Spectrum of Respiratory Viruses Circulating in Eastern India: Prospective Surveillance Among Patients With Influenza-Like Illness During 2010–2011

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In developing countries, viruses causing respiratory disease are a major concern of public health. During January 2010–December 2011, 2,737 patients with acute respiratory infection from the outpatient departments as well as patients admitted to hospitals were screened for different respiratory viruses. Nasal and or throat swabs were collected and transported to the laboratory where initial screening of influenza A and influenza B viruses was performed. The samples were tested further for influenza C virus, parainfluenza viruses 1–4, human rhinovirus, metapneumovirus and respiratory syncytial virus by conventional RT-PCR. The study revealed that the majority of the patients were under 5 years of age; both due to their higher susceptibility to respiratory infections and presentation to hospitals. Out of 2,737 patients enrolled in this study, 59% were found positive for one or more respiratory viruses. Influenza B infection was detected in 12% of patients followed by influenza A (11.7%), respiratory syncytial virus (7.1%), parainfluenza virus-2 (6%), metapneumovirus (3%), parainfluenza virus-3 (1%), parainfluenza virus-4 (0.6%), parainfluenza virus-1 (0.3%), influenza C (0.2%) and human rhinovirus (0.2%). Distinct seasonal infection was observed only for influenza A and influenza B viruses. **J. Med. Virol.** 85:1459–1465, 2013. © 2013 Wiley Periodicals, Inc.

KEY WORDS: influenza like illness; respiratory viruses; Surveillance in eastern India; parainfluenza virus; metapneumovirus

INTRODUCTION

Acute respiratory infections are a leading cause of morbidity and mortality in infants and children, especially in developing countries. According to the

World Health Organization (WHO), acute respiratory infections account for approximately 20% of all deaths among children under 5 years [WHO, 2010], and 70% of these deaths occur in Africa and Southeast Asia [Williams et al., 2002]. Viruses are a leading cause of acute respiratory infections with epidemiological variability that depends on the climate [Armstrong et al., 1999]. To date, most of the known data are based on studies with children and infants only [Choi et al., 2006; Costa et al., 2006; Kusel et al., 2006; Ma et al., 2006; Ordás et al., 2006; Pierangeli et al., 2007] whereas the etiology remains largely undetermined for infections in the elderly [Fine et al., 1999; Garbino et al., 2002; File, 2003]. In children, respiratory syncytial virus and influenza viruses induce bronchiolitis, asthma exacerbation and pneumonia, leading to high rates of hospital admission [El-Hajje et al., 2008; Di Carlo et al., 2009]. Human rhinovirus, believed previously to cause only mild upper respiratory illnesses, has also been found in association with acute and chronic lower respiratory tract infections, including asthma exacerbations and chronic obstructive pulmonary disease (COPD); although the role of human rhinovirus as a cause has only been established for asthma [Papadopoulos et al., 2002; Friedlander and Busse, 2005; Xatzipsalti et al., 2005; Pierangeli et al., 2007; Singh and Busse, 2007; Matthew et al., 2009]. It was reported that metapneumovirus accounted for approximately 5–10% of all acute respiratory infections in children and adults [van den Hoogen et al., 2001] whereas adenoviruses were responsible for

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5–10% of acute respiratory infections only in children [Chen et al., 2004]. In addition, infections caused by other respiratory viruses such as parainfluenza viruses and coronaviruses occur worldwide. Furthermore, coronavirus NL63 [van der Hoek et al., 2004], coronavirus HKU [Fouchier et al., 2004; Woo et al., 2005] and bocavirus [Allander et al., 2007] are also responsible for acute respiratory infections in pediatric, elderly and immunosuppressed patients. Most studies based on laboratory diagnosis in hospitals are still restricted to influenza and respiratory syncytial virus. On the other hand, emerging respiratory viruses have been a subject of concern because of the risk of rapid spread and high fatality rates due to lack of both diagnosis and effective antiviral therapy.

In developing countries where diagnosis of viruses causing respiratory disease is restricted to a few laboratories, the etiology is undefined in a significant proportion (>60%); which undermines the impact of acute respiratory infections on health and economic burden [Monto, 1994; Henrickson et al., 2004]. In eastern India, frequency and genetic diversity of influenza viruses during 2005–2009 have been reported [Agrawal et al., 2010], but no information is available from the Indian subcontinent regarding other respiratory viruses. The present study aimed to identify common circulating respiratory viruses in addition to influenza during January 2010 through December 2011 in the eastern region of India.

MATERIALS AND METHODS

Sample Collection and Transportation

Nasal and/or throat swabs were collected from patients with influenza like illness attending the outpatients departments of hospitals in Kolkata, West Bengal, as described previously [Agrawal et al., 2009a]. In addition, swabs from patients with severe respiratory illness admitted to different hospitals in West Bengal were also referred to the laboratory for diagnosis. [Mukherjee et al., 2010]. Informed consent forms and detailed case histories were recorded before the collection of a specimen. The study was approved by the Institutional Ethical Committees.

RNA Extraction

Viral RNA was extracted by using commercially available QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany) as per manufacturer's instructions.

Screening for Influenza A/B Viruses by Real-Time PCR

For initial detection of influenza viruses, amplification of the matrix (M) gene of influenza A and influenza B viruses were carried out by real-time RT-PCR as described previously [Agrawal et al., 2009a].

Screening for Other Respiratory Viruses by Conventional RT-PCR

Respiratory viruses other than influenza were detected using a multiplex RT-PCR technique. First-strand cDNA synthesis was achieved using random primers and reverse transcription system (Invitrogen, Carlsbad, CA). The resulting cDNA was then subjected to multiplex PCR to enable simultaneous detection of multiple viruses. Primer sequences used in the study are described in Table I. The amplified products were then separated by agarose gel electrophoresis and visualized under UV after ethidium bromide staining. All samples were tested independently three times, using negative and positive controls to exclude cross-contamination of samples.

Statistical Analysis

Distribution frequencies of respiratory viruses were compared using Pearson's chi square test and Fisher's exact test. Continuous variables for population parameters such as age, laboratory investigations and other parameters were compared using one-way analysis of variance.

RESULTS

Demographic Characteristics of Outpatients

From January 2010 through December 2011, 2,737 swab samples from patients with respiratory infections were obtained; 1,574 (57.5%) of the patients were

TABLE I. Primer Pairs Used for Amplification of Different Respiratory Viral Genes

Strain	Primer name	Sequence (5'-3')	Position	Product size (bp)
Parainfluenza virus-1	HPIV-1 (F)	(+)CCG GTA ATT TCT CAT ACC TAT G	2–21	317
	HPIV-1 (R)	(–)CCT TGG AGC GGA GTT GTT AAG	298–318	
Parainfluenza virus-2	HPIV-2 (F)	(+)AAC AAT CTG CTG CAG CAT TT	713–732	489
	HPIV-2 (R)	(–)ATG TCA GAC AAT GGG CAA AT	1,182–1,201	
Parainfluenza virus-3	HPIV-3 (F)	(+)CTC GAG GTT GTC AGG ATA TAG	632–652	195
	HPIV-3 (R)	(–)CTT TGG GAG TTG AAC ACA GTT	806–826	
Parainfluenza virus-4	HPIV-4 (F)	(+)CTG AAC GGT TGC ATT CAG GT	1,969–1,988	442
	HPIV-4 (R)	(–)TTG CAT CAA GAA TGA GTC CT	2,391–2,410	
Influenza C	Inf-C (F)	(+)ACA CTT CCA ACC CAA TTT GG	676–695	485
	Inf-C (R)	(–)CCT GAC AGC AAC TCC CTC AT	1,141–1,160	
Human rhinovirus	HRV (F)	(+)GGG ACC AAC TAC TTT GGG TGT CCG TG	304–323	113
	HRV (R)	(–)GCA TCT GGY ARY TTC CAC CAC CAN CC	397–416

Fig. 1. Percent positivity of human respiratory viruses; circulating in eastern India during 2010–2011.

males and 1,163 (42.5%) were females (ratio M/F = 1.35). Out of these 2,737 patients 49.4% were obtained from patients under the age of 5 years old. Remaining 50.6% patients were of 5–15 years (14.2%), 15–35 years (19.4%), 35–45 years (6.1%), 45–60 years (7.3%) and >60 years (3.7%), respectively.

Prevalence of Respiratory Viruses

Out of 2,737 samples, 1,616 (59%) were positive for one or more respiratory viruses; of which 11.7% were positive for influenza A; which comprised both influenza A(H1N1)pdm09 (6.7%) and influenza A(H3N2) (5%), influenza B was detected in 12% cases, influenza C (0.2%), parainfluenza virus-1 (0.3%), parainfluenza virus-2 (6%), parainfluenza virus-3 (1.0%), and parainfluenza virus-4 (0.6%), respiratory syncytial virus (7.1%), metapneumovirus (3.0%) and human rhinovirus were found in 0.2% cases (Fig. 1).

Co-Infection of Respiratory Viruses

During the study period, 64 samples were found to be co-infected with respiratory syncytial virus and influenza B viruses. Metapneumovirus was found together with respiratory syncytial virus (n = 16),

influenza A (n = 1), and influenza B (n = 3) viruses. Mixed infection with influenza A and influenza B was detected in 13 cases.

Age Distribution

The prevalence of viral infection in different age groups has been shown in Figure 2. Infections due to influenza A(H3N2), influenza B, metapneumovirus and respiratory syncytial virus was high in children under 5 years of age followed by 5–15 years and 15–35 years; whereas, adults (15–35 years) and the elderly people were infected mainly with influenza A (H1N1)pdm09 virus. Influenza C virus infection predominantly occurred in children under the age of 5 years; although the frequency is very low. Similarly higher percentage of parainfluenza virus-1 infection was observed among children (under 5 years of age) followed by 15–35 years of age. In other age groups parainfluenza virus-1 was not observed in this study. Elderly persons (>60 years of age) were at high risk for infection with parainfluenza virus-2 and -4, followed by the patients of 45–60 and patients under 5 years of age; whereas the infection rate for parainfluenza virus-3 was high among the patients under

Fig. 2. Distribution of age of patients infected with human respiratory viruses during the study period (2010–2011).

5 years of age. For human rhinovirus infection, the risk group was 35–45 years followed by 45–60 years. During this study, all mixed infections were observed only in children aged less than 5 years.

Seasonal Distribution

The correlation of meteorological variations with the prevalence of respiratory viruses in eastern India is shown in Figure 3. Due to the pandemic in 2009, only influenza A(H1N1)pdm09 strain was found in 2010, however influenza A(H3N2) emerged again in 2011. Influenza B was found mainly after the monsoon season (October–December). Seasonal fluctuations for influenza C virus, parainfluenza virus 1–4, metapneumovirus and respiratory syncytial virus infection have not been observed during this study.

DISCUSSION

In 2004, surveillance for influenza viruses was expanded to India, as part of the global influenza surveillance network. However specific diagnosis which requires laboratory tests, were not widely available in eastern India. Hence the information on epidemiology and clinical features of respiratory virus infection in India is based entirely on research studies and the disease burden or seasonal prevalence of respiratory viruses remains largely undefined. This study initiated to complete the information on circulating respiratory viruses among patients attending the outpatients departments of different hospitals with acute respiratory infections in the eastern region of India during 2010 through 2011.

Fig. 3. Correlation of meteorological variations with prevalence of (A) influenza A, influenza B, influenza C, respiratory syncytial virus, metapneumovirus and (B) parainfluenza virus [1–4] during 2010–2011 in eastern India.

During this surveillance, influenza B infection was found to be most prevalent followed by influenza A. In 2010, only influenza A(H1N1)pdm09 virus circulated whereas in 2011, only influenza A/H3N2 was found. This was in contrast with the previous report from eastern India, where higher prevalence of influenza A was observed compared to influenza B [Agrawal et al., 2010]. Increased frequency of influenza B infection could be due to the post-pandemic effect of influenza A(H1N1)pdm09 following which seasonal influenza A/H1N1 and influenza A/H3N2 viruses disappeared during 2009–2010. This is consistent with studies from Vietnam [Do et al., 2011] and other Asian countries [Mathisen et al., 2010]. Metapneumovirus has been associated with both upper and lower respiratory tract infections in children (3–13%) [van den Hoogen et al., 2001; Bosis et al., 2005; Gerna et al., 2005; Choi et al., 2006; Sarasini et al., 2006; Boivin et al., 2007]. During this study period, metapneumovirus was found in only 3% samples. This finding corroborates with reports from Canada, England and the USA, where the infection rate due to metapneumovirus varies from 2% to 4.5% [Stockton et al., 2002; Falsey et al., 2003; Boivin et al., 2004], but is significantly less compared to the prevalence in Netherlands (10%), Australia (9.7%), and Chile (5.4%) [van den Hoogen et al., 2001; Luchsinger et al., 2005; Mackay et al., 2006]. Respiratory syncytial virus which is the most common etiological agent of viral lower respiratory tract infections in infants and young children in the world caused 33.8 million new episodes in children <5 years of age [Nair et al., 2010] and is also associated with hospital admission of 3–9 per 1,000 children below 1 year [Pancer et al., 2011]. In Thailand alone, 417.1/100,000 incidences of pneumonia per year are attributed to respiratory syncytial virus [Olsen et al., 2010]. Worldwide, respiratory syncytial virus has been identified as the cause of 3.4 million cases of acute lower respiratory infections requiring hospital admission. Among all age groups, 7.1% cases were found positive for respiratory syncytial virus during 2010–2011 in this study. Following respiratory syncytial virus, parainfluenza virus-2 was the most predominant virus (6%) compared to parainfluenza virus-1, -3 or -4.

Consistent with surveillance results during 2006–2009, in eastern India [Agrawal et al., 2010; Roy et al., 2011] and in Bangladesh [Zaman et al., 2009] children under 5 years old were found to be most vulnerable to infections due to influenza B and influenza A (H3N2) viruses. Whereas, influenza A (H1N1)pdm09 affected adults and the elderly which is consistent with findings from other studies [Cauchemez et al., 2009; John and Moorthy, 2010; Mukherjee et al., 2010]. Similar to influenza viruses, metapneumovirus infection was also detected mainly in children (under 5 year of age), suggesting early acquisition of infection [van den Hoogen et al., 2001; Lu et al., 2011; Zappa et al., 2011]. During

2007–2008, only 8.7% of respiratory syncytial virus infection was found among children [Agrawal et al., 2009b]; whereas in the same geographical region, 13.7% samples were found to be positive for respiratory syncytial virus in children (under 5 years) during this study period (2010–2011). The higher prevalence of respiratory syncytial virus during this study period could be due to lower activity of influenza A viruses compared to the activity of influenza A viruses in 2007–2008 [Agrawal et al., 2009b]. Among children with acute respiratory infections, co-infection with one or more respiratory viruses has been observed [Bonzel et al., 2008; Canducci et al., 2008] which correlates with results of present study, where predominant mixed infections were observed only in children.

Unlike temperate countries where the prevalence of seasonal influenza may reach epidemic proportions during the winter months [John and Moorthy, 2010], in tropical countries like India, year round circulation of strains has been reported, though the infection peaks during rainy season (June–September) [Agrawal et al., 2009a, 2010]. There could be several factors such as socio-economic, environmental, education, overcrowding and other factors, which could affect the seasonal incidence and distribution of viral infections. In eastern India, influenza B was found to be prevalent after the monsoon season as well as in the winter months [Roy et al., 2011], and influenza A viruses predominated during the monsoon (June–July) [Agrawal et al., 2009a; Mukherjee et al., 2010]. During 2010–2011 seasonal infection due to influenza A [influenza A(H1N1)pdm09 or influenza A/H3N2] correlated with previous reports. However the seasonal pattern of infection with respiratory syncytial virus during 2010–2011 was found to be different from the previous reports from Kolkata, India [Agrawal et al., 2009a,b] and Bangladesh [Huq et al., 1990], where respiratory syncytial virus infection was found more commonly in the winter months or the dry seasons. Such variations in seasonal incidence are difficult to explain, as virus infection could be affected by a large number of different factors [Weber et al., 1998]. Previous studies in temperate climates showed that parainfluenza virus-1 and -2 infection occurs annually [Laurichesse et al., 1999; Karron and Collins, 2007], parainfluenza virus-4 infection occurs twice-yearly during the late fall and winter [Aguilar et al., 2000; Vachon et al., 2006; Lau et al., 2009] and parainfluenza virus-3 infection occurs mainly during late spring and summer [Laurichesse et al., 1999]. These differences may be attributed to different geographical regions and study years. However, as the numbers of positive cases were very low in the present study, the seasonal prevalence of influenza C, parainfluenza viruses and human rhinovirus could not be determined. Hence large-scale studies over a broader geographical range and longer time period are required to understand the seasonal infection pattern of respiratory viruses in India.

REFERENCES

- Agrawal AS, Sarkar M, Chakrabarti S, Rajendran K, Kaur H, Mishra AC, Chatterjee MK, Naik TN, Chadha MS, Chawla-Sarkar M. 2009a. Comparative evaluation of real-time PCR and conventional RT-PCR during 2 year surveillance for influenza and respiratory syncytial virus among children with acute respiratory infections in Kolkata, India, reveals a distinct seasonality of infection. *J Med Microbiol* 58:1616–1622.
- Agrawal AS, Sarkar M, Ghosh S, Chawla-Sarkar M, Chakraborty N, Basak M, Naik TN. 2009b. Prevalence of respiratory syncytial virus group B genotype BA-IV strains among children with acute respiratory tract infection in Kolkata, Eastern India. *J Clin Virol* 45:358–361.
- Agrawal AS, Sarkar M, Ghosh S, Roy T, Chakrabarti S, Lal R, Mishra AC, Chadha MS, Chawla-Sarkar M. 2010. Genetic characterization of circulating seasonal Influenza A viruses (2005–2009) revealed introduction of oseltamivir resistant H1N1 strains during 2009 in eastern India. *Infect Genet Evol* 10:1188–1198.
- Aguilar JC, Pérez-Breña MP, García ML, Cruz N, Erdman DD, Echevarría JE. 2000. Detection and identification of human parainfluenza viruses 1, 2 3, and 4 in clinical samples of pediatric patients by multiplex reverse transcription-PCR. *J Clin Microbiol* 38:1191–1195.
- Allander T, Jartti T, Gupta S, Niesters HG, Lehtinen P, Osterback R, Vuorinen T, Waris M, Bjerkner A, Tiveljung-Lindell A, van den Hoogen BG, Hyypiä T, Ruuskanen O. 2007. Human bocavirus and acute wheezing in children. *Clin Infect Dis* 44:904–910.
- Armstrong GL, Conn LA, Pinner RW. 1999. Trends in infectious disease mortality in the United States during the 20th century. *JAMA* 281:61–66.
- Boivin G, Mackay I, Sloots TP, Madhi S, Freymuth F, Wolf D, Shemer-Avni Y, Ludewick H, Gray GC, LeBlanc E. 2004. Global genetic diversity of human metapneumovirus fusion gene. *Emerg Infect Dis* 10:1154–1157.
- Boivin G, De Serres G, Hamelin ME, Côté S, Argouin M, Tremblay G, Maranda-Aubut R, Sauvageau C, Ouakki M, Boulianne N, Couture C. 2007. An outbreak of severe respiratory tract infection due to human metapneumovirus in a long-term care facility. *Clin Infect Dis* 44:1152–1158.
- Bonzel L, Tenenbaum T, Schrotten H, Schildgen O, Schweitzer-Krantz S, Adams O. 2008. Frequent detection of viral coinfection in children hospitalized with acute respiratory tract infection using a real-time polymerase chain reaction. *Pediatr Infect Dis J* 27:589–594.
- Bosis S, Esposito S, Niesters HG, Crovari P, Osterhaus AD, Principi N. 2005. Impact of human metapneumovirus in childhood: Comparison with respiratory syncytial virus and influenza viruses. *J Med Virol* 75:101–104.
- Canducci F, Debiaggi M, Sampaolo M, Marinozzi MC, Berrè S, Terulla C, Gargantini G, Cambieri P, Romero E, Clementi M. 2008. Two-year prospective study of single infections and co-infections by respiratory syncytial virus and viruses identified recently in infants with acute respiratory disease. *J Med Virol* 80:716–723.
- Cauchemez S, Donnelly CA, Reed C, Ghani AC, Fraser C, Kent CK, Finelli L, Ferguson NM. 2009. Household transmission of 2009 pandemic influenza A (H1N1) virus in the United States. *N Engl J Med* 361:2619–2627.
- Chen HL, Chiou SS, Hsiao HP, Ke GM, Lin YC, Lin KH, Jong YJ. 2004. Respiratory adenoviral infections in children: A study of hospitalized cases in southern Taiwan in 2001–2002. *J Trop Pediatr* 50:279–284.
- Choi EH, Lee HJ, Kim SJ, Eun BW, Kim NH, Lee JA, Lee JH, Song EK, Kim SH, Park JY, Sung JY. 2006. The association of newly identified respiratory viruses with lower respiratory tract infections in Korean children 2000–2005. *Clin Infect Dis* 43:585–592.
- Costa LF, Yokosawa J, Mantese OC, Oliveira TF, Silveira HL, Nepomuceno LL, Moreira LS, Dyonisio G, Rossi LM, Oliveira RC, Ribeiro LZ, Queiróz DA. 2006. Respiratory viruses in children younger than five years old with acute respiratory disease from 2001 to 2004 in Uberlândia, MG, Brazil. *Mem Inst Oswaldo Cruz* 101:301–306.
- Di Carlo P, Romano A, Salsa L, Gueli A, Poma A, Fucà F, Dones P, Collura M, Pampinella D, Motisi D, Corsello G. 2009. Epidemiological assessment of Respiratory Syncytial Virus infection in hospitalized infants, during the season 2005–2006 in Palermo, Italy. *Ital J Pediatr* 35:11.
- Do AH, van Doorn HR, Nghiem MN, Bryant JE, Hoang TH, Do QH, Van TL, Tran TT, Wills B, Nguyen VC, Vo MH, Vo CK, Nguyen MD, Farrar J, Tran TH, de Jong MD. 2011. Viral etiologies of acute respiratory infections among hospitalized Vietnamese children in Ho Chi Minh City, 2004–2008. *PLoS One* 6:e18176.
- El-Hajje MJ, Moulin F, de Suremain N, Marc E, Cosnes-Lambe C, Pons-Catalano C, Lorrot M, Chalumeau M, Rozenberg F, Raymond J, Lebon P, Gendrel D. 2008. Respiratory syncytial virus in hospitalized children. A 3-year study. *Presse Med* 37:37–43.
- Falsey AR, Erdman D, Anderson LJ, Walsh EE. 2003. Human metapneumovirus infections in young and elderly adults. *J Infect Dis* 187:785–790.
- File TM. 2003. Community-acquired pneumonia. *Lancet* 362:1991–2001.
- Fine MJ, Stone RA, Singer DE, Coley CM, Marrie TJ, Lave JR, Hough LJ, Obrosky DS, Schulz R, Ricci EM, Rogers JC, Kapoor WN. 1999. Processes and outcomes of care for patients with community-acquired pneumonia: Results from the Pneumonia Patient Outcomes Research Team (PORT) cohort study. *Arch Intern Med* 159:970–980.
- Fouchier RA, Hartwig NG, Bestebroer TM, Niemeyer B, de Jong JC, Simon JH, Osterhaus AD. 2004. A previously undescribed coronavirus associated with respiratory disease in humans. *Proc Natl Acad Sci USA* 101:6212–6216.
- Friedlander SL, Busse WW. 2005. The role of rhinovirus in asthma exacerbations. *J Allergy Clin Immunol* 116:267–273.
- Garbino J, Sommer R, Gerber A, Regamey C, Vernazza P, Gennè D, Dür P, Rothen M, Unger JP, Lew D. 2002. Prospective epidemiologic survey of patients with community-acquired pneumonia requiring hospitalization in Switzerland. *Int J Infect Dis* 6:288–293.
- Gerna G, Campanini G, Rovida F, Sarasini A, Lilleri D, Paolucci S, Marchi A, Baldanti F, Revello MG. 2005. Changing circulation rate of human metapneumovirus strains and types among hospitalized pediatric patients during three consecutive winter-spring seasons. Brief report. *Arch Virol* 150:2365–2375.
- Henrickson KJ, Hoover S, Kehl KS, Hua W. 2004. National disease burden of respiratory viruses detected in children by polymerase chain reaction. *Pediatr Infect Dis J* 23:S11–S18.
- Huq F, Rahman M, Nahar N, Alam A, Haque M, Sack DA, Butler T, Haider R. 1990. Acute lower respiratory tract infection due to virus among hospitalized children in Dhaka, Bangladesh. *Rev Infect Dis* 8:S982–S987.
- John TJ, Moorthy M. 2010. 2009 Pandemic influenza in India. *Indian Pediatr* 47:25–31.
- Karron RA, Collins PL. 2007. Parainfluenza viruses. In: Fields BN, Knipe DM, Howley PM, editors. *Fields virology*. 5th edition. Philadelphia: Lippincott Williams & Wilkins. pp 1497–1520.
- Kusel MM, de Klerk NH, Holt PG, Kebadze T, Johnston SL, Sly PD. 2006. Role of respiratory viruses in acute upper and lower respiratory tract illness in the first year of life: A birth cohort study. *Pediatr Infect Dis J* 25:680–686.
- Lau SK, Li KS, Chau KY, So LY, Lee RA, Lau YL, Chan KH, Lim WW, Woo PC, Yuen KY. 2009. Clinical and molecular epidemiology of human parainfluenza virus 4 infections in Hong Kong: Subtype 4B as common as subtype 4A. *J Clin Microbiol* 47:1549–1552.
- Laurichesse H, Dedman D, Watson JM, Zambon MC. 1999. Epidemiological features of parainfluenza virus infections: Laboratory surveillance in England and Wales, 1975–1997. *Eur J Epidemiol* 15:475–484.
- Lu G, Gonzalez R, Guo L, Wu C, Wu J, Vernet G, Paranhos-Baccalà G, Wang J, Hung T. 2011. Large-scale seroprevalence analysis of human metapneumovirus and human respiratory syncytial virus infections in Beijing, China. *Virol J* 8:62.
- Luchsinger V, Escobar C, Avendaño LF. 2005. Detection of human metapneumovirus in children hospitalized for acute lower respiratory infection in Santiago, Chile. *Rev Med Chil* 133:1059–1064.
- Ma X, Endo R, Ishiguro N, Ebihara T, Ishiko H, Ariga T, Kikuta H. 2006. Detection of human bocavirus in Japanese children with lower respiratory tract infections. *J Clin Microbiol* 44:1132–1134.

- Mackay IM, Bialasiewicz S, Jacob KC, McQueen E, Arden KE, Nissen MD, Sloots TP. 2006. Genetic diversity of human metapneumovirus over 4 consecutive years in Australia. *J Infect Dis* 193:1630–1633.
- Mathisen M, Strand TA, Sharma BN, Chandyo RK, Valentiner-Branth P, Basnet S, Adhikari RK, Hvidsten D, Shrestha PS, Sommerfelt H. 2010. Clinical presentation and severity of viral community-acquired pneumonia in young Nepalese children. *Pediatr Infect Dis J* 29:e1–e6.
- Matthew J, Pinto Pereira LM, Pappas TE, Swenson CA, Grindle KA, Roberg KA, Lemanske RF, Lee WM, Gern JE. 2009. Distribution and seasonality of rhinovirus and other respiratory viruses in a cross-section of asthmatic children in Trinidad, West Indies. *Ital J Pediatr* 35:16.
- Monto AS. 1994. Studies of the community and family: Acute respiratory illness and infection. *Epidemiol Rev* 16:351–373.
- Mukherjee A, Roy T, Agrawal AS, Sarkar M, Lal R, Chakrabarti S, Chawla-Sarkar M. 2010. Prevalence and epidemiology of pandemic H1N1 strains in hospitals of Eastern India. *J Pub Health and Epidemiol* 2:171–174.
- Nair H, Nokes DJ, Gessner BD, Dherani M, Madhi SA, Singleton RJ, O'Brien KL, Roca A, Wright PF, Bruce N, Chandran A, Theodoratou E, Sutanto A, Sedyaningsih ER, Ngama M, Munywoki PK, Kartasasmita C, Simões EA, Rudan I, Weber MW, Campbell H. 2010. Global burden of acute lower respiratory infections due to Respiratory syncytial virus in young children: A systematic review and meta-analysis. *Lancet* 375:1545–1555.
- Olsen SJ, Thamthitiwat S, Chantra S, Chittaganpitch M, Fry AM, Simmerman JM, Baggett HC, Peret TC, Erdman D, Benson R, Talkington D, Thacker L, Tondella ML, Winchell J, Fields B, Nicholson WL, Maloney S, Peruski LF, Ungchusak K, Sawanpanyalert P, Dowell SF. 2010. Incidence of respiratory pathogens in persons hospitalized with pneumonia in two provinces in Thailand. *Epidemiol Infect* 138:1811–1822.
- Ordás J, Boga JA, Alvarez-Argüelles M, Villa L, Rodríguez-Dehli C, de Oña M, Rodríguez J, Melón S. 2006. Role of metapneumovirus in viral respiratory infections in young children. *J Clin Microbiol* 44:2739–2742.
- Pancer K, Ciaccka A, Gut W, Lipka B, Mierzejewska J, Milewska-Bobula B, Smorczewska-Kiljan A, Jahnz-Rózyk K, Dzierzanowska D, Madaliński K, Litwińska B. 2011. Infections caused by RSV among children and adults during two epidemic seasons. *Pol J Microbiol* 60:253–258.
- Papadopoulos NG, Moustaki M, Tsolia M, Bossios A, Astra E, Prezerakou A, Gourgiotis D, Kafetzis D. 2002. Association of rhinovirus infection with increased disease severity in acute bronchiolitis. *Am J Respir Crit Care Med* 165:1285–1289.
- Pierangeli A, Gentile M, Di Marco P, Pagnotti P, Scagnolari C, Trombetti S, Lo Russo L, Tromba V, Moretti C, Midulla F, Antonelli G. 2007. Detection and typing by molecular techniques of respiratory viruses in children hospitalized for acute respiratory infection in Rome, Italy. *J Med Virol* 79:463–468.
- Roy T, Agrawal AS, Mukherjee A, Mishra AC, Chadha MS, Kaur H, Chawla-Sarkar M. 2011. Surveillance and Molecular characterization of human influenza B viruses during 2006–2010 revealed co-circulation of Yamagata-like and Victoria-like strains in eastern India. *Infect Genet Evol* 11:1595–1601.
- Sarasini A, Percivalle E, Rovida F, Campanini G, Genini E, Torsellini M, Paolucci S, Baldanti F, Marchi A, GraziaRevello M, Gerna G. 2006. Detection and pathogenicity of human metapneumovirus respiratory infection in pediatric Italian patients during winter-spring season. *J Clin Virol* 35:59–68.
- Singh AM, Busse WW. 2007. Human rhinovirus models in asthma. *Contrib Microbiol* 14:12–20.
- Stockton J, Stephenson I, Fleming D, Zambon M. 2002. Human metapneumovirus as a cause of community-acquired respiratory illness. *Emerg Infect Dis* 8:897–901.
- Vachon ML, Dionne N, Leblanc E, Moisan D, Bergeron MG, Boivin G. 2006. Human parainfluenza type 4 infections, Canada. *Emerg Infect Dis* 12:1755–1758.
- van den Hoogen BG, de Jong JC, Groen J, Kuiken T, de Groot R, Fouchier RA, Osterhaus AD. 2001. A newly discovered human pneumovirus isolated from young children with respiratory tract disease. *Nat Med* 7:719–724.
- van der Hoek L, Pyrc K, Jebbink MF, Vermeulen-Oost W, Berkhout RJ, Wolthers KC, Wertheim-van Dillen PM, Kaandorp J, Spaargaren J, Berkhout B. 2004. Identification of a new human coronavirus. *Nat Med* 10:368–373.
- Weber MW, Mulholland EK, Greenwood BM. 1998. Respiratory syncytial virus infection in tropical and developing countries. *Trop Med Int Health* 3:268–280.
- World Health Organization. 2010. Acute respiratory infections in children. Available: http://www.who.int/fch/depts/cah/resp_infections/en/print.html. Accessed February 10, 2011.
- Williams BG, Gouws E, Boschi-Pinto C, Bryce J, Dye C. 2002. Estimates of world-wide distribution of child deaths from acute respiratory infections. *Lancet Infect Dis* 2:25–32.
- Woo PC, Lau SK, Chu CM, Chan KH, Tsoi HW, Huang Y, Wong BH, Poon RW, Cai JJ, Luk WK, Poon LL, Wong SS, Guan Y, Peiris JS, Yuen KY. 2005. Characterization and complete genome sequence of a novel coronavirus, coronavirus HKU1, from patients with pneumonia. *J Virol* 79:884–895.
- Xatzipsalti M, Kyrana S, Tsolia M, Psarras S, Bossios A, Laza-Stanca V, Johnston SL, Papadopoulos NG. 2005. Rhinovirus viremia in children with respiratory infections. *Am J Respir Crit Care Med* 172:1037–1040.
- Zaman RU, Alamgir AS, Rahman M, Azziz-Baumgartner E, Gurley ES, Sharker MA, Brooks WA, Azim T, Fry AM, Lindstrom S, Gubareva LV, Xu X, Garten RJ, Hossain MJ, Khan SU, Faruque LI, Ameer SS, Klimov AI, Rahman M, Luby SP. 2009. Influenza in outpatient ILI case-patients in national hospital-based surveillance, Bangladesh, 2007–2008. *PLoS ONE* 4:e8452.
- Zappa A, Canuti M, Frati E, Pariani E, Perin S, Ruzza ML, Farina C, Podestà A, Zanetti A, Amendola A, Tanzi E. 2011. Co-circulation of genetically distinct human metapneumovirus and human bocavirus strains in young children with respiratory tract infections in Italy. *J Med Virol* 83:156–164.