Original Article

What We Have Learned from the Influenza A pH1N1 2009/10 Pandemic: High Clinical Impact of Human Metapneumovirus and Respiratory Syncytial Virus in Hospitalized Pediatric Patients

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SUMMARY: The influenza pandemic in 2009/2010 shifted public awareness to respiratory tract infections caused by the influenza virus. A prospective study was conducted during the influenza pandemic from November 2009 through April 2010 to determine the causative pathogens and clinical symptoms present in all children and adolescents admitted to the University Children's Hospital, Duesseldorf, Germany, with signs and symptoms of respiratory tract infection. A total of 272 children and adolescents were admitted with symptoms of acute respiratory tract infection (ARI) or influenza-like illness. Viral pathogens were detected in 80% (218/272). However, influenza A pH1N1 infection was only detected in 11% (30/272) of children. Human metapneumovirus (HMPV) and respiratory syncytial virus (RSV) were the predominant identified pathogens that led to the admission of young tachypneic children with pneumonia in the post pandemic phase and the requirement for more intense treatment. During the pandemic and early post-pandemic phase the clinical impact of other respiratory viruses, such as HMPV and RSV, led to a higher clinical disease burden than pH1N1. Consequently, HMPV testing should be performed as routinely as RSV testing in patients hospitalized for ARI. Even while preparing for pandemics, the awareness of other respiratory viruses must be maintained.

INTRODUCTION

In 2009, the spread of pandemic influenza (pH1N1) virus led to a drastically increased number of children and adolescents presenting to emergency departments (EDs) with influenza or influenza-like illness (ILI). The pH1N1 virus rapidly infected over 40% of the susceptible population and became endemic by replacing the previously circulating human seasonal H1N1 virus (1). This occurred despite extensive preparations for the pandemic worldwide and readily available molecular diagnostics (2). Diagnosis of acute respiratory tract infection (ARI) and discrimination between bacterial and viral infection is not straight-forward. The diagnosis, clinical management, and understanding of the pathogenesis of a child presenting with symptoms of viral infection can be readily improved by using multiplex PCR to determine the presence or absence of a viral respiratory pathogen (3,4).

In general, the clinical course of viral respiratory tract infections is affected by virus type, viral load, and viral or bacterial co-infections (4,5). During the early pandemic phase the information available on the clinical course of pH1N1 infection in children was very sparse or non-existent. Therefore, children presenting with signs and symptoms of ARI during the early pandemic phase represented an invaluable source of clinical information and prevalence data for respiratory viruses.

In 2009 from weeks 43 to 51, the incidence of pH1N1 infection in Germany was estimated to be 468 and 1,110 per 100,000 in children aged 1–4 years and 5–17 years, respectively (6). Previous investigations in the study area have shown a winter prevalence of 40% for respiratory syncytial virus (RSV), 4% for human metapneumovirus (HMPV), and 3% for influenza infections (3). Both HMPV and RSV, important pathogens for respiratory tract infections in children, circulate predominantly in late winter and spring. Peaks of activity for both viruses coincide with a phase of high influenza disease burden (7,8).

The aims of this study were to investigate the occurrence and disease burden of respiratory viruses, with a special focus on HMPV, RSV, and pH1N1, and to examine the clinical characteristics of children presenting with signs and symptoms of respiratory tract infections in the early pandemic and post-pandemic phases in 2009/2010.

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MATERIALS AND METHODS

Prospective study design: We performed a prospective study among children and adolescents aged 0–18 years who were admitted to the University Children's Hospital Duesseldorf, Germany, with signs and symptoms of acute upper or lower respiratory tract infection and/or ILI symptoms during 6 consecutive months from November 2009 to April 2010. Patients presented with ≥ 1 of following symptoms: cough, sore throat, myalgia, chills, fatigue, wheezing, dyspnea, tachypnea, nasal flaring, distractions, fever, or rhinitis. Classification tables for the integrated management of childhood illnesses were taken into account when classifying cough or difficulty breathing (9). Patients with vomiting, diarrhea, or abdominal pain in the absence of abdominal disease were only included when respiratory tract infection was likely to be present based on the clinical judgment of the examining pediatrician. Patients who were not admitted after evaluation in the ED were not included. Patients with primary or secondary immunosuppression, and patients or parents who did not provide written informed consents were excluded. The study was approved by the Institutional Review Board of the Medical Faculty of the Heinrich-Heine-University of Dueseldorf.

After admission, clinical, laboratory, and radiological data were collected and studied as routinely needed. After written informed consent was obtained, standardized questionnaires were administered to the patients' parents or guardians at enrolment. Chest radiograph was not mandatory for study participation. Sample collection and diagnostic procedures were conducted in accordance with the guidelines of good clinical practice. Within 48 h of admission, deep nasal swabs or pharyngeal swabs were taken, placed in liquid universal viral transport media and delivered to the Department of Virology within 12 h for further testing. Blood cultures were obtained depending on the clinical judgment of the attending pediatrician, however, in general, before the initiation of antibiotic therapy a blood culture was obtained. Blood samples were obtained as clinically indicated. Standard bacterial cultures were not performed routinely, but were obtained based on clinical judgment. If a bacterial culture was required, a second throat swab was taken and was plated on Columbia, chocolate, and MacConkey agars for aerobic and anaerobic cultures.

The medical records of hospitalized subjects were reviewed after or directly before discharge, and data were extracted using the same questionnaire. Demographic data, clinical signs and symptoms, pre-existing medical conditions, history of vaccination with seasonal influenza vaccine including pandemic vaccine, laboratory and imaging results, and medical management (including treatments, requirement for supplemental oxygen, steroids, inhalers, and pediatric intensive care unit (PICU) admission) were recorded. Hypoxemia was defined as $\text{SpO}_2 < 92\%$ on admission. Hospitalization was defined as admission for > 24 h to a pediatric ward or PICU.

PCR testing: All samples were submitted to the Institute of Virology of the University Hospital of Dueseldorf. Aliquots were analyzed within 24 h or stored at

-80°C until further testing. Nucleic acids from clinical samples were obtained with the EZ 1 Virus Mini Kit (No. 955338; Qiagen, Hilden, Germany). Quantitative real-time PCR was used to detect HMPV genotype A (HMPV-A), HMPV genotype B (HMPV-B), RSV genotype A (RSV-A), RSV genotype B (RSV-B), pH1N1 and seasonal influenza virus, and other respiratory viruses including adenoviruses, coronaviruses 229E, OC43 and NL63, enterovirus, rhinoviruses, influenza virus A/B, human bocavirus, and parainfluenza viruses types 1, 2, and 3 (3).

Statistics: The results are expressed as percentages for discrete variables, or as mean and standard deviation for continuous variables. Age and days of hospitalization data are described by their median. Clinical characteristics and laboratory variables are compared using the Pearson's χ^2 test, Relative Risk (RR), and Mann-Whitney U test as appropriate. Tests were performed using SigmaPlot for Windows Version 12.0 (Systat Software, San Jose, CA, USA). In general, a P-value (P) of < 0.05 was considered to be statistically significant.

RESULTS

During the study period, 285 patients met the inclusion criteria to participate in the study. Of these, 4 parents declined participation, 7 verbally accepted but did not sign the informed consent form later, and in 2 eligible patients the clinical data was incomplete because the parents left the clinic with their children before being evaluated for discharge by a pediatrician. Therefore, a total of 272 (95.4%) children and adolescents aged 0–18 years were enrolled in the study (man 57%, woman 43%) between November 2009 and April 2010 (week 43 in 2009 to week 17 in 2010). The median age was 17 months (range 0–218 months). In total, 78% (212/272) of patients were younger than 48 months of age. An underlining condition was reported for 25% (69/272) of admitted patients, including premature birth (7%), cardiac abnormalities (2%), and severe pulmonary conditions (cystic fibrosis; 1%; Table 1). Within the study group, 8% (22/272) of patients were vaccinated against pH1N1 with at least one dose of the German pandemic influenza vaccine (Pandemrix), 16% (44/272) of patients had received the seasonal influenza vaccine, and 4% (11/272) had received both vaccines. None of the recruited children had received antiviral medication prior to admission. Nineteen percent (52/272) of children received antibiotic therapy had been initiated by the primary care physician prior to admission.

Viral respiratory pathogens were found in 80% (218/272) of the children. The majority (62%, 170/272) had a mono-infection with one respiratory virus, and 18% (48/272) had at least 2 respiratory viruses (co-infection). No viral pathogens were detected in 20% (54/272) of participants. The influenza (pH1N1) virus was detected in 11% (30/272) of children. Of these patients, 4 were co-infected with other respiratory viruses, (coronavirus 229E, $n = 2$; rhinovirus, $n = 2$). In 47% (128/272) of patients, infection with HMPV (15%, 41/272) or RSV (32%, 87/272) was detected; in 4 individuals, both of these viruses were present. The most frequently detected virus other than influenza A

Table 1. Clinical characteristics

	Number (%)
All patients	272 (100)
Man	155 (57)
Woman	117 (43)
Age (in months)	17 (0–218) ¹⁾
<48 months	212 (78)
One or more virus detected	218 (80)
No virus found	54 (20)
Days with symptoms prior to admission	3 (0–49) ¹⁾
Chest radiograph	169 (62)
Radiologic confirmed pneumonia	117 (43)
Radiologic signs of bronchitis	37 (14)
Underlying conditions	69 (25)
Prematurity	19 (7)
Cardiac abnormality	6 (2)
Cystic fibrosis	3 (1)
Mucopolysaccharidosis	1 (<1)
Inherited disorders of metabolism	10 (4)
Spherocytosis	1 (<1)
Central diabetes insipidus	1 (<1)
First or recurrent seizure episodes	8 (3)
Diarrhea	10 (4)
Syndromal disease	10 (4)
Wheezing episodes previously recorded	122 (45)
Asthma previously recorded	5 (2)

¹⁾: Median (range).

pH1N1, was RSV A/B in 32% (87/272) of patients (RSV-A, 56 cases; RSV-B, 31 cases), followed by rhinovirus (18%, 49/272), HMPV-A/B (15%, 41/272), human bocavirus (7%, 20/272), and adenovirus (7%, 19/272; Table 2).

The median age of the children was 17 months (range, 0–218 months; 1st and 3rd inter quartile range, 6 and 41.8, respectively). The groups containing the youngest children were infected with HMPV (median age, 14 months; range 5.3–38.8) and RSV (median age, 7 months; range, 3.0–22.0). In contrast, the oldest groups of children were infected with influenza or with no virus. Patients infected with pH1N1 were significantly older (median age, 55 months; range, 12.5–143.3) compared to HMPV or RSV mono-infected patients (median age 8 months; $P < 0.05$). In children >24 months of age, pH1N1 infection was detected more commonly than in children <24 months of age (6.6%, 18/272 vs. 2.9%, 8/272). Before presenting to the ED, the median duration of symptoms prior to admission was 3 days (range, 0–49 days; Table 1).

Patients who suffered from pH1N1 infection had a significantly shorter period of symptoms prior to admission than patients with HMPV, RSV, or any other viral mono-infection (median, 1 vs. 3 days, $P < 0.001$). Patients infected with pH1N1 were more likely to present with fever >38.4°C (pH1N1, 85%; other viral pathogens, 59%; $P = 0.02$). However, there were no significant differences in absolute peak temperature or duration of fever. The clinical course after admission varied and was a function of age and disease-causing pathogen. Hypoxemia ($\text{SpO}_2 < 92\%$) was diagnosed in 46 patients, and subsequently, oxygen therapy was initiated. Patients with HMPV or RSV mono-infection

Table 2. Distribution of viral pathogens found in children presenting with signs and symptoms of acute respiratory tract infection or influenza like illness from November 2009–April 2010

	All Total, 272 n (%)	Mono- infection ¹⁾ n (%)	Co- infection ¹⁾ n (%)	Influenza A pH1N1	RSV A/B	HMPV	Parainfluenza 1/2/3	Corona virus	Rhinovirus	Enterovirus	Adenovirus	Bocavirus
Influenza A pH1N1	30 (11.0)	26 (86.7)	4 (13.3)	—	0	0	0	2	2	1	0	0
RSV A/B	87 (32.0)	68 (78.2)	19 (21.8)	—	—	4	1	2	6	2	5	5
HMPV	41 (15.1)	28 (68.3)	13 (31.7)	—	—	—	1	2	4	0	2	3
Parainfluenza 1/2/3	10 (3.7)	4 (40.0)	6 (60.0)	—	—	—	—	0	3	0	2	1
Corona Virus	13 (4.8)	3 (23.1)	10 (76.9)	—	—	—	—	—	2	1	1	1
Rhinovirus	49 (18.0)	25 (51.0)	24 (49.0)	—	—	—	—	—	—	6	7	2
Enterovirus	11 (4.0)	4 (36.4)	7 (63.6)	—	—	—	—	—	—	—	3	1
Adenovirus	19 (7.0)	4 (21.1)	15 (78.9)	—	—	—	—	—	—	—	—	4
Bocavirus	20 (7.4)	8 (40.0)	12 (60.0)	—	—	—	—	—	—	—	—	—

¹⁾: In 11 patients, co-infections with ≥3 viruses were detected, one of them in the pH1N1 group, 2 in the HMPV, and 4 in the RSV group, respectively. HMPV and RSV co-infection was seen in 4 individuals.

Table 3. Clinical data of children with HMPV, RSV, and pH1N1 mono-infection, or mono-infection of another viral pathogen, any co-infection, or no identified causative virus

	All eligible children	Influenza pH1N1	RSV	HMPV	Other viral mono-infection	Any viral co-infection	No virus	Patient ≤ 5 years of age	Patient > 5 years of age
Number (%)	272	26	68	28	48	48	54	236 (87)	36 (13)
Age [months, median]	17	55 ¹⁾	7 ¹⁾	14 ¹⁾	18	15	42	12	115
Man (%)	156 (57)	15 (58)	38 (56)	18 (64)	24 (50)	30 (63)	31 (57)	138 (58)	18 (50)
LOS [days, median]	4	4	4	4	3	4	4	4	4
Fever >38.4°C (%)	174 (64)	22 (85)	38 (56)	18 (64)	18 (64)	35 (73)	32 (59)	150 (64)	24 (67)
Tachypnea (%)	90 (33)	2 (8) ²⁾	28 (41) ²⁾	12 (43)	17 (35)	15 (31)	16 (30)	84 (36)	6 (17)
Wheezing (%)	17 (6)	2 (8)	4 (6)	2 (7)	6 (13)	2 (4)	1 (2)	15 (6)	2 (6)
CRP >4.9 mg/dl (%)	54 (20)	3 (12)	9 (13)	7 (25)	11 (23)	5 (10)	17 (31)	43 (18)	11 (31)
Leucocytosis (%)	52 (19)	1 (4)	5 (7)	1 (4)	12 (25)	12 (25)	21 (39)	47 (20)	5 (14)
SpO ₂ min [%, mean]	91.1	92.0	90.6	90.5	91.3	90.7	92.2	91.2	92.0
Chest radiograph (%)	169 (62)	10 (39)	43 (63)	19 (68)	31 (65)	31 (65)	35 (65)	146 (62)	23 (64)
Pneumonia (%)	117 (43)	6 (23) ²⁾	36 (53) ²⁾	14 (50)	21 (44)	16 (33)	25 (46)	101 (43)	16 (44)
O ₂ -suppl. [h, mean]	7.5	2.8 ¹⁾	10.9 ¹⁾	16.1 ¹⁾	12.0	4.0	0.7	8.0	3.0
Salbuterol (%)	180 (66)	9 (35)	56 (82)	21 (75)	31 (65)	34 (71)	29 (54)	164 (70)	16 (44)
Systemic steroids (%)	69 (25)	4 (15)	20 (29)	12 (43)	14 (29)	13 (27)	6 (13)	63 (27)	6 (17)
Seasonal infl. vaccine (%)	44 (16)	6 (23)	6 (9)	4 (14)	8 (17)	8 (17)	12 (22)	32 (14)	12 (33)
Pandemrix (%)	22 (8)	1 (4)	3 (4)	3 (11)	5 (10)	4 (8)	6 (11)	16 (7)	6 (17)

¹⁾: $P < 0.05$, Influenza single infection vs. RSV and HMPV single infection.

²⁾: $P < 0.05$, Influenza single infection vs. RSV single infections.

Leucocytosis, leukocyte count > age adjusted normal value. LOS, length of stay (two children required mechanical ventilation).

Table 4. Radiologic diagnosis in pediatric patients admitted for signs and symptoms of acute respiratory disease

Virus (mono-infection)	Number	Chest radiograph (%)	Pneumonia/ Chest radiograph %
Influenza pH1N1	26	10 (39)	60
RSV	68	43 (63)	84
HMPV	28	19 (68)	74
Other virus	48	31 (65)	68
Total	170	103 (61)	75

received supplemental oxygen for a significant longer period of time compared to children with pH1N1 infection (12.4 h vs. 2.8 h; $P < 0.05$). HMPV-infected patients received supplemental oxygen for a mean duration of 16.1 h (Table 3).

Systemic steroids were administered in 25% (69/272) of children. The observation that steroids were more administered more frequently in HMPV- or RSV-infected children compared to children without viral infection (33%, 32/96 vs. 13%, 6/54) is of limited value since stringent criteria were not applied for the use of steroids.

Patients with RSV or HMPV infection were more likely to develop pneumonia compared to patients with pH1N1 infection (RR 2.3; 95% confidence interval [CI], 1.1–4.7) (Table 4). There were no significant differences in leukocyte counts between children with different viral infections (pH1N1, HMPV, or RSV). C-reactive protein (CRP) levels >4.9 mg/dl were recorded in 25% (7/28) of HMPV-infected children. CRP levels >4.9 mg/dl were seen more frequently in children without a viral pathogen (31%, 17/54). Blood cultures were obtained in 14% (38/272) of patients, however, a

positive result was only observed in 3 children. Five children suffered from acute otitis media.

DISCUSSION

This prospective study was performed to evaluate the respiratory virus distribution and clinical characteristics in pediatric inpatients during the 2009/2010 influenza pandemic and early post-pandemic. A total of 272 of children and adolescents admitted with clinical signs of respiratory tract infection and/or ILI symptoms were enrolled. This high number of recruited patients reflects the local disease burden, as well as a higher demand for testing of the underlying pathogens of respiratory disease in the midst of a pandemic. Admission numbers and testing frequency were remarkably higher than during ARI seasons in the same local area in previous years (3,4). Although influenza testing was requested frequently during the reported period, the test results mainly identified respiratory viruses other than influenza in children with respiratory tract infections. HMPV and RSV infections had a high clinical impact, and viral co-infections were also seen at a high rate in non-influenza cases. The overall number of admissions due to non-influenza pathogens was higher than the number of admissions due to influenza virus infections during the peak phase of the pandemic. This observation is in accordance with other reports of children presenting to EDs with ILI during the influenza pandemic (10). A study of virus distribution in outpatients (including adults) in Belgium found pH1N1 to be the most common virus among children under the age of 5 years during the pandemic, probably reflecting a correlation between ILI case definition and actual influenza diagnosis. However, this group was frequently co-infected by other respiratory viruses (11). The rate of

negative results in our study with respect to viral etiology was only 20%. This is especially low compared to 76% found by Chang et al. (10). This study included any patient with a temperature of $\geq 38.2^{\circ}\text{C}$ either at the time of presentation or documented by the parents, and ≥ 2 ILI symptoms. In a study in Brazil that assessed only hospitalized children, 27% of patients were found to be negative for the five respiratory viruses tested, which is readily comparable to our results (12).

A high prevalence of HMPV mono-infections were recorded (15% of virus-positive patients). This rate was higher than previously reported for children in the local area (4% in 2005/2006, and 5% in 2006/2007 and 2007/2008). Former investigations in the study area have demonstrated a prevalence for 40% for RSV, 4% for HMPV, and 3% for influenza infections. However, the highest prevalence of respiratory viruses besides RSV and Influenza was found for rhinovirus. While high seasonal infection frequencies are expected for rhinovirus and RSV infection (32%) (e.g. up to 44% and 51% in previous reports), the number of children positive for HMPV was unexpectedly high (3,4). Hom-brouck et al. also found a remarkably high HMPV infection rate (14% of positives) during the pandemic; in contrast to our study, they excluded hospitalized patients (11).

The higher frequency of HMPV infections found in this study could represent the clinical phenomenon that HMPV infection leads to children having more severe symptoms and being readily admitted. The comparably low pH1N1 frequency in admitted children could be explained by a better clinical condition of these children. The coincidence of the pH1N1 pandemic and high frequency of HMPV infections raises the question of a putative cumulative pathogenic effect, and consequently, greater spread of HMPV infection in the population. Outbreaks of HMPV disease are reported to be a local phenomenon, while 2 or 3 influenza virus strains spread across the globe each year. HMPV circulates predominantly in late winter and spring, and the peak of activity at any given location often coincides with or follows the peak of RSV activity. The peak of HMPV detection was previously reported to be in February and March or from March to May. In our study, HMPV peaked in February followed by RSV in March (Fig. 1). Large outbreaks of HMPV have been reported to alternate with small outbreaks in a regular, biannual pattern in successive epidemic seasons, while HMPV virus epidemics were anti-cyclical to RSV epidemics (7,13–15). No clear relationship between influenza and HMPV infections could be established; however, the unusual increase in HMPV infection observed in the 2009 winter season in our study group might be linked to viral interference by pandemic influenza or other circulating viruses, as well as the emergence of new genotypes (16–20). This study included children and adolescents admitted to the hospital solely based on clinical criteria. Reasons for admittance were disease severity as judged by the pediatrician on duty and requests for observation, e.g. to rule out pH1N1 infection. One important reason for presentation to the ED was the fear of pH1N1 infection rather than the severity of the clinical presentation, predominantly in older children brought in by anxious parents. This observation changed during

Fig. 1. Monthly distribution of percent admissions of children infected with HMPV, RSV, influenza pH1N1 viruses, and no viral pathogen detected cases from week 45/2009 to week 17/2010 (from November to April; $n = 218$). Total of 41 HMPV-positive, 87 RSV-positive, 30 pH1N1-positive cases, and 54 cases detected with no viral pathogen are illustrated. The percentage of positive (or negative) specimens /total positive cases during each period for each virus is indicated.

the second half of the study period when a shift to younger children presenting to the ED occurred. These children had more severe clinical symptoms not reflecting the impact of pH1N1 infection but infection with other respiratory viruses, such as HMPV, RSV, and rhinovirus.

Using a different study design, the incidence of pH1N1 infection in children was reported to show hemagglutination inhibition titers $>1:10$ in 53.5% (95% CI, 50.9–56.2) of German children when determining pH1N1 infection serologically (6). The markedly lower frequency of pH1N1 in our study group is most likely explained by only assessing hospitalized patients. This might also explain the lower median age of 17 months compared to the higher age group of the Belgian outpatient cohort (82%; >6 years old) and corresponding German epidemiological data (1,6,8).

Manoha et al. reported the sex ratio of HMPV infection favoring male patients to be 2.2 (21), consistent with the ratio of 1.8 found in this study. Tachypnea was observed in nearly half of the HMPV infected children (43%) indicating lower respiratory tract infection (LRTI). HMPV was detected in 18% of LRTI cases (clinically characterized by wheezing, rales, tachypnea, dyspnea, and signs of lower airway involvement on chest radiograph). This is close to the frequency of 20% for HMPV lower airway disease reported by Kahn (7). Further underlining the disease severity, HMPV infected children received oxygen treatment for a longer duration than any other patient subgroup in this study, and had the lowest oxygen saturation (mean, 90.5%) at the time of admission. It has been reported that the presence of HMPV increased the risk of $\text{SaO}_2 < 92\%$ on admission almost 6-fold (22). The high prevalence of HMPV and RSV infections in admitted children led to a higher disease burden, and to the presence of a more critically ill patient group admitted to the hospital not caused by influenza infections. It was virtually impossible to clinically identify influenza infections. This un-

derscores the need for virological testing. A broad panel of diagnosed viruses gives insight into the nature of the infection and the overall role of individual viruses in respiratory infections. This emphasizes the need to test for a variety of viral respiratory pathogens in hospitalized children even during seemingly local or even global predominance of one respiratory virus to estimate the actual severity and progression of the disease.

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Conflict of interest None to declare.

REFERENCES

1. Cheng VC, To KK, Tse H, et al. Two years after pandemic influenza A/2009/H1N1: what have we learned? *Clin Microbiol Rev.* 2012;25:223-63.
2. Kumar S, Henrickson KJ. Update on influenza diagnostics: lessons from the novel H1N1 influenza A pandemic. *Clin Microbiol Rev.* 2012;25:344-61.
3. Bonzel L, Tenenbaum T, Schrotten H, et al. Frequent detection of viral coinfection in children hospitalized with acute respiratory tract infection using a real-time polymerase chain reaction. *Pediatr Infect Dis J.* 2008;27:589-94.
4. Franz A, Adams O, Willems R, et al. Correlation of viral load of respiratory pathogens and co-infections with disease severity in children hospitalized for lower respiratory tract infection. *J Clin Virol.* 2010;48:239-45.
5. Tregoning JS, Schwarze J. Respiratory viral infections in infants: causes, clinical symptoms, virology, and immunology. *Clin Microb Rev.* 2010; 23:74-98.
6. Von Kries R, Weiss S, Falkenhofst G, et al. Post-pandemic seroprevalence of pandemic influenza A (H1N1) 2009 infection (swine flu) among children <18 years in Germany. *PLoS One.* 2011;6:e23955.
7. Kahn JS. Epidemiology of human metapneumovirus. *Clin Microbiol Rev.* 2006;19:546-57.
8. Feuillet F, Lina B, Rosa-Calatrava M, et al. Ten years of human metapneumovirus research. *J Clin Virol.* 2012;53:97-105.
9. Gove S. Integrated management of childhood illness by outpatient health workers: technical basis and overview. The WHO Working Group on Guidelines for Integrated Management of the Sick Child. *Bulletin of the World Health Organization.* 1997;75 Suppl 1:7-24.
10. Chang ML, Jordan-Villegas A, Evans A, et al. Respiratory viruses identified in an urban children's hospital emergency department during the 2009 influenza A(H1N1) pandemic. *Pediatr Emerg Care.* 2012;28:990-7.
11. Hombrouck A, Sabbe M, Van Casteren V, et al. Viral aetiology of influenza-like illness in Belgium during the influenza A(H1N1)2009 pandemic. *Eur J Clin Microbiol Infect Dis.* 2012; 31:999-1007.
12. Watanabe AS, Carraro E, Moreira L, et al. Respiratory virus infections among hospitalized patients with suspected influenza A H1N1 2009 virus during the first pandemic wave in Brazil. *Braz J Infect Dis.* 2011;15:220-4.
13. Edwards KM, Zhu Y, Griffin MR, et al. Burden of human metapneumovirus infection in young children. *N Engl J Med.* 2013; 368:633-43.
14. Miller EK, Gebretsadik T, Carroll KN, et al. Viral etiologies of infant bronchiolitis, croup, and upper respiratory illness during 4 consecutive years. *Pediatr Infect Dis J.* 2013;32:950-5.
15. Williams JV, Edwards KM, Weinberg G, et al. Population-based incidence of human metapneumovirus infection among hospitalized children. *J Infect Dis.* 2010;201:1890-8.
16. Yoshida A, Kiyota N, Kobayashi M, et al. Molecular epidemiology of the attachment glycoprotein (G) gene in respiratory syncytial virus in children with acute respiratory infection in Japan in 2009/2010. *J Med Microbiol.* 2012;61(Pt 6):820-9.
17. Zlateva KT, Vijgen L, Dekeersmaeker N, et al. Subgroup prevalence and genotype circulation patterns of human respiratory syncytial virus in Belgium during ten successive epidemic seasons. *J Clin Microbiol.* 2007;45:3022-30.
18. Aberle JH, Aberle SW, Redlberger-Fritz M, et al. Human metapneumovirus subgroup changes and seasonality during epidemics. *Pediatr Infect Dis J.* 2010;29:1016-8.
19. Rafiefard F, Yun Z, Orvell C. Epidemiologic characteristics and seasonal distribution of human metapneumovirus infections in five epidemic seasons in Stockholm, Sweden, 2002-2006. *J Med Virol.* 2008;80:1631-8.
20. Regev L, Meninger T, Hindiyeh M, et al. Increase human metapneumovirus mediated morbidity following pandemic influenza infection. *PLoS One.* 2012;7:e34750.
21. Manoha C, Espinosa S, Aho SL, et al. Epidemiological and clinical features of hMPV, RSV and RVs infections in young children. *J Clin Virol.* 2007;38:221-6.
22. Marguet C, Lubrano M, Gueudin M, et al. In very young infants severity of acute bronchiolitis depends on carried viruses. *PLoS One.* 2009;4:e4596.