MOLECULAR IDENTIFICATION REVEALS A HIGH PREVALENCE OF HUMAN BOCAVIRUS 1 INFECTION IN HOSPITALIZED PEDIATRIC PNEUMONIA PATIENTS, THAILAND

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Abstract. Human bocavirus 1 (HBoV1) infection is associated with lower respiratory tract diseases in children. However, clinical significance of HBoV1 dissemination in pediatric patients with pneumonia is unclear. This study investigated the presence of HBoV1 DNA in various sample types in pediatric patients hospitalized with lower respiratory tract infections (LRTI). Nasopharyngeal aspirate, nasopharyngeal swab, blood and feces were collected from 147 pediatric patients with LRTI. HBoV1 DNA, identified by a nested-PCR assay was detected in 35% of respiratory samples and in 17% of both respiratory and blood samples. Eighty-two percent of HBoV1 DNA-positive patients were less than one year of age. HBoV1 prevalence was 37% and 45% in the summer and winter season, especially in March and October, respectively. HBoV1 DNA sequences from different samples taken from the same patient were usually identical, indicating that disseminated infection might have occurred. In conclusion, HBoV1 is able to cause systemic infection that may have disseminated from a respiratory site to the rest of the body.

Keywords: disseminated HBoV1, human bocavirus 1, lower respiratory tract disease, pediatric pneumonia

INTRODUCTION

Lower respiratory tract infections (LRTI), including pneumonia, are the major causes of morbidity and mortality in children below the age of 5 years worldwide (Rudan *et al*, 2008; Benet *et al*, 2017; Wong-Chew *et al*, 2017). Viral infections are the most common cause of LRTI, in particular human bocavirus (HBoV) infection in infants and younger children (Hasan *et al*, 2014).

HBoV was first discovered in 2005 to be a cause of LRTI and is also associated with gastrointestinal disease (Allander *et al*, 2005; Allander, 2008). Furthermore, HBoV has also been reported as a causative agent of pneumonia in infants and young pediatric patients (Longtin *et al*, 2008; Korner *et al*, 2011; Eskola *et al*, 2017). HBoV is a member of family Parvoviridae,

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subfamily Parvovirinae, genus *Bocavirus*. The virus is non-enveloped and contains single-stranded linear DNA genome of ~5.3 kb and is the second parvovirus known to infect humans, the first being B19 (Qiu *et al*, 2017). Four genotypes of HBoV have been classified, namely, HBoV1-4 (Broccolo *et al*, 2015), with HBoV1 being the first discovered genotype. This genotype is most commonly detected in respiratory and fecal samples, while HBoV2-4 are more common in fecal samples (Chuang *et al*, 2011; Paloniemi *et al*, 2014; Tran *et al*, 2014).

Apart from causing LTRI and pneumonia (Longtin *et al*, 2008; Korner *et al*, 2011; Eskola *et al*, 2017), HBoV1 has recently been found in blood of pediatric patients with respiratory disease (Bubshait *et al*, 2015), suggesting it is able to potentially cause systemic infection. However, the clinical significance of HBoV1 dissemination in pediatric patients hospitalized with LRTI is unclear. Prevalence of this virus ranges 1.5-33% worldwide (Allander *et al*, 2005; Allander, 2008; Tabasi *et al*, 2016), especially in tropical countries (Misigo *et al*, 2014; Tran *et al*, 2014; Silva *et al*, 2018). Prevalence of HBoV in Thailand is 3.9-27% among hospitalized child. (Fry *et al*, 2007; Chieochansin *et al*, 2008; Deerojanawong *et al*, 2013; Hasan *et al*, 2014). HBoV can be found in many different sample types, such as blood, feces and respiratory tract (Lu, 2010; Khamrin *et al*, 2012; Bubshait *et al*, 2015). However, there have been few reports of the detection of HBoV in different types of sample from the same patient (Li *et al*, 2016).

Hence, this study investigated the presence of HBoV1 in nasopharyngeal aspirate, nasopharyngeal swab, blood, and feces from the same pediatric patients hospitalized with LRTI, and the correlation between presence of HBoV1 DNA in blood and clinical characteristic data.

MATERIALS AND METHODS

Patients and sample collection

A total of 147 pediatric patients admitted to Srinagarind Hospital or Khon Kaen Hospital, Khon Kaen Province, Thailand, during the period from November 2014 to October 2015 were enrolled in the study. Inclusion criteria were (i) pediatric patients (newborn to 14 years of age) and (ii) diagnosed with lower respiratory tract infections (*ie*, pneumonia and bronchiolitis) by an attending physician. Severely immunocompromised individuals were excluded. Clinical and demographic data, including history and medical records, were obtained from the Division of Medical Records and Statistics, chest radiograph data from the Department of Radiology and data relating to bacterial, fungal and viral infections from the Clinical Microbiology Laboratory of both Hospitals.

A total of 467 samples [nasopharyngeal swab (NPS), n = 136; blood, n = 134; nasopharyngeal aspirate (NPA), n = 119; and feces, n =78] were collected from each patient. All four sample types were able to be collected from only 49 (33%) patients. NPA samples were collected using a mucus extractor, and NPS samples using a flocked nasal swab with phosphate-buffered saline pH 7.2 (PBS). Blood samples were collected in EDTA tubes and then centrifuged at 700g for 10 minutes to obtain plasma. All fecal samples were prepared as 10% suspensions in sterile normal saline (Khamrin *et al*, 2012). All collected samples were stored at -20 ºC until used.

The study protocol was approved by Khon Kaen University Ethics Committee for Human Research (Ref. no. HE571131) and

the Khon Kaen Hospital Ethics Committee for Human Research (ref. no. KE57036). Prior written informed consents were obtained from all individual participants or their legal guardians.

Detection of HBoV1 DNA and phylogenetic tree construction

Viral nucleic acid was extracted from NPA, NPS and plasma samples using QIAamp MinElute Virus Spin Kit (QIAGEN, Hilden, Germany). For fecal samples, 140 μl aliquot of the 10% fecal suspensions was extracted using QIAamp Viral RNA Mini Kit (QIAGEN) as previously described (Kapoor *et al*, 2010). HBoV DNA was detected by nested-PCR using pan-bocavirus primers (Kapoor *et al*, 2010; Khamrin *et al*, 2012). First-round PCR primers (yielding a 611-bp amplicon) were AK-VP-F1 (5′-CGCCGTGGCTCCTGCTCT-3′) and AK-VP-R1 (5′-TGTTCGCCATCA-CAAAAGATG TG-3′). Second-round PCR primers (yielded a 576-bp amplicon) were AK-VP-F2 (5′-GGCTCCTGCTCTAG-GAAATAAAGAG-3′) and AK-VP-R2 (5›-CCTGCTGTTGGTC TTGTTGTATGT-3′) that targeted VP1 region of both HBoV1 and HBoV2. Positive control DNA used in this study was kindly provided by Prof Niwat Maneekarn, Chiang Mai University, Thailand. Reaction mixture (50μl) contained 20 pmol of each forward and reverse primers, 2.5U *Taq* DNA polymerase (NEB, Brown, MA), 2.5 mM dNTPs, 1.1X Thermopol reaction buffer (NEB), and 2.5 μl of extracted DNA template in the first round or 1 μl of first round PCR product as template in the second round. First round thermocycling (conducted in Thermal Cycler**,** Gene Technologies, England) was as follows: 95˚C for 35 seconds; followed by 30 cycles of 95˚C for 30 seconds, 54˚C for 45 seconds and 72˚C for 45 seconds; with a final step at 72˚C for 10 minutes. Similar conditions were used in the second round PCR. Amplicons were separated by1.5% agarose gel-electrophoresis and visualized by staining with ethidium bromide. Amplicons were purified using Gene pHlow™ Gel/PCR Kit (Geneaid, Taipei, Taiwan) and subjected to DNA sequencing (Macrogen, Seoul, Korea). Sequences were aligned using Clustal W2 as implemented in BioEdit version 7.0.5 and deposited at GenBank (accession no. MH837090-MH837092 and MH884863-MH884894).

Phylogenetic tree was constructed using the maximum likelihood method (Abdel-Moneim *et al*, 2017). Tamura 3-parameter model (Tamura, 1992) was identified by Molecular Evolutionary Genetics Analysis version 7.0 (MEGA 7) (Kumar *et al*, 2016) as being the most appropriate to use. Bootstrap values are determined by 1,000 pseudo replicates. The tree with the highest log likelihood (-389.61) was constructed. Fifty nucleotide sequences from the study and another 10 reference sequences from GenBank were analyzed. All positions containing gaps and missing data were eliminated. Evolutionary analysis was performed using MEGA7.

Statistical analysis

IBM SPSS Statistics 19 program (IBM, Armonk, NY) was used for statistical analysis. Chi-square test was employed where appropriate. A p-value <0.05 is considered statistically significant.

RESULTS

LRTI in hospitalized pediatric patients

Based on clinical microbiology results and physical examination of 147 pediatric patients, bacterial infection was most commonly found (n = 44; 30%) to be the primary cause of LRTI, followed by viral infection (n = 41; 28%) and fungal infection (n = 7; 5%) (Table 1). Co-infection of bacteria and fungi, bacteria and virus, fungi

Table 1
Clinical demographic data of hospitalized pediatric cases with lower respiratory tract infection (LRTI) at Srinagarind and Khon Kaen Hospitals, Khon Kaen Province, Thailand from November 2014 to October 2015.

Characteristic	LRTI cases		
	HBoV1 DNA-positive, *n* (%)	HBoV1 DNA-negative, *n* (%)	*p*-value
Total	51 (35[a])	96 (65[a])	
Gender			
Female	20 (39[b])	35 (36[c])	NS
Male	31 (61[b])	61 (64[c])	NS
Age			
Newborn – 1 year	42 (82[b])	75 (78[c])	NS
>1 – 3 years	4 (8[b])	9 (9[c])	NS
>3 – 5 years	2 (4[b])	2 (2[c])	NS
≥5 years	3 (6[b])	10 (10[c])	NS
Mean (month)	16	18	NS
Symptoms at presentation			
Wheezing	45 (88[b])	70 (73[c])	0.032
Diarrhea	15 (29[b])	28 (29[c])	NS
Respiratory failure	23 (45[b])	40 (42[c])	NS
Hospitalization			
Intensive care unit admission	13 (25[b])	37 (39[c])	NS
Hospital stay, day, mean	26	32	NS
Intensive care unit stay, day, mean	37	45	NS
Respirator requirement	24 (47[b])	50 (52[c])	NS
Duration of oxygen requirements, day, mean	22	27	NS
Death	5 (10[b])	5 (5[c])	NS
Chest radiograph features			
Alveolar and/or interstitial infiltration	17 (33[b])	25 (26[c])	NS
Other pathogens			
Single infection			
Bacteria	12 (23[b])	32 (33[c])	NS
Virus	21 (41[b])	20 (21[c])	0.012
Fungi	1 (2[b])	6 (6[c])	NS
Co-infection			
Bacteria + fungi	7 (14[b])	13 (14[c])	NS

Table 1 (continued)

Characteristic	LRTI cases		
	HBoV1 DNA-positive, *n* (%)	HBoV1 DNA-negative, *n* (%)	*p*-value
Bacteria + virus	2 (4[b])	4 (4[c])	NS
Fungi + virus	1 (2[b])	0	NS
Mixed infection	2 (4[b])	1 (1[c])	NS
No data	5 (10[b])	20 (21[c])	NS

[a]Percent LRTI cases ($n = 147$). [b]Percent positive for HBoV1 ($n = 51$). [c]Percent negative for HBoV1 ($n = 96$). HBoV1, human bocavirus1; NS, not significant.

and virus, and bacteria, fungi and virus was detected in 14%, 4%, 1%, and 2% of the patients, respectively. Unknown causes of LRTI constituted 17 % of the cases.

Detection of HBoV1 DNA in the four sample types

HBoV1 DNA was detected by nested-PCR in 51 (35%) cases and HBoV2 DNA in only one case (data not shown). Viral DNA was mainly found in NPA samples (37/119, 31%), followed by NPS (38/136, 28%), blood (30/134, 22%), and fecal (11/78, 14%) samples (Table 2). All 51 patients were DNA-positive for at least one of the respiratory samples (NPA and/or NPS). Interestingly, HBoV1 DNA was found in all four types of specimen in four patients. In 25 cases, viral DNA was detected in both respiratory and blood samples, indicating HBoV1 dissemination. HBoV1 DNA was detected less frequently in feces (8/51, 16%) in the same patients. Interestingly, in cases where LRTI was diagnosed as due to viral pneumonia, HBoV1 DNA was found in only 51% of respiratory samples (21/41).

Relationship between presence of HBoV1 DNA and clinical demographic data

The average age of HBoV1-infected patients was 16 months, with highest prevalence among patients < 12 months of age (42/51, 82%) (Table 1). Wheezing was the most common symptom ($n = 45$; 88%), which was positively associated with presence of HBoV1 DNA in blood (Table 3). There are no statistical relationships of HBoV1 DNA detection with other symptoms or findings, *viz*. chest radiographs, diarrhea and other medical conditions.

Seasonable distribution pattern of HBoV1 infection

HBoV1 DNA-positive patients were found throughout the year, with the highest infection numbers (15, 29%) in March and October (14, 27%), but there are no statistically significances in numbers among the three seasons, namely, winter (October to January; 45%), summer (February to May; 37%), and rainy (June to September; 18%) (Fig 1).

Phylogenetic analysis of HBoV1 sequences

In order to investigate whether genotypes of HBoV1 were similar among different sample types from the same patient, a phylogenetic tree was constructed from VP1 partial sequences. There was one major clade (containing 30/50 of HBoV1 strains from the study) and several minor clades (Fig 2). Sequences of multiple

Table 2
Details of human bocavirus1 (HBoV1) DNA-positive hospitalized pediatric cases with lower respiratory tract infection at Srinagarind and Khon Kaen Hospitals, Khon Kaen Province, Thailand from November 2014 to October 2015.

Pattern no.	Sample ID	Gender	Age	Length of stay in hospital (day)	Types of sample				Bacterial and fungal infections		Diagnosis
					NPA	NPS	Blood	Feces	Respiratory sample	Blood sample	
1	060*	Male	7 months	3	Positive	Positive	Positive	Positive	No specimen	NG	Viral pneumonia
	061*	Male	8 months	3	Positive	Positive	Positive	Positive	No specimen	No specimen	Viral pneumonia
	125*	Male	8 months	3	Positive	Positive	Positive	Positive	No specimen	No specimen	Bacterial pneumonia, iron deficiency
	138	Female	10 months	3	Positive	Positive	Positive	Positive	TS = NG	NG	Viral pneumonia, acute respiratory failure, sepsis with septic shock
2	147*	Female	6 months	11	Positive	Positive	Positive	Negative	No specimen	No specimen	Pneumonia, bronchopulmonary dysplasia, respiratory failure
	026*	Male	3 months	7	Positive	Positive	Positive	NA	No specimen	NG	Bacterial pneumonia
	062*	Male	11 months	8	Positive	Positive	Positive	NA	No specimen	No specimen	Bacterial pneumonia, asthma
	063	Male	6 months	4	Positive	Positive	Positive	NA	No specimen	No specimen	Viral pneumonia
	064	Female	1 month	2	Positive	Positive	Positive	NA	No specimen	No specimen	Pneumonia
	066*	Male	8 months	25	Positive	Positive	Positive	NA	TS = (F) *C. albicans*	NG	*C. albicans* pneumonia, bacterial meningitis, severe hydrocephalus
	073*	Male	11 months	2	Positive	Positive	Positive	NA	No specimen	No specimen	Bacterial pneumonia
	137	Male	1 month	40	Positive	Positive	Positive	NA	No specimen	NG	Complete atrioventricular septal defect, progress to pneumonia, acute respiratory failure
	145*	Male	8 months	14	Positive	Positive	Positive	NA	TS = NG	NG	Pneumonia, respiratory failure with hypoxia, upper gastrointestinal hemorrhage
	146*	Male	9 months	1	Positive	Positive	Positive	NA	No specimen	No specimen	Viral pneumonia
3	128	Male	8 years	78	Positive	Positive	Negative	Positive	TS = (F) *K. pneumoniae*, (F) *A. baumannii*	*C. albicans*	Pneumonia, viral encephalitis, acute respiratory failure, meningoencephalitis, sepsis
4	111*	Female	10 months	5	NA	Positive	Positive	Positive	No specimen	No specimen	Bronchitis, viral pneumonia, acute bronchiolitis

Table 2 (continued)

Pattern no.	Sample ID	Gender	Age	Length of stay in hospital (day)	Types of sample				Bacterial and fungal infections		Diagnosis
					NPA	NPS	Blood	Feces	Respiratory sample	Blood sample	
5	027	Male	4 years	45	Positive	Positive	Negative	Negative	TS = (F) *S. maltophilia*	NG	Pneumonia, gastrointestinal bleeding, mitochondrial encephalopathy with lactic acidosis and stroke-like episodes syndrome (MELAS syndrome)
	055*	Male	4 months	5	Positive	Positive	Negative	NA	No specimen	No specimen	Bacterial pneumonia
	056	Male	4 months	5	Positive	Positive	Negative	NA	No specimen	No specimen	Bacterial pneumonia, persistent pneumonia twin 36 weeks preterm
	065*	Female	14 years	10	Positive	Positive	Negative	NA	TS = (F) *A. baumanii*	NG	Respiratory failure, hospital -acquired pneumonia, pulmonary edema, hypokalemia, acute respiratory distress syndrome
	129*	Male	2 years	101	Positive	Positive	Negative	NA	TS = (F) *P. aeruginosa, A. baumanii, C. albicans*	NG	Pneumonia, acute respiratory failure
	131*	Female	1 month	3	Positive	Positive	Negative	NA	No specimen	NG	Viral pneumonia
	139	Male	7 months	2	Positive	Positive	Negative	NA	No specimen	No specimen	Viral pneumonia
	135*	Female	3 months	13	Positive	Positive	NA	Negative	No specimen	No specimen	Viral pneumonia
	140*	Female	1 month	8	Positive	Positive	NA	NA	No specimen	No specimen	Viral pneumonia, heart failure with ventricle septum with defect patent ductus arteriosus, hyperkalemia
6	047	Male	6 months	5	Negative	Positive	Positive	Negative	No specimen	NG	Viral pneumonia
	059*	Male	9 months	3	Negative	Positive	Positive	Negative	No specimen	No specimen	Viral pneumonia, acute bronchiolitis, acute respiratory failure
	123*	Female	1 year	40	Negative	Positive	Positive	Negative	TS = (F) normal flora, (R) *C. albicans*	NG	Pneumonia with viral croup U/D, hydrocephalus S/P VP shunt, acute bronchiolitis
	001	Female	4 months	2	ND	"Positive"	"Positive"	Negative	No specimen	NG	Acute bronchiolitis, viral croup, mild dehydration
	141	Male	7 months	7	Negative	"Positive"	"Positive"	ND	No specimen	NG	Viral pneumonia, acute viral gastroenteritis

Table 2 (continued)

Pattern no.	Sample ID	Gender	Age	Length of stay in hospital (day)	Types of sample				Bacterial and fungal infections		Diagnosis
					NPA	NPS	Blood	Feces	Respiratory sample	Blood sample	
7	005*	Female	1 year	21	"Positive"	Negative	"Positive"	Negative	TS = (N) *Enterobacter* spp, (N) *S. maltophilia* (MDR)	NG	Lennox-Gastaut Syndrome (LGS) with delayed development, aspiration pneumonia, global delayed development, communicating hydration
	006*	Female	4 years	85	"Positive"	Negative	"Positive"	Negative	TS = (N) *P. aeruginosa*, (N) *S. maltophilia* (MDR), (N) *A. lwoffii*	NG	Respiratory syncytial virus pneumonia, aspirated pneumonia, sepsis, epilepsy
	015	Female	5 months	277	Positive	Negative	Positive	Negative	No specimen	NG	Preterm with respiratory failure, hospital acquired pneumonia
	050	Male	2 months	3	Positive	Negative	Positive	Negative	No specimen	NG	Pneumonia
	144	Male	3 months	11	Positive	Negative	Positive	Negative	No specimen	NG	Bacterial pneumonia, hospital acquired pneumonia
8	002	Female	5 months	7	Positive	NA	Negative	Positive	TS = (F) *S. maltophilia*, (F) *A. baumannii* (MDR), (F) *K. pneumoniae* (ESBL)	No specimen	Pneumonia
	033	Female	6 months	155	Positive	Negative	Negative	Positive	TS = (N) *P. aeruginosa* (MDR), (M) *A. baumannii*	NG	Nosocomial pneumonia, acute respiratory failure, slow fetal growth
9	008	Female	10 months	10	Positive	Negative	Negative	Negative	Sputum = NG	NG	Pneumonia with respiratory failure, Down syndrome, atrial septal defect, patent ductus arteriosus
	109	Male	9 months	3	Positive	Negative	Negative	Negative	No specimen	NG	Viral pneumonia, community acquired pneumonia
	117	Female	7 years	19	Positive	Negative	Negative	Negative	TS = (N) *P. aeruginosa*, (F) *Moraxella* spp	NG	Aspirated pneumonia with respiratory failure, upper airway obstruction
	076	Male	2 months	1	Positive	Negative	Negative	NA	No specimen	NG	Viral pneumonia
	090	Female	6 months	177	Positive	Negative	Negative	NA	No specimen	NG	Nosocomial pneumonia, acute respiratory failure, cardiac catheterization
	127	Male	1 year	32	Positive	Negative	NA	Negative	TS = (M) *A. baumannii*	Coagulase-negative staphylococci	Viral pneumonia, community acquired pneumonia, respiratory failure

Table 2 (continued)

Pattern no.	Sample ID	Gender	Age	Length of stay in hospital (day)	Types of sample				Bacterial and fungal infections		Diagnosis
					NPA	NPS	Blood	Feces	Respiratory sample	Blood sample	
10	072	Male	7 months	5	Negative	Positive	Negative	Negative	No specimen	No specimen	Viral pneumonia, acute diarrhea, febrile convulsion
	085	Male	9 months	9	Negative	Positive	Negative	Negative	No specimen	NG	Bacterial pneumonia, respiratory failure, bronchopulmonary dysplasia, cholestatic jaundice
	136	Male	3 months	19	Negative	Positive	Negative	Negative	TS = NG	NG	Bacterial pneumonia, acute respiratory failure, upper airway obstruction
	067	Male	1 year	7	Negative	Positive	Negative	NA	TS= (F) *P. aeruginosa*	NG	Bacterial pneumonia, acute gastroenteritis, respiratory failure
	071	Female	3 months	11	Negative	Positive	NA	Negative	TS = NG	NG	Atypical pneumonia, atrial septal defect, VACTERL syndrome
	110	Male	2 years	4	NA	Positive	Negative	Negative	No specimen	NG	Respiratory syncytial virus pneumonia
	126	Male	2 years	7	NA	Positive	Negative	Negative	No specimen	NG	Respiratory syncytial virus pneumonia, epilepsy
	048	Female	1 month	3	Negative	Positive	NA	NA	No specimen	No specimen	Viral pneumonia

NA, no data available; NG, no growth; TS, tracheal suction; NPA, nasopharyngeal aspirate; NPS, nasopharyngeal swab. *Cases yielding good quality DNA and for which sequences were included in the phylogenetic tree.

Table 3
Clinical demographic data of hospitalized pediatric cases with lower respiratory tract infection and with or without human bocavirus 1 (HBoV1) DNA in blood samples at Srinagarind and Khon Kaen Hospitals, Khon Kaen Province, Thailand from November 2014 to October 2015.

Characteristic	HBoV1 DNA-positive cases (n = 46)*		
	Positive in blood, n (%)	Negative in blood, n (%)	p-value
Total	25 (54[a])	21 (46[a])	
Symptoms at presentation			
Wheezing	25 (100[b])	15 (71[c])	0.004
Diarrhea	9 (36[b])	4 (19[c])	NS
Respiratory failure	10 (40[b])	12 (57[c])	NS
Medical condition			
Asthma/reactive airway disease	16 (64[b])	12 (57[c])	NS
Condition from other diseases	9 (36[b])	9 (43[c])	NS
Chest radiograph feature			
Alveolar and/or interstitial infiltration	10 (40[b])	6 (29[c])	NS

*Data were not available for 5/51 HBoV1 DNA-positive cases. NS, not significant.
[a]Present HBoV1 DNA-positive cases (n = 46). [b]Present positive inblood (n = 25). [c]Present negative inblood (n = 21).

samples from the same patient were usually identical, *eg* case no. 60.

DISCUSSION

Although HBoV1 infection has been identified in various human sample types, there has been little study of sample types from the same patient (Li *et al*, 2016). This study identified HBoV1 DNA in various specimens (blood, feces, NPA, and NPS) from the same pediatric patients at two hospitals in Khon Kaen Province, Thailand. HBoV1 DNA was detected in respiratory secretions of 35% of pediatric cases with LRTI. In 17% of the cases, identical HBoV1 VP1 partial sequences were identical in both respiratory and blood samples, indicating HBoV1 could be disseminated from the respiratory system to blood, resulting in a viremic condition. HBoV1 dissemination was associated with wheezing symptom. Male patients and those <1 year of age were most likely to be infected.

The infection rate of HBoV1 DNA in various sample types from pediatric patients with LRTI in our study is similar to that 70 patients (59%) of a study in Seattle, Washington from 119 children with respiratory disease attending daycare centers (Martin *et al*, 2010). However, other studies in Asia, Australia, Europe, and USA reported lower infection rates (1.5-33%) in pediatric patients (Allander *et al*, 2005; Allander e*t al*, 2007; Tabasi *et al*, 2016). Possibly, differences in prevalence of HBoV1 infection might be due to geographical location, assay sensitivity and

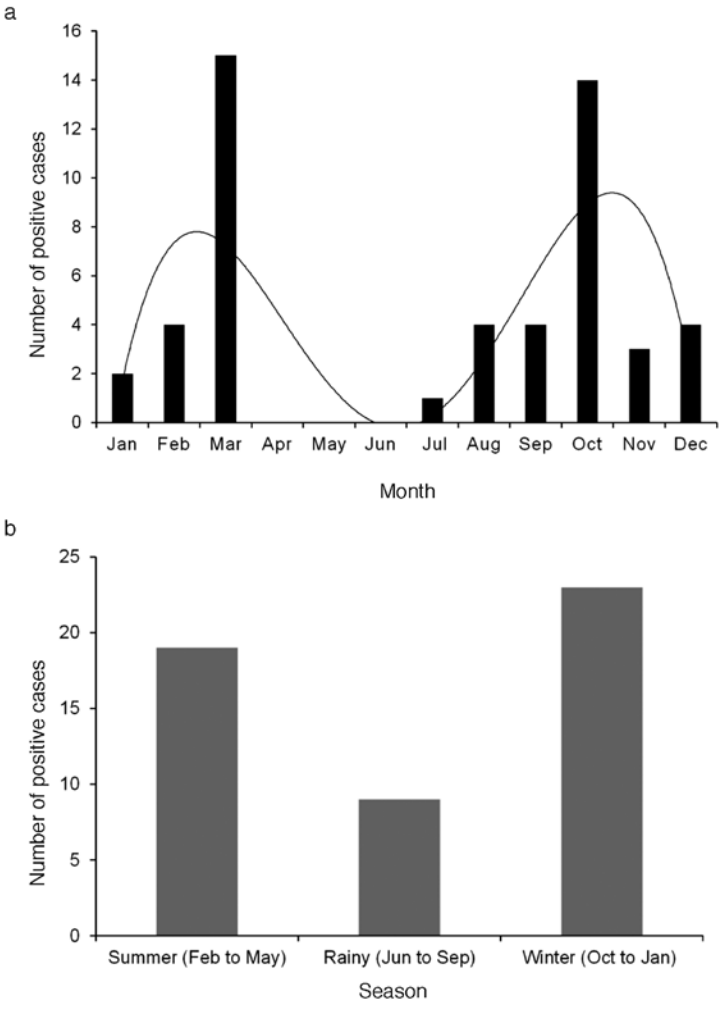

Fig 1-Seasonal distribution of human bocavirus 1 (HBoV1) in pediatric patients with lower respiratory tract infection at Srinagarind and Khon Kaen Hospitals, Khon Kaen Province, Thailand from November 2014 to October 2015. HBoV1 in various types of samples was identified by nested-PCR. (a) Number of positive cases in each month. (b) Number of positive cases during each season. Solid line indicates trend.

study population.

Viral infection was present throughout the year, although there were peaks in March (summer) and October (winter). Similar to earlier survey in Thailand, Fry *et al* (2007) reported the distribution of HBoV1 infection in children from 2004 to 2005 peaks in March but did not identify HBoV1 genotypes.

HBoV1 infection has been reported in many sample types, such as respiratory secretions (Zhou *et al*, 2014), feces (Lasure and Gopalkrishna, 2017) and blood (Christensen *et al*, 2010) but not from different types of sample from the same patient. Thus, these previous reports are not able to demonstrate the possibility of a single HBoV1 genotype infection

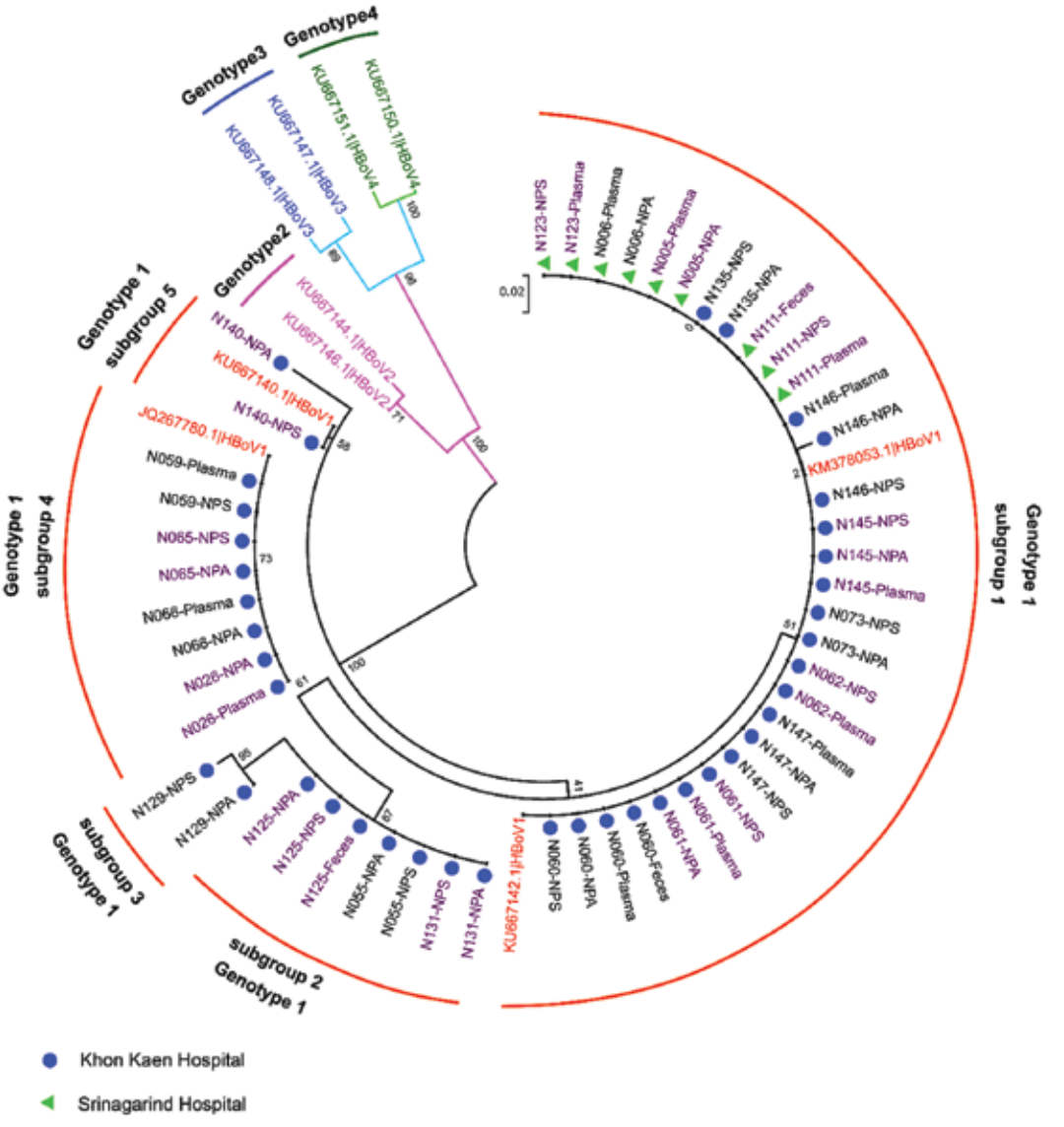

Fig. 2-Phylogenetic analysis of fifty human bocavirus 1 (HBoV1) VP1 partial sequences from pediatric patients with lower respiratory tract infection at Srinagarind and Khon Kaen Hospitals, Khon Kaen Province, Thailand from November 2014 to October 2015. The phylogenetic tree was constructed using the maximum likelihood method based on the Tamura 3-parameter model (Tamura, 1992). Support for each node was estimated using 1,000 bootstrap pseudo replicates. Reference strains were from GenBank database. The percent associated taxa clustered together is shown next to the branch node. The tree is drawn to scale, with branch length according to the number of substitutions per site. Scale denotes number of substitutions per site. Reference HBoV genotype 1, 2, 3 and 4 is shown in red, pink, blue and green, respectively.

spreading throughout the human body. In this study, we deliberately obtained multiple types of sample from the same patients as far as possible. Positive respiratory samples were the most frequent, followed by blood and fecal samples. Although HBoV1 DNA was detected in the four types of samples in four cases, VP1 partial sequences from three cases could not be included in the phylogenetic tree because of their poor quality. The finding of (nearly) identical viral DNA sequences in multiple sample types from a single individual suggests that HBoV1 infection can become systemic, as in the case with related bocaviruses of animals, such as bovine parvovirus and canine minute virus (Tijssen, 1999). However, it has to be acknowledged that variation among HBoV1 VP1 partial sequence was low in our study, so identical sequences from multiple samples from the same individual could come from different genotypes. It is worth noting that HBoV1 DNA was detected in blood and fecal samples from 5 cases and 3 cases, respectively but not in the respiratory tract samples. It is possible that HBoV1 initially established in the bloodstream or GI tract might not necessarily spread to the respiratory system.

The main clinical symptom of HBoV1 infection was wheezing, which is also the most common presenting symptom in lung disease. LRTI patients with wheezing is a symptom reported in previous studies of patients with respiratory tract infection (Allander *et al*, 2007; Zhou *et al*, 2014). Wheezing occurred in all cases in which HBoV1 DNA was found in blood samples, confirming the significant association between disseminated infection and wheezing symptom. However, co-infection data showed other pathogens combined with HBoV1 infection in nearly a quarter of the cases. Further investigation of many of these points should form the basis of a larger cohort and long-term study.

In conclusion, the study reveals a high prevalence of human bocavirus 1 DNA in blood of patients with lower respiratory tract infection who had wheezing symptoms. The finding of identical or nearly identical HBoV1 sequences from different types of samples, namely, blood, feces and respiratory secretion, from the same individual suggests that HBoV1 can cause systemic infection that may spread from respiratory site to the rest of the body.

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CONFLICTS OF INTEREST

The authors declare that no conflicts of interest.

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