

MOLECULAR EPIDEMIOLOGY OF HUMAN BOCAVIRUS IN CHILDREN WITH ACUTE RESPIRATORY INFECTIONS IN MALAYSIA

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ABSTRACT

The objective of this study is to determine the prevalence of human bocavirus (HBoV) in Malaysia. Respiratory samples from 125 hospitalised children with Acute Respiratory Disease (ARD) were analysed for the presence of human bocavirus in the year 2012 and HBoV was detected in 5.6% (7/125) of the cases. Molecular analysis based on the partial NP1 region revealed that all 7 belonged to HBoV1. All our HBoV showed a nucleotide substitution from A to T at position 2683.

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INTRODUCTION

Human Bocavirus (HBoV) was first identified by Allander *et al.* in 2005 from nasopharyngeal aspirate samples in children with respiratory infections in Sweden and belongs to the genus *Bocavirus* in the *Parvoviridae* family [1]. There are four known strains of human HBoV; HBoV1, HBoV2, HBoV3 and HBoV4. Various studies reported that HBoV1 is more frequently found to cause ARD primarily in children, with detection rate ranges from 1.5% to 33.0% [3,8,11,17]. However, study done by Liu *et al.* [16] showed that HBoV was also found in adults with ARD especially in elderly patients. In addition to the respiratory samples, the virus was also detected in stool samples from children experiencing gastroenteritis [2]. In 2009, Arthur *et al.* reported the discovery of HBoV type 2 (HBoV2) and type 3 (HBoV3) from stool samples among Australian children with diarrhea [5]. Subsequently, a new strain named HBoV4 was detected by Kapoor *et al.* (2009) in stool samples of children and adults [13]. Except for HBoV1, the

other 3 HBoVs were detected in stool samples and studies revealed that detection rate of HBoV2 in stool were higher compared to HBoV3 and HBoV4 [17].

Several genomic studies were conducted to determine strains variation of HBoV and from the analysis performed, results indicated that HBoV2, HBoV3 and HBoV4 showed 80% similarity with HBoV1 [19]. The NS1 and NP1 genes do not vary among different strains but the VP1 and VP2 show variation in the genes sequences [18].

The first case of HBoV in Malaysia was reported in 2011 in a 13-month-old boy diagnosed with pneumonia and also known to have asthma [7]. After this report, there was no further report on HBoV cases in Malaysia. Thus, in this study we attempted to determine the prevalence of HBoV in Malaysian children ≤ 3 years of age by analyzing partial of NP1 region.

Respiratory samples received at the Institute for Medical Research (IMR) in 2012, consisting of nasopharyngeal

aspirate, nasopharyngeal secretion, throat swab and tracheal aspirate from 125 hospitalised ARD cases (≤ 3 years of age), were included in this study. These samples were tested negative for adenovirus, influenza A and B viruses, parainfluenza viruses (type 1-3) and respiratory syncytial virus by viral isolation and immunofluorescence antibody technique (IFAT).

DNA was extracted using High Pure Viral Nucleic Acid Kit (Roche, Germany). PCR was performed using MyFi™ Mix Kit (Bioline) and primers set as described by Xiaoming *et al.* [21]. All amplified products were sequenced and data obtained were aligned and compared with other sequences obtained from GenBank using the Basic Local Alignment Search Tool (BLAST). Sequence analysis and editing were done using ChromasPro Software. Nucleotide sequences alignment was generated by GeneDoc Software and phylogenetic tree was constructed using Mega 5.05 Software. The 7 partial NP1 gene sequences have been deposited in GenBank (Accession Numbers KF444214 to KF444222).

In this study, 5.6% (7/125) of the cases were found to be positive for HBoV and 352bp nucleotide length of NP1 region were sequenced. The constructed phylogenetic tree showed that the Malaysian virus belongs to HBoV1 and their sequences were highly homologous to strains detected from other countries such as Thailand, Taiwan, Germany, Japan, Hong Kong and Sweden. Based on 352bp length of NP1 sequences (Figure 1), all the Malaysian strains were clustered together with prototype strains.

Further analysis showed that the Malaysian HBoV1 had 2 nucleotide differences. Compared to the 2 reference prototypes (DQ000495/ST1 & DQ000496/ST2), all our HBoV showed a nucleotide substitution from A to T at position 2683. However, sample RP449/12 had an additional substitution from G to A at position 2645 (Figure 2).

World Health Organization in September 2012 reported that underlying pneumonia or other acute respiratory infections as one of the leading causes of death in post-neonatal children [20]. Acute respiratory infections include upper respiratory tract infections or lower respiratory tracts infections and most of the respiratory tract diseases have a viral etiology. Human rhinoviruses and respiratory

syncytial virus (RSV) take a leading role in the list followed by influenza A viruses, influenza B viruses, parainfluenza viruses, adenoviruses and the latest identified viruses which were human metapneumovirus (hMPV) and human bocavirus [9-10,14].

HBoV is difficult to grow in culture system, so far the only successful culture attempt was reported by Dijkman *et al.* [6] using pseudostratified human airway epithelium. However, the success of HBoV identification by Allander group using molecular method has managed to decrease the percentage of undetected etiological of respiratory diseases since virus has been known as the main cause of acute respiratory tract infections especially among children.

All the positive samples in this study were detected between May to August 2012 and from nasopharyngeal and tracheal aspirates (Table 1). Based on this data, HBoV was found to be circulating in Malaysia and the incidence appears to occur during the dry season. Majority of the cases (57.1%, 4/7) were from Johor, southern state in Peninsular Malaysia. Compared to other countries that have reported cases of HBoV, the prevalence of HBoV in Malaysia is still low.

From the sequencing data, there is no significant difference in partial NP1 region of the Malaysian strain compared to the prototype strains and strains from other countries. The highly conserved sequence among the strains has been reported also by Arnold *et al.* and Lin *et al.* [4,15].

Even though HBoV has been identified almost a decade ago, most of the studies published were on the prevalence of this virus. However, recently a few studies on the association of HBoV with diseases have reported. It has been noted that HBoV has hardly been detected in adult patients except in immunosuppressed hosts [2]. A report has also been published on the possible association of this virus with hepatitis in an immunosuppressed child [12]. However, the presence of HBoV in these patients does not necessarily warrant that HBoV is the cause of patient's disease since immunosuppressed patients are prone to several types of infectious agents.

In conclusion, even though there is no reported fatal case caused by HBoV to date, more extensive studies need to be conducted on the association of the virus with diseases,

immunogenicity and pathogenicity including the level of severity the virus can incur.

Table 1: Characteristics of the positive cases for Human Bocavirus

Sample ID	Age (mth)	Sample type	Clinical History	Date of received sample	Location
RP405/12	10	Tracheal aspirate	Fever, cough, acute asthmatic attack	20 May 12	Alor Setar, Kedah
RP449/12	13	NPA	chronic lung disease, nososomal pneumonia, clinical GERD, tachypnea	9 June 12	Kuala Lumpur
RP458/12	8	NPA	Fever, cough, running nose	12 June 12	Johor Bharu, Johor
RP475/12	12	NPA	Fever, right lung crepitation	15 June 12	Johor Bharu
RP462/12	27	NPA	Fever, cough, tachypnea	30 May 12	Johor Bharu, Johor
RP585/12	17	NPA	bronchopneumonia, severe bronchospasm	5 July 12	Putrajaya
RP708/12	6	NPA	fever, cough, runny nose, tachypnea	7 Aug 12	Muar, Johor

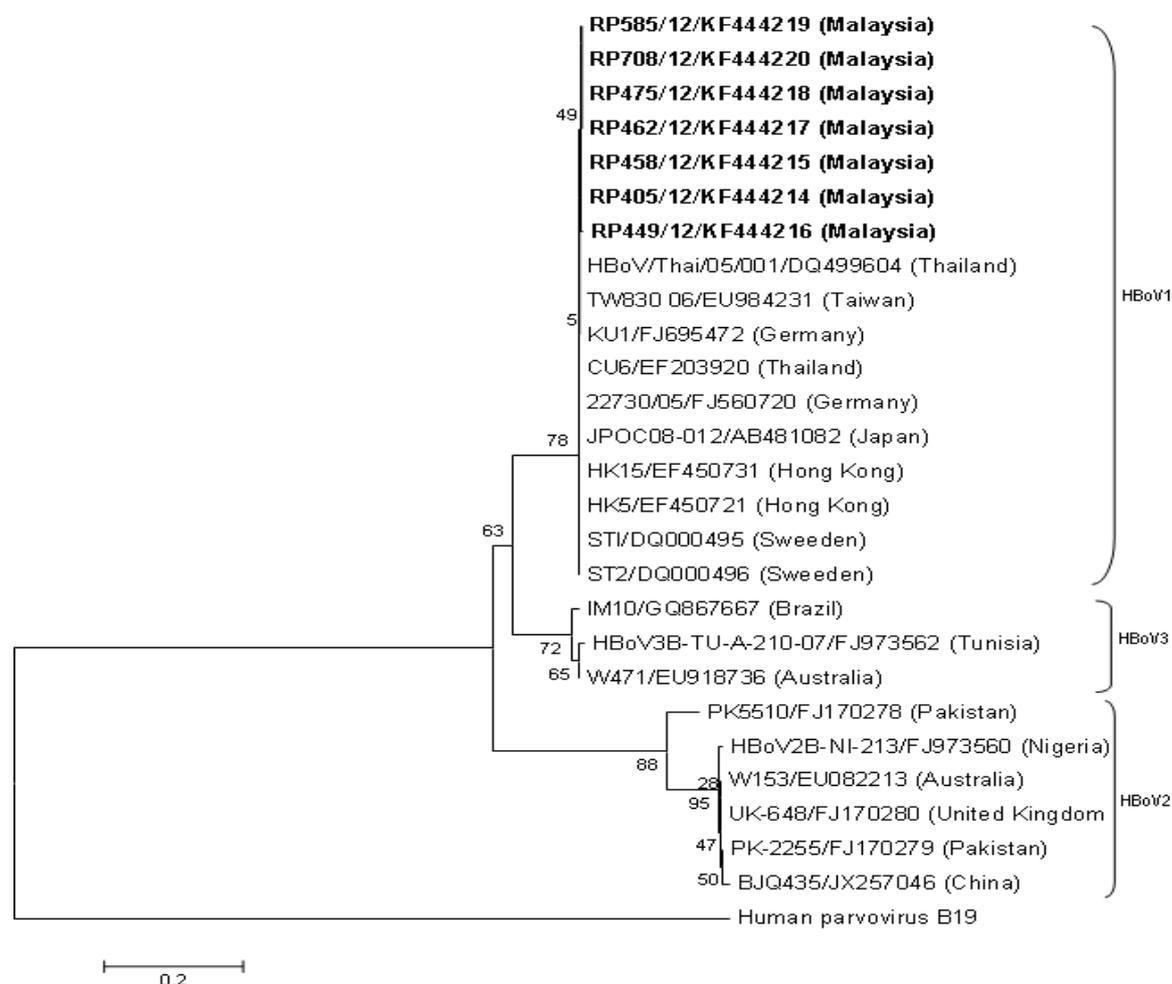


Figure 1. Phylogenetic tree of the partial nucleotide sequence encoding NP1 region of HBoVs which was generated using Neighbour-Joining method from MEGA 5.05 software. The evolutionary distances were computed using the Maximum Composite Likelihood method. Human Bocavirus group are indicated by bracket with Human parvovirus B19 as an outgroup. Seven HBoV isolated in this study are indicated in **boldface**.

	*	2640	*	2660	*	2680	*	2700	
RP405/12	:	: 351
RP449/12	:	A.....	: 353
RP458/12	:	: 351
RP462/12	:	: 352
RP475/12	:	: 353
RP585/12	:	: 353
RP708/12	:	: 353
ST1	:	: 2631
ST2	:	: 2701
HK15	:	: 2701
HK5	:	: 2701
CZ643	:	: 2699
CU6	:	: 2701
22730/05	:	: 2460
KU1	:	: 2701
HBoV/Thai0	:	: 1213
TW830_06	:	: 2686
JPOC08-012	:	: 2701
		ATGAAGACGAGGGAGAGTACATCGGGGAAAAAAGACAATAGAACAATAATCCATAC CTGTATTTCAGTCAACACA							

Figure 2. Alignment of partial NP1 nucleotide sequences for all positive HBoV compared with published sequences of HBoV in GenBank; prototype strain, ST1 (DQ000495) and ST2 (DQ000496) from Sweeden, KU1 (FJ695472) & 22730/05 (FJ560720) from Germany, HK5 (EF450721) & HK15 (EF450731) from Hong Kong, JPOC08-012 (AB481082) from Japan, TW830-06 (EU984231) from Taiwan, HBoV/Thai01 (DQ499604) & CU6 (EF203920) from Thailand and CZ643 (DQ457413) from China. *Data showed only the alignment block which consists of nucleotide differences.*

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