Clinical and Epidemiological Profiles of Lower Respiratory Tract Infection in Hospitalized Children due to Human Bocavirus in a Subtropical Area of China

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Lower respiratory tract infection is a major cause of morbidity and mortality in children. *Human bocavirus* (HBoV) is confirmed to have an association with pediatric lower respiratory tract infection. Seasonal and meteorological factors may play a key role in the epidemiology of HBoV. The purpose of this study was to ascertain the frequency, season, and clinical characteristics of hospitalized children with HBoV infection. In addition, an evaluation of the effects of meteorological factors on the incidence of HBoV in a subtropical area in China will be conducted. Children were <14 years in age and hospitalized for lower respiratory tract infection between January 1, 2009 and December 31, 2012 in the Respiratory Disease Department at the Children's Hospital affiliated to Soochow University. Multi-pathogens were detected in nasopharyngeal aspirate samples. The association between HBoV activity and regional meteorological conditions was analyzed. The average incidence of HBoV infection was 6.6% (502/7,626). Of the 502 HBoV positive children, the median age was 13 months (range 1–156 months). The HBoV infection rate was highest among the 7–12 months groups (12.9%, 163/1,267). Seasonal distribution of HBoV was noted during June to November, especially during the summer season (June to August). HBoV activity was associated with temperature and humidity although the lag effect between temperature and HBoV activity observed. HBoV is one of the most common viral pathogens in children with lower respiratory tract infection. HBoV infection occurs throughout the year with a peak during the summer. Temperature and humidity may affect the incidence of HBoV. *J. Med. Virol. 86:2154–2162, 2014.*
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INTRODUCTION

Acute respiratory tract infection is a major cause of morbidity and mortality, especially for infants and young children under 5 years old [United Nations Children's Fund, 2011]. The incidence of community-acquired childhood pneumonia in low- and middle-income countries in the year 2010, using World Health Organization's definition, was about 0.2 (interquartile range [IQR] 0.1–0.5) episodes per child-year, with 11.5% (IQR 8.0–33.0%) of cases progressing to severe episodes [Rudan et al., 2013]. A variety of viruses and atypical pathogens, including *influenza*

viruses (IV), *respiratory syncytial virus* (RSV), *para-influenza virus* (PIV), *adenovirus* (ADV), *human metapneumovirus* (hMPV), and *Mycoplasma pneumoniae* have all been associated with acute respiratory tract infection in children. *Human bocavirus* (HBoV), belonging to the family *Parvoviridae*, subfamily *Parvovirinae*, and genus *Bocavirus*, was first identified from pooled nasopharyngeal aspirate specimens by large-scale molecular virus screening [Allander et al., 2005].

Evidence supporting HBoV role as an etiologic agent in upper and lower respiratory tract infection in children under 5 years has begun to emerge [Manning et al., 2006; Weissbrich et al., 2006; Allander et al., 2007; Canducci et al., 2008; Huang et al., 2008; Deng et al., 2012]. Global HBoV activity varies substantially from year-to-year among different populations and regions. Furthermore, epidemiological data shows that HBoV is present year-round with different incidence rates from 2.2% to 19% in children with lower respiratory tract infection [Manning et al., 2006; Weissbrich et al., 2006; Allander et al., 2007; Canducci et al., 2008]. Recent studies conducted in several countries have reported that the incidence of HBoV fluctuates based on seasonal attributes with infection being higher during winter months [Allander et al., 2005; Manning et al., 2006; Weissbrich et al., 2006]. However, in Japan the infectious rate of HBoV increases during the spring and summer seasons leading to the belief that regional, in addition to seasonal, factors play a role in the rate of disease transmission [Moriyama et al., 2010]. While this suggests that seasonal changes may influence the development of HBoV infection [Naghipour et al., 2007], meteorological factors (temperature, humidity, and precipitation) may play the greatest role in the epidemics of HBoV based on research conducted in Brazil [do Amaral de Leon et al., 2013]. Although few studies have investigated the epidemiological files of HBoV infection in children with lower respiratory tract infection in subtropical area in China, a possible relationship between meteorological factors and incidence of HBoV is currently unknown.

The purpose of this study was to ascertain the frequency, seasonal, and clinical characteristics in hospitalized children with lower respiratory tract infection and evaluate the effects of meteorological factors on the incidence of HBoV in a subtropical region of China.

MATERIALS AND METHODS

Study Design

In this retrospective study, subjects were <14 years in age and hospitalized for lower respiratory tract infection between January 1, 2009 and December 31, 2012 in the Respiratory Department at Soochow University affiliated Children's Hospital. The demographic and clinical characteristics of all subjects were collected for analysis. This tertiary teaching hospital in Suzhou is the only hospital that provides special care to pediatric patients and the participation rate was ~60%. The city of Suzhou is located in east of China and has a subtropical climate. Informed consent was obtained from parents or legal guardians. This study was conducted with the approval of the Institutional Human Ethical Committee of Soochow University.

Study Patients

Patients were considered eligible for enrollment if they had a clinical and radiological diagnosis of community-acquired lower respiratory tract infection including acute bronchiolitis, bronchitis, and pneumonia. Chest radiography was performed using standard equipment and radiographic techniques, and reviewed by the radiologists in digital format. Before hospital discharge, an attending physician recorded information such as age, gender, clinical diagnosis, underlying chronic diseases, clinical manifestation, and peripheral blood routine test. Children with a history of chronic lung disease, underlying immunodeficiency, or preexisting cardiac, renal, neurologic, or hepatic dysfunction, or bronchopulmonary malformation were excluded from the study.

Lower respiratory tract infection was defined as the presence of wheezing, tachypnea, chest retractions, abnormal auscultatory findings (wheezing and crackles), the presence of fever and radiologic evidence indicative of a lower respiratory tract infection. Pneumonia was defined as the presence of focal infiltration (bronchopneumonia) or consolidation (lobar pneumonia) in the lung by chest radiography. Very severe pneumonia was defined as the presence of (i) central cyanosis (ii) inability to breastfeed or drink without vomiting (iii) convulsions, lethargy, or unconsciousness (iv) severe respiratory distress as defined by the World Health Organization.

Respiratory Tract Aspirates Preparation and Nucleic Acid Extraction

Nasopharyngeal aspirate samples were obtained from all patients within 24 hr of admission. This involved passing a suction catheter through the nose with the intent of passing it into the lower part of the pharynx. The depth of penetration for the nasopharyngeal aspirate catheter was set at 5–10 cm. A total 2 ml nasopharyngeal aspirate sample was obtained and centrifuged at $500 \times g$ for 10 min and resuspended in 2 ml saline and divided into two aliquots for pathogen detection using direct immunofluorescence assay (DFA) and PCRs as described previously [Chen et al., 2013b]. One of the equally divided samples of nasopharyngeal aspirate was centrifuged at $12,000 \times g$ for 5 min, followed by extraction of DNA and RNA from a 400-μL sample using DNA-EZ Reagents (Sangon Biotech, Shanghai, China) or TRIzol Reagent (Life Technologies, Carlsbad, CA) in accordance with the manufacturer's instructions. A final 200 μL of

DNA or RNA was eluted and DNA sample was divided into two aliquots for HBoV and *Mycoplasma pneumoniae* gene amplification via PCR. RNA sample was used for hMPV gene detection.

Detection of the HBoV NP1 Gene by Real-Time PCR

Primers were designed using sequence information from the NP1 gene sequence available from the GenBank database. Primers and probe were synthesized using the following sequences: HBoV-F:5′-TGA-CATTCAACTACCAACAACCTG-3′;HBoV-R:5′-CAGAT-CCTTTTCCTCCTCCAATAC-3′;HBoV-probe:AGCAC-CACAAAACACCTCAGGGG-TAMRA (Sangon Biotech). PCR was performed in a volume of 25 μL using iQ5^{TM} BIO-RAD iCycler (BIO-RAD, Carlsbad, CA). The 25 μL amplification reaction contained 3 μL of sample DNA, 0.25 μL of TaqMan (Promega, Madison, WV), DEPC treated water 14.75 μL, buffer solution 2.5 μL, 25 mM MgSO$_4$ 2 μL, dNTP 1 μL, forward, reverse primers and probe 0.5 μL, respectively. Amplification was performed with the following settings: 94°C for 30 sec, 56°C for 30 sec, 72°C for 30 sec for 40 cycles. Positive and no-template controls were included in each run. Positive samples containing the target genes were used as positive controls for all four HBoV subtypes and were constructed by Shanghai Sangon Biotech Co., Ltd. The concentration of each detected sample was then calculated automatically according to standard curve.

Detection of Other Common Viruses and *Mycoplasma pneumoniae*

Samples were also analyzed for seven common viruses, including RSV, IV-A and IV-B, PIV-1, 2, 3, and ADV using DFA with virus-specific fluorescence-labeled monoclonal antibodies (Diagnostic HYBRIDS, Athens, OH) and ultraviolet light microscopy. hMPV and *Mycoplasma pneumoniae* were detected by reverse transcription PCR and real-time PCR, respectively. Briefly, for hMPV detection, primers were designed to specifically amplify the N gene (213 bps). The forward and reverse primers were 5′-AACCGTGTACTAAGT-GATGCACTC-3′ and 5′-CATTGTTTGACCGGCCCCA-TAA-3′, respectively. Reverse transcription reactions were performed with M-MLV reverse transcriptase (Promega) and random hexamers for cDNA synthesis according to the manufacturer's specifications. PCR was performed in a volume of 25 μL and the PCR conditions were as follows: denaturation at 95°C for 5 min, then 45 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 30 s, and extension at 68°C for 30 s, followed by a final extension at 68°C for 7 min as described previously [Wang et al., 2013]. For *Mycoplasma pneumoniae* detection, another fluorescent real-time PCR was performed to identify the P1 adhesion protein gene of *Mycoplasma pneumoniae* as described previously [Chen et al., 2013a,b]. Briefly, A 21 μL PCR master mixture (Daan gene, Guangzhou, China) containing the

primers and probes was combined with 3 μL of the sample DNA and 1 μl of the GoTaq® DNA Polymerase (Promega) for the PCR reactions. Real-time PCR was performed using the iQ5^{TM} BIO-icycler (BIO-RAD), and the cycling conditions were as follows: 2 min at 37°C; 10 min at 94°C, and 40 cycles of 10 s at 94°C, 30 s at 55°C, and 40 s at 72°C. The quantitation curves were plotted using several concentrations of standard control samples, which were purchased from Daan gene Co. Positive samples were defined with a concentration of DNA $>2.5 \times 10^3$ copies/ml in case of *Mycoplasma pneumoniae* colonization.

Meteorological Data Collection

Meteorological data for Suzhou, including daily mean temperature (°C), mean relative humidity (%), total monthly rainfall (mm), sum of sunshine (h), and mean wind velocity (m/s), were obtained from Suzhou Weather Bureau at longitude 120°6′ east and latitude 31°3′ north, which is located 8 km away from the hospital. Meteorological data were obtained hourly, and average daily values were calculated. Monthly means were calculated using the daily means for temperature, relative humidity, and wind velocity. Total rainfall and hours of sunshine were calculated as a total measurement for the month.

Statistical Analysis

Values were expressed as percentages for discrete variables, as mean and standard deviation for continuous variables. The continuous variables were compared using the Student t-test or Mann–Whitney U test if the data were abnormal in distribution. Categorical data were analyzed using the Mentel-Haenszel, chi-squared (χ^2), or Fisher's exact tests.

Correlations of incidence of HBoV with meteorological factors were evaluated using Pearson's or Spearman rank correlation. Because of colinearity between meteorological factors, associations and lag effects between meteorological factors and HBoV incidence were also analyzed using Linear Regression.

For the final goal of predicting the incidence of HBoV on the basis of meteorological data and season, a time series analysis utilizing a seasonal model was established for the time period between January 2009 and December 2011 (estimation period). It was evaluated by comparing the predicted versus the observed incidence of HBoV during the period between January 2012 and December 2012 (evaluation period). The R^2 autoregression coefficient was calculated to judge the fitness of the model.

RESULTS

Frequency Distribution of Viruses and Atypical Pathogens

From January 2009 to December 2012, a total of 8,288 children with acute respiratory tract infection were admitted to our hospital. Nasopharyngeal

aspirate samples were not taken from 510 hospitalized children due to a refusal to participate from their parent or guardian. One hundred fifty-two hospitalized children were excluded on account of congenital heart disease, pulmonary tuberculosis, Down's syndrome or bronchopulmonary malformation. Finally, a total of 7,626 nasopharyngeal aspirate samples were collected, 3,491(45.8%) of which were positive for at least one virus or *Mycoplasma pneumoniae*. The most commonly identified pathogen was RSV (15.7%, 1,197/7,626), followed by *Mycoplasma pneumoniae* (14.3%, 1,094/7,626), HBoV (6.6%, 502/7,626), PIV-3 (6.1%, 464/7,626), hMPV (4.2%, 318/7,626), IV-A (2.8%, 166/7,626), ADV (1.3%, 97/7,626), IV-B (0.8, 62/7,626), PIV-2 (0.1%, 8/7,626), and PIV-1 (0.03%, 2/7,626).

A total of 388 nasopharyngeal aspirate samples (5.1%, 388/7,626) were detected as containing at least two viruses or co-infection with *Mycoplasma pneumoniae* and HBoV. The latter combination was one of the most common co-infections accounting for 37.6% (146/388) of total co-infected samples as well as 29.1% (146/502) of total HBoV positive samples. A total of 356 samples (4.7%, 356/7,626) were detected single HBoV infection and RSV (27.4%, 40/146) and *Mycoplasma pneumoniae* (25.3%, 37/146) were most common co-infection pathogens with HBoV (Table I).

Demographic and Clinical Characteristics of Children Infected by HBoV With or Without Co-infection

Of the 502 HBoV positive children, the median age was 13 months (range 1–156 months). HBoV infection incidence of different age groups are as follows: 1–6 months (3.5%, 71/2,005), 7–12 months (12.9%, 163/1,267), 13–36 months (8.1%, 170/2,109), 37–60 months (5.6%, 65/1,155), and >60 months (3.0%, 33/1,090), respectively. The infectious rate of HBoV was highest among the 7–12 months old group compared with the other four groups (all $P < 0.05$) and lowest among the 1–6 and >60 months old group when compared to the other three groups (all $P < 0.05$) as shown in Figure 1. The ratio of male to female children with HBoV infection was 1.67:1 and no gender difference was observed in the incidence of HBoV infection compared with total children with lower respiratory tract infection (male to female, HBoV infected children 1.7 vs. total children 1.7, $P > 0.05$).

The demographics and clinical information of hospitalized children with single HBoV infection or co-infection are summarized in Table II. No significant differences were observed in the demographic characteristics, clinical presentations, and laboratory tests between single HBoV infection and co-infection groups except for diagnosis of lobar pneumonia. Children with co-infection presented with a higher rate of lobar pneumonia compared to single HBoV infection group ($\chi^2 = 5.2$, $P = 0.02$).

Seasonal Distribution of HBoV Infection

To determine the seasonal distribution of HBoV infection, we assessed incidence over a 4-year period from January 2009 to December 2012. The incidence of infection for 2012 (4.2%, 93/2,240) was significantly lower than that of 2009 (8.1%, 144/1,779), 2010 (7.6%, 122/1,599), and 2011 (7.1, 143/2,008) (all $P < 0.05$). HBoV could be detected every year-round except for December 2010. A seasonal distribution of HBoV was noted during June to November, especially during the months of summer (June to August) which accounted for 37.8% (190/502) of the total HBoV cases. However, variations in this trend were observed from 1 year to another. Interestingly, in 2009 two peaks of HBoV activity occurred, one in June (11.8%) and the other in November (15.7%). This was also seen in 2010, however, the peaks occurred during April (11.8%) and July (14.0%) as shown in Figure 2.

Associations Between Meteorological Factors and HBoV Infection

The Suzhou area has a typical subtropical monsoon climate. From 2009 to 2012, the monthly mean temperature was 17.1 ± 9.0 (mean ± standard deviation) °C, relative humidity was $68.4 \pm 6.3\%$, total rainfall was 83.6 ± 69.7 mm, sum of sunshine was 148.4 ± 47.3 h, and wind velocity was 1.8 ± 0.4 m/s. The monthly mean data for these meteorological variables over the course of this study are shown in Figure 2.

The associations of HBoV activity with meteorological factors were performed using Pearson's or Spearman correlations. As shown in Table III, HBoV

TABLE I. Frequency of HBoV Infection in Children With Lower Respiratory Tract Infection

Single or co-infection groups	Positive number	%
HBoV-single infection	356	4.7
HBoV-co-infection	146	1.9
Total HBoV infection	502	6.6
Pathogen distribution of co-infection with HBoV		
RSV	40	27.4
Mycoplasma pneumoniae	37	25.3
hMPV	21	14.4
PIV-3	15	10.3
IV-A	9	6.2
ADV	9	6.2
IV-B	3	2.1
RSV+IV-A	3	2.1
PIV-3+MP	3	2.1
RSV+hMPV	2	1.4
PIV-3+hMPV	2	1.4
RSV+PIV-3	1	0.7
Mycoplasma pneumoniae + IV-A	1	0.7
Total co-infection	146	100

HBoV, human bocavirus; RSV, respiratory syncytial virus; hMPV, human metapneumovirus; PIV-3, parainfluenza virus type 3; IV-A, influenza virus type A; ADV, adenovirus.
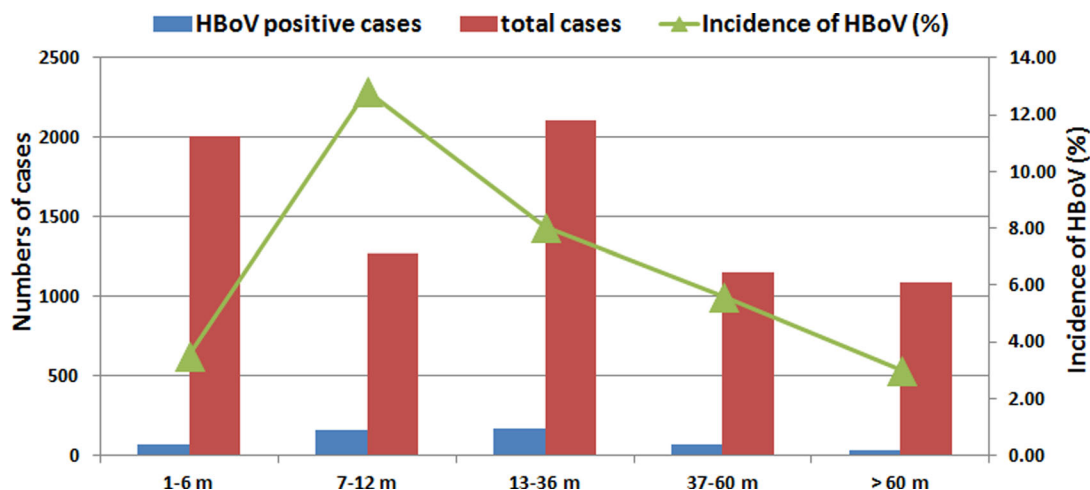
Fig. 1. Age distribution in hospitalized children with lower respiratory tract infection due to *human bocavirus* (HBoV) infection. HBoV infection incidence of different age groups is 1–6 months group (3.54%, 71/2,005), 7–12 months group (12.87%, 163/1,267), 13–36 months group (8.06%, 170/2,109), 37–60 months group (5.63%, 65/1,155), and >60 months group (3.03%, 33/1,090), respectively.

activity was associated with mean temperature and relative humidity. Because of colinearity between meteorological factors (data not shown), associations between meteorological factors and HBoV incidence were also analyzed using Linear Regression based on a stepwise procedure. In Model 0, which had no lag time for analyzing HBoV activity and meteorological factors, HBoV activity is positively associated with mean temperature and relative humidity. When the lag time was taken into consideration, the M1 model was a better fit, with a higher R^2 value of 0.370 when compared to the M0 and M2 models that had R^2 values of 0.308 and 0.230, respectively. These indicated HBoV activity could be better explained according the meteorological factors. Specifically, this study shows that HBoV activity is associated with mean temperature and a lag time effect (Supplementary Table SI).

Time series analysis was also performed to predict the HBoV activity in hospitalized children with lower respiratory tract infection. The R^2 and P were 0.8

TABLE II. Demographic and Clinical Characteristics of Children Infected by HBoV With or Without Co-infection

Parameters	Single infection	Co-infections	P value
Age, median (25–75%)	13 (9–25.5)	14 (8–36.3)	0.455[a]
Sex, male (%)	63.8	59.6	0.380
Median duration of stay in hospital, days	8.2 ± 2.9	8.7 ± 4.0	0.486[a]
Clinical manifestation			
Cough, n (%)	356 (100)	146 (100)	1
Wheezing, n (%)	169 (47.5)	63 (43.2)	0.378
Rhinorrhea, n (%)	109 (30.6)	49 (33.6)	0.519
Fever, n (%)	195 (54.8)	81 (55.5)	0.885
Tachypnea, n (%)	148 (41.6)	61 (41.8)	0.966
Dyspnea, n (%)	80 (22.5)	37 (25.3)	0.49
Cynosis, n (%)	28 (7.9)	15 (10.3)	0.381
Clinical diagnosis			
Bronchiolitis, n (%)	102 (28.7)	37 (25.3)	0.452
Bronchitis, n (%)	56 (14.6)	14 (9.6)	0.131
Bronchopneumonia, n (%)	165 (46.3)	69 (47.3)	0.852
Lobar pneumonia n (%)	24 (6.7)	19 (13.0)	0.023
Very severe pneumoniae, n (%)	13 (3.7)	7 (4.8)	0.552
Blood routine examination			
White blood cells ($\times 10^9$/ml)	10.5 ± 4.3	9.9 ± 4.6	0.168
Neutrophils (%)	42.6 ± 19.0	43.1 ± 18.4	0.806
Platelets ($\times 10^9$/ml)	327 ± 105	336 ± 112	0.468
C-reaction protein, mg/l, median (25–75%)	1.3 (0.2–7.6)	1.5 (0.3–8.6)	0.320[a]

HBoV, human bocavirus.
$P < 0.05$ indicates significant difference.
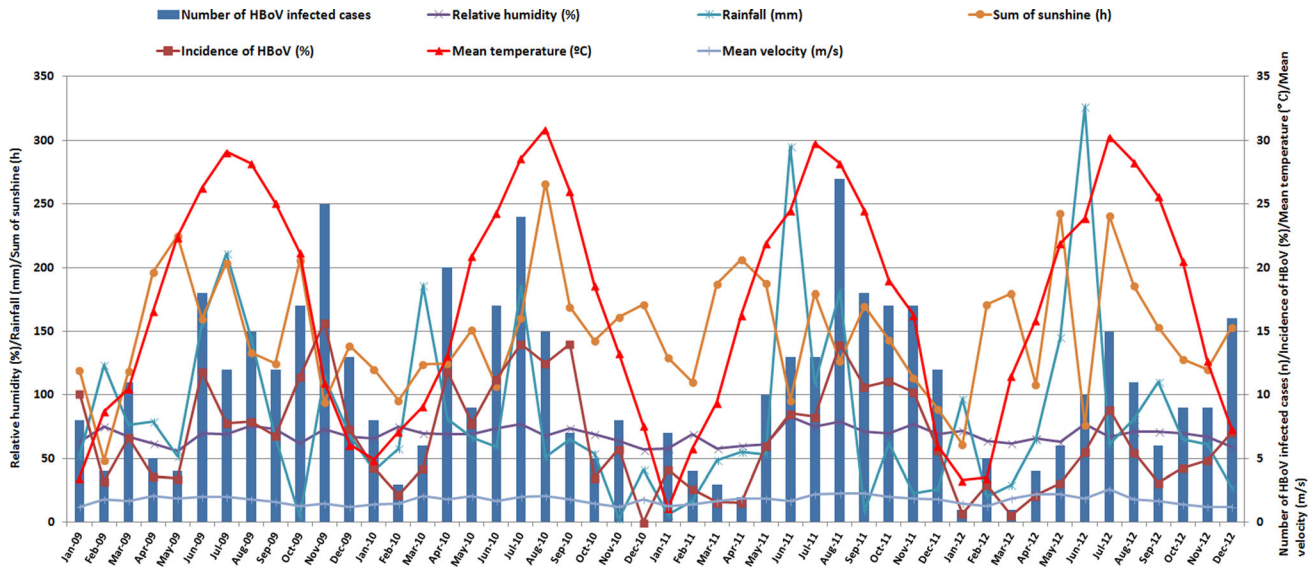[a]Mann–Whitney U test.

Fig. 2. Seasonal and monthly distribution of *human bocavirus* (HBoV) infection and meteorological factors for a 4-year period from January 2009 to December 2012.

and 0.006, respectively, which showed a good fitness for this simple seasonal model. Taken together, HBoV activity in hospitalized children with lower respiratory tract infection could be predicted based on periodicity and season (Fig. 3).

DISCUSSION

The findings of this study confirmed the hypothesis that HBoV is a common pathogen in children with lower respiratory tract infection. In addition, co-infection frequently occurs with RSV and *Mycoplasma pneumoniae*. Children with co-infection were prone to lobar pneumonia compared to children infected with HBoV only. To our knowledge, this is the first time that an association between HBoV activity and meteorological factors were taken into consideration by a 4-year respiratory virus surveillance in a subtropical region of China. The present study shows that mean temperature was the main meteorological factor associated with HBoV activity and this influence also had a month lag time effect.

TABLE III. Associations of HBoV Activity and Meteorological Factors

Meteorological factors	Correlation coefficient	P value
Mean temperature (°C)	0.474	0.001
Mean relative humidity (%)	0.457	0.001
Total rainfall (mm)	0.212	0.148[a]
Sum of sunshine (h)	0.049	0.738
Mean wind velocity (m/s)	0.186	0.205

HBoV, human bocavirus.
$P < 0.05$ indicates significant difference.
[a]Spearman correlation.

Based on the data from this study, HBoV was the third most common pathogen after RSV and *Mycoplasma pneumoniae* with an incidence of 6.6% in all hospitalized children with lower respiratory tract infection in Suzhou area. This is consistent with recent reports in Shanghai area (7.0%, 39/554) [Zhao et al., 2013] and Lanzhou area (7.1%, 29/406) [Zheng et al., 2010]. Although this data does differ when looking at the Gansu area (2.2%) [Huang et al., 2013] and Beijing area (33.8%) in China [Zhang et al., 2013]. Both serology and PCR methods could be applied to detect the HBoV infection in children. Certainly, PCR methods have a higher sensitivity when compared to the IgM detection method using ELISA (100% vs. 81.1%) [Zaghloul, 2011].

Most studies have shown that the incidence of infection with HBoV is highest among young infants less than 3 years of age [Nascimento-Carvalho et al., 2012; Zhao et al., 2013; Abdel-Moneim et al., 2013]. In the present study, the median age was 13 months, and the age group of 7–12 months and 13–36 months accounted for 66.3% of the total cases. Furthermore, a higher incidence rate (12.9% and 8.1%, respectively) compared to that of the other age groups was observed. This parallels the findings from studies conducted in Tokyo, Japan and Salvador, Brazil [Moriyama et al., 2010; Nascimento-Carvalho et al., 2012].

Several previous studies have reported that RSV, hMPV, rhinovirus, and human coronavirus are principal causes of co-infection with HBoV. Presumably, this is because the seasonal distribution of HBoV and these other viruses appear to overlap [Allander et al., 2007; Koseki et al., 2012; Xu et al., 2012]. Indeed, the reported prevalence of co-infections with other respiratory viruses varies from 18.3% to 71.1%
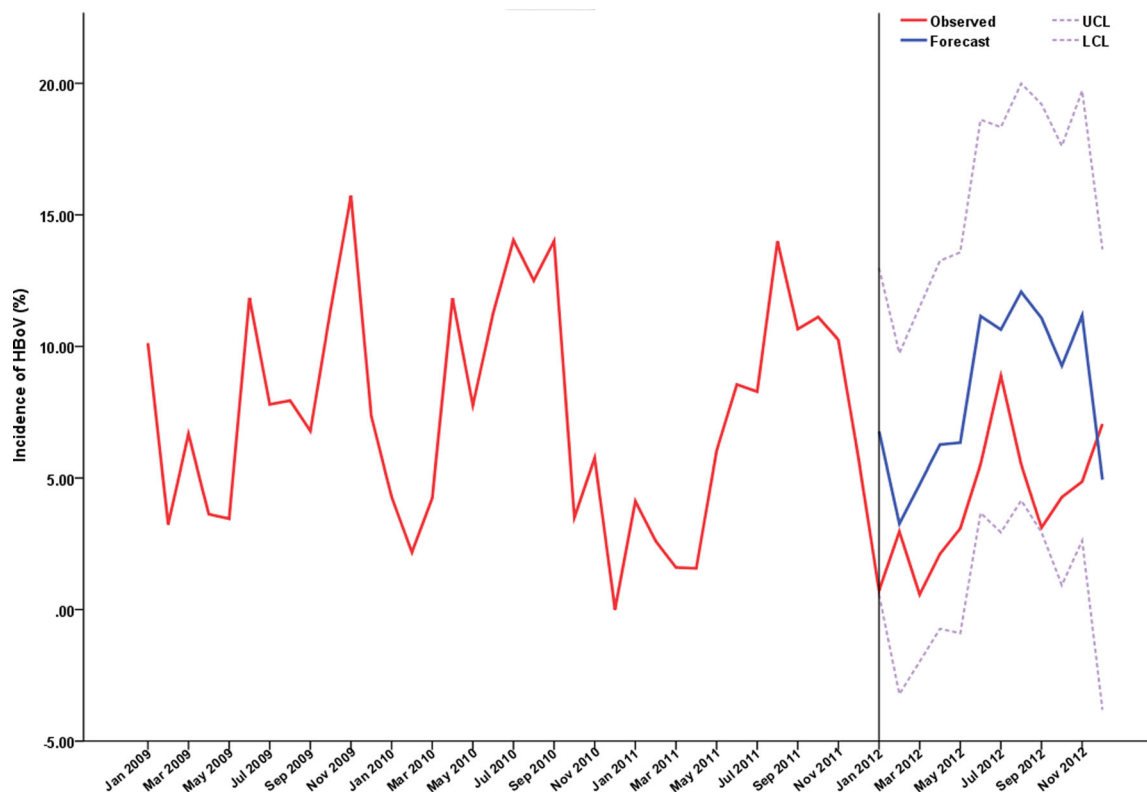
Fig. 3. Prediction of *human bocavirus* (HBoV) with a simple seasonal model including meteorological factors. Good agreement was found between observed and predicted incidence of HBoV infection. LCL, lower confidence interval; UCL, upper confidence interval.

[Weissbrich et al., 2006; García-García et al., 2007; Brieu et al., 2008; Kim et al., 2011]. Co-infection was also common in this study and accounted for 29.1% (146/502) of the total HBoV positive samples. Interestingly, *Mycoplasma pneumoniae* was a major pathogen that co-infected with HBoV after RSV and accounted for 25.3% (37/146) of total HBoV co-infection cases.

The major presenting clinical picture of lower respiratory tract infection due to HBoV was similar to other common respiratory virus infections like RSV and hMPV including cough, fever, wheezing and tachypnea and no characteristic symptom was found. The present study indicated pneumonia and bronchiolitis were the most frequent diagnoses associated with HBoV infected children, which is consistent with previous study [Allander et al., 2007; García-García et al., 2007; Moriyama et al., 2010]. There was no difference in clinical manifestation, laboratory test and proportion of severe pneumonia between single HBoV infection and co-infection groups. Co-infections were similar to simple infections in children with HBoV except that hypoxia was slightly more frequent ($P = 0.038$) in children with co-infection [García-García et al., 2007]. On the contrary, another study suggested that the association between HBoV and other respiratory viruses may be of clinical importance, and that infection with HBoV alone appears to produce no major symptoms in infants seen in emergency departments [Esposito et al., 2008]. It might have contributed to a lower severity of this present study because of the exclusion of children with underlying diseases. However, a recent study showed that children with single HBoV infection had a higher viral load compared to children with co-infection and there was a direct correlation of high viral load ($>10^6$ copies/ml) with increasing disease severity in children co-infected with HBoV but not in children with single HBoV infection [Zhao et al., 2013]. Meanwhile, high viral load ($>10^4$ copies/ml) of HBoV correlates with the duration of wheezing in children with severe lower respiratory tract infection [Deng et al., 2012]. Nevertheless, a study reported that there was no apparent association between the viral load of HBoV and co-infection or disease severity [Zheng et al., 2010]. Taken together, the role of co-infection with HBoV is still not clear and further studies need to be taken to explore these clinical observations. Interestingly, children with co-infection had a higher rate of lobar pneumonia compared to children with single HBoV infection. It is hypothesized that co-infection with *Mycoplasma pneumoniae* maybe play a role in such phenomenon due to it being the most common cause of lobar pneumonia in children according our 8 year surveillance (data not shown).

It has been described in earlier studies that HBoV infection seems to have a seasonal distribution. HBoV positive patients were most prevalent during January to May and the peak occurred during April to May in Japan [Ma et al., 2006] while the seasonal distribution is November to June with peaks occurring during March to April in France [Jacques et al., 2008]. In Hong Kong, the peak seasons were in fall and winter [Chieochansin et al., 2008]. In the Guangzhou area of China with similar subtropical climate, HBoV activity was year-round and the peak season was June [Xu et al., 2012], similar to what is seen in the present study. In India, however, which has a tropical climate, HBoV appeared to have no seasonal distribution [Bharaj et al., 2010]. These studies suggest that different areas with different climates have their own seasonal distribution of HBoV incidence. However, how a seasonal epidemic of HBoV starts is currently unknown.

Factors such as climate may impact the survival and spread of infectious disease indicating that the environment may contribute to an epidemic and the seasonal outbreaks of respiratory viruses [du Prel et al., 2009]. In the present study, HBoV activity was positively associated with mean temperature and relative humidity, which was consistent with another recent report [do Amaral de Leon et al., 2013]. The temperature and relative humidity during the summer was significantly higher than the other three seasons (shown in Fig. 2). To our interest, a lag time effect existed between HBoV activity and mean temperature especially for 1 month lag and no lag effect was found between HBoV activity and relative humidity. Taking into account that HBoV causing lower respiratory tract infection possesses an incubation period, several days of medical consultation, and an average of 5–7 days medical treatment before admission to our hospital (data not show), we presume that the lag time between climate parameters and HBoV, as confirmed by real-time PCR, is 2–3 weeks. This is consistent with the results of 1 month lag effect analyzed using linear regression in present study. This explains why the M1 model including mean temperature has a higher R2 value than the M0 or M2 models. Taken together, this study suggests that high temperature plays a more important role than high relative humidity in HBoV seasonal activity. The data on predicting HBoV activity could help in geographical areas where detection of HBoV infection may be difficult.

Some limitations of this study should be noted. First of all, It is difficult to confirm HBoV infection depending solely upon PCR because of certain bacterial colonization in healthy children although positive samples were defined with a concentration of DNA $>2.5 \times 10^3$ copies/ml. Secondly, the data analysis alone may not serve as a conclusive interpretation, since any associations with meteorological factors may be an indication of other social or environmental factors that also vary with the seasons. What's more, our study was based on a single center for data, which might have potential biases.

Despite these limitations, this study indicates that HBoV is a common cause of lower respiratory tract infection in children less than 3 years. Understanding the impact of meteorological factors, especially temperature, on HBoV activity can be useful and important in predicting seasonal outbreaks of lower respiratory tract infection.

ACKNOWLEDGMENTS

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