Document heading   doi:

Burden of pediatric influenza A virus infection post swine−flu H1N1 pandemic in Egypt

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ARTICLE INFO

ABSTRACT

Objective: To screen children with influenza like illness or with symptoms of acute respiratory tract infections for influenza A virus infection—post swine flu pandemic era—using rapid influenza diagnostic tests. **Methods:** During two years (2010 & 2011), 1 200 children with influenza like illness or acute respiratory tract infections (according to World Health Organization criteria) were recruited. Their ages ranged from 2−60 months. Nasopharyngeal aspirates specimens were collected from all children for rapid influenza A diagnostic test. **Results:** Influenza A virus rapid test was positive in 47.5% of the children; the majority (89.6%) were presented with lower respiratory tract infections. Respiratory rate and temperature were significantly higher among positive rapid influenza test patients. **Conclusions:** Influenza A virus infection is still a major cause of respiratory tract infections in Egyptian children. It should be considered in all cases with cough and febrile episodes and influenza like symptoms even post swine flu pandemic.

1. Introduction

Respiratory tract infection is a leading cause of morbidity and mortality in children; however, the actual global burden of diseases attributable to influenza virus in children is unknown[1].

Nevertheless, influenza A viral infection propensity is attributed to frequent antigenic shifts and drifts as well as possessing a capacity to cause annual epidemics and occasional pandemics[2]. Prompt and appropriate diagnosis and therapy affect individual patients as well as whole society, because local outbreaks may be detected and control measures can be initiated[3].

Moreover, it is difficult to diagnose influenza clinically, because its symptoms are nonspecific and a variety of diseases share similar symptoms. A symptom complex for influenza−like illness (ILI) has been used as a predictive tool for the diagnosis of influenza infection at the primary−care level[4]. Thus, recent appearance and worldwide spread of novel influenza A virus have highlighted the need to commercially available, widely used rapid influenza diagnostic tests (RIDTs) for their ability to detect these viral antigens in respiratory clinical specimens[5].

Although on 10 August, 2010, the Director General of the World Health Organization (WHO) has announced that the world is no longer in phase 6 of influenza pandemic alert and we are now moving into the post−pandemic period, the virus transmission is still highly active in many parts[6]. However, the situation in Egypt was not yet clear. Thus, the objective of this study—during the post−pandemic period of swine flu—was to provide information regarding the actual burden of influenza A viral infections in Egyptian children.

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2. Subjects and methods

2.1. Study design

This cross sectional pilot observational study was performed in two Egyptian governorates, Gharbbia (North) and Fayoum (South), during two years 2010 & 2011 (post swine–flu H1N1 pandemic). One thousand and two hundreds children (760 males & 460 females) with either ILI or with symptoms of acute respiratory tract infections defined by WHO[6] were recruited from primary health centers. Their ages ranged from 2–60 months. Distribution according to residence showed that 920 children were rural inhabitants, while 280 were urban.

An informed consent was obtained from the parents of children who provided specimens. The study protocol was approved by the Research Ethics Committee of Ain Shams University. Nasopharyngeal aspirates specimens were collected and transported immediately to the laboratory for RIDT.

2.2. Definition of influenza virus infection

ILI was defined according to the criteria proposed by WHO: the presence of fever plus at least one upper–respiratory symptom (cough, sore throat, or rhinorrhea) and one constitutional symptom (headache, malaise, or myalgia)[6]. Children with ILI were considered to have influenza A infection if the RIDT was positive.

2.3. Clinical severity score of acute respiratory tract infection

Based on variables reported in previous studies[10,11], a severity index was defined by assigning 1 point to each of the following: use of supplemental oxygen, duration of hospital stay $\geqslant$5 days, and admission to an intensive care unit.

2.4. Data collection

Demographic data, underlying medical conditions known to be risk factors for influenza–related complications (e.g., cardiac diseases, bronchial asthma, diabetes mellitus, and obesity)[13], clinical features at presentation, laboratory test results, radiographic findings, influenza–related complications, and treatment course of each patient were collected from their medical records.

2.5. Rapid influenza test

Nasopharyngeal swabs from children were tested by QuickVue Influenza A rapid influenza antigen test (Quidel Corp., San Diego, CA, USA), according to the manufacturer's instructions and as mentioned in previous study[12]. This test provides rapid visual qualitative immunochromatographic assay for influenza type A nucleoprotein antigen extracted from the nasal swab specimen by double antibody sandwich method. Patients were considered to have influenza A virus when the test was positive.

2.6. Statistics

Statistical analyses were performed using the SPSS software v.15 (SPSS, Inc., Chicago, IL, USA). Student's t–test was used to compare continuous variables and Fisher's exact test was used to compare dichotomous variables. Moreover, variables that were significant in univariate analyses were entered into a multivariate analysis using a logistic regression model to identify independent factors associated positively with influenza virus infection. Two–tailed P values <0.05 were considered to indicate statistical significance.

Receiver operating characteristic curves were plotted for single symptoms and various symptom combinations. The diagnostic accuracy of single and combined symptoms was assessed by calculating area under the receiver operating characteristic curves. Assessment of positive predictive value (PPV), negative predictive value (NPV), specificity, and sensitivity were done to identify best predictors of an influenza infection. PPVs were compared for individual symptoms, as well as combinations of symptoms.

PPV indicates positive predictive value, the probability of having laboratory–confirmed influenza when the symptom is present; NPV, negative predictive value, the probability of not having laboratory–confirmed influenza when the symptom is not present; sensitivity, the probability of having the symptom when the patient has laboratory–confirmed influenza; and specificity, the probability of not having the symptom when the patient does not have laboratory–confirmed influenza (i.e., when the test result for influenza is negative).

3. Results

Children's demographic and clinical data are presented in Table 1. RIDTs for influenza A virus (H1N1) infection were positive in 47.5% (570/1 200). The most frequent symptoms were fever and respiratory symptoms (Table 2), while gastrointestinal symptoms, including nausea/vomiting, diarrhea, and abdominal pain, were observed in 36.8% of these pediatric patients.

Most (83%) children were less than 5 years of age with mean age of (24.76±16.27) months. The mean age of children with influenza A virus (H1N1) infection was greater than that of children with non–influenza illnesses (P<0.05). From total recruited children, 61.7% were males (male sex predominates) and also majority of children were recruited from rural areas (76.7%).

Most of the positive cases (510/570; 89.6%) were diagnosed to have lower respiratory tract infections, while 10.4% had only upper respiratory tract infections with ILI. Those with lower respiratory tract infections were admitted to the hospital. The mean length of hospital stay was 4.3 days. Eighty one children with lower respiratory tract infections (81/510; 15.68%) had influenza–associated complications in the form of secondary bacterial pneumonia. Ten children required admission to the intensive care unit. Two hundreds children had leukocyte counts <5 000/μL and only fifty had leukocyte counts >15 000/μL. Children with positive influenza A virus (H1N1) infection were more likely to have

leucopenia when compared to children with non–influenza illness (*P*<0.05).

According to the guidelines[6], oseltamivir was recommended to be prescribed to children at the age of 5 years old or less who had ILI and also had a positive RIDT result, complicated influenza or at–risk conditions for complications as defined by the WHO. Thus, antiviral therapy with oseltamivir was administered to 842/1 200 (70%) children, the youngest of whom was 15 month of age. Three hundreds and eighty five children with ILI (31.6%) have received parenteral or oral antibacterial therapy.

Table 1
Demographic and clinical data of studied children.

Item	Patients according to rapid test		*P* value
	Positive (*n*=570)	Negative (*n*=630)	
Age (Months)	26.76±6.276	18.40±7.672	0.001*
Male:Female	400:170	340:290	0.553
Rural:Urban	430:140	490:140	0.605
Duration of symptoms (days)	3.02±1.334	2.87±1.563	0.557
Weight % for age and sex[a]	49.70±31.28	53.70±21.134	0.513
Height % for age and sex[a]	41.58±27.378	48.72±28.759	0.070
Passive smokers	451/570 (78.9%)	129/630 (20.6%)	0.001*
Respiratory rate (breaths per minute)	40.45±7.229	35.52±5.603	0.013*
Clinical severity score	3±1	0±1	0.001*
Hospital admissions	510/570 (89.6%)	200/630 (31.7%)	0.001*
Length of hospital stay (days)	4.3±2.6	2.0±1.5	0.046*

Age, duration of symptoms, weight % for age and sex, height % for age and sex, respiratory rate, clinical severity score, and length of hospital stay are expressed as mean±SD. *Statistically significant. [a]Weight & Height are expressed as "Percentile" for the same age and sex (Those are done for standardization of growing children of different ages and sex).

Univariate regression analysis (Table 2) showed that children with positive influenza A virus (H1N1) infection were more likely to have fever (*P*=0.048), cough (*P*=0.001), headache (*P*=0.001), malaise (*P*=0.001), myalgia (*P*=0.001), and sore throat (*P*=0.001), compared with those who did not have influenza.

Table 2
Univariate predictive analysis of influenza–like illness (ILI) symptoms for influenza A infection in the Egyptian children.

| Symptom	Manifestation	Patients according to rapid test (%)		*P* value
		Positive (*n*=570)	Negative (*n*=630)	
Fever		100.0	92.1	0.048*
Respiratory symptoms	Cough	94.7	82.5	0.001*
	Coryza	71.9	63.4	0.203
	Sore throat	57.9	19.0	0.001*
	Any	98.2	87.3	0.002
Constitutional symptoms	Headache	47.3	15.8	0.001*
	Malaise	22.8	6.3	0.001*
	Myalgia	57.9	19.0	0.001*
	Any	77.2	31.7	0.001*
Gastrointestinal symptoms	Nausea/vomiting	22.8	28.6	0.334
	Abdominal pain	12.3	14.3	0.811
	Diarrhea	35.1	44.4	0.074
	Any	36.8	46.0	0.121

*Statistically significant.

Multivariate regression analysis showed that sore throat and fever significantly increased the odds of having influenza A virus infection by more than 3–fold predictivity index (OR= 3.9; 95% CI: 1.92–7.35) and 7–fold predictivity index (OR= 7.5; 95% CI: 4.01–14.32), respectively.

We further evaluated the performance of individual symptoms and symptom combinations as well as the rapid diagnostic test for the diagnosis of influenza infection (Table 3). Predictive

Table 3
Sensitivity and specificity of predictive symptoms among influenza A virus infected children.

Symptom	Manifestation	PPV	NPV	Sensitivity (%)	Specificity (%)
Single symptom	Fever	77.84	47.12	76.0	60.0
	Cough	69.41	60.90	94.0	21.3
	Coryza	76.58	49.24	72.0	34.2
	Sore throat	85.43	40.33	58.0	82.4
	Any respiratory symptom	71.14	69.89	88.2	13.1
	Headache	83.40	41.13	43.7	86.0
	Malaise	62.87	71.46	22.8	95.0
	Myalgia	86.43	79.86	58.9	79.0
	Any constitutional symptom	78.94	67.12	77.7	67.0
Combined symptoms	Fever+cough	72.41	53.32	67.0	64.0
	Fever+any respiratory symptom	81.45	48.21	66.7	72.3
	Fever+cough+any constitutional	79.02	46.81	65.5	68.4
	Any ILI	79.96	40.02	76.8	77.4

PPV indicates positive predictive value, the probability of having laboratory–confirmed influenza when the symptom is present; NPV, negative predictive value, the probability of not having laboratory–confirmed influenza when the symptom is not present; sensitivity, the probability of having the symptom when the patient has laboratory–confirmed influenza; and specificity, the probability of not having the symptom when the patient does not have laboratory–confirmed influenza (*i.e.*, when the test result for influenza is negative).

symptoms showed a sensitivity of 76.8% and a specificity of 77.4% in identifying influenza A virus infection from non-influenza illness.

Moreover, another particular interest was the PPV proportion of those with a symptom who were confirmed positive for influenza as shown in Table 3. The PPV for baseline fever and cough was 72.41%, with sensitivity and specificity values of 67.0% and 64.0%, respectively. The PPV for baseline cough and fever was even higher (PPV=84.6%) in children with time from onset of 36 to 48 hours. However, combining other symptoms did not increase the PPV substantially. However, as illustrated in Figure 1, the probability of a child having confirmed influenza increased with increasing baseline temperature and it was higher in patients with cough than in those without cough. The proportion of children who were confirmed to be positive for influenza RIDT exceeded 80% when baseline temperature was above 38 ℃.
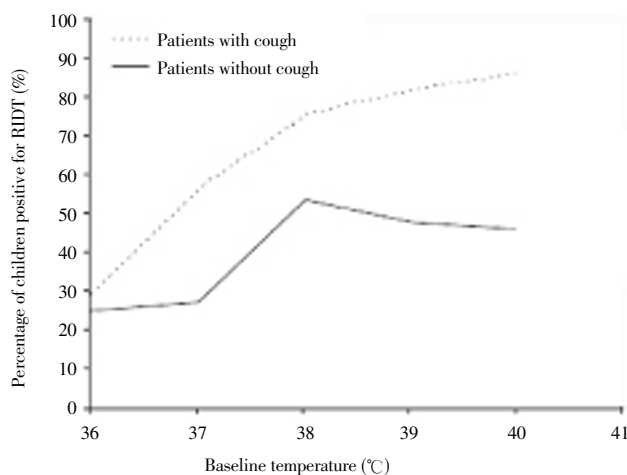
Figure 1. Frequency of positive rapid influenza A diagnostic test (RIDT) in patients in relation to fever and cough

4. Discussion

Influenza is a major health threat throughout the world, yet many countries lack even rudimentary influenza surveillance systems. Furthermore, influenza surveillance in developing countries often includes only major cities due to limited laboratory resources in outlying areas and poor infrastructure, e.g., lack of electrical power. Consequently, data are lacking about the types of influenza viruses that are circulating and the burden of influenza-related disease in the developing world[8].

Though reverse transcriptase-polymerase chain reaction and viral isolation are the preferred diagnostic methods, rapid influenza tests are valuable for their ease of use and laboratory-independence[12,14]. The precise origins for acute respiratory illnesses are rarely identified[15,16]. Hence, in most respiratory viral diseases, establishment of the specific viral cause is neither necessary (i.e., does not direct therapy) and thus is not cost-effective. However, it is different for

influenza virus infection, for which specific antiviral therapy has been available for many years. The introduction of the new neuraminidase inhibitors has made the need for influenza diagnosis a challenge[16,17].

This work was designed to screen the incidence of influenza A virus infection in Egyptian children with ILI in two consecutive post winter seasons (post swine-origin H1N1 pandemic) in Gharbia and Fayoum governorates using one step influenza A rapid test. Among 1 200 children with ILI and/or symptoms of acute respiratory tract infection, 47.5% were positive in RIDT for influenza A infections.

Parallel to our research, the latest unpublished Egyptian Influenza report 2012 by the Egyptian Ministry of Health (EMOH) reported that mean age for documented children cases of H1N1 infections in Egypt from June 2009 to the end of 2011 was 8.2 years with standard deviation 4.5 (13 782 cases) with case fatality rate 0.5% (71 deaths). Primary school age group (6–12 years) showed the highest case fatality rate (39.8%, 27 deaths), followed by pre-school age group (32.5%, 25 deaths), and the last was 12–18 year age group (27.6%, 19 deaths). Distribution of Egyptian children with mortality related to H1N1 infection showed that 56.3% of cases were males and 68.7% were from urban area. Most of cases were 67.9% during the year 2009 (46 deaths), then 26.1% (20 deaths) during 2010 and just 6% (5 deaths) during 2011.

However, our study concentrated upon the pre-school age children, which seems to be the 2nd most affected age group in Egypt (according to the EMOH report). Our results still show that influenza A virus group was still highly disseminated (around half of ILI affected children were positive). Nevertheless, H1N1 virus was not tested in particular, but this may point to its persistent high prevalence. On the other hand, documented reduction of children deaths due to H1N1 may be explained by developed immunity to the virus year after year. This may explain the result in our study that 10 children were admitted to intensive care unit, but none of the study children died.

Similarly, high prevalence of male sex affection in our study is parallel to that in the EMOH's documents. However, most of the documented H1N1 deaths in Egypt were from urban cities and towns. Our study spot lighted that more rural children were presented with ILI. This discrepancy could be explained by better diagnostic reports related to urban childhood deaths. Our study may point out that even rural deaths due to influenza related infections may be much more than urban deaths but better attention for diagnosis and reporting.

However, human influenza incidence peaks in the Northern and Southern Hemispheres during their respective winters[18], yet despite recognition of this phenomenon for at least a hundred years[19]. The mechanisms driving influenza seasonality are not well understood[20–22]. Several competing hypotheses have been proffered, including biological, sociological and environmental explanations, but none have been definitively established[22]. The pattern of influenza seasonality in humans appears different in tropical and subtropical areas, with high year-round circulation

and semi–annual peaks in incidence[23–25]. However, in the tropics, understanding the seasonal pattern of influenza in humans is further hampered by a lack of routinely collected incidence data[26].

For that, in our study, Egyptian children with ILI were recruited all through the year even in spring and summer with relative disappearance of the well evident peak of incidence in autumn and winter. This also shows a novel pattern of influenza seasonality in Egypt.

In agreement with the objects included by Ji et al[27], most children in our study were less than 5 years of age with mean age of (24.76±16.27) months. Nevertheless, the mean age of children with influenza A virus infection was greater than that of children with non–influenza illnesses. In contrast to Zaraket et al who screened influenza virus infections in Lebanese children with influenza like symptoms, the average age of their studied children was (5.5±4.6) years old[28]. This difference may be due to screening in winter and school season.

Smoking is believed to exacerbate respiratory diseases by harming respiratory defense mechanisms[29]. Young children in day care or those exposed to tobacco smoke can have as many as 10 episodes of infections over 1 year, even with a healthy immune system[30]. Moreover, children of smokers had more childhood respiratory infections and hospitalizations for respiratory illness compared with children of nonsmokers also; passive smoking for two cigarettes increased the likelihood of experiencing respiratory symptoms[30]. The present study reported that 451/570 (78.9%) among positive influenza A infections were passive smokers. In contrast to van Gageldonk–Lafeber et al who studied the environmental risk factors for acute respiratory tract infections in Netherlands, they reported only 35% were passive smokers[31].

This work aimed also to determine if there are certain clinical symptoms and signs that might help clinician to discriminate influenza infection from illness due to other respiratory viruses. The most common presenting symptoms among individuals with influenza A positive were cough, myalgia, and weakness, consistent with previous studies[32,33].

In this population, the PPV of cough and fever was 72.41%, with 67.0% sensitivity and 64.0% specificity. The PPV rose even further as the height or degree of fever at recruitment increased. Thus, during periods when influenza virus is circulating within a community, a high index of suspicion is warranted in patients who are seen with acute onset of cough and fever (body temperature ≥37.8 ℃)[32]. This also agrees with Ohmit et al. who reported that, despite the absence of one commonly accepted definition for high–probability ILI, using a definition of fever >38.2 ℃ with cough, it had a high positive predictive value (83%) for laboratory–confirmed influenza[33]. However, the selection of children with defined symptoms of ILI like fever may have overestimated PPV that would actually be observed among practicing physicians in the community.

The sensitivity and predictive values of ILI criteria for the diagnosis of influenza have varied among studies, with PPV ranging from 23% to 81%[32,33]. Our study reported that "ILI" symptoms PPV of influenza A infections was 79.96% with a sensitivity of 76.8%.

The burden of laboratory confirmed influenza infection in our study population of individuals with defined ILI was 47.5%. Also, Arifeen et al showed that 58% of the studied children had influenza A in a population–based surveillance for influenza virus among children under 5 years of age with acute infectious respiratory illness between April 2004 and November 2005[34]. That is to say, influenza A family of viruses seems to be accused for about half of the ILI in children at different populations and within different circumstances of place and time.

Moreover, these findings come in consistent with reported seasonality of influenza infection from the population based surveillance in Bangladesh, where the peak season was April to September. This seasonality is in contrast to temperate zones, where peak transmission occurs between September and March[35].

Little is known about the seasonality of influenza viruses in tropical and sub–tropical countries, where temperature fluctuations are generally less extreme than in temperate regions. Longitudinal studies in Nicaragua[24] and Hong Kong[23] have demonstrated the importance of influenza year–round as a cause of respiratory infections in children, but there is an evidence that seasonal peaks in incidence of human influenza do occur and appear to vary in number and timing between countries and from year to year.

In Egypt, all meteorological variables (temperature, precipitation, relative humidity and absolute humidity, measured as vapor pressure) exhibited statistically significant seasonal patterns with period length 1.18 years/cycle or 14 months/cycle[26], which may explain unseasonality and respectable burden of influenza A and its circulation in whole year–round.

In conclusion, although swine flu influenza pandemic is over, evidence of predominate influenza A virus infection in Egyptian children is still present. Developed immunity to the newly developed virus has reduced the ugly face of the infection mortality and morbidity. However, epidemiologists must stay alert for any new mutation to develop in influenza A virus family as it affects around half of the childhood with ILI cases. This will allow the timely detection of an epidemic or pandemic, detect changes in behavior of circulating influenza A virus family, inform decisions on health policy and clinical management, and guide the selection of strains for vaccines in Egypt.

Conflict of interest statement

The authors declare they have no conflict of interest.

Acknowledgments

References

[1] Nair H, Abdullah B, Katz M, Roca A, Berkley JA, Madhi SA, et al. Golbal burden of respiratory infections due to seasonal influenza in young children: a systemic review and meta analysis. *Lancet* 2012; **10**(1): 19–32.

[2] Amato–Gauci A, Zucs P, Snacken R, Ciancio B, Lopez V, Broberg E, et al. Surveillance trends of the 2009 influenza A(H1N1) pandemic in Europe. *Euro Surveill* 2011; **16**(26): pii=19903.

[3] Call SA, Vollenweider MA, Hornung CA, Simel DL, McKinney WP. Does this patient have influenza? *JAMA* 2005; **23**(3): 987–997.

[4] Boivin G, Hardy I, Tellier G, Maziade J. Predicting influenza infections during epidemics with use of a clinical case definition. *Clin Infect Dis* 2000; **31**(11): 1166–1169.

[5] Dawood FS, Jain S, Finelli L. Emergence of a novel swine–origin influenza A (H1N1) virus in humans. *N Engl J Med* 2009; **27**(9): 1605–1615.

[6] World Health Organization (WHO). Clinical management of pandemic (H1N1) 2009 virus infection–For the countries of Eastern Mediterranean region. *Interim Guidance from Expert Consultation* [Online]. Avaible from: http://www.who.int/wer. [Accessed on 15 October 2009]

[7] Earn DJD, Dushoff J, Levin SA. Ecology and evolution of the flu. *Trends Ecol Evol* 2002; **17**(4): 334–340.

[8] Ruef C. Diagnosing influenza–clinical assessment and/or rapid antigen testing? *Infection* 2007; **35**(2): 49–50.

[9] Schoub BD, McAnerney JM, Besselaar TG. Regional perspectives on influenza surveillance in Africa. *Vaccine* 2002; **20**(Suppl 2): S45–S46.

[10]Gilca R, De Serres G, Tremblay M, Vachon ML, Leblanc E, Bergeron MG, et al. Distribution and clinical impact of human respiratory syncytial virus genotypes in hospitalized children over 2 winter seasons. *J Infect Dis* 2006; **193**: 54–58.

[11]Martinello RA, Chen MD, Weibel C, Kahn JS. Correlation between respiratory syncytial virus genotype and severity of illness. *J Infect Dis* 2002; **186**: 839–842.

[12]Agoritsas K, Mack K, Bonsu BK, Goodman D, Salamon D. Evaluation of the Quidel Quick Vue test for detection of influenza A and B viruses in the pediatric emergency medicine setting by use of three specimen collection methods. *J Clin Microb* 2006; **44**: 2638–2641.

[13]McGeer A, Green KA, Plevneshi A, Shigayeva A, Siddiqi N. Antiviral therapy and outcomes of influenza requiring hospitalization in Ontario, Canada. *Clin Infect Dis* 2007; **45**: 1568–1575.

[14]Grijalva CG, Poehling KA, Edwards KM, Weinberg GA, Staat MA. Accuracy and interpretation of rapid influenza tests in children. *Pediatrics* 2007; **119**: e6–e11.

[15]Wat D. The common cold: a review of the literature. *Eur J Intern Med* 2004; **15**(1): 79–88.

[16]Karevold G, Kvestad E, Nafstad P, Kvaerner KJ. Respiratory infections in schoolchildren: co–morbidity and risk factors. *Arch Dis Child* 2006; **91**: 391–395.

[17]Koch A, Molbak K, Homoe P, Sorensen P, Hjuler T, Olesen ME, et al. Risk factors for acute respiratory tract infections in young Greenlandic children. *Am J Epidemiol* 2003; **158**: 374–384.

[18]Earn DJD, Dushoff J, Levin SA. Ecology and evolution of the flu. *Trends Ecol Evol* 2002; **17**(3): 334–340.

[19]Huntington E. The control of pneumonia and influenza by the weather. *Ecology* 1920; **1**(1): 6–23.

[20]Lowen AC, Mubareka S, Steel J, Palese P. Influenza virus transmission is dependent on relative humidity and temperature. *PLoS Pathog* 2007; **3**: 1470–1476.

[21]Dushoff J, Plotkin JB, Levin SA, Earn DJD. Dynamical resonance can account for seasonality of influenza epidemics. *Proc Natl Acad Sci USA* 2004; **101**(48): 16915–16916.

[22]Lofgren E, Fefferman NH, Naumov YN, Gorski J, Naumova EN. Influenza seasonality: underlying causes and modeling theories. *J Virol* 2007; **81**: 5429–5436.

[23]Chiu SS, Lau YL, Chan KH, Wong WHS, Peiris JSM. Influenza–related hospitalizations among children in Hong Kong. *N Engl J Med* 2002; **347**: 2097–2103.

[24]Gordon A, Ortega O, Kuan G, Reingold A, Saborio S, Balmaseda A, et al. Prevalence and seasonality of influenza–like illness in children, Nicaragua, 2005–2007. *Emerg Infect Dis* 2009; **15**(3): 408–14.

[25]Viboud C, Alonso WJ, Simonsen L. Influenza in tropical regions. *PLoS Med* 2006; **3**: e89.

[26]Murray EJ, Morse SS. Seasonal oscillation of human infection with influenza A/H5N1 in Egypt and Indonesia. *PLoS One* 2011; **6**(9): e24042.

[27]Ji W, Zhang T, Zhang XL, Jiang LF, Ding YF, Hao CL, et al. The epidemiology of hospitalized influenza in children, a two year population–based study in the People's Republic of China. *BMC Health Serv Res* 2010; **10**: 82–89.

[28]Zaraket H, Dbaibo G, Salam O, Ssito R, Suzyki H. Influenza virus infections in Lebanese children in 2007–2008 season. *Jpn J Infect Dis* 2009; **62**: 137–138.

[29]Murin S, Bilello KS. Respiratory tract infections: another reason not to smoke. *Cleve Clin J Med* 2005; **72**: 916–920.

[30]Peat JK, Keena V, Harakeh Z, Marks G. Parental smoking and respiratory tract infections in children. *Paediatr Respir Rev* 2001; **2**(5): 207–213.

[31]van Gageldonk–Lafeber AB, van der Sande MB, Heijnen MA, Peeters MF, Bartelds AM, Wilbrink B. Risk factors for acute respiratory tract infections in general practitioner patients in the Netherlands: a case–control study. *BMC Infect Dis* 2007; **7**(1): 35–46.

[32]Monto AS, Gravenstein S, Elliott M, Colopy M, Schweinle J. Clinical signs and symptoms predicting influenza infection. *Arch Intern Med* 2000; **160**: 3243–3247.

[33]Ohmit SE, Monto AS. Symptomatic predictors of influenza virus positivity in children during the influenza season. *Clin Infect Dis* 2006; **43**: 564–568.

[34]Arifeen SE, Akhter T, Chowdhury HR, Rahman KM, Chowdhury EK. Causes of death in children under five years of age. *Bangladesh Demographic and Health Survey 2009*. Dhaka: National Institute of Population Research and Training Report 2010; **22:** 125–133. [Online] Avaible from: http://www.univdhaka.edu/research.php. [Accessed on 15 September 2012]

[35]Centers for Disease Control and Prevention (CDC). Prevention and control of influenza with vaccines: Recommendations of the Advisory Committee on Immunization Practices ACIP 2011; **60**(33): 1128–1132. *Morbidity and Mortality Weekly Report* (MMWR). [Online] Available from: http://www.cdc.gov/mmwr/preview/mmwrhtml/mm6033a3.htm.[Accessed on 15 September 2012]