HEV has been associated with consumption of undercooked pork products (Said *et al.,* 2014), with the possibility of several thousand people being infected each year HEV has also been detected in mussels, shellfish and other bivalves (Koopmans & Duizer, 2004; Cook & Rzezutka, 2006).

Molecular techniques like RT–PCR, SYBR-Green real-time RT–PCR and quantitative Real-time RT-PCR are used in most laboratories for detection of HAV and HEV in shellfish (Pintó *et al.,* 2009; Namsai *et al.,* 2011;, Bigoraj *et al.,* 2014; Polo *et al.,* 2015). HAV and HEV occurence in shellfish has been investigated in many countries (Hansman *et al.,* 2008; Namsai *et al.,* 2011; Crossan *et al.,* 2012; Bigoraj *et al.,* 2014; Suffredini *et al.,* 2014; Polo *et al.,* 2015) but there have been no similar studies performed in Turkey.

Therefore, this study was performed to detect and investigate the frequency of HAV and HEV in mussels collected from the Bosphorus, Istanbul, Turkey.

MATERIAL AND METHODS

Collection of mussels and RNA extraction

Raw shelled mussels were collected between 2014 and 2016 along the coast of the Bosphorus (Kumkapı, Rumelikavağı, Sarıyer, Ambarli) from the fish distributors in Istanbul. Sampling was the modification of the methods as described by others (Boxman *et al.,* 2006; Yilmaz *et al.,* 2010). Digestive tissues were removed from each mussel, and these materials taken from 8 mussels were pooled. By this method, 92 mussel pools were formed representing 736 mussels. Approximately 2 grams of pooled digestive tissue were homogenised and supernatant was used for RNA extraction. Viral RNA was extracted from test samples by using RNeasy® Plant Mini Kit (Qiagen, Turkey) as described by the manufacturer. Norovirus GII spiked samples were used as extraction control.

Reverse transcription and detection of HAV and HEV by quantitative real time RT-PCR assay

Reverse transcription was performed in two steps as decribed previously (Yilmaz *et al.,* 2011). The location and target region of the primers used for Quantitative real time RT-PCR are summarised in Table 1. The method used for the quantitative real time RT-PCR for HAV (Costafreda *et al.,* 2006) and HEV (Jothikumar *et al.,* 2006) were similar as reported previously. Norovirus GII, HAV and HEV positive serum were used as positive control. A reaction mixture with nuclease free water in place of template were included as negative control.

After the real time RT–PCR, the products were visualised by agarose gel (1.5%) electrophoresis. If a product is present, it was sequenced to confirm to be HAV by using SYBR-Green RT-PCR.

Table 1. Primers and probe used in this study

Primers/ probes	Sequences (5'-3')	Product size	Target gene	Target sequences	References
HAV68	TCACCGCCGTTTGCCTAG	174 bp	5'UTR	68-85[a]	Costafreda *et al.,* 2006
HAV150	FAM-CCTGAACCTGCAGGAAT TAA–MGB			150-169	
HAV240	GGAGAGCCCTGGAAGAAAG			241-223	
JVHEVF	GGTGGTTTCTGGGGTGAC	70 bp	capsid	5,237-5,254[b]	Jothikumar *et al.,* 2006
JVHEVP	FAM-TGATTCTCAGCCCTTCGC-BHQ			5,260-5,277	
JVHEVR	AGGGGTTGGTTGGATGAA			5,306-5,289	

[a]NC-001489; [b]NC001434.

RESULTS

Among 92 pooled mussel samples (representing 736 mussels), 3 (3.2 %) were found to be positive for HAV. HEV was not detected in any of the mussel sample. A HAV positive signal was obtained using Quantitative real time RT-PCR in 3 mussel pools collected in December and November 2014 and July 2015 (CT values 34, 35 and 38, respectively). A 174 bp product was observed on gel elctrophoresis with HAV positive samples detected by real-time RT-PCR. This product was sequenced and confirmed to contain HAV sequences by alignment using the data previously reported to GenBank.

The real-time PCR for NoV Genogroup II virus showed threshold cycle (CT) value between 25 and 28 and a 100-bp amplicon by agarose gel. CT of 27 and 28 was obtained with HAV and HEV positive controls. No CT value was obtained with negative controls.

DİSCUSSİON

HAV and HEV are important food and water-borne viruses causing hepatitis in many countries in the World (Koopmans & Duizer, 2004, Cook & Rzezutka, 2006, Rodríguez-Lázaro *et al.*, 2012). In Turkey, mussels are frequently consumed and they are either fried or steam cooked. Both of these cooking methods may not result in a temperature in the mussels digestic organs high enough to inactivate viruses (Kirkland *et al.,* 1996; McDonnell *et al.,* 1997; Croci *et al.,* 2005). Therefore, such mussels may pose risk to human health in Turkey, however this had not been investigated at present. Therefore, this study was aimed to investigate the frequency of HAV and HEV in mussels collected from Bosphorus, Turkey.

The presence of HAV and HEV in shellfish has been investigated in many countries by using RT-PCR or real time PCR probe assay (Formiga-Cruz *et al.,* 2002; Hansman *et al.,* 2008; Namsai *et al.,* 2011; Crossan *et al.,* 2012; Bigoraj *et al.,* 2014, Suffredini *et al.,* 2014; Polo *et al.,* 2015). Detection of HAV was performed in shellfish collected from four European countries – Greece, Spain, United Kingdom - and HAV was detected in 4, 3 and 1 sample respectively, but not in Sweden (Formiga-Cruz *et al.,* 2002). In

summary, the frequency of detecting HAV in masssels in Spain, Italy, Poland, USA, Tailand, Japan, China were between 0.9–14.1% (Bosch *et al.,* 2001; Macaluso *et al.,* 2006; Hansman *et al.,* 2008; Pintó *et al.,* 2009; Manso *et al.,* 2010; DePaola *et al.,* 2010; Namsai *et al.,* 2011; Bigoraj *et al.,* 2014; Ming *et al.,* 2014; Polo *et al.,* 2015). In the present study, 3 (3.2%) of 92 mussel pools representing 736 mussels were found to be positive for HAV. Results of this study is similar to what was found in the USA, China (DePaola *et al.,* 2010, Ming *et al.,* 2014) but different than in Spain, Poland and Thailand (Bosch *et al.,* 2001; Pintó *et al.,* 2009; Manso *et al.,* 2010; Namsai *et al.,* 2011; Bigoraj *et al.,* 2014; Polo *et al.,* 2015). The difference in detection rate may be associated with viral RNA extraction methods, the test used, the season the samples were taken and the place of samples taken (high or low contaminated areas) (Bosch *et al.,* 2001; Costafreda *et al.,* 2006; Manso *et al.,* 2010; Uhrbrand *et al.,* 2010; Polo *et al.,* 2015).

In comparison to HAV, the detection rate reported for HEV is either absent or low in many countries (Namsai *et al.,* 2011; Crossan *et al.,* 2012; La Rosa *et al.,* 2012; Krog *et al.,* 2014; Grodzki *et al.,* 2014; Gao *et al.,* 2015). Similarly, no HEV was detected in any of the samples analysed in the present study. Results of some studies (Namsai *et al.,* 2011; La Rosa *et al.,* 2012; Grodzki *et al.,* 2014; Krog *et al.,* 2014), and this study indicate that mussels does not seem to be playing a major role in transmission of HEV to people. In contrast, HEV has been associated with consumption of undercooked pork products (Said *et al.,* 2014).

In conclusion, HAV was found 3.2% of pooled mussel samples collected from Bosphorus in Istanbul and may pose risk to public health. HEV was not detected in any of the mussel samples tested, indicating that mussels may not be a significant vehicle for this virus in this region of Turkey.

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