Viral etiology in hospitalized children with acute lower respiratory tract infection

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SUMMARY: Hatipoğlu N, Somer A, Badur S, Ünüvar E, Akçay-Ciblak M, Yekeler E, Salman N, Keser M, Hatipoğlu H, Şiraneci R. Viral etiology in hospitalized children with acute lower respiratory tract infection. Turk J Pediatr 2011; 53: 508-516.

This study was performed to investigate the viral etiological agents, age distribution and clinical manifestations of lower respiratory tract infection (LRTI) in hospitalized children. The viral etiology and clinical findings in 147 children (1 month to 5 years of age) hospitalized with acute LRTI were evaluated. Cell culture was used for isolation of influenza viruses and direct fluorescent antibody assay for parainfluenza viruses (PIVs), respiratory syncytial virus (RSV) and adenoviruses (ADVs). Reverse-transcriptase polymerase chain reaction was employed for human metapneumovirus (hMPV). One hundred and six of all patients (72.1%) were male, and 116 children (79.8%) were ≤2 years. A viral etiology was detected in 54 patients (36.7%). RSV was the most frequently isolated (30 patients, 55.6%), and PIV (27.8%), hMPV (13%), influenza-A (9.3%), and ADV (5.6%) were also shown. Dual infection was detected in six patients. There were no statistically significant differences between the two groups (with isolated virus or no known viral etiology) with respect to symptoms, clinical findings, laboratory work-up, or radiological data. Length of hospital stay was also not different. Determination of the etiology of acute LRTI in children less than 5 years of age seems impossible without performing virological work-up, whether viral or nonviral in origin.

Key words: bronchiolitis, metapneumovirus, pneumonia, respiratory syncytial virus, respiratory viruses.

Respiratory tract infections (RTIs) are a major cause of morbidity and mortality worldwide[1]. Acute RTI is most common in children under five years of age, and represents 30-50% of the pediatric medical admissions, as well as 20-40% of hospitalizations in children[2]. Respiratory infections cluster during winter and early spring months. Viral pathogens are responsible for 30-40% of RTIs[3]. The leading viral agents include respiratory syncytial virus (RSV), influenza A and B (INF-A, INF-B) viruses, parainfluenza viruses (PIVs), and adenoviruses (ADVs). The recently identified types, such as human metapneumovirus (hMPV), have been added to the etiologic agent list[4].

Acute RTIs are classified as upper and lower RTI, according to the involved anatomic localization. Upper RTIs (URTIs) cause non-severe but widespread epidemics that are responsible for continuous circulation of pathogens in the community. Lower RTIs (LRTIs) have been classified as frank pneumonia and bronchiolitis with clinical, radiological and etiological features that usually overlap. Viruses are again the foremost agents of LRTI; they are often considered as bacterial in origin and hence treated with antibiotics unnecessarily[5].

The clinical trials on the viral etiology of LRTI in Turkish children that investigate many viruses are insufficient. The present study was conducted to determine the prevalence of LRTIs due to respiratory viruses in hospitalized children under five years and to evaluate the risk factors, clinical and radiological work-up and therapeutic approach.

Material and Methods

Study Group and Follow-Up

This study was a prospective and descriptive clinical study. The viral etiology and clinical features were evaluated in children between one month and five years of age, who were admitted to İstanbul University İstanbul Faculty of Medicine, Pediatric Infectious Diseases Department, with acute LRTI between 1 October 2006 and 31 March 2007. The local ethical committee of İstanbul University İstanbul Faculty of Medicine approved the study protocol. An informed consent was received from one of the parents.

Demographic features of the patients were registered on a questionnaire form. Patients included in the study were those hospitalized and who suffered from LRTI during the previous week before admission and which did not develop during the hospital stay due to other reasons. Patients with no LRTI symptoms, duration of longer than one week or nosocomially acquired LRTI and complicated cases like pleural empyema or lung abscess were excluded from the study.

Lower respiratory tract infection (LRTI) was diagnosed with at least one of the following: fever during the last 48 hours, coughing, runny nose, plus one of the following: wheezing, tachypnea, dyspnea, cyanosis, intercostal retractions, congestion, and/or crepitations on lung auscultation[6,7]. Discrimination between bronchiolitis and pneumonia was done according to clinical and radiological findings:

• Overinflation, flattening of costal bones and diaphragm, reticular image, brightening of parenchyma, and atelectasis in chest X-ray, and mild to moderate fever, rapid breathing, wheezing, and prolonged expiration were considered as bronchiolitis[6,7].

• Moderate to high fever, respiratory distress, absence of respiratory sounds, crackles, and lobar/lobular/patchy consolidation of lung fields on chest X-ray were diagnosed as pneumonia[6,7].

Total leukocyte counts with differential and C-reactive protein (CRP) levels were studied in all patients with acute LRTI. Chest radiographs of all cases were evaluated by a pediatric radiologist. Proper antibiotic therapy, inhaled steroid and/or bronchodilators were administered if necessary. Duration of hospitalization and any developed complications (need for assisted ventilation, antibiotherapy switch due to nosocomial infection) were recorded.

Specimen Collection for Viral Analysis and Processing

Specimens were collected from the nasopharyngeal cavity by a special swab[8], placed into viral transport medium and delivered to the virology laboratory at İstanbul University İstanbul Faculty of Medicine located within the same campus as the pediatric clinic. Influenza viruses were cultured on Madin-Darby canine kidney (MDCK) cell culture and immunocapture ELISA test was used for the detection of INF-A and INF-B. Reverse-transcriptase polymerase chain reaction (RT-PCR) was performed for hMPV, and direct fluorescent antibody (DFA) tests were used for the other viruses. Detection of the viral agents was done with standard laboratory studies published elsewhere[3,9].

No community-acquired bacterial or atypical bacterial (mycoplasmal or chlamydial) agent was studied microbiologically as an etiology of LRTI, as this was not the aim of this study.

Statistics

Study cases caused by any viral pathogen formed the main group, and those with negative viral work-up were considered as the control group. Statistical analyses were performed with the Statistical Package for the Social Sciences (SPSS) for Windows 10.0. Chi-square and Fisher's exact tests were utilized for comparison of categorical variables. Statistical significance was defined as $p<0.05$.

Results

A total of 165 patients fulfilled the inclusion criteria for enrollment. Nasopharyngeal sample was taken only once from each case. However, only 147 (89.1%) cases were eligible for laboratory testing.

One hundred and six of all patients (72.1%) were males. The average age was 14.7 ± 15.8 months (range: 1-60 months, median: 9 months), and a majority (116 children, 79.8%) were ≤2 years of age (Table I). The average

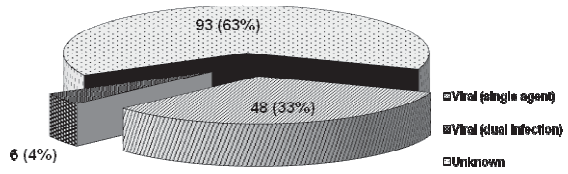

Fig. 1. Etiology of LRTI in 154 hospitalized children.

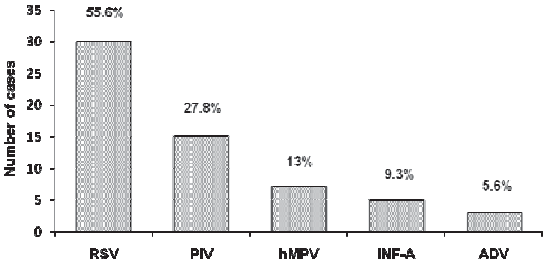

Fig. 2. Distribution of viral pathogens isolated in 54 cases.
RSV: Respiratory syncytial virus. PIV: Parainfluenza virus. hMPV: Human metapneumovirus. INF-A: Influenza-A virus. ADV: Adenovirus.

weight of the study group was 11.3 ± 4.2 kg (range: 4-19 kg), and the average height was 76 ± 25 cm (range: 52-113 cm).

One or more viral pathogens were detected in 54 patients (36.7%) of 147 cases from whom nasopharyngeal swab specimens were collected (Fig. 1). RSV was the most frequently detected virus (55.6%, 30 patients), followed by PIV (15 patients, 27.8%) (Fig. 2). All five of the influenza viruses isolated were A (H3N2). More than one virus was detected in six patients (11.1%) concomitantly: RSV and hMPV, RSV and PIV, and hMPV and PIV in two patients each.

Viral etiology was detected in 40 males out of 54 positive cases (74.1%) and in 46 (85.2%) children ≤2 years of age. The average age was 13.6 ± 15.9 months (range: 1-60 months, median: 7 months) in this group. There was no statistical difference between age or sex distribution of patients with positive and negative viral etiology (p>0.05) (Table I).

The most frequently isolated viruses from children aged 1-24 months were RSV (27 cases, 58.7%) and PIV (11 cases, 23.9%), while in the older age group (25-60 months old), the most common agent was PIV (4 cases, 50%).

From October-March, viral detection was most common (37%) in cases admitted to the hospital in December (Fig. 3). RSV and PIV peaked in incidence in December (Fig. 4).

The prominent symptoms of the patients in whom a virus was isolated were cough (88.9%) and wheezing (72.2%). As with complaints and physical findings, chest X-rays, mean leukocyte counts, leukocyte differentials, and CRP levels of patients studied on admission were all statistically insignificant between viral-positive and -negative study cases (p>0.05). Peribronchial thickening and hilar lymphadenopathy were recorded noticeably in both groups. There was an additional morbidity (most frequently a congenital cardiac anomaly) in 13 (24.1%) patients with a known viral etiology (Table II). Length of stay was between 3 to 40 days (median: 7.9±5.8 days) for a known viral agent and 3 to 30 days (median: 7.7±5.3 days) in the unknown agent group (Table I). Treatment modality and duration of hospitalization were also statistically not different (p>0.05).

Bronchiolitis was diagnosed in 55.6% and pneumonia in 44.4% of patients in whom a viral agent was recovered. The rates of both diagnoses were comparable in both known and unknown viral cause groups (p>0.05). In patients with a documented respiratory virus, bronchiolitis was caused predominantly by RSV (40.7%), which is parallel to the finding that this agent was the most frequent pathogen in this study (Table II).

Inhaled bronchodilator therapy was given more frequently (83.3%) to the patients whose nasopharyngeal specimen tested positive. Twenty-four patients with LRTI due to RSV and almost all of the rest in the positive group received beta-agonists. On the other hand, 26 patients (48.1%) in this group were treated with antibiotics due to very young age, bacterial LRTI coinfection or follow-up in the

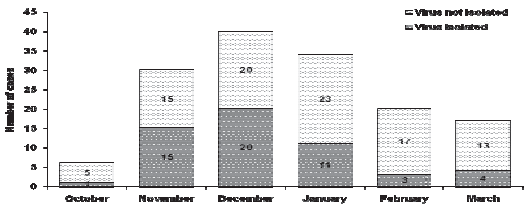

Fig. 3. Distribution of cases according to months and a viral pathogen detected or not detected.

intensive care unit (ICU). Two patients with LRTI due to influenza virus were treated with oseltamivir. Six patients in the presented study required intensive care and assisted ventilation support, with three cases caused by RSV, PIV or INF-A viruses (Table III).

Three patients with severe other morbidities with no isolated viral etiology died in the ICU. All of them were very young (≤6 months old), with a defined cardiac anomaly, metabolic problem and/or adult respiratory distress syndrome, which was relevant to the long

Table 1. Demographic and clinical characteristics of 154 hospitalized children with LRTI.

Characteristics	Number of cases				*P* value[a]
	Virus isolated	(%)	Virus not isolated	(%)	
Total cases	54	-	93	-	-
Sex					
Male	40	(74.1)	66	(71)	NS
Female	14	(25.9)	27	(29)	NS
Age (months)					
1-6	26	(48.2)	31	(33.3)	NS
7-24	20	(37)	39	(41.9)	NS
25-60	8	(14.8)	23	(24.8)	NS
Type of LRTI					
Bronchiolitis	30	(55.69	49	(52.7)	NS
Pneumonia	24	(44.4)	44	(47.3)	NS
Symptoms and signs[b]					
Cough	48	(88.9)	68	(73.1)	NS
Wheeze	39	(72.2)	43	(46.2)	NS
Fever (>38.3°C)	26	(48.1)	36	(38.7)	NS
Crackles	22	(40.7)	40	(43)	NS
Dyspnea	27	(50)	33	(35.5)	NS
Retractions	25	(46.3)	35	(37.6)	NS
Acute phase response[c]					
Leukocyte (/mm^3)	11,100	-	12,700	-	NS
Polymorphonuclears (%)	46	-	56	-	NS
CRP (mg/L)	10	-	14,5	-	NS
Chest X-ray findings					
Peribronchial thickening	41	(75.9)	55	(59.1)	NS
Hilar lymphadenopathy	29	(53.7)	45	(48.4)	NS
Hyperaeration	17	(31.5)	25	(26.9)	NS
Consolidation	12	(22.2)	11	(11.8)	NS
Normal	9	(16.7)	21	(22.6)	NS
Comorbidity					
Congenital cardiac anomaly	4	(7.4)	4	(4.3)	NS
Prematurity	2	(3.7)	6	(6.5)	NS
Neurologic problem	2	(3.7)	3	(3.2)	NS
Inborn error of metabolism	2	(3.7)	3	(3.2)	NS
Immune deficiency	2	(3.7)	3	(3.2)	NS
Renal problem	1	(1.9)	1	(1.1)	NS
Treatment					
Beta agonists	45	(83.3)	76	(81.7)	NS
Inhaled steroid	24	(44.4)	54	(58.1)	NS
Antibiotics	26	(48.1)	67	(72)	NS
Complications					
Mechanical ventilation	3	(5.6)	3	(3.2)	NS
Death	0	(0)	3	(3.2)	NC
Duration of hospitalization (days)[c]	7.9	-	7.7	-	NS

NS: Nonsignificant. LRTI: Lower respiratory tract infection. CRP: C-reactive protein. NC: Not calculated.

[a]*P* value was considered when >0.05.

[b]Patients might have suffered from more than one symptom or sign.

[c]The median value was used.

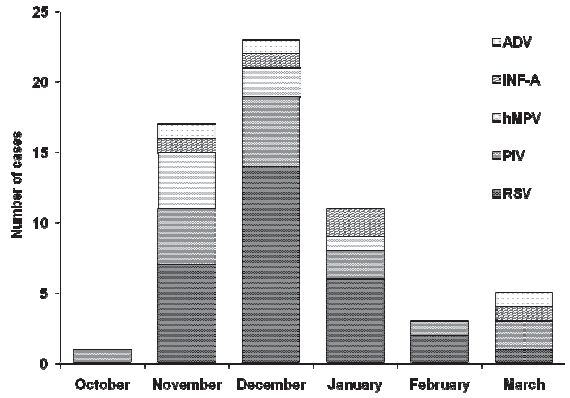

Fig. 4. Distribution of viral pathogens according to months.
ADV: Adenovirus. INF-A: Influenza-A virus. hMPV: Human metapneumovirus. PIV: Parainfluenza virus. RSV: Respiratory syncytial virus.

hospital stay. No patient with a documented viral cause was lost during hospitalization.

Discussion

When disease burden and mortality in the Turkish population (10,11) are considered, LRTIs rank second, at a rate of 10.5%, after perinatal causes (27.6%). These data suggest that LRTI can be considered as an important public health issue in our country, especially in children younger than five years of age, as in other developing countries.

In the present study, almost four out of five children (78.9%) hospitalized due to LRTI were infants under two years old, while those with a documented viral etiology constituted even a larger group (85.2%).

Respiratory syncytial viruses (RSVs), PIV, influenza, measles, and varicella viruses in endemic regions and hMPV are the most commonly detected agents in LRTI in pediatric ages. All children by the age of two years have had at least one episode of RSV and/or PIV illness, which have a severe course in patients with a chronic respiratory or cardiac condition.

Studies on the epidemiology in viral LRTI for many viruses concomitantly in developing countries are underway (2,4,12). Yilmaz et al. (13) reported viral positivity in nasopharyngeal aspirates (NPAs) of infants with LRTI as 41%, and found rates of 39.2% for RSV, and 1.2%, 3.6% and 2.4% for PIV, ADV and influenza viruses, respectively, in Turkey. In another national prospective study performed from 1998-2000, NPAs and sputum samples were collected from 76 children to investigate atypical bacteria and viruses (14). Viral positivity was found to be 27.6%, with RSV predominating, whereas ADV, PIV1-2, INF-A and INF-B were rare. The rate of virus isolation was 29.8% in 201 nasal swab specimens from outpatients with URTIs during 2005-2006 autumn-spring seasons according to a recent study reported by Ünüvar et al. (3). Similar to rates reported in the literature, a viral cause was shown in our study group in 36.7% of cases.

Human MPV is a member of the Metapneumovirus genus within the Pneumo-viridae subfamily of the Paramyxoviridae family. This novel virus has been reported to cause both upper and lower RTI in susceptible individuals. There has been a bulk of epidemiological work on hMPV. Many have shown that the prevalence in pediatric ages in industrialized nations ranges from 3.5% (15,16) to 11.3% (17) and 21% (18). It is more interesting that the frequency of hMPV infection can vary substantially even in the same population during two consecutive seasons, from 4.7 % up to 25.3% (19).

In neighboring countries of Turkey, hMPV was detected in 12.8%, 16% and 25% of children with LRTI at or under five years of age in Greece, Bulgaria and Romania, respectively (20-22). The incidence of hMPV infection is more frequent in children younger than two years (15), and up to 33.6% of patients at this age were found positive in an Iranian study (23). Similarly, in the presented trial, six out of seven cases positive for hMPV were below two years of age.

Studying many viruses in LRTI is costly and is difficult because of lack of necessary facilities in routine laboratories, rendering these tests hard to perform widely. hMPV has not been studied extensively in Turkey. To our knowledge, this is the first national study investigating hMPV together with the other viruses as an etiologic agent in children under five years of age. The active surveillance of hMPV, together with other viral agents in respiratory infections, will uncover the impact of this virus on the disease burden in Turkey in the future.

Respiratory syncytial virus (RSV) formed the largest fraction (58.7%) of LRTI infants aged

Table II. Demographic and Clinical Characteristics of 54 Patients with a Documented Respiratory Viral Pathogen

Characteristics	Virus isolated				
	RSV	PIV	hMPV	INF-A	ADV
Sex					
Male	25	11	5	3	2
Female	5	4	2	2	1
Age (months)					
1-6	14	6	2	1	3
7-24	13	5	4	2	-
25-60	3	4	1	2	-
Type of LRTI					
Bronchiolitis	22	8	2	1	2
Pneumonia	8	7	5	4	1
Symptoms and signs[a]					
Cough	25	15	7	4	3
Wheeze	22	14	7	1	1
Fever (>38.3°C)	7	9	5	4	1
Crackles	10	7	4	4	1
Dyspnea	15	7	3	3	1
Retractions	11	9	3	3	1
Treatment					
Beta agonists	24	14	6	5	2
Inhaled steroid	14	8	4	4	-
Antibiotics	14	9	5	3	1
Complications					
Mechanical ventilation	1	1	-	1	-
Death	-	-	-	-	-
Total cases	30	15	7	5	3

RSV: Respiratory syncytial virus. PIV: Parainfluenza virus. hMPV: Human metapneumovirus. INF-A: Influenza-A virus. ADV: Adenovirus. LRTI: Lower respiratory tract infection.

[a]Patients might have suffered from more than one symptom or sign.

1-24 months, higher than the rates of 25-36% reported by other Turkish studies (24-28). Influenza is the only viral illness against which treatment and vaccination are available, but the rate found was only 9.3%, which was lower than previously reported (3,29). On the other hand, PIV infection has been known to be the leading viral LRTI after the age of two (30), which was similar in our report.

Dual viral LRTIs in children have been described with an incidence ranging from 3.7% up to 34.1%, and induce a more severe bronchiolitis, up to 3-to-10 fold, for admission to the ICU compared to single-agent infections (13,24,31,32). Viral coinfection was found in 6 patients (11.1%) in our study, indicating that studying one virus may not be enough when cohorting hospitalized patients in the face of the possibility of mixed infection.

In accordance with previous studies (33), in our study, radiological findings together with clinical signs did not allow for reliable differentiation between a known viral agent versus unknown LRTI agent without laboratory testing for the viral etiology.

Epidemics of respiratory viruses have been observed in winter and early spring[1,15,30,34-36]. A majority (85.2%) of all positive viral cases were admitted in November, December and January, suggesting that routine polyviral work-up in these patients might be cost-effective and would hinder the overuse of unnecessary antibiotic therapy at least during this period.

There were no statistically significant differences in the number of bronchiolitis and pneumonia diagnoses between the group with a confirmed viral agent and the other group. The association between respiratory syndromes and infecting agents has been established (37). Bronchiolitis was caused predominantly by RSV, accounting for 40.7% of cases. However, hMPV and INF-A viruses were usually responsible for pneumonia. Interestingly, all patients suffered from pneumonia when hMPV was one of the viral agents in mixed infection. As noted, hMPV causes a variety of RTIs; however,

Table III. Characteristics of Cases Admitted to the Intensive Care Unit

No. of patients	Age*	Sex	Length of stay in hospital	Symptoms / signs	Comorbidity	Chest X-ray	Lab. Results (leukocytes / CRP)**	Diagnosis	Treatment	Outcome
1	4	M	15	Fever, cough, dyspnea, wheeze	-	Hyperaeration	8900 / 32	Bronchiolitis	β-agonist, antibiotic	Recovery
2	4	F	12	Fever, cough, dyspnea, wheeze, retractions, crackles	Inborn error of metabolism, Reye syndrome	Consolidation	14,000 / 50	Pneumonia	β-agonist, antibiotic	Recovery
3	58	M	30	Fever, cough, dyspnea, wheeze, retractions, crackles	Congenital cardiac anomaly, renal problem	Consolidation	13,600 / 10	Pneumonia	β-agonist, antibiotic, steroid	Recovery
4	6	F	6	Fever, cough, dyspnea, wheeze, retractions, crackles	Congenital cardiac anomaly, inborn error of metabolism	Peribronchial thickening, atelectasis	17,100 / 32	Pneumonia	β-agonist, antibiotic	Exitus
5	6	F	40	Fever, dyspnea, retractions,	Congenital cardiac anomaly	Hyperaeration, diffuse consolidation, ARDS	12,600 / 21	Bronchiolitis	β-agonist, antibiotic, steroid	Exitus
6	2	F	30	Cough, dyspnea, crackles	Congenital cardiac anomaly, inborn error of metabolism, renal problem	Diffuse consolidation	20,000 / 60	Pneumonia	β-agonist, antibiotic	Exitus

M: Male. F: Female. CRP: C-reactive protein. ARDS: Adult respiratory distress syndrome. *Age defined as months. **Leukocytes as $/mm^3$, CRP as mg/L.

bronchiolitis and pneumonia were the most common diagnoses encountered in children (16,19,23). Clinical findings related to hMPV respiratory disease can be any of the complaints and signs seen during a RSV infection[15,19]. The number of patients with hMPV infection was limited in our study population, though all had cough and wheezing.

Outbreaks of pneumococcal pneumonia have been observed during RSV and influenza epidemics[38]. In other terms, viral respiratory infections can precede secondary bacterial superinfections. Demonstrating a viral cause in LRTI would reduce inappropriate antibiotic use.

The overall mortality rate was 3.2% and was recorded in patients with severe comorbidities. As reported, congenital cardiac anomalies or metabolic disorders have been related with poor prognosis[35]. Although combined respiratory viral infections are linked to a more serious course, death associated with any studied virus (even in mixed infection) did not occur in the present study, although 5.6% of the patients in the positive viral group were followed in the ICU.

Our study was limited to only one season and one center. Nevertheless, this study is the first in which all five respiratory viruses, including hMPV, were evaluated as the causes of LRTIs in hospitalized Turkish children under five years of age. None of the parameters differed in documented and non-documented viral etiological groups, emphasizing that further research projects are warranted to draw an epidemiological map of our country and to shape the management of LRTI in young pediatric ages. Evaluating common and atypical bacterial agents microbiologically other than the viruses would further aid in understanding the role of mixed etiology in LRTI, which should be referred to in further studies. If combination of agents had not been evaluated in our patients, it could be claimed that the respiratory viruses identified were the only factors responsible for the entire clinical picture.

In conclusion, the clinical trials about LRTI in children in which viral agents are evaluated simultaneously are insufficient in Turkey. It was the strength of this study that several respiratory viruses were assessed in all patients. Continuous surveillance of respiratory viruses,

including those defined recently, will give more information about the current status in our country.

REFERENCES

1. Monto AS. Epidemiology of viral respiratory infections. Am J Med 2002; 112(Suppl): 4S–12S.
2. Lin TY, Huang YC, Ning HC, Tsao KC. Surveillance of respiratory viral infections among pediatric outpatients in northern Taiwan. J Clin Virol 2004; 30: 81–85.
3. Ünüvar E, Yıldız İ, Kılıç A, et al. Viral etiology and symptoms of acute upper respiratory tract ınfections in children. Turk J Med Sci 2009; 39: 29–35.
4. Williams JV. The clinical presentation and outcomes of children infected with newly identified respiratory tract viruses. Infect Dis Clin North Am 2005; 19: 569–584.
5. Nuezil KM, Mellen BG, Wright PF, Mitchel EF, Griffin MR. The effect of influenza on hospitalizations, outpatient visits, and courses of antibiotics in children. N Engl J Med 2000; 342: 225–231.
6. Yalçın E, Karadağ B, Uzuner N, et al. Turkish Thoracic Society – consensus report for the diagnosis and management of acute bronchiolitis. Turk Thoracic J 2009; 10(Suppl): 1–7.
7. Kocabaş E, Ersöz DD, Karakoç F, et al. Turkish Thoracic Society – consensus report for the diagnosis and management of community-acquired pneumonia. Turk Thoracic J 2009; 10(Suppl): 1-24.
8. Abu-Diab A, Azzeh M, Ghneim R, et al. Comparison between pernasal flocked swabs and nasopharyngeal aspirates for detection of common respiratory viruses in samples from children. J Clin Microbiol 2008; 46: 2414–2417.
9. Ciblak MA, Hasoksuz M, Escuret V, et al. Surveillance and oseltamivir resistance of human influenza A virus in Turkey during the 2007-2008 season. J Med Virol 2009; 81: 1645-1651.
10. Hancıoğlu A, Alyanak İY. Infant and pediatric mortality. In: Hacettepe University Population Studies Institute, Turkish Population and Health Research–2003. Ankara, Turkey: Ministry of Health of Turkey; 2004: 109–118.
11. Yardım N, Mollahaliloğlu S, Hülür Ü, et al. Health at a glance–Turkey 2007. The Ministry of Health of Turkey, Refik Saydam Hygiene Center Presidency, School of Public Health, Ankara, Turkey, 2008.
12. Williams J, Harris P, Tollefson SJ, et al. Human metapneumovirus and lower respiratory tract disease in otherwise healthy infants and children. N Engl J Med 2004; 350: 443–450.
13. Yilmaz G, Uzel N, Isik N, Baysal SU, Aslan S, Badur S. Viral lower respiratory tract infections in children in Istanbul, Turkey. Pediatr Infect Dis J 1999; 18: 173.
14. Kaygusuz S, Koksal I, Aydin K, Caylan R. Investigation of atypical bacteria and virus antigens in respiratory tract infections by use of an immunofluorescence method. Jpn J Infect Dis 2004; 57: 33–36.
15. Heikkinen T, Osterback R, Peltola V, Jartti T, Vainionpää R. Human metapneumovirus infections in children. Emerg Infect Dis 2008; 14: 101–106.
16. Williams JV, Edwards KM, Weinberg GA, et al. Population-based incidence of human metapneumovirus infection among hospitalized children. J Infect Dis 2010; 201: 1890–1898.
17. Cilla G, Oñate E, Perez-Yarza EG, Montes M, Vicente D, Perez-Trallero E. Hospitalization rates for human metapneumovirus infection among 0- to 3-year-olds in Gipuzkoa (Basque Country), Spain. Epidemiol Infect 2009; 137: 66–72.
18. Døllner H, Risnes K, Radtke A, Nordbø SA. Outbreak of human metapneumovirus infection in Norwegian children. Pediatr Infect Dis J 2004; 23: 436–440.
19. Caracciolo S, Minini C, Colombrita D, et al. Human metapneumovirus infection in young children hospitalized with acute respiratory tract disease: virologic and clinical features. Pediatr Infect Dis J 2008; 27: 406–412.
20. Gioula G, Chatzidimitriou D, Melidou A, Exindari M, Kyriazopoulou-Dalaina V. Contribution of human metapneumovirus to influenza-like infections in North Greece, 2005-2008. Euro Surveill 2010; 15: 19499.
21. Pavlova S, Hadzhiolova T, Abadjieva P, Kotseva R. Application of RT-PCR for diagnosis of respiratory syncytial virus and human metapneumovirus infections in Bulgaria, 2006-7 and 2007-8. Euro Surveill 2009; 14: 19233.
22. Tecu C, Orăşanu D, Sima A, et al. First detection of human metapneumovirus in children with respiratory infections in Romania. Roum Arch Microbiol Immunol 2007; 66: 37–40.
23. Arabpour M, Samarbafzadeh AR, Makvandi M, et al. The highest prevalence of human metapneumovirus in Ahwaz children accompanied by acute respiratory infections. Indian J Med Microbiol 2008; 26: 123–126.
24. Dereli D, Ertem E, Serter D, Sadiment M, Coker M, Tanaç R. Detection of respiratory syncytial virus in children in the 1993-94 winter season in Izmir, Turkey, by two diagnostic methods. APMIS 1994; 102: 877–880.
25. Yarkın F, Alhan E, Kibar F, Karabay A, Köksal F. Seroepidemiologic analysis of viral agents in children with lower respiratory tract infections. Mikrobiyoloji Bülteni 1995; 29: 149–156.
26. Gökalp C, Gökahmetoğlu S, Deniz ES, Güneş T. Investigation of respiratory syncytial virus by three different methods in children with lower respiratory tract infection. Mikrobiyol Bul 2009; 43: 433–438.
27. Yüksel H, Yilmaz O, Akçali S, et al. Common viral etiologies of community acquired lower respiratory tract infections in young children and their relationship with long term complications. Mikrobiyol Bul 2008; 42: 429–435.
28. Kanra G, Tezcan S, Yilmaz G; Turkish National Respiratory Syncytial Virus (RSV) Team. Respiratory syncytial virus epidemiology in Turkey. Turk J Pediatr 2005; 47: 303–308.
29. Carhan A, Altaş AB, Albayrak N, Uyar Y. [Influenza

surveillance results in 2007-2008 winter season in nine provinces of Turkey.] Mikrobiyol Bul 2009; 43: 235–241. Glezen WP, Frank AL, Taber LH, Kaer JA. Parainfluenza virus type 3: seasonality and risk of infection and reinfection in young children. J Infect Dis 1984; 150: 851–857.

30. Richard N, Komurian-Pradel F, Javouhey E, et al. The impact of dual viral infection in infants admitted to a pediatric intensive care unit associated with severe bronchiolitis. Pediatr Infect Dis J 2008; 27: 213–217.
31. Semple MG, Cowell A, Dove W, et al. Dual infection of infants by human metapneumovirus and human respiratory syncytial virus is strongly associated with severe bronchiolitis. J Infect Dis 2005; 191: 382–386.
32. Nohynek H, Valkeila E, Leinonen M, Eskola J. Erythrocyte sedimentation rate, white blood cell count and serum C-reactive protein in assessing etiologic diagnosis of acute lower respiratory infections in children. Pediatr Infect Dis J 1995; 14: 484–490.
33. Mullins JA, Lamonte AC, Bresee JS, Anderson LJ. Substantial variability in community RSV season timing. Pediatr Infect Dis J 2003; 22: 857–862.
34. Iwane MK, Edwards KM, Szilagyi PG, et al. Population-based surveillance for hospitalizations associated with respiratory syncytial virus, influenza virus, and parainfluenza viruses among young children. Pediatrics 2004; 113: 1758–1764.
35. Osterhaus A, Fouchier R. Human metapneumovirus in the community. Lancet 2003; 361: 890–891.
36. Murphy TF, Henderson FW, Clyde WA Jr, Collier AM, Denny FW. Pneumonia: an eleven-year study in a pediatric practice. Am J Epidemiol 1981; 113: 12–21.
37. Talbot TR, Poehling KA, Hartert TV, et al. Seasonality of invasive pneumococcal disease: temporal relation to documented influenza and respiratory syncytial viral circulation. Am J Med 2005; 118: 285–291.