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The Incidence of Human Bocavirus Infection Among Children Admitted to Hospital in Singapore

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Human bocavirus (HBoV) is a parvovirus, belonging to the genus *Bocavirus*. The virus was identified recently in Sweden, and has now been detected in several different countries. Although it is associated with lower respiratory tract infections in pediatric patients, the incidence of HBoV infection in a developed country in South East Asia, has not been examined. The objective of this study was to determine the importance of HBoV as a cause of lower respiratory tract infections among children admitted to hospital in Singapore. Five hundred nasopharyngeal swabs were collected from anonymized pediatric patients admitted to the Kandang Kerbau Women's and Children's Hospital for acute respiratory infections. The specimens were tested for the presence of HBoV using polymerase chain reactions. HBoV was detected in 8.0% of the patients tested, and a majority of these HBoV patients exhibited lower respiratory tract infections. A significant level of coinfection with respiratory syncytial viruses and rhinoviruses was also observed in these HBoV patients. The data suggest that HBoV is an important cause of lower respiratory tract infections among children admitted to hospital in Singapore, and is the first study examining the incidence of HBoV infection in a developed country in South East Asia. ***J. Med. Virol. 81:82–89, 2009.***

KEY WORDS: *Bocavirus*; coinfections; pediatric patients; lower respiratory tract infections; Singapore,

INTRODUCTION

Respiratory syncytial virus (RSV), parainfluenza viruses (PIV), adenovirus, rhinovirus (RHV), and influenza viruses are the most common etiological agents responsible for acute respiratory infections in children, causing either lower or upper respiratory tract infections. Recently, several new viruses have been discovered that are associated with respiratory infections in children [see reviews by Kahn, 2007; Sloots et al., 2008]. The human metapneumovirus was first discovered in Netherlands [Van der Hoogen et al., 2001], and is now reported to be an important global cause of lower respiratory tract infections in children. Similarly, human coronavirus (HCoV) NL63 and HKU1 were first isolated in the Netherlands [Van der Hoek et al., 2004], and Hong Kong [Woo et al., 2005], respectively, and have been reported to be associated with acute respiratory infections in children. Both strains have been detected subsequently in patients from other countries [see reviews by Van der Hoek et al., 2006; Pyrc et al., 2007].

A new parvovirus belonging to the genus *Bocavirus* was identified in Sweden [Allander et al., 2005], and its presence was associated with acute respiratory infections in pediatric patients [see reviews by Kahn, 2008; Schildgen et al., 2008]. This virus was referred to as human bocavirus (HBoV), and it was distinct genetically from the human parvovirus B19. Since its initial discovery, HBoV has been detected in children with lower respiratory tract infections in several different countries [see review by Allander, 2008]. In Asia, HBoV has been reported in Thailand [Fry et al., 2007], China [Qu et al., 2007], South Korea [Choi et al., 2006; Chung et al., 2006, 2007; Lee et al., 2007], Japan [Ma et al., 2006], and Hong Kong [Lau et al., 2007a]. In this study the incidence of HBoV was examined in pediatric

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patients admitted to hospital in Singapore. The data suggest that HBoV is a possible cause of lower respiratory tract infections among children admitted to hospital in Singapore.

MATERIALS AND METHODS

Specimen Collection

Between October 2005 and March 2007, nasopharyngeal swabs were obtained from pediatric patients admitted to Kandang Kerbau Women's and Children's Hospital for acute respiratory infections. The specimens were tested in the hospital's microbiology laboratory for the presence of influenza virus, RSV, adenovirus, HMPV and PIV as described previously [Loo et al., 2007]. In all cases, aliquots of the clinical specimens were stored at −80°C until they were tested for the presence of HBoV. This study was approved by the hospital's ethic committee, approval number EC/043/2004.

Extraction of Genetic Materials

The nasopharyngeal swabs were thawed and subjected to total nucleic acid extraction, using either the QIAamp viral RNA minikit or the QIAamp RNeasy minikit (Qiagen Inc., Valencia, CA), according to the manufacturer's instructions.

PCR Testing for HBoV, HCoV, and RHV

One to five microliters of the total extract were tested using PCR assays targeting HBoV [Sloots et al., 2006], HCoV [Escutenaire et al., 2007], and RHV [Hayden et al., 2003]. All primers were synthesized from ProOligo (Singapore). For the PCR testing of HBoV, the reaction was performed in a 50 μl reaction mixture containing 0.5 μM of each primer, 2.5 U Platinum Taq DNA polymerase (Invitrogen Corporation, Carlsbad, CA), 0.2 mM dNTPs, and 1.5 mM $MgCl_2$, in the conventional PCR machine. In the case of HCoV, the PCR reaction was carried out in a 25 μl reaction mixture containing SYBR Green RT-PCR reaction mix (Biorad, Hercules, CA) with 0.7 μM of each primer, and 0.5 μl of iScript reverse transcriptase (Biorad) in the I-Cycler (Biorad). At the end of the reaction, the products were subjected to a melting curve analysis by heating the products to 95°C for 1 min, and then cooling to 55°C for 45 sec, and heating back to 95°C at 0.5°C intervals. Positive products are represented by HCoV-specific melting peak as described [Escutenaire et al., 2007], and were confirmed by agarose gel analysis with visualisation in the presence of ethidium bromide staining. RHV identification was carried out with 0.5 μM of each forward and reverse primer, 0.1 μM of Taqman probe in Superscript reverse transcriptase/Platinum Taq enzyme reaction mix (Invitrogen Corporation) in the LightCycler machine (Roche Diagnostics, Mannheim, Germany). Positive products were represented by an exponential increase of fluorescence captured by the F2 channel of the LightCycler.

Sequencing Reactions

All amplified products were purified from agarose gel using the QIAquick gel extraction purification kit (Qiagen Inc.). The identity of the products were confirmed by DNA sequencing using the BigDye Terminator cycle sequencing kit (Applied Biosystems, Foster City, CA) with the same primers used for PCR testings.

Sequence and Phylogenetic Analyses

The DNA sequences were assembled using SeqMan (DNASTAR, Lasergene Version 7). The viral sequences were aligned using the algorithm CLUSTALW method in the program MEGALIGN (DNASTAR, Lasergene version 7). The percent sequence homology and phylogenetic trees were calculated and constructed using the neighbor-joining method at the nucleotide (nt) level, with bootstrap analysis performed on 1,000 replicates. The phylogenetic trees were viewed using TreeView version 1.6.6 [Page, 1996].

Nucleotide Accession Numbers

The partial sequences for both the Singapore HBoVs and HCoVs have been submitted to GenBank under the accession numbers EU014167 to 206, and EU370700 to 1.

RESULTS

Incident Rate of Respiratory Viruses in the Pediatric Patients

A total of 500 nasopharyngeal swabs were collected from pediatric patients admitted to Kandang Kerbau Women's and Children's Hospital with acute respiratory infections. The patients exhibited symptoms that were consistent with both lower respiratory tract infections (bronchiolitis, bronchitis, pneumonia, chronic lung disease, asthma, and wheezing) or upper respiratory tract infections (croup, infantile pyrexia, pharnygitis). The age group of the children ranged from 1 month to 12 years. Of the 500 specimens collected, 59 tested positive for RSV (11.8%), 4 for influenza A virus (0.8%), 2 for influenza B virus (0.4%), 4 for PIV1 (0.8%), 0 for PIV2; 8 for PIV3 (1.6%), 1 for adenovirus (0.2%) and 29 for HMPV (5.8%) (Table I). In addition, PCR analysis revealed that 40 patients were positive for HBoV (8.0%), and 3 tested positive for HCoV (0.6%).

Clinical Presentation and Age Distribution of Patients Infected With Respiratory Viruses

The clinical symptoms exhibited by the patients in which HBoV was detected were compared with patients in which RSV, HMPV and HCoV-NL63 were detected (Tables I and II). In the current study, 50% of the patients in which HBoV was detected presented symptoms that were consistent with lower respiratory tract infections, which compared with 57.6% and 48.3% for RSV and HMPV, respectively. These data placed HBoV

TABLE I. Number of Positives and Age Distribution of Patients Infected With RSV, HMPV, and HBoV

		Age distribution of patients[b] (%)					Respiratory infections[c]		
Viral agents tested	No. of positives[a] (%) (**N** = 500)	≤3 months (**N** = 85)	>3 to ≤6 months (**N** = 45)	>6 to ≤12 months (**N** = 141)	>12 to <24 months (**N** = 64)	>24 months (**N** = 165)	L (%)	U(%)	O (%)
Respiratory syncytial virus	59 (11.8)	12 (14.1)	11 (24.4)	18 (12.8)	7 (10.9)	11 (6.7)	34 (57.6)	13 (22.0)	12 (20.4)
Human metapneumovirus	29(5.8)	2 (2.4)	2 (4.4)	10 (7.1)	3 (4.7)	12 (7.3)	14 (48.3)	12 (41.4)	3 (10.3)
Human bocavirus	40 (8)	4 (4.8)	3 (6.7)	15 (10.6)	5 (7.8)	13 (7.9)	20 (50)	13 (32.5)	7 (17.5)
Influenza A virus	4(0.8)	0	0	0	2 (3.1)	2 (1.2)	0	2	2
Influenza B virus	2 (0.4)	0	0	1 (0.7)	0	1 (0.6)	0	0	2
Parainfluenza virus type 1	4(0.8)	0	0	3 (2.1)	1 (1.6)	0	3	0	1
Parainfluenza virus type 2	0	—	—	—	—	—	—	—	—
Parainfluenza virus type 3	8(1.6)	1 (1.2)	1 (2.2)	2 (1.4)	3 (4.7)	1 (0.6)	4	2	3
Adenovirus	1(0.2)	0	0	0	0	1 (0.6)	1	0	0
Coronavirus	3(0.6)	0	2 (4.4)	0	0	1 (0.6)	0	2	1

[a]The no. of positives refer to the samples that tested positive for each viral agent, relative to the total no. of samples collected for the study.
[b]The % age distribution of patients refer to the samples that tested positive for each viral agent, relative to N, representing the no. of samples collected for the respective age group.
[c]The respiratory infections are relative to the no. of samples that tested positive for each viral agent, and L refers to LRTI with symptoms for bronchiolitis, bronchitis, pneumonia, asthma, wheezing, and chronic lung disease; U to URTI with symptoms for croup or laryngotracheobronchilitis, and pharnygitis; and O to others with symptoms not defined as L or U.

TABLE II. Clinical Characteristics of Patients Infected With HBoV

Specimen	Age[a]	*L, U, O[b]	Co-infection
SIN05-NTU-12	1	U	
SIN05-NTU-22	10 months	O	
SIN05-NTU-46	9 months	L	
SIN05-NTU-79	1	O	
SIN05-NTU-86	5	L	
SIN05-NTU-104	10 months	L	
SIN05-NTU-150	2	O	
SIN06-NTU-159	1	L	RSV
SIN06-NTU-165	1	L	RSV
SIN06-NTU-167	1	U	
SIN06-NTU-193	4	L	
SIN06-NTU-194	6 months	U	RSV
SIN06-NTU-195	2	L	
SIN06-NTU-218	2	L	RHV
SIN06-NTU-234	2 months	U	RHV
SIN06-NTU-243	8 months	U	
SIN06-NTU-246	6	L	
SIN06-NTU-250	3 months	U	
SIN06-NTU-258	1 months	U	RSV
SIN06-NTU-263	11	L	RHV
SIN06-NTU-268	2	L	PIV3
SIN06-NTU-275	3	L	
SIN06-NTU-290	1	L	PIV3
SIN06-NTU-325	1	O	PIV1
SIN06-NTU-328	6 months	U	
SIN06-NTU-353	1	U	RSV
SIN06-NTU-371	5	L	
SIN06-NTU-374	13	U	
SIN06-NTU-375	2	L	
SIN06-NTU-399	1	L	
SIN07-NTU-421	3 months	U	RHV
SIN07-NTU-427	1	U	
SIN07-NTU-430	4	O	RSV
SIN07-NTU-432	4 months	L	
SIN07-NTU-441	3	O	RHV
SIN07-NTU-470	3	L	RHV
SIN07-NTU-494	1	U	
SIN07-NTU-496	12	L	
SIN07-NTU-497	10	L	RHV
SIN07-NTU-500	8	0	RHV

[a]refers to age of patients in years, unless otherwise stated.
[b]L refers to LRTI with symptoms for bronchiolitis, bronchitis, pneumonia, asthma, wheezing, and chronic lung disease; U to URTI with symptoms for croup or laryngotracheobronchilitis, and pharnygitis; and O to others with symptoms not defined as L or U. RSV, respiratory syncytial virus; PIV, parainfluenza virus; RHV, rhinovirus.

as the second most likely cause of lower respiratory tract infections. The incident rate for upper respiratory tract infections in patients in which HBoV was detected was 32.5%, which compared with 22.0% and 41.4% for RSV and HMPV, respectively.

The age distribution and clinical presentation of the patients infected with HBoV were examined and compared. Fifty-five percent of the HBoV-infected patients were 1 year old or younger, which compared with detection rates of 70% and 48% for RSV and HMPV respectively (Table I). Most reported studies on the detection of HBoV have been carried out in patients who exhibited respiratory symptoms, and there were few parallel studies on healthy children. Similarly, this current study did not examine the incidence of HBoV in healthy children, or other pediatric patients with non-respiratory symptoms. However, a recent report had clearly described the detection rate in healthy children to be less significant than that in children exhibiting clinical symptoms consistent with respiratory tract infections [Garcia-Garcia et al., 2008]. Several reports have also noted the presence of HBoV in fecal [Lau et al., 2007a; Lee et al., 2007; Vicente et al., 2007] and urine samples from children [Pozo et al., 2007], suggesting that the virus is associated with both enteric and respiratory infections. The current study did not find any symptoms of gastroenteritis presented by the HBoV patients. However, this does not exclude the possibility that the virus does not cause enteric infections as fecal samples were not collected.

Of the three patients in whom HCoV was detected, two tested positive for the HCoV-NL63, and one for HCoV-OC43. Both HCoV-NL63 patients exhibited croup, which is consistent with reports from Germany, Taiwan and South Korea [Van der Hoek et al., 2005; Han et al., 2007; Wu et al., 2008]. However, the small number of

patients in which HCoV was detected makes any clinical association with the presence of the virus and severity of infection impossible. It is possible that the assay used in the current study may not be sensitive enough to detect HCoV in some patients. This is unlikely to be the reason, since similar low rates of HCoV detection have been observed consistently elsewhere [Koetz et al., 2006; Chung et al., 2007; Kaplan et al., 2007; Pierangeli et al., 2007].

Coinfections of HBoV With Other Respiratory Viruses

Although HBoV can be the single cause of lower respiratory tract infection in children, several studies have reported that HBoV coinfections with other respiratory viruses resulted in an increased severity of infection [Allander et al., 2005; Choi et al., 2006; Chung et al., 2006; Foulongne et al., 2006; Manning et al., 2006; Sloots et al., 2006; Fry et al., 2007]. The 40 HBoV positive specimens were therefore examined for the presence of other respiratory viruses. In 23 of these HBoV patients, HBoV was the only virus detected, and lower respiratory tract infections was observed in 12 (52.1%) of single infections. Interestingly, in 17 (42.5%) of the patients infected with HBoV, the presence of RSV, PIV1 and 3, and RHV were also detected (Table II). The rate of HBoV coinfection was almost similar to the 40% coinfections reported in Thai pediatric patients [Chieochansin et al., 2008], but less than that reported elsewhere [Pozo et al., 2007; Hindiyeh et al., 2008]. In the current study, both RSV and RHV each accounted for 6 (35%) and 8 (47%) of the coinfections respectively, with PIV at 3 (17.6%). Of the 17 patients who showed evidence of coinfections with respiratory viruses, 47% exhibited lower respiratory tract infections, lower than that caused by single HBoV infections, while 29% of these exhibited upper respiratory tract infections. Four of the eight coinfected with RHV showed lower respiratory tract infections, compared to only two out of six patients coinfected with RSV. Coinfections with more than one of the other respiratory viruses were not detected.

Sequence and Phylogenetic Analyses of HBoV and HCoV

The HBoVs detected in this study were sequenced to determine the genetic relationship with other HBoVs reported elsewhere. A 245 bp region of the NS1 gene sequence was used to analyze the genetic relatedness of the Singapore isolates as described previously [Chung et al., 2006; Sloots et al., 2006]. The Singapore HBoV isolates showed nt identity ranging from 91% to 100% with published HBoV sequences. Approximately 60% of the Singapore HBoV isolates were identical at the nt sequence level, and the remaining HBoV isolates showed only minor nt differences. This highly conserved sequence identity is consistent with HBoV which has been isolated in other countries [Chung et al., 2006; Qu et al., 2007]. The HBoV sequences were aligned, and percent sequence homology was calculated and phylogenetic trees were constructed as described in Materials and Methods Section. The analysis showed that the majority of the Singapore HBoV strains clustered with the prototype virus, st1 strain, that was first detected in Sweden (Fig. 1). In addition, the Singapore HBoV strains appeared to be closely related to canine minute virus and bovine parvovirus than the human parvovirus

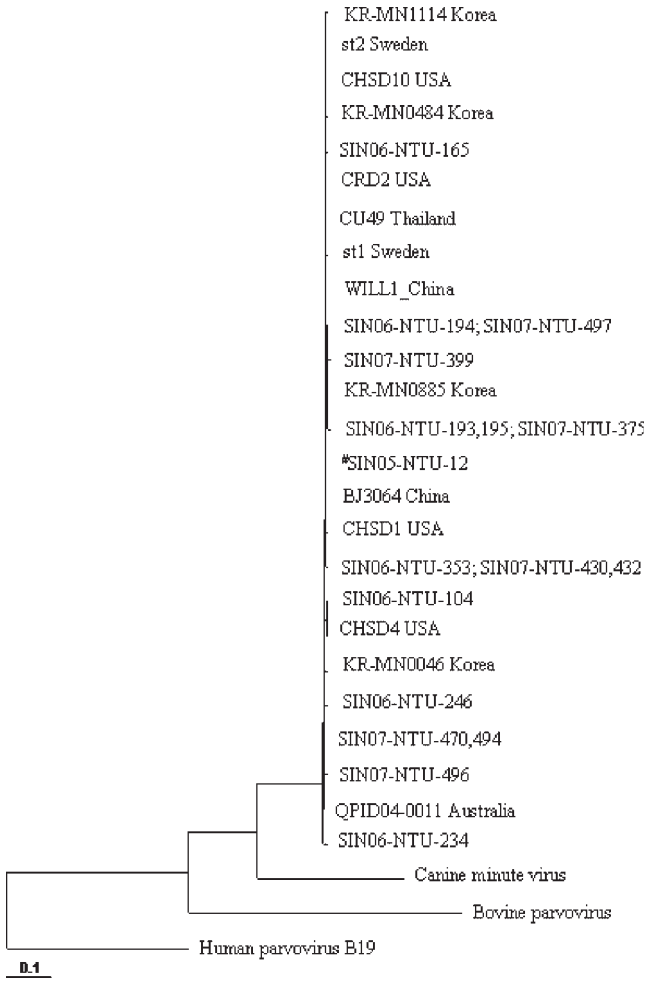

Fig. 1. Phylogenetic analysis of the partial NS1 gene region (245 bp) for HBoV detected from hospitalized pediatric patients. The phylogenetic tree was constructed using the neighbour-joining method and the bootstrap values were generated in 1,000 replicates. The viral sequences from the Singapore strains are represented by SIN05-NTU, followed by the specimen number. SIN represents Singapore and 05 represents the year the specimen was collected. #SIN05-NTU-12 is the representative strain for a cluster of 24 strains, comprising of specimen numbers SIN05-NTU-12, 22, 46, 79, 86, 150; SIN06-NTU-159, 167, 218, 243, 250, 258, 263, 268, 275, 290, 325, 328, 371, 374; and SIN07-NTU-421, 427, 441, and 500, with 100% sequence similarity at the nucleotide level. The Singapore HBoV sequences were analyzed with the two HBoV prototypes isolated in Sweden, st1_Sweden and st2_Sweden (DQ000495 and DQ000496), and published sequences from GenBank, whose strain names are reported next to their country of isolation. Their accession numbers are: QPID04-0011_Australia, DQ206702; WILL1_China, DQ778300; BJ3064_China, DQ988933; CU49_Thailand, EF203921; KR-MN0046_Korea, MN0484_Korea, KR-MN0885_Korea, KR-MN1114_Korea, DQ340225 to 8; CHSD1, 4 and 10_USA, DQ471812, DQ471814, and DQ471820; CRD2_USA, DQ340570; human parvovirus B19, DQ408301; canine minute virus, NC004442; and bovine parvovirus, NC001540.

B19, which is consistent with recent reports from other regions of the world [Chung et al., 2006; Foulongne et al., 2006; Sloots et al., 2006; Qu et al., 2007].

The main focus of this study was to detect the incidence rate of HBoV in the pediatric population in Singapore. As recent reports have indicated that HCoVs may have a possible role in respiratory infections, their presence was also assessed in the same cohort of patients. Only three HCoVs were detected from the 500 specimens. Their PCR amplicons was subjected to DNA sequencing to confirm their identities by blasting the sequences with published HCoV sequences. SIN06-NTU-259 was confirmed to be HCoV-OC43, whereas both SIN06-NTU-211 and SIN07-NTU-395 were HCoV-NL63. The Singapore HCoV-NL63 strains showed 100% nt identities with each other, and with the Dutch NL63 strains. Interestingly, the phylogenetic tree constructed at the partial orf 1b gene region showed that the Singapore HCoV-NL63 isolates, SIN06-NTU-211 and SIN07-NTU-395, clustered with the Dutch strains, away from the rest of the HCoVs including the recent strains of HCoV-NL63 from Australia and Japan (Fig. 2). SIN06-NTU-259, as expected, clustered with the HCoV-OC43. The clustering of the HCoVs was somewhat similar to that reported by Escutenaire et al. [2007].

DISCUSSION

The main focus of this study was to examine the incidence of HBoV infections among the pediatric patients in Singapore. Respiratory viruses were detected in 141 of the specimens, giving an incident rate of 28.2%. RSV was the most common virus detected in the current study (11.8%), followed by HBoV (8.0%), and HMPV (5.8%). The rate of HBoV detection in this study was similar to that reported in China [Qu et al., 2007] and South Korea [Chung et al., 2006], but was higher than the reported rates in Thailand [Fry et al., 2007], Japan [Ma et al., 2006], and in several non-Asian countries [Allander et al., 2005; Arnold et al., 2006; Bastien et al., 2006; Foulongne et al., 2006; Sloots et al., 2006]. One reason for the difference in incidence rates could be the criteria used in the sampling population. For example, in a report by Sloots et al. [2006] the study population included hospitalized and non-hospitalized patients exhibiting both lower respiratory tract infections and upper respiratory tract infections, with a wide age range from 7 days to 86 years. In contrast to the current study, and that reported from China and South Korea, the sampling population was confined to hospitalized children exhibiting respiratory tract infections.

Coinfections of HBoV with a range of respiratory viruses have been reported, with the highest rates at 69% and 60% in children admitted to hospital in Israel [Hindiyeh et al., 2008] and Spain, respectively [Pozo et al., 2007]. A high proportion of these HBoV coinfections have been associated with either RSV, HMPV, or PIV [Choi et al., 2006; Chung et al., 2006; Fry et al., 2007]. The Israeli study also reported a high rate of coinfection with adenoviruses [Hindiyeh et al., 2008]. Recent studies have also suggested a correlation between the severity of infection and the presence of coinfections involving HBoV [Allander et al., 2005; Choi et al., 2006; Chung et al., 2006; Foulongne et al., 2006; Manning et al., 2006; Sloots et al., 2006; Fry et al., 2007; see review by Schildgen et al., 2008]. For example, in a recent study in Thailand, wheezing was associated in patients infected with HBoV and coinfected with RSV, PIV, or RHV, compared to single infections with these viruses [Fry et al., 2007]. In the same study, a significant number of patients presented with lower respiratory tract infections in which HBoV was the only virus detected. This trend of coinfections with other respiratory viruses was also observed in the current study. In the current study, 50% of the coinfections with RHV caused lower respiratory tract infections in children, whereas in contrast, only 33% of the coinfections with RSV caused lower respiratory tract infections. Interestingly, a recent study described the association of HBoV coinfections with RHV-A, as well as a newly identified RHV species, designated as RHV-C by Lau et al. [2007b]. RHV is also an important cause of lower respiratory tract infections in children. Coinfections with HBoV may exacerbate the symptoms in children, giving rise to hospital admission with the requirement of intensive medical care. Coinfections with RSV and HMPV have also been associated with an increase severity in respiratory infections, although this correlation remains controversial. Some reports have described an increased severity in a high proportion of coinfections [Greensill et al., 2003; Semple et al., 2005], whereas in others, a low proportion of co-infections or no co-infections were found [Al-Sonboli et al., 2006; Mackay et al., 2006]. However, in the previous report [Loo et al., 2007] and the current study, only single HMPV infections were detected among the Singaporean children. The increased severity in coinfections with respiratory viruses remained to be challenged. A larger sampling population, and a longer duration for the study of coinfections will be required to confirm the correlation.

In conclusion, this report describes the first comprehensive study examining the prevalence of HBoV among the pediatric population in Singapore. Five hundred specimens were analyzed, and an infection rate for HBoV at 8% was reported, with 55% of these patients were 1 year old or younger. A significant level of coinfection was also detected in the patients with HBoV, with RSV and RHV being the most common viruses detected. 52% of the HBoV patients in which HBoV was the sole agent detected showed evidence of lower respiratory tract infection, which compared with 47% of the HBoV coinfections. This study, therefore, suggests that HBoV is a significant cause of lower respiratory tract infections among the pediatric population of Singapore. Furthermore, the current data suggest that single infection with HBoV is sufficient to cause severe respiratory infection, and the clinical significance of

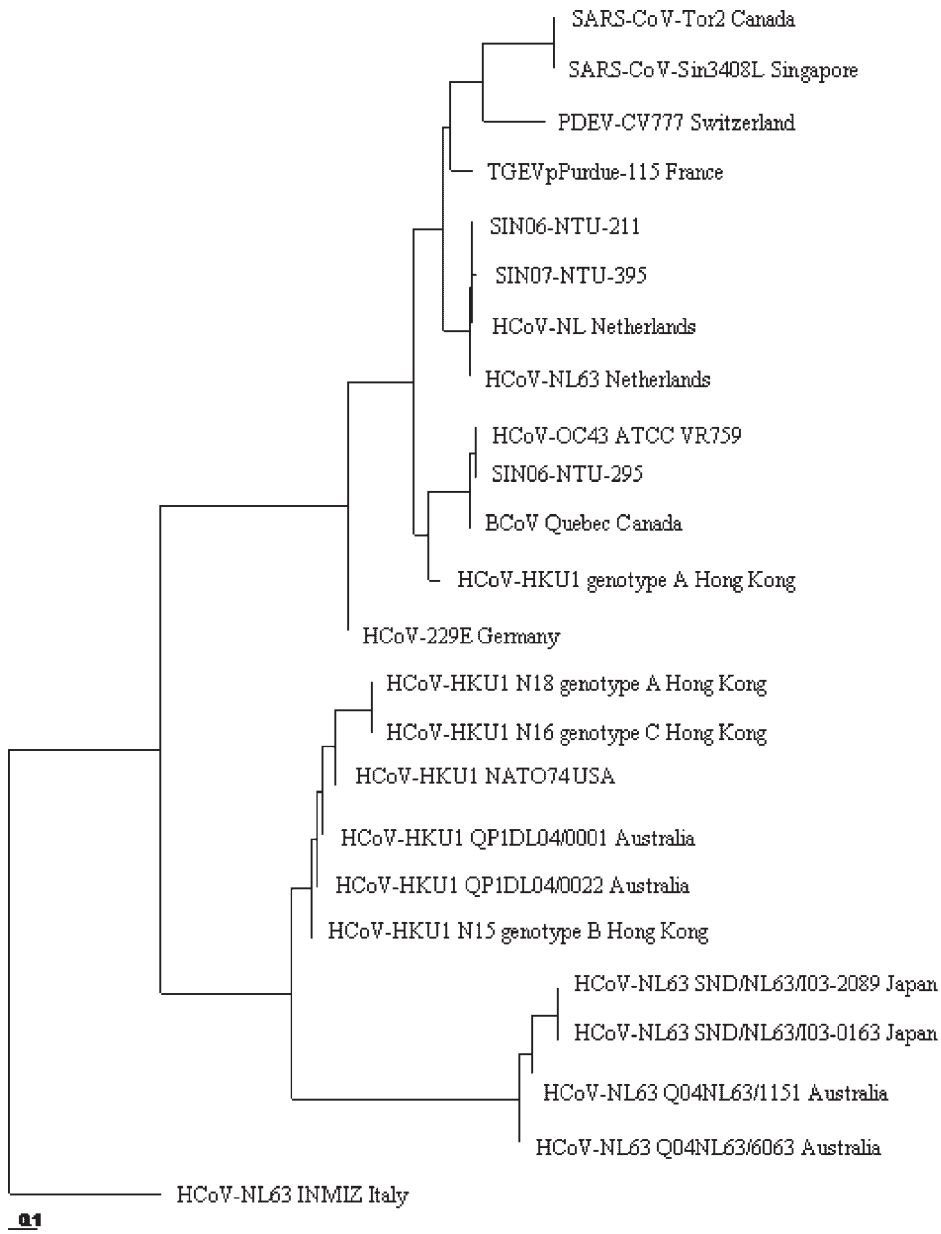

Fig. 2. Phylogenetic analysis of the partial orf 1b region (99 bp) for HCoV detected from hospitalized pediatric patients. The phylogenetic tree was constructed as described for Figure 1. The viral sequences from the Singapore strains are represented by SIN06-NTU, followed by the specimen number. SIN represents Singapore and 06 represents the year the specimen was collected. The Singapore sequences were analyzed with published sequences of coronviruses obtained from GenBank, whose strain names are reported next to their country or cities of isolation. Their accession numbers are: human coronavirus (HCoV)-NL63-004NL63/1151 Australia, AY600446; HCoV-NL63-Q04NL63/6063 Australia, AY600443; HCoV-HKU1 QP1D04\0001 Australia, DQ190472; HCoV-HKU1 QP1D04\0022 Australia, DQ206693; human coronavirus (HCoV)-229E Germany, AF304460; HCoV-HKU1 genotype A Hong Kong, AY597011; HCoV-HKU1 N18 genotype A Hong Kong, DQ415914; HCoV-HKU1 N15 genotype B Hong Kong, DQ415911; HCoV-HKU1 N16 genotype C Hong Kong, DQ415912; HCoV-NL63-INMIZ Italy, EU030685; HCoV-NL63-SND/NL63/I03-2089 Japan, AY662698; HCoV-NL63-SND/NL63/I03-0163 Japan, AY662694; HCoV-NL Netherlands, AY518894; HCoV-NL63 Netherlands, AY567487; HCoV-HKU1-NAT074 USA, EF077277; and HCoV-OC43 ATCC VR-759, AY391777; severe acute respiratory syndrome coronaviruses, (SARS-CoV)-Tor2 Canada, AY274119; SARS-CoV-SIN3408L Singapore, AY559097; bovine coronavirus (BCoV)-Quebec Canada, AF220295; porcine epidemic diarrhea virus (PEDV)-CV777 Switzerland, AF353511; transmissible gastroenteritis virus (TGEV)-Purdue-115 France, Z34093.

HBoV coinfections with other respiratory viruses in Singapore remains to be established.

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