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BURDEN OF INFLUENZA IN CHILDREN

by

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ABSTRACT

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Burden of influenza in children
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Background: Most children with influenza are treated as outpatients but, especially among young children, influenza-attributable illnesses often result in hospitalization. However, relatively scarce data exist on the clinical picture and the full disease burden of pediatric influenza. Prompt diagnosis of influenza could enable the institution of antiviral therapy and adequate cohorting of patients. Data are needed to help clinicians correctly suspect influenza at the time of hospital admission.

Aims and methods: We conducted a prospective 2-year cohort study of respiratory infections in children aged ≤13 years to determine the incidence of influenza in outpatient children and to assess the clinical presentation of influenza in various age groups seen in primary care. We also determined the rates of different complications attributable to influenza and the absenteeism of the children and their parents due to the child's influenza infection. We then conducted a further 16-year retrospective study of children ≤16 years of age, hospitalized with virologically confirmed influenza. We estimated the population-based rates of hospitalizations and determined the primary admission diagnoses of the hospitalized children in different age groups.

Results: The average annual rate of influenza was highest (179 / 1000) among children <3 years old. In this age group, acute otitis media was diagnosed as a complication of influenza in 40% of children. High fever was the most prominent sign of influenza, and 20% of children <3 years of age had a fever ≥40°C. Most children had rhinitis already during the first days of the illness. The average annual incidence of influenza-related hospitalization was highest (276 / 100,000) among infants <6 months of age, of whom 52% were primarily admitted due to sepsis-like illnesses. Respiratory symptoms accounted for 38% of the hospitalizations.

Conclusions: Influenza causes a substantial burden of illness on outpatient children and their families. The clinical presentation of influenza is most severe in children <3 years of age. The high incidence of influenza-associated hospitalizations among infants aged <6 months calls for more effective ways to prevent influenza in this age group. The clinical manifestations of influenza vary widely in different age groups of children at the time of hospital admission. Awareness of this phenomenon is important for the early recognition of the illness and the potential initiation of effective antiviral treatment of these patients.

Keywords: influenza, children, outpatient, signs and symptoms, hospitalization, admission diagnosis

TIIVISTELMÄ

Heli Silvennoinen
Influenssan lapsille aiheuttama tautitaakka
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Tausta: Vaikka suurin osa influenssaa sairastavista lapsista hoidetaan avohoidossa, erityisesti pikkulapset joutuvat influenssan vuoksi usein sairaalahoitoon. Influenssan kliinisestä kuvasta ja kokonaistautitaakasta lapsipotilailla on kuitenkin olemassa suhteellisen vähän tutkimustietoa. Influenssan nopea tunnistaminen mahdollistaisi niin viruslääkehoidon aloittamisen ajoissa kuin sairaalapotilaiden oikeanlaisen sijoittelun. Influenssan varhaisesta oirekuvasta sairaalahoitoa tarvitsevilla lapsilla tarvitaan lisää tutkimustietoa.

Tavoitteet ja menetelmät: Selvitimme kaksivuotisessa seurantatutkimuksessa ≤13-vuotiaiden lasten sairastuvuutta influenssaan sekä influenssan kliinistä taudinkuvaa eri-ikäisillä avohoidossa hoidettavilla lapsilla. Tutkimme myös influenssan aiheuttamien komplikaatioiden sekä taudista aiheutuvien lasten ja heidän vanhempiensa poissaolojen esiintyvyyttä. Retrospektiivisen, väestöpohjaisen tutkimuksen avulla selvitimme ≤16-vuotiaiden lasten influenssasta johtuvia sairaalahoitoja 16 vuoden seurantajakson aikana. Selvitimme niin influenssaan liittyvien sairaalahoitojen esiintyvyyttä kuin influenssan oirekuvaa sairaalaan tulovaiheessa eri ikäryhmissä.

Tulokset: Influenssan ilmaantuvuus oli suurinta (179/1000 lasta) alle 3-vuotiaiden ryhmässä. Akuutti välikorvatulehdus todettiin tässä ikäryhmässä noin 40 %:lla influenssaan sairastuneista lapsista. Korkea kuume oli influenssan tyypillisin oire, ja 20 %:lla alle 3-vuotiaista lapsista kuumetta oli ≥40˚C. Suurimmalla osalla lapsista esiintyi nuhaa jo taudin ensipäivistä alkaen. Influenssan aiheuttamien sairaalahoitojen ilmaantuvuus oli suurinta alle 6 kuukauden ikäisillä lapsilla, joista 52 %:lla pääasiallinen sairaalaan joutumisen syy oli sepsisepäily. Hengitystieoireet olivat tärkein sairaalahoidon syy 38 %:lla influenssaan sairastuneista lapsista.

Päätelmät: Influenssa aiheuttaa lapsille ja heidän perheilleen merkittävän tautitaakan. Influenssan kliininen kuva on vakavin alle 3-vuotiailla lapsilla. Koska influenssan aiheuttamat sairaalahoidot ovat yleisimpiä alle 6 kuukauden ikäisillä lapsilla, influenssan ehkäisyyn tarvittaisiin tehokkaampia keinoja nimenomaan tässä ikäryhmässä. Influenssan oirekuvassa esiintyy suurta vaihtelua eri ikäryhmissä sairaalaan tulovaiheessa. Tämän huomioon ottaminen on tärkeää influenssan nopeaksi tunnistamiseksi ja mahdollisen viruslääkehoidon aloittamiseksi ajoissa.

Avainsanat: influenssa, lapset, avohoito, oireet ja löydökset, sairaalahoito, diagnoosi

TABLE OF CONTENTS

ABBREVIATIONS

AOM	Acute otitis media
CDC	Centers for Disease Control and Prevention
CFR	Case-fatality ratio
CNS	Central nervous system
CRP	C-reactive protein
EMA	European Medicines Agency
FDA	U.S. Food and Drug Administration
HA	Hemagglutinin
HPAI	Highly pathogenic avian influenza
ICD	International Classification of Diseases
IF	Immunofluorescence
ILI	Influenza-like illness
LAIV	Live attenuated influenza vaccine
M1	(Influenza) matrix 1 protein
M2	(Influenza) matrix 2 protein
NA	Neuraminidase
NI	Neuraminidase inhibitor
NP	Nucleoprotein
NPA	Nasopharyngeal aspirate
PCR	Polymerase chain reaction
PEP	Post-exposure prophylaxis
PICU	Pediatric intensive care unit
RNA	Ribonucleic acid
RSV	Respiratory syncytial virus
RT-PCR	Reverse transcriptase polymerase chain reaction
SBI	Serious bacterial infection
THL	Finnish National Institute for Health and Welfare
TIV	Trivalent inactivated (influenza) vaccine
WHO	World Health Organization

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following publications, which are referred to in the text by the Roman numerals I-IV. The original publications have been reproduced with the kind permission of the copyright holders.

I Heikkinen T, Silvennoinen H, Peltola V, Ziegler T, Vainionpää R, Vuorinen T, Kainulainen L, Puhakka T, Jartti T, Toikka P, Lehtinen P, Routi T, Juvén T. Burden of influenza in children in the community. *J Infect Dis 2004;190:1369-73.*

II Silvennoinen H, Peltola V, Lehtinen P, Vainionpää R, Heikkinen T. Clinical presentation of influenza in unselected children treated as outpatients. *Pediatr Infect Dis J 2009;28:372-5.*

III Silvennoinen H, Peltola V, Vainionpää R, Ruuskanen O, Heikkinen T. Incidence of influenza-related hospitalizations in different age groups of children in Finland. A 16-year study. *Pediatr Infect Dis J 2011;30:e24-8.*

IV Silvennoinen H, Peltola V, Vainionpää R, Ruuskanen O, Heikkinen T. Admission diagnoses of children 0-16 years of age hospitalized with influenza. *Eur J Clin Microbiol Infect Dis 2012;31:225-31.*

1 INTRODUCTION

Previously, influenza had mainly been considered as a disease of the elderly, since most excess deaths due to seasonal epidemics occur in people over 65 years of age. This misconception was further strengthened by the generally good outcome of influenza in healthy children. However, during the past two decades influenza has been gradually recognized as an important illness in the pediatric population, and accumulating data suggest that influenza places a considerable health toll on children worldwide (Izurieta et al. 2000, Neuzil et al. 2000a, Chiu et al. 2002, Quach et al. 2003, Poehling et al. 2006b, Rojo et al. 2006, Ploin et al. 2007, Ajayi-Obe et al. 2008). Regardless of the season or circulating strain, the attack rates are constantly highest in the pediatric population (Monto and Sullivan 1993), and the rates of hospitalizations attributable to influenza among the youngest children have been demonstrated to be comparable to those for adults with high-risk illnesses (Izurieta et al. 2000, Neuzil et al. 2000a, Neuzil et al. 2002a). Although the burden of influenza due to increased pediatric hospitalizations is already quite well established, substantially fewer data are available on the illness burden and the socioeconomic consequences of influenza in the outpatient setting, even though the great majority of children with influenza are treated as outpatients. Assessment of the full burden of influenza in children is essential for the evaluation of influenza vaccination strategies of children in any country.

One of the main difficulties when estimating the true burden of influenza is that the traditional indicators of the impact of influenza have relied on indirect measures such as excess rates of hospitalizations or deaths attributable to influenza-like illness during confirmed influenza activity, lacking virologic confirmation of the illness. However, in children it has been well demonstrated that even during seasonal outbreaks, the majority of influenza-like illnesses are caused by other viruses than influenza (Zambon et al. 2001); therefore the validity of these indirect estimates is questionable in the pediatric population (McIntosh and Lieu 2000). Furthermore, since the intensity of an influenza epidemic can vary substantially from season to season, the results obtained during a very short period of time may not be well generalizable. Monitoring the characteristics of influenza for multiple seasons is crucial for reliable evaluation of the true impact of seasonal influenza.

The clinical spectrum of influenza ranges widely from a subclinical infection to a fulminant, life-threatening disease. The significant benefits afforded by prompt initiation of specific antiviral treatment have enhanced the need for early recognition of influenza in children (Whitley et al. 2001, Heinonen et al. 2010). Furthermore, the fundamental role of children in the transmission of influenza in the community makes awareness of the clinical features of the illness in children a topic of major importance (Glezen and Couch 1978, Loeb et al. 2010).

This study was undertaken to estimate the full burden of influenza in children. We determined the attack rates of influenza in outpatient children, and the incidences of influenza-related hospitalizations in different age groups of children. We also described the clinical characteristics and typical complications of influenza among outpatients and hospitalized children. Furthermore, we evaluated the socioeconomic aspects of pediatric influenza in the community.

2 REVIEW OF THE LITERATURE

2.1 Influenza virus

The term "influenza" has probably been derived from the Italian word "influentia" in the mid-1300s, indicating that the disease was believed to result from astrological influences. In the beginning of the 19th century, the causative agent of influenza was thought to be the bacterial species *Haemophilus influenzae*. In 1931, Richard Shope demonstrated that swine influenza could be transmitted with filtered mucus, indicating that the causative agent was a virus (Shope 1931). The influenza A virus from humans was first isolated by Wilson Smith et al. in 1933 (Smith et al. 1933). Influenza B was discovered in 1940 and influenza C in 1947 (Taylor 1949). The first diagnostic test for influenza was introduced in 1941, and the production of influenza vaccines began in 1947 (Duin and Sutcliffe 1992). The first tests of vaccine efficacy were conducted in military populations and in prisons in the 1930s and 1940s (Hirst et al. 1944).

2.1.1 Types and subtypes

Influenza viruses belong to the family of *Orthomyxoviridae*, and are originally classified into three distinct types – A, B, and C – based on the absence of antigenic cross-reactivity between their nucleoprotein (NP) and matrix 1 (M1) protein.

Influenza A viruses are further divided into subtypes depending upon the antigenic relationship of their two main surface glycoproteins, *hemagglutinin* (HA), and *neuraminidase* (NA). To date, 17 different H antigens (H1 to H17) and nine different N antigens (N1 to N9) have been detected (Fouchier et al. 2005, Tong et al. 2012). The latest HA subtype was identified in 2009 from bats in Guatemala (Tong et al. 2012). Sequence analyses of the HA subtypes have further divided these 17 HAs into two major groups with five different clades (Air et al. 1981). Aquatic birds are considered the primary biotic reservoir for influenza A viruses (Horimoto and Kawaoka 2001). Usually these viruses cause little or no disease in those birds, but are subsequently shed in the faeces to infect other birds and maintain the enzootic cycle. Besides humans, many influenza A virus subtypes have been isolated from a variety of other mammals, including pigs, horses, seals, whales, cats, dogs, and non-human primates (Webster et al. 1992).

The influenza B virus is almost exclusively a human pathogen; the only other animals known to be susceptible to influenza B infection are the seal and the ferret. Influenza B viruses mutate at a rate 2-3 times lower than type A (Nobusawa and Sato 2006) and are consequently less genetically diverse, with only one sero-/subtype. Nonetheless, mutations occur rapidly enough to prevent the host from acquiring lasting immunity (Webster et al. 1992). Due to the reduced rate of antigenic change, and the inability for cross species antigenic shift, influenza B viruses are considered to be unable to cause pandemic outbreaks.

Influenza C viruses infect humans, pigs, and dogs. In contrast to the two other influenza types, the influenza C virus has a single surface glycoprotein, and is divided into six antigenic and genetic groups or lineages. Even though globally distributed, the influenza C virus is regarded as less important to humans than influenza A or B, as it causes only localized epidemics and relatively mild symptoms. However, in young children a clinical picture similar to that of influenza A and B has been described (Moriuchi et al. 1991, Matsuzaki et al. 2006).

Of the numerous strains of influenza A viruses, only four subtypes have been reported to cause epidemics in humans (H1N1, H1N2, H2N2, H3N2), and six others (H5N1, H7N2, H7N3, H7N7, H9N2 and H10N7) have caused sporadic outbreaks without confirmed sustained human-to-human transmission. Except for the highly pathogenic avian influenza (HPAI) H5N1 virus, infections with these viruses have resulted in mild symptoms and very little severe illness (Peiris et al. 1999, Fouchier et al. 2004, Koopmans et al. 2004, Tweed et al. 2004, Beigel et al. 2005, Butt et al. 2005).

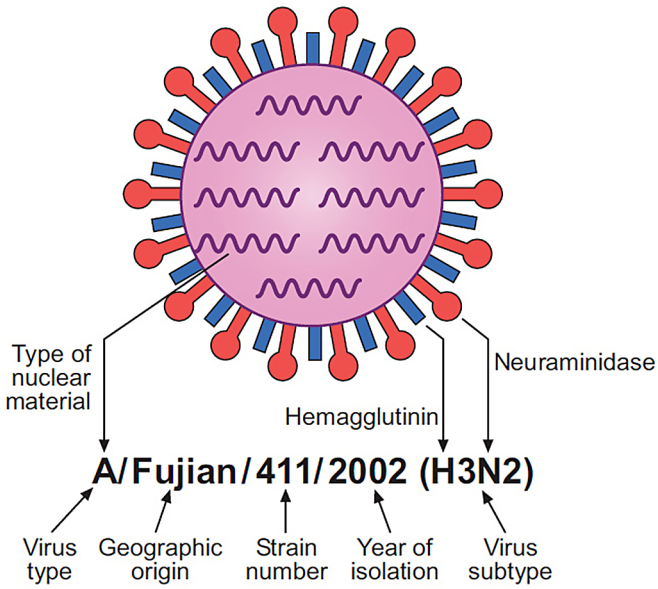

Figure 1. Structure and classification of the influenza A virus. Specific influenza strain isolates are classified by a standard nomenclature specifying virus type, geographical location where first isolated, sequential number of isolation, year of isolation, and, for influenza A, HA and NA subtype. The recent A/H1N1 pandemic virus was identified as A/California/04/09 (H1N1).

2.1.2 Structure and replication

Unusually for a virus, the genome of influenza A and B viruses is not a single piece of nucleic acid; instead, it contains eight pieces of segmented single stranded negative-sense RNA which encode 10 and 11 different proteins in influenza A and B viruses, respectively. In contrast, the genome of the influenza C virus consists of seven RNA segments, encoding 9 proteins. The total genome length is 12000-15000 nucleotides. A characteristic feature

of influenza virus particles is their external lipid layer of approximately 500 spike-like projections, representing the envelope glycoproteins HA (rod-like shape) and NA (mushroom-shaped) (Wilschut et al. 2006). The envelope also contains the matrix 2 (M2) protein, which is an integral membrane tetramer, functioning as an ion channel.

Hemagglutinin is responsible for the attachment of the virus to specific receptors, terminal sialic acids of glycoproteins and glycolipids, on the target cell surface. HA also mediates a fusion reaction between the viral envelope and cell membrane, enabling virus entry into cells (Bullough et al. 1994). Cleavage of the HA into two subdomains (HA1 and HA2) is required to make the virus infectious. The cell imports the virus by endocytosis, after which the viral RNA is replicated and viral proteins are synthesized, leading to the production of thousands of new virus particles per cell. As a result, progeny viruses are released back to the airways. Eventually, the cell dies as a result of the infection. HA represents the major antigenic determinant of influenza types A and B and induces neutralizing antibodies, which in turn determines much of the host's susceptibility to reinfection by related virus strains (Gerhard 2001).

Neuraminidase, the second major antigenic determinant of influenza, is a receptor-destroying enzyme that, like HA, also recognizes sialic acids of cell-surface glycoproteins and glycolipids (Weis et al. 1988). Following virus replication, neuraminidase removes sialic acid from infected cell surfaces so that newly made viruses are released to infect more cells (Compans et al. 1969, Wagner et al. 2002). The NA also prevents viral aggregation, and facilitates dispersion of the virus through the mucus that coats the respiratory tract epithelium. Neuraminidase inhibitors block NA's activity and subsequently prevent the spreading of the new progeny virus.

2.1.3 Transmission and viral shedding

Human-to-human transmission of influenza occurs by three routes: aerosols, large droplets, and direct contact with secretions (or fomites); however, these three routes are not mutually exclusive (Tellier 2006, Brankston et al. 2007). Coughing or sneezing generates a large quantity of particles, mainly aerosols <5-10um in diameter (Nicas et al. 2005), which are expelled to the environment and can subsequently be inhaled into the lower respiratory tract. Droplet (>10 um in diameter) transmission occurs over a relatively short distance, when droplets come into contact with another person's conjunctiva, mouth, or nasal mucosa. Experimental as well as observational studies have suggested that all three mechanisms of transmission are possible, yet there is still much controversy about the relative importance of the three main transmission routes (Tellier 2006, Brankston et al. 2007, Weber and Stilianakis 2008).

In laboratory settings, influenza viruses can be cultured from surfaces at room temperature for up to 3 days (Bean et al. 1982, Thomas et al. 2008). However, the amount of viable viruses reduces quickly (in minutes) after experimental inoculation of hands (Bean et al. 1982).

There is evidence that influenza-infected children have higher viral loads than adults (Hall et al. 1979). As young children do not always practice good hand hygiene, it is possible that direct or indirect transmission modes (environmental contamination) via hands or fomites may play a more important role in the pediatric population than among adults. Boone and Gerba (2005) showed that the influenza virus could be detected on over 50% of the fomites tested in day care centers and homes during the influenza season. Consistently in a recent study from Thailand, it was demonstrated that surfaces contaminated with influenza virus RNA were significantly more common in homes with children <8 years of age than in homes with older children (Simmerman et al. 2010).

Occasionally, the RNA of influenza viruses has been detected in faecal excretion of infected pediatric patients (Wootton et al. 2006, Chan et al. 2009), but it is not considered as a significant mode of transmission of seasonal influenza viruses (Tamura et al. 2010).

Viral shedding can be counted as a surrogate marker for infectiousness of an individual. According to a study by Couch and co-workers (1971), virus replication begins within 6 hours of influenza infection, and continues at least 24 hours before the onset of symptoms. In a large meta-analysis of 56 different studies of healthy adult volunteers challenged with wild-type influenza viruses, the duration of viral shedding in 375 patients was an average of 4.8 days with no significant differences between viral types or subtypes (Carrat et al. 2008). On average, viral shedding peaked on day 2, and illness symptoms on day 3, after inoculation. Across all studies included in the meta-analysis, the mean generation time (i.e., average time between new infection and transmission to another susceptible) was 2.5 days (Carrat et al. 2008). In a recent study from Hong Kong, however, it was demonstrated that there are differences in viral shedding patterns between different influenza types: viral loads for influenza A had already peaked at the time of symptom onset and decreased more rapidly than those for influenza B, whereas in influenza B infections the peak was reached only 3 days after the onset of illness, with viral shedding at substantial levels for 5 days from the illness onset (Lau et al. 2010).

Compared to adults, children are reported to shed the influenza virus earlier, before the illness begins, and for longer periods once the illness starts (Hall et al.1979, Frank et al. 1981, Longini et al. 1982, Whitley et al. 2001); indeed viral shedding for up to three weeks in young children has been reported (Munoz et al. 1999). Severely immunocompromised children have been reported to shed the influenza virus for even longer (Evans and Kline 1995). In a study from Japan, of 63 children hospitalized for influenza-attributable illnesses, the duration of positive virus isolation was 6.8 days in influenza A and 6.2 days in influenza B infections in untreated patients (Sato et al. 2005).

There is controversy about to what degree asymptomatic individuals can transmit influenza infection to others (Ferguson et al. 2006, Patrozou and Mermel 2009). In a recent study from Hong Kong only 14% of influenza infections with detectable viral shedding were asymptomatic, and shedding was low in these cases (Lau et al. 2010),

indicating that asymptomatic or presymptomatic individuals do not play as important a role in influenza transmission as previously thought.

As for the pandemic A/H1N1 influenza, incubation periods ranging from 1 to 7 days have been demonstrated in studies combining adult and pediatric patients (Cao et al. 2009, Dawood et al. 2009). In one recent study (To et al. 2010), younger age was associated with prolonged shedding in the respiratory tract and higher viral load in the stool. In another study (Li et al. 2010), no correlation with viral load and age was detected. In general, the transmission dynamics in the recent A/H1N1 pandemic seem broadly similar to that of seasonal influenza (Cowling et al. 2010).

2.1.4 Antigenic drift and shift

The influenza virus's evolutionary strategy takes two forms – antigenic drift and antigenic shift. Replication of the influenza genome requires RNA polymerase activity. Since this enzyme lacks proof-reading activity, it has limited potential to correct mistakes during RNA transcription, resulting in a high gene mutation rate of approximately one error per replicated genome (Drake 1993). Antigenic drift occurs when the genes encoding the viral surface antigens, the HA and NA, undergo random stepwise mutations. Eventually, these proteins on the virus particle become sufficiently different, so host antibodies are no longer capable of neutralizing the virus, and the new variant is able to cause illness through evasion of the pre-existing immunity of the host (Wilschut et al. 2006). Repeated infections of people with variant forms of the same virus subtype result in continuing selection of the virus in populations with widespread immunity to its earlier variants (Bush et al. 1999).

Occasionally, an entirely new influenza A virus subtype of avian origin emerges in the human population, causing a pandemic outbreak of influenza. This is called antigenic shift. There are three main ways by which such new human virus subtypes may arise:

1. An avian virus may be transmitted directly to humans and subsequently adapt to the new host by mutation. The 1997 A/H5N1 bird flu outbreak in Hong Kong confirmed, for the first time, the possibility of direct transmission of an avian influenza virus to humans; before that, the H5N1 virus had not been isolated from other species than birds (Claas et al. 1998, Subbarao et al. 1998). The highly pathogenic H5N1 virus was originally isolated from a three-year-old child who subsequently died with Reye's syndrome, implying that the virus had adapted to the human host. Even though the first outbreak was controlled by the depopulation of 1.5 million chickens in Hong Kong farms and markets, the A/H5N1 virus re-emerged in 2003, subsequently resulting in a major outbreak in 2003 in Vietnam (Peiris et al. 2004). The virus has since spread within many Eastern countries, as well as to Europe and Africa (WHO 2012). By May 2 2012, the WHO had reported 603 confirmed human cases and 356 deaths in a total of 15 countries since the first case was reported in 2003 (WHO 2012). Luckily, there has not yet been sustainable human-to-human transmission of the HPAI H5N1 virus; nevertheless, its continuing circulation and extreme lethality is still a major concern to humans as well as for poultry (Capua and

Alexander 2010). It has since become evident that the 1918 Spanish flu virus was also an avian virus and not a human-avian reassortant (Gamblin et al. 2004).

2. G*enetic reassortment* can occur between an avian and human influenza virus, when a host cell is simultaneously infected with two different influenza A viruses. In this process, the RNA segments from the two strains can get mixed together and a third "new" viral strain can be produced with elements from both of the original viruses. It has long been assumed that genetic reassortment would occur exclusively in pigs. The pig provides an ideal "mixing vessel", since pigs are readily susceptible to infection with both human and avian viruses due to the presence of both α2,3- and α2,6-linked cellular receptors in their respiratory epithelium (Ito et al. 1998). Nonetheless, there is increasing evidence that other species, including humans, might also serve as a 'mixing vessel' (Webster et al. 1992). This may be the case in those rare occasions when HPAI viruses cross the species barrier to humans with subsequent co-infection of host cells with an avian and human influenza virus. It is well established that genetic reassortment has been the basis of the formation of the 1957 and 1968 pandemic viruses (Ito et al. 1998).

3. An "old" strain of a previously epidemic influenza A virus can re-emerge unchanged after being hidden for some time, for example in a frozen state (Webster et al. 1992). The waning immunity in the population to that strain enables new emergence and a pandemic outbreak. Due to the lack of pre-existing immunity in the population, this re-appearance is also regarded as a form of antigenic shift, even though no new virus has been created in the process. The appearance of the Russian flu (H1N1) in 1977 supports the idea of reintroduction of a previous strain: the virus was found to be identical in all of its genes to the H1N1 virus that circulated before 1957 (Nakajima et al. 1978).

Theoretically, all influenza viruses which are novel to the immune system of the human population today possess the potential to initiate an influenza pandemic if their ability to enter human cells and transmit efficiently evolves. Nonetheless, historically, only viruses of three HA and NA subtypes have established efficient transmission in humans: H1N1, H2N2, and H3N2. A key barrier to an avian flu becoming a human pandemic is its inefficient human-to human transmission, which requires a switch of receptor specificity from α2,3- to α2,6-linked receptors in sialic acid residues (Ito et al. 1998). This binding to a specific receptor is a primary determinant of viral tropism.

2.2 Epidemiology

2.2.1 Seasonal influenza

2.2.1.1 Seasonality

Influenza epidemics typically occur in the winter months in temperate climate zones: from October to April in the northern hemisphere, and from April to October in the southern hemisphere. In rare conditions, however, summer outbreaks have been reported

(Wolf et al. 2004). The length of a certain epidemic can vary greatly: it can be a matter of few weeks, or last for several months (Neuzil et al. 2000a, Heikkinen et al. 2003, Peltola et al. 2003). The mean length of an influenza season during 1999-2007 in Europe was 15.6 weeks (Paget et al. 2007).

It is still not fully understood why there is such seasonal regularity in the occurrence of influenza epidemics. Various theories have been proposed to explain how seasonal change might stimulate influenza activity: transmission rates might increase during school terms and winter crowding, the stability of the virus might be enhanced by cooler temperatures, or host immunity might decline during colder weather (Dowell 2001). Indeed, winter school breaks have been shown to reduce influenza transmission to children by approximately 25% (Cauchemez et al. 2008), supporting the theory of the effect of behavioural changes on influenza activity. The effect of seasonal climate change on immune function and host susceptibility has also been documented (Shephard and Shek 1998).

On the other hand, studies of ambient temperature and low relative humidity (Hemmes et al. 1960, Lowen et al. 2007) contributing to the seasonal effects of influenza are not consistent with the reports of significant influenza burden in some subtropical regions with warm, humid climates (Chiu et al. 2002, Wong et al. 2006). Similarly, the hypothesis of short days and limited sun exposure in relation to seasonal patterns of influenza is challenged by the same argument. A recent publication on the subject (Shaman et al. 2010) suggests that absolute humidity drives seasonal variations of influenza transmission in temperate regions. It is possible that a combination of factors may synergistically contribute to the seasonality of influenza (Lipsitch and Viboud 2009).

2.2.1.2 Prediction of epidemics

Regardless of their annual seasonal character, influenza epidemics are unpredictable. It is difficult to predict when a certain epidemic will start, how long it will last, and how virulent it will be (Russell et al. 2008). The severity of an epidemic in any given year is a result of the interplay between the waning immunity in the population, the extent of antigenic variation of the virus, and the intrinsic virulence of the new virus variant (Cox and Subbarao 2000, Munoz 2003).

In 1947, the WHO established an international Influenza Surveillance Network of laboratories to monitor the emergence and spread of new influenza strains around the world, and thus to predict the circulating strains to be presented in a vaccine (Wilschut et al. 2006). This activity is co-ordinated in the WHO Collaborating Centres based in Atlanta, London, Melbourne, and Tokyo together with over 120 national influenza centres located in 94 different countries. Global surveillance of influenza viruses has shown that antigenic variation and the consequent epidemiologic behaviour of influenza A viruses follow a relatively uniform pattern. Each successive antigenic variant replaces its predecessor, so the co-circulation of distinct antigenic variants of a given subtype occurs for relatively short periods (Cox and Subbarao 2000).

During the past decade, new epidemic variants of influenza are often first detected in East and Southeast Asia before spreading to other locations (Russell et al. 2008). Besides the global migration of influenza viruses, seasonal epidemics can also result from the rapid emergence of new drift variants in small geographic areas. For these reasons, accurate prediction of which viruses are expected to be circulating in the forthcoming season in a certain area is challenging. A recent study conducted in Taiwan demonstrated that the dominantly circulating subtype of the influenza virus in the coming winter season could be predicted by a low seroprotection rate, against a specific locally circulating influenza strain, among pediatric patients (Su et al. 2010). Recently, the sequencing of whole influenza genomes has provided important additional information on the genesis and spread of reassortment viruses, their rapid migration, and the co-circulation of multiple lineages (Nelson et al. 2006, Russell et al. 2008).

Influenza strains circulating during a seasonal epidemic may be influenza type A strains A/H1N1, A/H3N2, strains of influenza B lineages B/Victoria or B/Yamagata, or any combination of these. According to North American surveillance data from 1976 to 1999, 15% of influenza illnesses during the observation period were caused by A/H1N1, 60% by A/H3N2, and 25% by B viruses (Thompson et al. 2003). In general, influenza B viruses have tended to be prominent every 2-4 years (Belshe 2010). Conventionally, influenza seasons with A/H3N2 subtype predominance are associated with a more severe clinical illness than seasons with influenza A/H1N1 or influenza B (Wright et al. 1980, Frank et al. 1985, Simonsen et al. 1997).

2.2.1.3 Methods for measuring the impact of influenza

When the behaviour of influenza viruses in populations is examined, it is important to recognize whether cases are identified by virus identification or serology, or, as is most commonly the case, by epidemiologic means (Monto 2008). Traditionally, the impact of influenza epidemics and pandemics have been measured by excess mortality and/or excess hospitalizations either due to any cause or due to pneumonia and influenza during an influenza epidemic, (or excess outpatient visits), compared to peri-influenza season, when influenza viruses are not circulating. This method is, however, especially susceptible to bias in child populations due to various other respiratory viruses cocirculating at the same time as influenza (Zambon et al. 2001, Heikkinen et al. 2003). In particular, respiratory syncytial virus – which is considered the most important viral cause of hospitalizations in young children (Fisher et al. 1997, Bourgeois et al. 2009) – places a serious obstacle to reliable estimations of the burden of pediatric influenza in studies without virologic confirmation of the illness (McIntosh and Lieu 2000). Direct documentation of the virus on an individual level allows for exact calculations of the incidence of the illness, but the method is both expensive and time-consuming.

2.2.1.4 Morbidity

The fundamental data exploring the burden of influenza in pediatric population were generated by a series of prospective, longitudinal family and community studies conducted between the 1960s and 1980s in the USA, all of which combined clinical surveillance with attempts at virus isolation and serological studies. In Tecumseh, Michigan, between 100 and 300 families with at least one child were studied continuously for six years from 1966 to 1971 – a period which included the emergence of the pandemic influenza A/H3N2 in 1968 (Monto and Cavallaro1971). In Seattle, Washington, a similar study took place between the years 1965 and 1969 and then 1975 to 1979, involving over 215 families with young children (Hall et al. 1973, Fox et al. 1982a). In Houston, Texas, a comparable observation period of the Houston Family Study spanned eight years (from 1976 to 1984), including two influenza B epidemics (Glezen 1996). The findings of these major studies have consistently shown that, in any season, the highest attack rates for influenza occur in children, with rates ranging from approximately 15% to 40%, compared with adult rates ranging from 12–20%. Depending on the study and circulating influenza type, the peak attack rate has been observed in either school-aged or preschool-aged children (Hall et al. 1973, Glezen and Couch 1978, Frank et al. 1981, Fox et al. 1982a, Monto and Sullivan 1993). Later, Hurwitz et al. (2000) observed even higher attack rates (up to 50%) of influenza in daycare-children during regular influenza seasons.

In these studies, asymptomatic infections in children were fairly common: serological attack rates are reported to be 20-30% higher than clinical attack rates (Fox et al. 1982a, Glezen et al. 1997). The studies also revealed that seroconversion to the influenza virus continued to occur between epidemics, implying the persistence of viral activity throughout the year (Hall et al. 1973, Glezen and Couch 1978).

The course of an influenza epidemic follows a certain pattern with regard to the impacted age group and the stage of the epidemic: typically, school children predominate among persons presenting for health care during the early stage of an influenza epidemic (Glezen 1982, Olson et al. 2007). Concurrently, school absenteeism occurs in the first part of an epidemic (Glezen and Couch 1978), while employee absenteeism occurs later. Young infants and the elderly are usually at the end of the transmission chain (Glezen 1996). These observations, together with similar findings of some other studies (Taber et al. 1981, Longini et al. 1982) support the concept that preschool-aged and school-aged children act as introducers and spreaders of influenza infections in communities and individual households, apparently because they mix more often and shed viruses in higher titers and for longer periods than adults. This central role of children in the transmission of influenza in the community was nicely demonstrated in a pivotal study by Glezen and Couch (1978), who showed an upwards shift in the age distribution of influenza-infected persons (from children aged 5–19 years towards adults aged 20-44 years) as two influenza A epidemics progressed over time. Similarly, in the same setting, the numbers of pediatric admissions for pneumonia peaked about two weeks earlier than adult admissions for pneumonia did. When interpreting these studies it must be

recognized, however, that the day-care of children has significantly increased since 1980s, making the role of younger children as spreaders of influenza infection in the community even more important.

Several studies have shown that vaccination of school children against influenza lead to the reduction of influenza-related mortality and morbidity in the community (Monto et al. 1970, Rudenko et al. 1993, King et al. 2006, Glezen et al. 2010). In Japan, from the mid-1970s through to the late 1980s, influenza vaccination was required by law for all school children aged 6-15 years, resulting in a strong reduction of influenza morbidity and wintertime excess mortality in older age groups. After the government's decision to discontinue mass vaccinations, the vaccination coverage of school-aged children rapidly dropped to almost zero, which consequently resulted in a clear increase (up to the pre-program levels) in the overall mortality of the elderly population in subsequent years (Reichert et al. 2001). Similarly, in a recent randomised trial conducted in a rural community in western Canada it was demonstrated that vaccination of 83% of children aged 3 to 15 years with the trivalent influenza vaccine conferred 61% indirect protection against influenza among persons who did not receive the study vaccine (Loeb et al. 2010). The data presented above are examples of so called herd immunity, which means indirect protection of illness by vaccinating one group to reduce exposure of another. Since schools provide a ready conduit for the spread of influenza in the community, yet school children respond well to influenza vaccines (Glezen et al. 1997, Jefferson et al. 2008), these results support selective influenza immunization of this age group to interrupt influenza transmission (Loeb et al. 2010).

The impact of influenza B viruses is conventionally thought to be greatest on school children and teenagers with relative sparing of the youngest children (Monto and Sullivan 1993). In a study carried out in New York during 5 influenza seasons (from 2001 to 2006), the influenza B/Victoria lineage was shown to mainly affect those aged 5-17 years, while the circulating A/H3N2 strain had a similar impact across all age groups (Olson et al. 2007). On the other hand – as demonstrated in a 2-year Italian prospective study of children ≤14 years of age with influenza presenting to pediatric emergency units – random mutations of influenza B giving rise to new strains may occasionally give the virus the potential to circulate effectively in other age groups as well (Gasparini et al. 2007).

2.2.1.5 Mortality

Worldwide, influenza infection is estimated to cause 250 000-500 000 fatalities annually (WHO 2009). Most deaths occur in adults over 65 years with underlying cardiopulmonary conditions that place them at increased risk of pneumonia (Barker and Mullooly 1980, Barker 1986). In the United States, during an observation period from 1990 to 1999, the estimated average rates of influenza-associated pulmonary and circulatory deaths per 100 000 persons per year were 0.4-0.6, 7.5, and 98.3 among persons aged 0-49 years, 50-64 years, and ≥65 years, respectively (Thompson et al. 2003). In Europe, an estimated

number of excess deaths attributable to influenza ranges between 40 000 and 200 000 per year depending on the severity of the epidemic (ECDC 2009, Mereckiene et al. 2010). In Finland, an average influenza epidemic results in an estimated half a million infections and 1000 excess deaths per year, mostly among the elderly and those with underlying medical conditions (THL 2011).

Influenza-associated deaths are uncommon among children; nevertheless they represent a substantial proportion of vaccine-preventable deaths. In general, the estimates of annual mortality rates for children caused by seasonal influenza are less than one per 100 000 persons (Neuzil et al. 2000a, Thompson et al. 2003, Montes et al. 2005, Bhat et al. 2005). During the 2003-2004 season in the United States, 153 laboratory-confirmed pediatric deaths were reported, with the annual mortality rate highest among children less than 6 months (0.88 per 100 000) (Bhat et al. 2005). Half of the children were previously healthy. In another study conducted in California, USA, during the 2003-04 and 2004-05 seasons, 51% of children <18 years with laboratory-confirmed influenza who died, and 40% of those who required admission to intensive care units, had no underlying medical conditions (Louie et al. 2006). These findings demonstrate that although children with risk factors for influenza complications are at a higher risk of death, up to half of the fatal cases occur among children with no known high-risk conditions (Bhat et al. 2005).

Influenza A/H3N2 virus infections are associated with higher mortality than influenza A/H1N1 or B virus infections in all age groups (Simonsen et al. 1997, Thompson et al. 2003). In a recent study from Portugal, in which excess mortality due to influenza was estimated in eight age groups during a 24-year period, rates were 3.3–6.1 times higher for seasons dominated by A/H3N2 viruses compared to seasons dominated by influenza B or A/H1N1, depending on the outcome studied (Nunes et al. 2011). In that study, the average excess mortality rate for the children aged 0–4 years was 2.6 per 100 000, which is clearly higher than findings from previous studies. The authors acknowledge, however, that their estimates of mortality in the youngest age groups can be confounded by the co-circulation of RSV; information for which they had no data. In general, RSV is associated with higher hospitalization rates and excess mortality in infants compared to influenza (Zambon et al. 2001, Iwane et al. 2004).

2.2.1.6 Outpatient visits

The natural result of the high attack rates of influenza in child populations is that the number of visits by children to outpatient clinics, which are attributable to influenza infection, is also high. Virologic surveillance studies from the USA in the 1970s and ‘80s already show that the annual peak in the number of visits for acute respiratory disease in children always coincides with the peak of influenza activity (Glezen et al. 1987a). This phenomenon was further confirmed in a comprehensive surveillance study conducted in the United States during 19 consecutive years with a finding that the excess number of outpatient visits in children with influenza was approximately three times as high as that

of the adult population, with estimated excess consultation rates ranging from 6 to 15 for every 100 children under one year of age (Neuzil et al. 2000a). In another American surveillance study comprising 6 years (from 1994 to 2000), the annual rate of outpatient visits attributable to influenza was estimated to be 8.5 visits per 100 children under 17 years of age (O'Brien et al. 2004).

When it comes to virologic confirmation of influenza illness in individual cases, there are relatively few studies on the burden of influenza in healthy children in the outpatient setting, and most of these come from the US. In 2002, Neuzil et al. (2002a) reported results of a 25-year study of laboratory-confirmed influenza in 289 children under 5 years of age with annual rates of 9.3 outpatient visits per 100 children aged <1 years and 11 per 100 among one to two year olds. Subsequently, in an extensive population-based study of outpatient children <5 years of age, carried out in three different geographical regions in the United States, laboratory-confirmed influenza accounted for 10.2% and 19.4% of weekly clinic visits and 5.9% and 28.8% of weekly emergency department visits during the influenza seasons of 2002-03 and 2003-04, respectively (Poehling et al. 2006b). These mean annual rates of outpatient visits were approximately 10, 100, and 250 times as high as hospitalization rates for children aged <6 months, 6-23 months, and 2-4 years, respectively. The estimated rate of outpatient visits attributable to influenza were highest in the age group of 6 months-<2 years, with 5.2-12.5 visits per 100 children (Poehling et al. 2006b). Consistently, in a prospective 2-year community study of children aged ≥6 months to <14 years in Greece, during the 14-week period of an epidemic each year, influenza accounted for approximately 40% of all febrile respiratory infections in the children seeking acute medical care, and for 13.5% of all outpatient pediatric visits (Tsolia et al. 2006). Even though both influenza seasons were considered relatively mild, it was demonstrated that during the peak 4 weeks of the yearly outbreak influenza accounted for 25% of all outpatient visits. Of note was the finding that none of the 90 influenza-positive patients examined in primary care were admitted to hospital (Tsolia et al. 2006).

These studies confirm the fact that, while hospitalization rates can be counted as a measure of disease severity and thus provide important information on the burden of influenza, they only represent the tip of the iceberg when considering the total impact of influenza in children. The majority of influenza-positive children are treated as outpatients, and it is plausible that this group accounts for the greater part of the total disease burden in children (Poehling et al. 2006b, Heikkinen 2006).

2.2.1.7 Hospitalizations

First attempts to quantify influenza-related hospitalizations in children were made in the 1980s in the United States when Mullooly and Barker (1982) estimated the excess hospitalization rate seen in epidemic years (1968-69 and 1972-73) compared with non-epidemic years in Oregon, using retrospective data. A few years later, Perrotta et al. (1985) published a similar study conducted in Texas, USA between the years 1978-

1981. Both of these studies revealed that the greatest increase in hospitalizations during influenza epidemics occurred among young children; in the first study, the excess rate of hospitalization in previously healthy children aged ≤4 years was estimated at 100 per 100,000, and this rose to 470 per 100,000 in children 0-4 years of age with high-risk conditions. In line with these studies, Glezen and co-workers (1987b) showed that during three consecutive influenza epidemics from 1978 to 1981, the rate of hospitalization in children younger than 5 years of age was nearly as high as that of the elderly population. The significant finding from this study was that most of the hospitalized children did not have any chronic medical condition, in contrast to older inpatients, most of whom had one or more high-risk conditions.

Two major studies published in 2000 strengthened the earlier findings of the role of young age as a risk factor for hospitalization: Neuzil et al. (2000a) examined records for a 19-year time period among healthy children in Tennessee, USA, and found that children younger than one year of age had the highest rate of excess hospitalization from influenza, with average rates of more than 1000 per 100,000 for infants younger than 6 months of age. The rates for those <2 years regularly exceeded those for individuals 65 years of age and older. For comparison, the average number of excess hospitalizations in the age group of 5- to 15-year-old children was 40 per 100,000. Similarly, Izurieta et al. (2000) revealed high hospitalization rates in children <2 years of age in a study conducted in California among children enrolled in managed care programs. The rate for those <2 years of age was about 200 per 100,000 per month, similar to that for high-risk children aged 5-17 years. In contrast, the hospitalization rate was 90% less in low-risk 5- to 17-year-old children.

The problem with these studies was that the clinical data was derived from administrative data (excess rates of hospitalizations and outpatient visits during confirmed influenza activity), thus lacking virologic confirmation of the influenza illness on an individual level. However, particularly in children, it is known that RSV coincides with influenza, and even during a peak of an influenza epidemic, a major part of influenza-like illnesses are caused by viruses other than influenza (Zambon et al. 2001, Heikkinen et al. 2003, Peltola et al. 2005). Therefore, there remains uncertainty as to whether influenza has been responsible for all excess morbidity attributed to it in these studies (McIntosh and Lieu 2000).

A more precise estimate of hospitalizations in children was provided a few years later in a prospective population-based surveillance study by Poehling et al. (2006b) with laboratory confirmation of influenza illness in children <5 years of age followed through a 4-year period from 2000 to 2004 (**Table 1**). A total of 2979 children were enrolled in the study, 160 of which (6%) had influenza. The rate of hospitalization for all children up to 5 years of age was 90 per 100,000. Nearly half of those hospitalized were younger than 6 months of age (450 per 100,000) and 80% were younger than 2 years. Somewhat lower rates of infant admissions due to virologically confirmed seasonal influenza were

represented in two other US studies (253 and 153 per 100,000 in children under 6 months of age) (Ampofo et al. 2006, Dawood et al. 2010a) (**Table 1**).

In Europe, incidence data of influenza-related pediatric hospitalizations is available from five different countries (**Table 1**). Only two of the studies have assessed the population-based incidence of influenza-related hospitalizations in the age group of 0-<6 month-old children (Montes et al. 2005, Ajayi-Obe et al. 2008), with rates comparable to those of Poehling et al. (2006b).When it comes to South-East Asia, the reported hospitalization rates of children in subtropical regions like Hong Kong are consistently clearly higher than those from the US or Europe (Izurieta et al. 2000, Neuzil et al. 2000a, Chiu et al. 2002, Montes et al. 2005, Chiu et al. 2009, Sakkou et al. 2011). There may be several explanations for this phenomenon, one of them being the different circulation pattern of seasonal influenza viruses in subtropics compared to temperate regions (year-round versus sharp seasonality) (Griffin and Neuzil 2002).

The weak point of all the hospitalization studies presented in the table 1 is that the observation period is fairly short, with an average of three influenza seasons. Only one of the studies is comprised of more than five seasons (Rojo et al. 2006), but unfortunately in that study detailed data on rates of hospitalizations of children in different age groups (other than those under 3 years of age) are not available. Furthermore, few studies have determined the rates of hospital admissions according to influenza type. In the prospective study by Weigl et al. (2002), the incidence of hospitalization attributable to influenza A in children younger than one year of age was 149 per 100,000, whereas in the Hong Kong study it was 2 to 7 times higher (Chiu et al. 2009), depending on the year and circulating strain **(table 1**). As for influenza B, the population-based rates in the German study were also highest for the youngest children, although the overall incidence was much smaller than for influenza A; while in the Hong Kong study, during the three study seasons there were no influenza B cases among children younger than 2 years of age. Generally, children hospitalized with influenza B are older than those hospitalized with type A infection (Monto and Sullivan 1993, Peltola et al. 2003). Further to which, children admitted for influenza B seem to have an underlying disease more often than those admitted for influenza A (Liou et al. 1987, Peltola et al. 2003).

Children with underlying medical conditions have rates of hospitalization for influenza-related illnesses 2-6 times higher than in otherwise healthy children (Mullooly and Parker 1982, Neuzil et al. 2000b). Particularly, children under one year of age with an underlying chronic disease are in the highest risk for hospitalization (Neuzil et al. 2000b). Asthma is the most common underlying condition in children with influenza in developed countries. Other conditions that place children at higher risk for acquiring severe influenza include immunodeficiencies; malignancies; cardiac, renal and neurologic diseases; prematurity; hemoglobinopathies; and metabolic disorders like diabetes mellitus. The most common reason for admission in children at risk is lower respiratory tract disease (Neuzil et al. 2000b). Nosocomial influenza infection is also a concern for high-risk children, including infants less than one year of age as well as premature babies (Sagrera et al. 2002).

Table 1. Population-based studies of hospitalization rates in children with laboratory-confirmed influenza

Country	Germany	USA	Spain	USA	USA	Spain	England	Hong Kong	USA	Greece
Author	Weigl et al. 2002	Iwane et al. 2004	Montes et al. 2005	Poehling et al. 2006	Ampofo et al. 2006	Rojo et al. 2006	Ajayi-Obe et al. 2008	Chiu et al. 2009	Dawood et al. 2010	Sakkou et al. 2011
Number of patients	116	20	49	160	325	146	59	147	4015	161
Age group (years)	<16	<5	<5	<5	<19	<3	<6	<18	<18	0.5-<14
High-risk condition (%)	18(A) 43(B)	30	16	21	37	41	7	NA	47	30
Enrollment, years	Prospective 1996-2001	Prospective 2000-2001	Retrospect 2001-2004	Prospective 2000-2004	Retrospect 2001-2004	Retrospect 1996-2003	Prospective 2002-2004	Prospective 2003-2006	Retrospect 2003-2008	Prospective 2002-2005
Number of seasons	4	1	3	4	3	7	2	3	5	2
% of influenza A/B	89/11	NA	92/8	89/11	NA	95/5	98/2	69/31	74/14 (12 unknown)	83/14 (2 both)
INCIDENCE (per 100 000)										
0-6 mo	NA	240	410	450	253	NA	440	NA	153	NA
6 mo-<1 yr	NA	100	80	NA	113	NA	230	NA	NA	NA
<1 yr	149 (A) 49 (B)	170	240	NA	183	NA	NA	389-1038 (A) 0 (B)	NA	NA
1-2 yrs	161 (A) 31 (B)	50	70	NA	96	NA	230 (0.5-2 yrs)	409-955 (A) 0 (B)	NA	NA
<2 yrs	NA	NA	160	90 (0.5-2 yrs)	140	NA	280	NA	78	NA
2-<5 yrs	101 (A) 24 (B)	20	50	30	33	NA	NA	381-595 (A) 85-423 (B)	21	NA
<5 yrs	123 (A) 30 (B)	60	90	90	78	NA	NA	NA	46	NA

2.2.1.8 Economic impact on the society

The impact of pediatric influenza goes beyond the clinical illness it causes to the child. The related socioeconomic burden includes direct costs like medications, outpatient visits, and hospitalizations, and indirect costs including absence from school or daycare and missed workdays, either because of home care of a sick child or secondary illness of the caregiver. Recent studies based on laboratory-confirmed influenza cases have shown that direct costs of pediatric influenza, both community-managed and hospital-managed, are higher than previously calculated (Lambert et al. 2008, Fairbrother et al. 2010). The mean cost for an influenza infection of a child under 5 years of age requiring hospitalization in the US is calculated to be 5402 € (Fairbrother et al. 2010). In a population-based prospective Australian study of 234 preschool children, influenza was the most costly among the common respiratory viral infections encountered (Lambert et al. 2008).

However, health care use represents only a minor portion of the cost due to pediatric influenza as, in most children, influenza is a self-limiting illness without the need for medical intervention. Still, most of these cases cause absenteeism from daycare or school, subsequently leading to parental absence from work for taking care of the ill child (and possible secondary illness of the parent due to household transmission). In a prospective survey study of school children in Seattle, only one fourth of those with a symptomatic influenza infection made a health care visit; the school days missed by children and work days missed by parents far outnumbered outpatient visits for these illnesses (Neuzil et al. 2002b). For every 100 children followed up in the study, influenza-attributable illness accounted for an estimated 20 days of work missed by the parents. Likewise, according to two European prospective outpatient studies, an average of 1.3 and 1.4 working days were lost by parents in order to care for the influenza-infected child at home (Principi et al. 2003, Tsolia et al. 2006). In a recent Finnish study of children aged 1-3 years, a median duration of parental absence from work due to a child's influenza was 2.0 days (Heinonen et al. 2010).

In a Finnish cost-effectiveness analysis based on virologically confirmed influenza cases, the total costs of influenza in Finnish children aged 6 months to 13 years were estimated at 39 million € (with an average annual attack rate of 16%) (Salo et al. 2006). More than two thirds of the costs came from work absenteeism of the parents of a sick child, even though only 35% of influenza illnesses in children under 5 years of age were assumed to result in parental work absenteeism. Moreover, it is worth noting that infants under 6 months of age were excluded from these calculations, yet it is acknowledged that infants have the highest hospitalization rates for influenza, a fact that further increases the total costs of pediatric influenza (Izurieta et al. 2000, Neuzil et al. 2000a, Poehling et al. 2006b).

2.2.2 Pandemic influenza

Along with the antigenic shift of the influenza virus, a novel subtype with a new hemagglutinin and/or neuraminidase is introduced into the immunologically naïve human population, resulting in a rapid global epidemic referred to as a pandemic. There are convincing reports of at least eight influenza pandemics during the 18th and 19th centuries (Beveridge 1991). However, this review focuses on the last five pandemics that have occurred since the beginning of the 20th century.

2.2.2.1 1918 H1N1 – Spanish flu

The influenza pandemic of 1918 hit at the end of the First World War, and was exceptional in both breadth and depth. It spread across the world in three consecutive waves between 1918-1919, starting from Kansas, USA, and infecting an estimated one-third of the world's population: i.e. 500 million people. In Europe, the first infections were reported in Madrid in May 1918, hence the name "Spanish flu". The disease was exceedingly severe, with mortality rates of more than 2.5%, killing approximately 50 million people in ten months (Johnson and Mueller 2002). Further, it had an extraordinary toll on healthy young adults: nearly half of all influenza-related deaths during the pandemic were accounted for by 20 to 40-year-olds (Simonsen et al. 1998). The geographic origin of the 1918 pandemic is not clear.

Genomic sequencing of the Spanish influenza virus revealed an avian-like H1N1 virus that contains human-like signature amino acids and several proteins (Taubenberger et al. 1997). Interestingly, the virus lacks a multibasic HA cleavage site, a hallmark of highly pathogenic avian influenza viruses (Reid et al. 1999). Later, reverse genetics have allowed the re-creation of the full 1918 H1N1 influenza virus and its characterization, based on fragments of genetic material isolated from the remains of a victim of the Spanish flu pandemic, buried in the permafrost of Alaska (Tumpey et al. 2005). Further challenge studies on mice and non-human primates have confirmed the extreme lethality of the virus (Kash et al. 2006) – thus it is of considerable note that the 1918 H1N1 influenza virus continues to circulate in avian species (Reid et al. 1999).

2.2.2.2 1957 H2N2 – Asian flu

The H2N2 "Asian influenza" originated in Southern China in 1957, rapidly spreading around the globe in two waves. The highest attack rates during this pandemic, of 50%, occurred in children aged 5–19 (Glezen 1996). Other at-risk individuals who were particularly affected included people with underlying chronic disease of the heart and lungs and pregnant women, particularly those in the 3rd trimester (Kilbourne 2006). Ultimately, it affected 40-50% of population, with 25-30% suffering from clinical disease. Mortality was highest among the very young and the very old, exceeding 1.5 million.

2.2.2.3 1968 H3N2 – Hong Kong flu

In 1968, viruses of the H2N2 subtype were replaced by another human/avian reassortant, H3N2, which arose in Southeast Asia and acquired its appellation "Hong Kong flu" on the basis of the site of its emergence to western attention. It spread around the world in two waves in subsequent winters, affecting an estimated 30-40% of the population. Compared to the earlier pandemics of the century, the Hong Kong flu was relatively mild, probably as a result of immunity in the population against the N2 subtype neuraminidase which the new pandemic H3N2 virus shared with the circulating H2N2 strain. The mortality rate was estimated at one million (Wilschut et al. 2006).

2.2.2.4 1977 H1N1 – Russian flu

In May 1977 an influenza virus outbreak, affecting mainly young adults, was reported in China. It spread throughout Russia by December 1977 and other parts of the world within 1978. The outbreak was caused by influenza viruses of the H1N1 subtype that closely resembled viruses which had circulated in the early 1950s (Nakajima et al. 1978); thus, the pandemic was quite mild and confined almost entirely to children and teenagers with attack rates exceeding 50% (Fox et al. 1982b). There were suspicions that the virus had been accidentally released from a laboratory source (Webster et al. 1992).

Historically, the strain of influenza causing a pandemic becomes the strain causing subsequent seasonal epidemics of influenza. The H1N1 strain that caused the 1918 Spanish flu pandemic became the cause of seasonal influenza until it was replaced by the recombinant H2N2 in 1957, which was subsequently replaced by the H3N2 strain in 1968. The re-emerging H1N1 virus in 1977 was an exception: it could not replace the H3N2 viruses circulating at the time; therefore, both subtypes are co-circulating in humans to this day. Because of its milder nature, "Russian flu" is not always considered as pandemic but instead a severe seasonal epidemic.

2.2.2.5 2009 H1N1 – Swine flu

In March and April 2009, a previously undescribed influenza A virus H1N1 of swine origin was isolated from humans in Mexico (CDC 2009a) and the Unites States (CDC 2009b), causing the first pandemic in four decades. The novel pandemic virus proved to be somewhat of a surprise for the research community, who were focused on highly pathogenic avian viruses in poultry. The 2009 A/H1N1 pandemic influenza rapidly spread to more than 200 countries around the world. By June 2010 more than 18,000 confirmed deaths were associated with the pandemic worldwide (WHO 2010a) with approximately 2,900 deaths reported in Europe (ECDC 2010).

The sequence analysis indicated the virus to be a quadruple reassortant virus: the RNA of the H1N1 pandemic virus was originally derived from classical (North American)

swine viruses, human H3N2 viruses, avian viruses, and Eurasian avian-like swine viruses (Garten et al. 2009). Compared with seasonal H1N1 viruses the novel virus was, even though not a new subtype, genetically and antigenically very different from human H1N1 viruses that had been circulating for the preceding 60 to 70 years (Garten et al. 2009). Genetic and structural analyses also revealed that the 2009 pandemic virus was more closely related to the 1918 Spanish influenza and to the 1976 Fort Dix outbreak of swine viruses than to any other seasonal H1N1-type influenza viruses that have been isolated since the 1930s (Ikonen et al. 2010).

During the 2009 A/H1N1 influenza pandemic, epidemiologic studies in several countries indicated that the hospitalization rates and deaths among children and adults aged under 65 years of age exceeded those observed during typical winter seasonal influenza epidemics (Echevarria-Zuno et al. 2009, Jain et al. 2009, Libster et al. 2010, Mazick et al. 2010) – however, results to the contrary have also since been published (Morgan et al. 2011). On the other hand, there were reports of frequent asymptomatic infections, especially in children. For example, in London and Birmingham during the first wave of infection, in the summer of 2009, serological testing showed that over 30% of children were infected by the pandemic virus. This was ten times more than was estimated from clinical surveillance (McCaughey 2010). In the early stages of the pandemic, the case-fatality ratio (CFR) – a measure to estimate the virulence of the disease using confirmed cases as the denominator – was calculated to be approximately 0.5% (Nishiura 2010). However, later epidemiological studies estimated the symptomatic CFR as approximately 0.05% of all medically attended symptomatic cases, even though great variation according to age and risk-group existed, with children being disproportionately affected by the virus (Dawood et al. 2009, Lyytikäinen et al. 2010). In particular, in one retrospective case series from Argentina (Libster et al. 2010) conducted in May and July 2009, it was demonstrated that the pandemic H1N1 influenza resulted in as high as a 5% death rate among hospitalized children, which was 10 times higher than for seasonal influenza in previous years. In the United States, of the 272 patients with A/H1N1 infections who were hospitalized from April to mid-June 2009, almost half were under the age of 18 years (Jain et al. 2009). By contrast, the estimated number of hospitalizations and deaths among people over 65 years was below that observed in most seasonal epidemics (Viboud et al. 2010). This difference was attributed to a lower risk of infection associated with a higher prevalence of partial or full immunity among older persons, most likely as a result of exposures to antigenically similar influenza A viruses that circulated during the years preceding the Asian flu in 1957 (Wei et al. 2010). In approximately one third of individuals born before 1950, high titers of cross-reacting antibodies against 2009 A/H1N1 virus could be detected (Hancock et al. 2009), indicating pre-existing immunity.

The clinical features of the 2009 pandemic A/H1N1 influenza did not appear to be that different from seasonal influenza. In children, the reported symptoms have included fever, cough, rhinitis, sore throat, and muscle aches in older children (Dawood et

al. 2009, Hackett et al. 2009, Jain et al. 2009, Perez-Padilla et al. 2009). Vomiting and diarrhea have been reported in greater proportion than with seasonal influenza, both in children and adults (Dawood et al. 2009, Bettinger et al. 2010). The majority of children requiring admission to intensive care units had known comorbidities, including asthma (O'Riordan et al. 2010) and neurodevelopmental conditions (CDC 2009c). Obesity was also noted in some of these children (Jain et al. 2009, CDC2009d). It is noteworthy, however, that more than a fifth of all children who died due to the 2009 pandemic A/H1N1 influenza were previously healthy (CDC 2009d). Most deaths associated with this influenza occurred in the 20 to 49-year-old age group, although there was considerable variation depending on country or continent (Muscatello et al. 2010, Jain et al. 2009, Mazick et al. 2010). In the United States, according to a study by Viboud et al. (2010), the mean age of death from the novel A/H1N1 virus was half that of seasonal flu, at 37 years (the average age of influenza-associated fatality among those who died from seasonal influenza during 1979-2001 in the US was 76 years).

In Finland in 2009, A/H1N1 resulted in appoximately 7,700 laboratory-confirmed cases with 44 fatalities (median age 56 years; range 1-88): four of these were children (Lyytikäinen et al. 2010). Based on data on laboratory-confirmed cases, the morbidity was highest in children. The median age of the hospitalized patients was 32 years, which was also clearly lower than in seasonal influenza. 90% of those hospitalized were under the age of 65, and 43% of them had at least one chronic underlying illness. Obviously, the laboratory-confirmed cases represent a minor part of all those who became asymptomatically or symptomatically infected by the 2009 pandemic influenza A/H1N1 virus. Consistent with reports from other parts of the world, the majority of the children infected with pandemic influenza had a considerably mild, self-limiting disease without the need for any medical interventions (Lyytikäinen et al. 2010).

On 10th August 2010, the WHO finally declared the end of the pandemic (WHO 2010b). Data are still emerging on the overall impact of the pandemic in different parts of the world. For the first time, the global community was put to the test in terms of preparation, response, and deployment of resources toward a potentially deadly strain of influenza virus, which, fortunately this time, turned out to be milder than first assumed (Pada and Tambyah 2011). Furthermore, a more deadly second wave (as was seen in the Spanish pandemic) luckily did not materialize.

2.3 Pathogenesis

Pathogenicity is a measure of the extent to which a virus causes diseases. The primary targets for influenza viruses in humans are epithelial cells in the upper and lower respiratory tract, where they cause mucosal inflammation and lysis of respiratory epithelial cells and subsequent desquamation of the respiratory lining. This results in an

exudative process with increased mucus production, which can be seen as rhinorrhea, cough, and nasal congestion.

The balance between viral replication and host immune response determines the outcome of viral infection. Influenza infection induces a cascade of nonspecific and specific immune functions such as phagocytosis, natural killer and cytotoxic T lymphocyte activities, and the production of antibodies and various cytokines. While a number of cytokines have immunoregulatory and antiviral properties (like interferon-α/β/-δ and interleukin-2) that may be important in the control of influenza infections (preventing the spread of the virus outside of the respiratory tract), others (interleukin-1, tumor-necrosis-factor-α, and interleukin-6) are more likely to contribute to the systemic symptoms associated with influenza, such as fever, muscle aches, and malaise (Han and Meydani 2000).

The common circulating strains of the influenza virus normally remain restricted to the respiratory tract and escape only under exceptional circumstances. Consequently, the virus is rarely found circulating in the blood or other organs (Kuiken and Taubenberger 2008). The main reason is that the protease required for cleavage of the viral HA is restricted to the epithelium of the airways and lungs (Wilschut et al. 2006). In the case of an entirely new subtype, the situation is different. The severity of the disease may be markedly increased because of the complete lack of immunity in infected individuals. Analogously, the consequences of influenza infection are commonly more extensive in the naïve immune system of young children under 2 years of age, with the exception of possible protection by maternal antibodies in infants less than 6 months of age.

The primary marker for resistance to, and recovery from, influenza virus infection is that of humoral antibodies which are of complementary specificity to the HA and NA antigens of the virus. Hemagglutinin specifically attaches to the surface of a respiratory cell. Without this specific attachment the infection of the cell, and hence host, cannot be initiated. Specific antibodies to HA can block this attachment and confer immunity by neutralization of the virus (McCaughey 2010). NA antibodies, in turn, restrict virus spread by interfering with the release of newly replicated viruses from the host cell. Loss of complementary antibodies through natural diminution or by antigenic drift accounts for a reduction in resident humoral immunity (Hilleman 2002).

Cell-mediated immunity against influenza is less well defined than that of humoral immunity. It is based on class I CD8+ cytotoxic T cell responses which usually appear within 3-4 days after infection. CD8+ cytotoxic T cells detect and kill virus-infected host cells, possibly directing their specificity against more conserved epitopes than those regarding humoral immunity. In turn, CD4+ T helper cells facilitate humoral and cellular immune responses, and exert cytolytic effects (Hilleman 2002). Both humoral and cell-mediated immunities play essential roles in control of influenza infection.

2.4 Clinical picture

2.4.1 Signs and symptoms

2.4.1.1 Outpatients

Much of the knowledge of the clinical presentation of influenza in children is derived from hospital studies, which may emphasize the more severe forms of the illness. Few studies have been carried out to evaluate the clinical presentation of influenza infection in unselected groups of healthy children at the primary care level. In an American prospective study of 274 outpatient children under 5 years of age with influenza, the most common symptoms included fever (95%), cough (96%), and rhinitis (96%) (Poehling et al. 2006b). In another study of 58 influenza-positive emergency department patients aged <17 years, 55% of the children had a fever ≥39˚C, 83% had a cough, and 60% had rhinitis (Friedman and Attia 2004). There were no significant differences in clinical findings between children with influenza A of influenza B. Likewise, in a Finnish study of 683 influenza-positive children aged <17 years who were referred to emergency department of a tertiary hospital (43% were discharged to their homes after clinical examination), fever (94% and 89%), cough (67% and 60%), and rhinitis (66% and 56%) were the most common symptoms of children with influenza A and B, respectively (Peltola et al. 2003). As for the A/H1N1 pandemic influenza, similar symptoms have been observed in outpatient children (Smit et al. 2012). From these studies it can be concluded that virtually all healthy outpatients have fever, and most of them have upper respiratory symptoms, common to numerous other viral respiratory infections.

Headache and myalgia, which are common symptoms in adult influenza-positive patients (Boivin et al. 2000, Monto et al. 2000), are relatively rare in children: headache has been reported in 23-44% and muscle aches in 6-33% in pediatric outpatient or emergency department patients (Peltola et al. 2003, Friedman and Attia 2004, Ceyhan et al. 2012). However, it is noticeable that the interpretation of these symptoms is unreliable in young children, who cannot verbally describe their symptoms. The same is true with sore throat (pharyngitis), which is thought to be more common in school-aged children compared with younger age groups (Moore et al. 2006). Gastrointestinal complaints have been reported in approximately 10% of the outpatient children (Friedman and Attia 2004, Ceyhan et al. 2012). During the recent pandemic, however, gastrointestinal symptoms, particularly diarrhea, were unusually common and were recorded in up to 40% of the cases (McLean et al. 2010).

The duration of illness symptoms in outpatient children has mainly been studied in a few influenza antiviral treatment trials. In an American study of 235 children under 12 years of age, the median duration of influenza illness (after enrolment) was 5.7 days, and the median duration of fever was 2.8 days (Whitley et al. 2001). In a Finnish study of children aged 1-3 years, the corresponding figures of 51 unvaccinated children were 7.3 days and 3.5 days, respectively (Heinonen et al. 2010). It must be

noted, however, that even though an average child with uncomplicated influenza can return to normal activities after a week of illness, mild to moderate cough resulting from hyperreactivity of the airways due to influenza infection can persist for several weeks.

2.4.1.2 Hospitalized children

Like outpatient children, the majority of children hospitalized with influenza infections have a fever and some signs of respiratory tract infection (Quach et al. 2003, Moore et al. 2006, Rojo et al. 2006, Sakkou et al. 2011) (**table 2**). However, in young children, respiratory symptoms may be absent in the early phase of the illness, resulting in an isolated fever, which in turn may lead to suspicion of bacterial sepsis (Dagan and Hall 1984, Ploin et al. 2003, Bender et al. 2010). In two European studies, 22-25% of infants and children under 3 years of age with laboratory-confirmed influenza had an admission diagnosis of isolated fever (Ploin et al. 2003, Rojo et al. 2006). Correspondingly, in a Canadian study comprising 182 children < 18 years of age admitted with influenza, 31% of all children were admitted due to suspected sepsis (34% of the study children were under 6 months of age) (Quach et al. 2003). In a prospective surveillance study during one influenza season (2000-01) in Tennessee, USA, Iwane et al. (2004) found out that hospitalized children younger than 5 years of age with influenza had a significantly higher percentage of an admission diagnosis of fever or rule-out sepsis compared with those hospitalized with parainfluenza of RSV, even though this influenza season was considered particularly mild. Apart from fever, neonates with influenza infection may present with unspecific non-respiratory symptoms like decreased appetite, lethargy, or apneas (Hite et al. 2007).

Gastrointestinal manifestations are more common in hospitalized children than in outpatients. Vomiting has been recorded from between 28% to 37%, and diarrhea from 9% to 16%, of inpatients (Quach et al. 2003, Rojo et al. 2006, Moore et al. 2006).

Wheezing and laryngitis can be regarded as manifestations of the primary viral infection of the upper respiratory tract, rather than complications of influenza. Acute expiratory wheezing is fairly common in hospitalized children with influenza (**table 2**). In a Canadian study, 25% of 172 children aged 6 months to <2 years had expiratory wheezing during hospitalization, 62% of them being previously healthy (Moore et al. 2006). Consistently, asthmatic children are prone to acute exacerbations of asthma during influenza infection (Neuzil et al. 2000b). Laryngitis, while not as common as wheezing in children admitted with influenza (Ploin et al. 2007, Sakkou et al. 2011), can be more severe than that caused by parainfluenza viruses (Peltola et al. 2002).

Table 2. Clinical profile and complications of children hospitalized with seasonal influenza

Variable	Quach et al. 2003	Moore et al. 2006	Rojo et al. 2006	Sakkou et al. 2011
Country	Canada	Canada	Spain	Greece
Study period	1999-2002	2003-2004	1997-2003	2002-2005
No. of patients	182	505	146	161
Age group	<18 yrs	<18 yrs	<3 yrs	6 mo-<14 yrs
Influenza A/B (%)	79/21	99/1	95/5	83/14 (2% both)
Preexisting condition (%)	30	42	41	30
Asthma (%)		7	23	
Proportion of children (%)				
<6 months	34	23		0
<1 year			64	13 (6 mo-<1 yr)
<2 years	70	57		31 (0.5-<2 yrs)
Symptom (%)				
Fever	90	93	96	91
Cough	75	81		65
Rhinorrhea		53		66
Laryngitis / hoarseness		4	2	4
Wheezing / asthma	15	18	31	15
Myalgia				8
Headache				17
Vomiting	37	28	32	
Diarrhea	16	11	9	
Lethargy / ill appearance	33	30		
Complication / diagnosis (%)				
Suspected sepsis	31		31	
Positive blood culture	1	0.4		4.6
Pneumonia [on admission]	10	32	21 [9.4]	10
Febrile seizure [on admission]	13 [9]	9	3	19
Encephalopathy / encephalitis	1.6	1.2	0	0.6
Dehydration	5	32		
Myositis				2
Myocarditis		0.4		
Death	0	0.6		
Admission to intensive care	12	12	8.2	0.6
Mechanical ventilation	5.5	6	5	
Antiviral / antibiotic use		7 / 77		NA / 61
Mean length of hospital stay (days)	5	5.3	4.6	4.3

2.4.2 Complications

The clinical burden of influenza is not limited to the viral infection alone since influenza-infected children are susceptible to bacterial complications, and other complications of the illness. In particular, acute otitis media and pneumonia represent a substantial proportion of influenza-related morbidity in children.

2.4.2.1 Outpatients

Acute otitis media (AOM) is the most common complication in the pediatric outpatient population: for young children infected with culture-confirmed influenza, AOM has been shown to develop as a complication in roughly 20-70% of influenza cases (Heikkinen et al. 1991, Neuzil et al. 2002a, Poehling et al. 2006b, Ruuskanen et al. 1989, Tsolia et al. 2006, Heinonen et al. 2010). The risk of AOM is to a great extent dependent on the age of the child, being highest in those younger than two years (Neuzil et al. 2002a, Tsolia et al. 2006, Whitley et al. 2001). Influenza viruses have been isolated from the middle ear fluid of children with AOM and are considered to play a key role in the evolution of the infection by promoting the spread of nasopharyngeal bacteria to the middle ear, and modifying host immune and inflammatory responses (Heikkinen and Chonmaitree 2003). Usually, bacteria are isolated alongside influenza viruses, implying the presence of viral-bacterial co-infection (Heikkinen et al. 1999). Besides AOM, influenza virus infection in outpatient children may predispose to sinusitis, especially in older children (Tsolia et al. 2006).

2.4.2.2 Hospitalized children

Secondary bacterial pneumonia is the most common complication of influenza infection in hospitalized children, in both previously healthy children and those with underlying conditions (**table 2**). In a large, 5-year surveillance study of virologically confirmed influenza in hospitalized children aged ≤17 years, 38% of those who had chest x-rays taken had radiologic evidence of pneumonia, with the highest frequency among children aged 6 months to 4 years (Dawood et al. 2010b). *Streptococcus pneumoniae* and *Staphylococcus aureus* are the most significant bacterial agents causing influenza-related pneumonia in the pediatric population (Juvén 2000, Dawood et al. 2010b). In a case-control study carried out in Iowa, USA, it was demonstrated that preceding infection with influenza was a risk factor for severe pneumococcal pneumonia in children (O'Brien et al. 2000). Correspondingly, during the 2009 autumn pandemic in the United States, a significant increase in pneumococcal pneumonia hospitalizations were observed among school-aged children (Weinberger et al. 2012).

The clinical signs of pneumonia attributed to influenza in children may be subtle. In a Finnish study by Lahti et al. (2006**)** half of the 134 study children with radiologically verified pneumonia presented with no apparent clinical findings suggesting pneumonia. On the other hand, compared to the admitted children without pneumonia, those with

influenza-associated pneumonia have been demonstrated to have more a severe clinical course, including intensive care unit admission, respiratory failure, and even death (Dawood et al. 2010b).

Progressive primary viral pneumonia is the most severe pulmonary complication of influenza associated with a high mortality rate. While uncommon during seasonal epidemics, it has been observed particularly in the context of the pandemics, mainly due to lack of previous exposure of the population to an antigenically related influenza virus (Kuiken and Taubenberger, 2008). Primary viral pneumonia occurs when the viral infection is extended distally to the lung, resulting in damage to the alveolar epithelium. In contrast to damage to the tracheo-bronchial epithelium in uncomplicated influenza, viral pneumonia leads to severe impairment of the gas exchange function of the respiratory tract (Kuiken and Taubenberger 2008). The clinical picture is characterized by rapid progression of fever, cough, and dyspnea, followed by the development of acute respiratory distress syndrome in the most ominous cases (Cox and Subbarao 2000). The cytokine storm is believed to play a major role in the pathogenesis of this condition (Kuiken and Taubenberger, 2008).

Involvement of the central nervous system attributable to influenza has been well documented in children, with higher frequency than in adults (Studahl 2003). Children between the ages of 2 and 4 years, and those with pre-existing neurologic or neuromuscular disease, are most often affected (Chiu et al. 2001, Newland et al. 2007). The most common neurological complication in children is febrile convulsion. In different hospital settings (Chiu et al. 2001, Peltola et al. 2003, Quach et al. 2003, Moore et al. 2006, Rojo et al. 2006, Newland et al. 2007, Frobert et al. 2011), febrile convulsions have been reported to occur in 3-20% of influenza-positive children, and it can sometimes be the first manifestation of influenza infection (Chiu et al. 2001). Further, it is estimated that up to 35-44% of all convulsions during the peak influenza season are associated with influenza virus infection (Chiu et al. 2001). In a recent prospective 2-year study from Greece, during the 14 weeks of the yearly influenza outbreak influenza could be detected in one fourth of all children admitted with febrile convulsion (Sakkou et al. 2010).

Apart from febrile convulsions, a wide spectrum of other CNS manifestations have been described in association with both seasonal and pandemic influenza, including encephalitis, acute disseminated encephalomyelitis, Guillain-Barré syndrome, Reye syndrome, transverse myelitis, and acute necrotizing encephalopathy (Newland et al. 2007, Morishima et al. 2002, Studahl 2003). These complications are rare, and usually consistent with serious sequelae or death. Reports of influenza-attributable encephalitis/encephalopathy, especially among children, increased in Japan in the late 1990s (Morishima et al. 2002); however, the same kind of phenomenon was not observed elsewhere (Toovey et al. 2008). The incidence of Reye syndrome, an acute non-inflammatory encephalopathy with fatty degeneration of the liver, has markedly decreased during the past 20 years along with the avoidance of using acetylsalicylic acid in children with febrile illness (Studahl 2003).The pathogenesis of influenza-associated

CNS complications are still not fully understood, however, indirect autoimmune reactivity, metabolic disorders, and/or genetic susceptibility have been suggested as an underlying mechanism (Studahl et al. 2003, Toovey et al. 2008).

Influenza-related myositis is a rare complication affecting typically school-aged children. It usually presents in early convalescence with an acute onset of pain and tenderness in the gastrocnemius and soleus muscles that can be severe enough to prevent walking. Serum creatine phosphokinase levels are usually transiently raised. Complete recovery generally occurs in 3-4 days. Myoglobinuria and renal failure occur extremely rarely.

Cardiac muscle damage with associated electrocardiographic changes, disturbances of rhythm, and high concentrations of cardiac enzymes, has been reported after influenza virus infection. It can occur in both healthy children and those with underlying cardiac conditions. A recent case report of fulminant myocarditis in two healthy children secondary to the 2009 pandemic influenza infection underscores the nonspecific symptoms of this rare but potentially life-threatening complication (Gross et al. 2011).

Admission to intensive care

Admissions to pediatric intensive care units (PICU) are not infrequent in children with influenza-related complications, although naturally there are country-specific differences in access to health care, as well as in criteria for intensive care hospitalization. According to an extensive American population-based study during 5 consecutive influenza seasons in 10 US states, 12% of the hospitalized children required admission to an intensive care unit, and 5% of those required mechanical ventilation (Dawood et al. 2010a). In a recent German surveillance study of severe, laboratory-confirmed pediatric influenza cases, carried out during the relatively mild influenza seasons of 2005-2008, the most frequent complications leading to PICU admission were pneumonia, secondary bacterial infection, acute respiratory distress syndrome, and encephalitis /encephalopathy (Streng et al. 2011). As shown earlier (Bhat et al. 2005, Coffin et al. 2007) – and also in the German study – children younger than one year of age and those with underlying conditions, especially cardio-respiratory and neurological conditions, were at the greatest risk of severe disease (Streng et al. 2011).

Antibiotic treatment

Influenza infection is associated with excess and, in many cases, inappropriate antibiotic use. At the community level influenza is estimated to account for 3 to 9 courses of antibiotics per 100 children annually (Neuzil et al. 2000a). Otitis media is the most frequent reason for (adequate) use of outpatient antibiotic therapy (Peltola et al. 2003, Tsolia et al. 2006). In a Greek study of influenza-positive outpatients aged 0.5 to <14 years, 40% of the children received antibiotic treatment during their illness, despite the rapid test results available for the physicians (Tsolia et al. 2006). The percentage was even higher in a recent Italian study of 901 healthy children younger than 15 years presenting to the emergency department, where more than 70% of the children with

laboratory-confirmed influenza received antibiotic treatment (Esposito et al. 2011a). In a hospital setting, nearly 40% of those receiving antimicrobial treatment for suspected secondary infection of influenza have been estimated not to have an apparent indication for the therapy (Wilkes et al. 2009). The more widespread use of rapid influenza tests early in the course of the influenza illness is one possible way to lessen the inadequate use of antibiotics in the absence of evidence for bacterial complication (Bonner et al. 2003, Poehling et al. 2006a).

2.4.3 Differences in clinical features between influenza A and B

There are scarce data on the impact of different influenza types and subtypes on the clinical illness in children. In general, children with influenza B seem to be somewhat older than those with influenza A infection (Peltola et al. 2003, Hite et al. 2007, Esposito et al. 2011a). Myositis is found to be associated with influenza B more often than with type A strains (Peltola et al. 2003, Hu et al. 2004, Chi et al. 2008, Hite et al. 2007). Some studies have also found that certain other influenza-related symptoms, like gastrointestinal complaints and leukopenia, occur more frequently in children with influenza B than with influenza A (Chi et al. 2008, Peltola et al. 2003), whereas others have not been able to demonstrate any significant differences (except for muscle aches with influenza B) in the clinical picture between the two influenza types (Daley et al. 2000, Hite et al. 2007).

Infection with the A/H3N2 subtype is conventionally associated with more severe clinical illness than that with influenza A/H1N1 or B (Frank et al. 1985, Wright et al. 1980, Simonsen et al. 1997, Esposito et al. 2011a). In a recent report by Esposito et al. (2011a), during the influenza seasons of 2007-2009 wheezing and pneumonia were observed more often in children with influenza A/H3N2 than in those with influenza A/H1N1. Furthermore, illness attributable to the A/H3N2 subtype was associated with higher hospitalization rates and an overall greater socio-economic impact than that attributable to the seasonal A/H1N1 subtype. During the study period, the A/H1N1 subtype accounted for 22% of all influenza A illnesses. There is no consensus whether it is the different innate pathogenic potential of the virus, or merely epidemiological factors like prior circulation of the certain subtype in the community, that determine the greater impact of H3N2 compared to the H1N1 subtype on the overall burden of illness (Frank et al. 1985, Esposito et al. 2011a).

2.5 Diagnosis

2.5.1 Clinical diagnosis

Early and accurate diagnosis of influenza is essential for efficient antiviral management as well as for reduction of the disease spread. However, it is often challenging to distinguish influenza from other respiratory illnesses on clinical grounds alone, even in adult populations (Call et al. 2005). In children, correct identification of influenza is

substantially hindered by the frequent co-circulation of other respiratory viruses during an influenza epidemic. In a British, community-based study of children under the age of five who were diagnosed as having an influenza-like illness, only approximately one third had the influenza virus identified from samples, whereas respiratory syncytial virus was found as the etiological agent in 35% of the cases, and the rest were caused by other viruses (Zambon et al. 2001). In an American study it was demonstrated that for children with laboratory-confirmed influenza only 28% of hospitalized children and 17% of outpatients were correctly diagnosed by their treating physicians (Poehling et al. 2006b). The clinical diagnosis of children is further complicated in those under three years of age, who cannot verbally describe their subjective symptoms, like a sore throat, headache, or muscle aches.

In a hospital-based study of 58 children with predetermined criteria suggesting influenza infection, the symptom triad of cough, headache, and pharyngitis was noted to be a predictor of influenza infection (Friedman and Attia 2004). However, fewer than half of the children enrolled in the study really had an influenza infection. The difficulty of diagnosing children as having an influenza infection during an epidemic was further demonstrated in an oseltamivir treatment trial in 221 children aged 1-4 years with ILI symptoms, where neither cough nor fever were found to be successful predictors of influenza-virus positive status (Ohmit and Monto 2006). In line with this finding, in a Finnish study of outpatient children the overall sensitivity of the clinical diagnosis of influenza made by pediatricians or pediatric residents was only 38%, and the positive predictive value 32% (Peltola et al. 2005). Even during the peak weeks of the epidemic, the sensitivity of clinical diagnosis remained below 50%, and the accuracy of influenza diagnosis was remarkably poorer during the early and the late phases of the outbreak. These results clearly show that virologic methods are requisite to optimize the treatment of both inpatient and outpatient influenza in children

2.5.1.1 Surrogate markers

Influenza is associated with transient leukopenia in adults and children (Lupovitch 2005) and, in particular, lymphocytopenia or lymphocyte levels in the low normal range are a fairly common phenomenon during uncomplicated influenza infection. In a study by Peltola et al. (2003), mild leukopenia was more commonly seen in children with influenza B than in those with influenza A. In severe cases of influenza, marked leukopenia can sometimes be seen. On the other hand, the presence of an accompanying bacterial co-infection may result in polymorphonuclear leukocytosis (Rice and Resar 1998).

During the 2009 A/H1N1 pandemic, lymphocytopenia had been observed in 60-90% of adult patients (Perez-Padilla et al. 2009, Cunha et al. 2009). Some studies have reported the same phenomenon (defined as an absolute lymphocyte count <3,000/ml) in children (Cao et al. 2009), while others have not (Cunha et al. 2009). In a recent study from Italy (Chiappini et al. 2011), laboratory data from 37 children with A/H1N1 were compared

with 39 matched controls, and lymphocytopenia was observed significantly more often among A/H1N1–positive patients than among controls.

Serum C-reactive protein (CRP) is usually normal or only slightly elevated in uncomplicated influenza infection in children (Ruuskanen et al. 1985, Peltola et al. 2006). Peltola et al. found CRP-values of >40 mg/l in only 8% of children without acute otitis media or pneumonia (Peltola et al. 2003). Similarly, in a Spanish study of hospitalized children under 3 years of age with influenza (Rojo et al. 2006), in only 8 children out of 57 (14%) CRP was ≥80 mg/l. CRP may thus be helpful in the detection of bacterial co-infection in influenza-positive children (Peltola et al. 2003).

2.5.2 Microbiological diagnosis

Due to the nonspecific signs and symptoms of pediatric influenza, and the high frequency of various other respiratory pathogens during an influenza epidemic (Heikkinen et al. 2003, Zambon et al. 2001), virologic methods are required for definitive diagnosis to enable optimal patient care. There are several modalities to document influenza virus infections, which fall into four broad categories: virus isolation, detection of viral proteins, detection of viral nucleic acid, and serological diagnosis. The choice among these tests is dependent on the use and answers sought. Influenza test results are influenced by the level of influenza activity in the population being tested (i.e., the prevalence), the characteristics of a test compared to a gold standard, the clinical picture of the patient, and the sample collection and transport methods (Uyeki 2003).

The sampling methods most commonly used to detect influenza viruses include a nasopharyngeal aspirate (NPA) and a nasal swab. The sensitivity of nasal swabs has been demonstrated to be comparable to that of NPA (Heikkinen et al. 2002), and as the procedure for a nasal swab is easier and more comfortable that an NPA, it is a favorable method for sampling in most circumstances (Heikkinen et al. 2001). In general, the influenza virus can be isolated from samples obtained within 5 days of the onset of the illness (Harper et al. 2009); however, in children the time frame is usually longer due to the longer duration of viral shedding (Hall et al. 1979, Frank et al. 1981).

2.5.2.1 Viral culture

Viral isolation has been considered as the “gold standard” for detection of infection with human influenza viruses (Takahashi et al. 2010). Influenza viruses can be isolated in 10 to 11-day-old embryonated hens’ eggs, and in various primary, diploid, and continuous cell cultures. While the traditional viral culture takes 4-10 days to yield the result, the rapid culture assays that detect viruses in cultured cells by immunological techniques (specific monoclonal antibodies) allow the detection of viral antigens in 1-2 days (Espy et al. 1986, Waris et al. 1990). Although viral culture does not provide timely results, it is essential as a source of virologic data on strain characteristics, such as antigenic comparison to influenza vaccine strains, and antiviral susceptibility, that are important

for clinicians and public health. Moreover, viral culture is helpful for identifying influenza virus infection when other screening tests yield false-negative results, and as confirmation of a subset of negative rapid influenza test results, particularly in the context of an institutional outbreak (Harper et al. 2009). Characterization and detailed analyses of influenza viruses isolated during out-of-season activity are particularly important for surveillance purposes as it allows for the monitoring of antigenic drifts and shifts.

2.5.2.2 Antigen detection

Immunofluorescence (IF) microscopy, either direct or indirect antibody staining (of exfoliated nasal epithelial cells) for influenza antigen detection, is mainly used as a screening test (Harper et al. 2009). IF exhibits moderately high sensitivity (>80%) and high specificity in children (Doing et al. 1998, Spada et al. 1991), compared with viral culture, but requires good specimen collection techniques, a fluorescent microscope, and a trained clinical laboratory scientist (Uyeki 2003). Influenza antigens can also be detected in respiratory secretions by various immunological techniques, including enzyme, membrane- and fluoroimmunoassays. One-incubation time-resolved fluoroimmunoassay has been shown to be a faster and simpler method than the traditional immunofluorescence microscopy, and offers one option for diagnostic panel (Nikkari et al. 1989).

There are several rapid antigen detection tests commercially available that allow for diagnosis at the point of care, and in which the primary characteristics, sensitivity and specificity, are generally similar. Most of the tests detect both influenza A and B viruses, and some are able to distinguish between them. Generally, these tests are specific (95-100%), but sensitivity is modest in adults, ranging from approximately 20% to 65% (Uyeki et al. 2009, Rouleau et al. 2009, Hurt et al. 2007). Due to the higher viral loads in children, the sensitivities are clearly better in the pediatric population, ranging from approximately 63% to 85% (Grijalva et al. 2007, Heinonen et al. 2011b, Agoritsas et al. 2006). There are also differences with regard to influenza type; sensitivities being significantly lower with influenza B than for influenza A (Hurt et al. 2007, Heinonen et al. 2011b). In a recent Finnish study of children aged 1-3 years of age, the sensitivity of a rapid test for detecting influenza within 24 hours from the onset of illness was 90% for influenza A, but only 25% for influenza B (Heinonen et al. 2011b).

The major advantages of making a rapid diagnosis of influenza include reductions in inaccurate antibiotic use, in length of stay (both in the emergency department and in hospital), and in overall costs due to fewer diagnostic investigations needed (Bonner et al. 2003, Poehling et al. 2006a, Woo et al. 1997). Accurate diagnosis at an early stage of the illness is also essential for the rational use of antiviral drugs (Hedrick et al. 2000, Whitley et al. 2001, Heinonen et al. 2010).

2.5.2.3 Polymerase chain reaction (PCR)

Reverse-transcriptase polymerase chain reaction (RT-PCR) is currently the most sensitive testing modalities for influenza with results available within a few hours after specimen submission. Either clinical or cell culture specimens can be used. RT-PCR has a sensitivity approaching 100%, which is superior to that of cultures (Weinberg et al. 2004, Zitterkopf et al. 2006); therefore, the use of viral culture as the gold standard for sensitivity may be outdated (McGeer 2009). RT-PCR may be used as a confirmatory test, and it is useful for quickly differentiating between influenza types and subtypes. RT-PCR is also the preferred test for specimens obtained from persons with a history of exposure to animals with possible influenza illnesses (Harper et al. 2009). Along with the improvement in more automated techniques, RT-PCR is today largely available for clinical use.

2.5.2.4 Serology

Influenza viruses cause agglutination of erythrocytes due to the capacity of the viral HA to bind to sialic acid residues on the red blood cell surface. Anti-HA antibodies interfere with this process and the hemagglutination-inhibition test is based on these properties (Wilschut et al. 2006). A four-fold rise or greater increase in hemagglutination-inhibition antibodies is indicative of infection. Other serologic tests include enzyme-linked immunosorbent assay, complement-fixation, and neutralization tests. All these tests require paired acute- and convalescent-phase serum samples, and therefore are not helpful in acute clinical management. Paired serum specimens are only useful for retrospective diagnosis and for research purposes, for example in assessing the response to influenza vaccination (Harper et al. 2009).

2.6 Prevention and control

2.6.1 Vaccination

Influenza vaccines are the mainstay of efforts to reduce the health burden from seasonal influenza. Inactivated influenza vaccines have been available since the 1940s (Salk and Pearson 1945) and are administered via intramuscular injection. Live attenuated, cold-adapted influenza vaccines (LAIV) were developed in the 1960s (Beare et al. 1969), but were not licensed until 2003, and are administered via nasal spray. Both vaccines are trivalent preparations generally produced from viruses grown on embryonated eggs.

Due to the extensive capacity of the influenza virus to evolve, and thus evade the immune response, the composition of the influenza vaccine has to be updated annually to provide vaccines that are antigenically well matched to the influenza virus strains that are expected to cause epidemics in the subsequent season. Each year, in September in the Southern hemisphere and in February in the Northern hemisphere, the WHO predicts

which influenza viruses will be prevalent in different regions, based on data from the Global Influenza Surveillance Network. These forecasts are then used to select strains for influenza vaccine manufacture (Belshe 2010). Current influenza vaccines are trivalent, containing representative influenza A/H3N2, A/H1N1, and B viruses (either Yamagata or Victoria lineage). However, due to frequent mismatching of influenza vaccine compositions with circulating influenza B viruses, a quadrivalent influenza vaccine containing both lineage strains of the influenza B virus has been under investigation by several manufacturers, and recently the first quadrivalent LAIV was approved by FDA for subjects aged 2 to 49 years for prevention of seasonal influenza (FDA 2012).

Influenza vaccination induces antibodies primarily against the major surface glycoproteins hemagglutinin and neuraminidase. Antibodies directed against the HA are most important for protection against illness, whereas antibodies directed against the NA may reduce the severity of the disease (Gerhard 2001). Antibodies against one subtype of influenza A confer little or no protection against different subtypes. Similarly, immunity against viruses from one of the two B influenza virus lineages provides little, if any, protection against the other. The immune response peaks at 2-4 weeks after one dose in primed individuals (Brokstad et al. 1995). In previously unvaccinated children, ≤9 years of age, two doses of the influenza vaccine are recommended (with the doses separated by four weeks), as some children in this age group have had limited or no prior infections from circulating types and subtypes of seasonal influenza. Serum antibody titers based on hemagglutination inhibition testing (HI) generally correlate with protection against influenza, and HI titers of 1:32 to 1:40 are often used as benchmarks for an adequate immune response to inactivated influenza vaccine (Monto et al. 1970, Demicheli et al. 2000).

In Finland, influenza vaccination is recommended and offered free of charge for adults and children with high-risk conditions including chronic cardiac, pulmonary, renal, neurologic, or metabolic disease; immunosuppression; or those receiving long-term salicylate treatment. Additionally, yearly immunizations are recommended, and offered free of charge, for elderly people ≥65 years of age, health care workers, pregnant women (starting from the influenza season 2010-11) (THL 2011), and since the autumn of 2007 to healthy children aged 6 to 35 months (Heikkinen et al. 2006).

In the European Union, only six countries officially recommend influenza vaccination for healthy children from the age of six months to two or three years (Mereckiene et al. 2010). However, at the moment Finland is the only European country in which the vaccination is included in the routine, fully reimbursed, childhood vaccination program (Heikkinen and Heinonen 2011).

In the United States, influenza vaccination of healthy children aged 6 to 23 months was officially recommended for the first time in 2003 and, starting from the 2009-2010 season, the recommendation has expanded to “universal”, to include all children aged 6 months and older (Fiore et al. 2008). In Ontario, Canada, universal vaccination was

already implemented in 2000, and has resulted in higher rates of vaccination, particularly among those less than 65 years of age (Kwong et al. 2008).

The cost effectiveness of influenza vaccination in children has been shown to be comparable with, or better than, several currently recommended pediatric vaccinations (Salo et al. 2006, Nichol 2011). In a recent 8-year study of the effectiveness of TIV against virologically confirmed influenza infection in children under 5 years of age, it was calculated that 3-11 children should be vaccinated to prevent one medically attended influenza illness, when the attack rate varies between 10 and 40% (Joshi et al. 2009). In a Finnish cost-effectiveness analysis it was demonstrated that investing 1.7 million euros in the vaccination of children <5 years of age produced savings of 2.7 million euros in health care costs alone (Salo et al. 2006). The vaccination was calculated to be cost saving in all age groups, even with an assumed vaccine efficacy of 60%. Inclusion of so called indirect effects of childhood vaccinations (e.g. reduced morbidity and mortality of the elderly population) in the model would additionally increase the total saving achieved (Salo et al. 2006).

2.6.1.1 Trivalent inactivated influenza vaccine (TIV)

The original influenza vaccines were formalin-inactivated whole-virus vaccines, and these highly immunogenic vaccines are still in use in some countries. However, the enhanced reactogenicity of whole-virus vaccines, especially in young children, led to the development of split virion vaccines in the 1960s, which are vaccines derived by disrupting whole virus particles with disinfectants, and thus better tolerated by children receiving the vaccine for the first time. The third form of inactivated influenza vaccines is a subunit form, which is prepared by enriching for the glycoproteins HA and NA following disruption of viral particles. Subunit vaccines represent the most highly purified vaccines, yet they are equally immunogenic in primed individuals (Wilschut et al. 2006).

Trivalent inactivated influenza vaccines (TIVs) are licensed for use in children aged six months and older. In most countries, doses recommended for inactivated influenza vaccines are 15 μg of HA for each vaccine strain for subjects ≥3 years of age, and 7.5 μg of HA per vaccine dose for children aged 6-35 months. However in Finland, all children ≥6 months of age are currently recommended to receive the full dose (THL 2011). As young children, especially those under two years of age, are generally considered to be poor responders to inactivated influenza vaccines (Jefferson et al. 2008), they could be assumed to benefit from an increased dose. In line with this assumption, a recent randomized, controlled study of dose-response to influenza vaccination among children aged 6 months to <2 years showed that administration of two full doses (0.5 ml, instead of two times 0.25 ml) improved immunogenicity without increasing reactogenicity in infants (Skowronski et al. 2011).

More than by the age of the recipient, the effectiveness of the inactivated influenza vaccine is affected by immune competence of the vaccinee and the antigenic relatedness of vaccine strains to circulating strains (Fiore et al. 2009, Heinonen et al. 2011a). In years with a suboptimal match, vaccine benefit is likely to be lower, even though the vaccine can still provide substantial benefit in most years, notably against more severe outcomes (Ritzwoller et al. 2005, Eisenberg et al. 2008). It is also important to recognize that influenza illness rates vary substantially from year to year and, in years with low attack rates, the power of smaller studies to detect vaccine effectiveness may be compromised, even when using laboratory-confirmed outcomes (Hoberman et al. 2003, Shuler et al. 2007, Szilagyi et al. 2008).

In a recent analysis by Heikkinen and Heinonen (2011), ten effectiveness studies including children younger than 5 years of age with virologically verified influenza were analyzed. In that report, during seasons with a good antigenic match between the virus and vaccine strain, the effectiveness of the vaccine against the influenza A virus was relatively good in this age group, ranging from 52% to 86%. In four studies out of ten, the effectiveness of the vaccine against influenza A was over 80%. In three studies included in the analysis, in which the effectiveness data were analyzed separately according to influenza type, the effectiveness of the vaccine was clearly poorer against influenza B, ranging from 43%-59%. This result can partly be explained by the mismatch between the vaccine and the circulating influenza strains (Heikkinen and Heinonen 2011). However, it has been demonstrated earlier that the immunogenicity of influenza B antigens in TIV is lower than that of influenza A vaccine antigens, as also seen in a lower vaccine-induced antibody response to vaccination (Englund et al. 2005, Vesikari et al. 2009).

Given the fact that the heaviest burden due to seasonal influenza is among infants and young children, it is worth noticing that there is remarkable paucity of effectiveness studies of TIV in children under two (Jefferson et al. 2008); only two studies have been conducted in that age group, a two-year follow-up study from the US (Hoberman et al. 2003) and a one-year study from Finland (Heinonen et al. 2011a). Both studies reported the overall effectiveness of 66% of the vaccine against (any) influenza during a regular influenza season in children aged 6 months to <2 years. However, in the US study, no vaccine effectiveness in the second season could be demonstrated due to exceptionally low influenza activity.

Existing evidence of traditional trivalent inactivated vaccines shows that these vaccines are generally well tolerated. Mild systemic symptoms like fever, irritability, and malaise have been reported in 4-16% of the children, usually after the first exposure to the viral antigens as part of the vaccine (Ruben 2004, Muhammad et al. 2010). Serious adverse reactions following seasonal influenza vaccination are extremely rare (Muhammad et al. 2010). As influenza vaccines may contain trace amounts of residual egg protein as a result of production system on embryonated chickens eggs, persons with a history of anaphylaxis to eggs should not receive either TIV or LAIV. Importantly, there is

no evidence for a causal relationship between TIV and demyelinating disease such as Guillain-Barré syndrome in children (Meissner 2007).

Adjuvants have been developed to improve the performance of vaccines, and MF59, an oil-in-water emulsion containing naturally occurring squalene, has been approved for human use since 1997 as an influenza subunit vaccine adjuvant for elderly adults (Schultze et al. 2008). Recent clinical studies have demonstrated that MF59-adjuvanted influenza vaccines induce higher and broader antibody responses than nonadjuvanted vaccines, especially in subjects with low prevaccination antibody titers, like young children (Vesikari et al. 2009, Vesikari et al. 2011, Esposito et al. 2011b). AS03 is similar to MF59, but in addition to squalene it also contains tochoferol (Waddington et al. 2010). Like MF59 it has been shown to increase the immunogenicity of an inactivated influenza vaccine, especially in young children, although at the expense of a slightly increased reactogenicity (Waddington et al. 2010). The clinical experience of using AS03 in children is limited to its use in the monovalent 2009 pandemic A/H1N1 vaccine. So far, neither MF59 nor AS03-adjuvanted vaccines are licensed for use in children.

In August 2010, reports of sudden onset narcolepsy cases in children and adolescents following H1N1 vaccinations were reported in Finland, raising concerns over the possible association between narcolepsy and the AS03 adjuvanted pandemic vaccine. Similar reports soon also emerged from Sweden, leading to recommended discontinuation of this vaccine in these countries, and a review of this vaccine within the EMA (EMA 2010a, Läkemedelsverket 2011). Slightly increased occurrences of narcolepsy cases following the winter of 2009-2010, and after H1N1 vaccination, were subsequently reported in the US, Canada, and France (Dauvilliers et al. 2010). A recent study from China found a 3-fold increase in narcolepsy onset following the 2009 pandemic; the correlation, however, being independent of H1N1 vaccination (Han et al. 2011). By August 15, 2010, 67 new confirmed cases of narcolepsy in children and adolescents aged 4 to 19 years had been diagnosed in Finland (Nohynek et al. 2012). In a recent Finnish study, it was confirmed that there was a link between the vaccine and narcolepsy, with subjects aged 4-19 years having a 12.7-fold risk of developing narcolepsy when compared to unvaccinated children in the same age group (Nohynek et al. 2012). No similar risk was observed among adults or children younger than 4 years of age. Narcolepsy is considered an immune-mediated illness and, interestingly, antibodies against the AS03 adjuvant component of the vaccine were detected in one quarter of the symptomatic children. Furthermore, all affected children were demonstrated to carry a genetic risk factor for the disease. In spite of these findings, the exact mechanisms for the development of narcolepsy in the vaccinated children and adolescents remain unsolved. Currently, extended epidemiological assessments of the association between narcolepsy and pandemic H1N1 influenza vaccinations are underway (Kurz et al. 2011).

Passive protection of infants by maternal immunization

Pregnant women, as well as young infants, are at a considerably higher risk of illness and hospitalization attributable to influenza infection (Dodds et al. 2007, Neuzil et al. 1998). Therefore, immunization of pregnant women before the influenza season is now widely recommended. Since there is not a licensed influenza vaccine available for infants under 6 months of age, vaccination of pregnant women, while protecting themselves against influenza illness and its consequences, offers a way to protect the neonates and infants via IgG antibodies of maternal origin (Englund 2003). Passively transferred maternal antibodies have been shown to protect the baby during the first months of life, a period of increased vulnerability to influenza and its complications (Zaman et al. 2008). Poehling et al. (2011) reported that infants younger than 6 months of age hospitalized with respiratory symptoms and/or fever, whose mothers had received TIV during pregnancy, were 45-48% less likely to have laboratory-confirmed influenza compared with infants of unvaccinated mothers. A recent study from Italy of 69 mother-infant pairs demonstrated that the immunization of pregnant women with the 2009 pandemic A/H1N1 MF59-adjuvanted influenza vaccine during the last trimester was able to induce protective antibody titers in both maternal and newborn samples, with protective antibody levels persisting in most infants for at least 5 months (Zuccotti et al. 2010).

The lack of harmful events resulting from influenza vaccination for both maternal and newborn health during and after pregnancy has been demonstrated in several large, longitudinal studies (Tamma et al. 2009). In line with earlier studies, recent results from 20-year and 7-year surveillance studies of adverse events attributable to the administration of TIV and LAIV, respectively, to pregnant women confirm that these vaccines are safe, with no unusual patterns of pregnancy complications or fetal outcomes (Moro et al. 2011). Nevertheless, the rates of influenza vaccination have remained low in most countries. Enhanced education of health care workers about the safety and efficacy of influenza vaccination of pregnant women is considered to be the key factor in increasing the rates of influenza immunization in pregnancy (Tamma et al. 2009).

Vaccination of infants under 6 months of age

Although not commercially available, TIV has been successfully administered in infants aged 6 weeks to 3-5 months of age in four different clinical trials (Groothuis et al. 1991, Halasa et al. 2008, Walter et al. 2009, Englund et al. 2010). In these studies, the safety and reactogenicity profiles of the vaccine have shown to be consistent with reports of older infants and children (Ruben 2004). The influenza vaccine in this age category is moderately immunogenic, with higher post-vaccination seroprotection rates in infants without pre-existing (maternal) antibodies (Halasa et al. 2008, Walter et al. 2009). However, the level of protective antibody concentrations in infants is not known. Moreover, the immaturity of the immune system of young infants is likely to impact the ability to receive adequate protection against influenza infection (Englund et al. 2010). Whether vaccination against influenza can still prime infants in the presence of increased concentrations of maternal antibodies remains to be shown (Englund et al. 2010).

2.6.1.2 Live attenuated (cold-adapted) influenza vaccine (LAIV)

In contrast to TIV, intranasally administered LAIV induces an immune response through viral replication, and is assumed to mimic wild-type virus infection. In addition to eliciting an antibody response in the serum, live intranasal vaccines also induce local IgA production in the nasal mucosa (Johnson et al. 1986). A live influenza vaccine was broadly used in the former Soviet Union (Kendal 1997). In the 1960s, a research program was set in motion in the US to develop LAIVs; however, it was not until 2003 that the approval for the first LAIV was gained in the United States where it is currently indicated for children and adults aged 2-49 years. LAIV has also been recently approved in Europe for subjects 2 to <18 years of age, and is expected to become commercially available in several European countries in the autumn of 2012 (EMA 2010b).

LAIVs are made by the reassortment of a temperature-sensitive, cold-adapted parent virus with the actual vaccine virus strain (Wilschut et al. 2006). The cold-adapted virus replicates at the reduced temperatures of the upper respiratory track but in the lungs, at 37°C, replication is inhibited. As a result, the virus loses its virulence in the human host and does not cause clinical influenza. LAIV is easier to administer than TIV and thus has better acceptability among young children than intramuscular influenza vaccines.

Several large, randomized clinical trials in children have demonstrated that LAIV is highly effective in preventing culture-confirmed influenza compared with both placebo and with TIV, with protective efficacy exceeding 90% (Belshe et al. 1998, Belshe et al. 2007, Bracco Neto et al. 2009, Vesikari et al. 2006, Ashkenazi et al. 2006). In a recent meta-analysis of nine placebo-controlled studies (Rhorer et al. 2009), the efficacy of two doses of LAIV against antigenically similar strains in previously unvaccinated children was 77%, and the mean efficacy of one dose in previously vaccinated children was 87%. In contrast to TIV, LAIV has also shown to have efficacy against B viruses of a lineage not matching the vaccine strain, the estimated efficacy being approximately 30% (Belshe et al. 2010). Furthermore, a single dose of LAIV has been shown to provide better cross-protection than TIV in children exposed to new variants (Halloran et al. 2007, Glezen et al. 2010).

LAIV has been shown to reduce the severity of breakthrough illness despite vaccination in children in terms of less febrile illnesses and fewer days of missed daycare or school compared with TIV (Ashkenazi et al. 2006). Consistently, a recent pooled analysis of multiple vaccine efficacy studies conducted in children aged between 6 months to <7 years demonstrated that LAIV recipients who contracted a breakthrough influenza illness regardless of vaccination developed AOM at a significantly lower rate than unvaccinated children who got influenza (Block et al. 2011).

Live attenuated influenza vaccines have been found to be as safe as inactivated influenza vaccines in children, the most common adverse events reported being runny nose, headache, tiredness, and decreased activity (Belshe et al. 1998, Vesikari et al. 2006).

However, in infants and toddlers under the age of 2 years, an increase in wheezing or reactive airway disease occurred in association with live attenuated influenza vaccines. Therefore, the use has been restricted to children over 2 years, and not recommended for children between 2 and 4 years with asthma or with recurrent wheezing (Belshe et al. 2007, Fiore et al. 2009).

2.6.2 Antiviral prophylaxis

While vaccination is the method of choice for influenza prophylaxis, under specific conditions where a person has not been or cannot be vaccinated, or is not fully protected by vaccination, the use of antiviral drugs should be considered for the prevention of influenza infection. Antiviral chemoprophylaxis can be divided into seasonal and post-exposure prophylaxis (PEP). The purpose of PEP is to prevent the development of influenza illness after exposure, thereby decreasing the spread of the virus within the community and, especially, in the family. Seasonal prophylaxis means a long-term use of the antiviral drug throughout the influenza season, usually in patients with serious underlying conditions that place them at an increased risk for influenza-related complications. Both neuraminidase inhibitors (NIs) oseltamivir and zanamivir can be used for seasonal as well as post-exposure prophylaxis.

The efficacy of oseltamivir as PEP in households was examined in a prospective, open-label study of households involving adults and children aged ≥ 1 year with 298 index influenza cases and 812 contacts. Households were randomized to receive 5 days of oseltamivir treatment in the case of clinical illness, or PEP for 10 days, commencing within 48 hours of the symptom onset of the index case. All index cases received a 5-day oseltamivir treatment. PEP with oseltamivir reduced the rate of secondary influenza infections by 84.5% (Hayden et al. 2004). When pediatric contacts aged 1-12 years were analyzed separately, the incidence of laboratory-confirmed influenza was reduced by 80.1%. The efficacy of oseltamivir in seasonal prophylaxis against laboratory-proven influenza was demonstrated in a six-week study of low-risk adults, with a 76% protective efficacy of oseltamivir 75 mg once daily compared to placebo (Hayden et al. 1999).

Zanamivir was shown to be 67% effective against virologically confirmed influenza when used for seasonal prophylaxis in healthy adults (Monto et al. 1999), and 79% effective when used in adults and children ≥5 years of age after household exposure, irrespective of concurrent treatment given to the ill index case patients (Hayden et al. 2000).

2.6.3 Non-pharmaceutical interventions

Non-pharmaceutical interventions include different physical means to reduce the spread of the influenza virus. The preventive methods may be focused on aerosol or large droplet spread (such as using masks and distancing measures) or contact spread (such as by

using hand washing, alcohol-based hand-rub preparations, and gloves). These kinds of public health measures were already broadly adopted during the "Spanish flu" of 1918-1919 (Bootsma and Ferguson 2007). The value of non-pharmaceutical prophylaxis is stressed during pandemics, when they can be instituted rapidly and may be independent of any specific type of novel virus; however, to some degree, these interventions can also be implemented during seasonal epidemics.

Although the benefits of the commonly used physical preventive methods seem self-evident, relatively scarce data exists on the efficacy of these measures. Grayson et al. (2009) demonstrated that simple hand washing with soap and water, as well as the use of alcohol-based hand rubs, effectively removed influenza viruses from hands, thereby preventing virus transmission from human to human. Similarly, in a recent study from Thailand it was demonstrated that in households with children increased hand washing was relatively efficient in reducing influenza contamination in households with secondary influenza infections (Simmerman et al. 2010).

Cluster randomized controlled trials from Hong Kong (Cowling et al. 2009) and Germany (Suess et al. 2012) have shown that when implemented early (within 36 hours after the symptom onset of an influenza illness) and used diligently, the use of facemasks and intensified hand hygiene can reduce household transmission of influenza, compared to placebo. According to the latest Cochrane review on the subject, both surgical and N95 respirators appear equally effective in preventing influenza dissemination from infected individuals. However, the higher cost and discomfort of N95 masks reduce their usefulness at a community level (Jefferson et al. 2011).

During a pandemic, a broad range of other mitigation strategies can be applied in combination with those used on an individual level. Pandemic preparedness plans include both voluntary and imposed changes in social patterns like school or work place closure, travel restrictions, case isolation, household quarantine, and border screening. Mitigation may simply delay the pandemic burden, or distribute it over longer time periods, thus alleviating the burden on the healthcare system and buying time for antivirals and vaccines to become available (Chowell et al. 2011). Following the emergence of the recent A/H1N1 2009 pandemic, school closure was implemented as an early containment intervention in many countries. The basis for recommendations for school closures is the evidence that school children are the main channel through which influenza is introduced into the community (Glezen and Couch 1978). In general, school closure periods were shown to be associated with a significant reduction in the ratio of school age to other cases, referring to a substantial transmission reduction (Chowell et al. 2011). Border restrictions as well as internal travel restrictions have been calculated to delay the spread of a pandemic influenza for as little as 2-3 weeks, unless more than 99% effective (Ferguson et al. 2006). Household quarantine may be effective at reducing attack rates in the community in case the compliance is high (Ferguson et al. 2006).

2.7 Antiviral treatment

Influenza-specific antiviral drugs are an important supplement in reducing the burden of influenza among children. During an influenza outbreak, antiviral treatment can prevent influenza illness, lessen symptoms, and prevent complications. Two classes of antiviral agents are currently licensed worldwide for the treatment of influenza: neuraminidase inhibitors (NIs), oseltamivir and zanamivir, and adamantanes amantadine and rimantadine. However, since all circulating influenza A viruses are currently resistant to adamantanes, they are not recommended for the treatment of influenza infections in the present situation (Garg et al. 2012). Recently, two new NIs, intravenous peramivir and inhaled laninamivir, have been approved in North Asia but are investigational elsewhere (Van der Vries et al. 2011). Furthermore, there are also investigational intravenous formulations of oseltamivir and zanamivir.

2.7.1 Adamantanes

Adamantanes (amantadine and rimantadine) were the first generation of influenza antivirals, and have been available since the 1970s and 1990s, respectively. They act by blocking the activity of the viral matrix 2 (M2) proton channel, thus preventing virus uncoating, and inhibiting the release of the viral genome into host cells. They are only effective against influenza A viruses and are associated with several toxic effects, particularly of the central nervous system (such as dizziness, anxiety, insomnia), and with rapid emergence of drug-resistant variants. Adamantane-resistant isolates of influenza A are genetically stable and can be transmitted from person to person (Moscona 2008). Despite a number of studies showing positive effects of amantadine in reducing fever and duration of illness symptoms in children and adults, no placebo-controlled studies of amantadine for treatment of influenza A exclusively among children exist (Uyeki 2003). Neither of the two M2 ion channel blockers is currently available in Finland.

2.7.2 Neuraminidase inhibitors

Neuraminidase is an enzyme that breaks down sialic acid on the host cell surface and mediates the release of newly formed virus particles from the surface of infected cells. Additionally, it facilitates viral invasion of the upper airways by cleaving the sialic acid moieties on the mucin that bathes the airway epithelial cells. The neuraminidase inhibitors are substrate analogues of sialic acid, which inhibit the enzymatic activity of NA, thus interfering with the release of progeny viruses from infected cells. The active enzyme site of influenza NAs is highly conserved, rendering neuraminidase inhibitors active against both influenza A and B.

Neuraminidase inhibitors zanamivir and oseltamivir were first approved by the FDA in 1999, and by European regulatory authorities in 1999 and 2002, respectively. Currently in Finland, zanamivir is indicated for the treatment and chemoprophylaxis of influenza A and B in subjects ≥5 years of age, and oseltamivir for those ≥1 years of age. During the

2009 pandemic, the FDA and EMA temporarily expanded the indication of oseltamivir under the Emergency Use Authorization called EUA to also include infants <1 year of age (FDA 2009).

Zanamivir is only available in dry powder inhalation, which limits its use for young children. Zanamivir is highly concentrated in the respiratory tract, and its bioavailability is low (Moscona 2005). Oseltamivir is available as a capsule or powder for oral suspension. It is administered as oseltamivir phosphate, an inactive prodrug which is metabolized in the liver to its active form oseltamivir carboxylate. The drug has high plasma levels and can thus act outside the respiratory tract (Moscona 2005). Although not officially approved by the authorities, intravenous zanamivir and oseltamivir have been approved for compassionate use to treat critically ill patients (EMA 2010c, EMA 2011).

The replication of influenza viruses peaks at 24–72 hours after the onset of symptoms, and the viral load correlates positively with the severity of symptoms (Moscona 2005). Due to the mechanism of action of NIs, early administration of these drugs is essential for a good clinical effect. Both zanamivir and oseltamivir treatments are advised to commence no later than 48 hours from the onset of symptoms (Fiore et al. 2011).

In children, zanamivir has been studied in one placebo-controlled study (Hedrick et al. 2000). In that study, zanamivir administered within 36 hours from the onset of illness was demonstrated to reduce the median time to symptom abatement by 1.25 days in the age group of 5-12 years.

Even though oseltamivir is currently the only recommended drug for treating influenza in children younger than 5 years of age, there are only two randomized controlled trials on the efficacy of oseltamivir in this age group. In the pivotal study by Whitley et al. (2001), treatment started within 48 hours of symptom onset shortened the median duration of illness by 1.5 days in children aged 1-12 years. Viral shedding was also reduced in the oseltamivir recipients by day 4, potentially limiting the spread of the influenza virus. In a recent Finnish study of children aged 1-3 years, who were treated with oseltamivir within 24 hours of the onset of symptoms, the corresponding shortening of symptoms was 3.5 days among children with influenza A (Heinonen et al. 2010). Moreover, early oseltamivir treatment was associated with a 3-day reduction in parental absence from work among children with influenza A.

In addition to treating and preventing influenza infection per se, oseltamivir has been demonstrated to lower the incidence of bacterial complications from influenza infection in pediatric patients. The incidence of otitis media was shown to decrease by 44-85% when oseltamivir was administered within 48 and 24 hours of the onset of symptoms, respectively (Whitley et al. 2001, Heinonen et al. 2010). In a retrospective study of inpatient children aged 1-12 years, with oseltamivir treatment initiated within one day of influenza diagnosis, the risk of pneumonia was 52% lower when compared with non-

recipients (Barr et al. 2007). Oseltamivir therapy has been demonstrated to significantly improve the outcomes of influenza-infected children with chronic medical conditions (Piedra et al. 2009). Correspondingly, observational data from the 2009 A/H1N1 pandemic suggests that children with the highest risk of complications from influenza clearly benefit from early antiviral therapy (Farias et al. 2010).

In a recent Finnish study, no efficacy of oseltamivir was demonstrated against influenza B infections (Heinonen et al. 2010). Similar reports on the reduced sensitivity of influenza B viruses have been published previously (Sugaya et al. 2007, Hatakeyama et al. 2007). The suggested explanations for this phenomenon have included the baseline higher mean inhibitory concentration of oseltamivir to influenza B, and/or the emergence of oseltamivir-resistant influenza B strains (Sheu et al. 2008, Hatakeyama et al. 2007).

Oseltamivir and zanamivir are generally well tolerated among children. The most common adverse events in children taking oseltamivir include vomiting and mild abdominal discomfort (Whitley et al. 2001, Heinonen et al. 2010). Retrospective studies of off-label use of oseltamivir in infants younger than one year of age have shown that oseltamivir treatment is also safe and effective in this age group (Tamura et al. 2005, Kimberlin et al. 2010). Even though transient neuropsychiatric events have been reported in postmarketing surveillance among adolescents treated with oseltamivir, a causal link has not been shown, and these events are more likely to be related to the influenza infection itself (Toovey et al. 2008). Zanamivir can, rarely, induce bronchospasm and is therefore not recommended as treatment for patients with asthma or chronic obstructive pulmonary disease (Fiore et al. 2011).

Peramivir and laninamivir are new, long-acting neuraminidase inhibitors that are currently under investigation in many countries. However, in Japan and South Korea, peramivir has already been approved for the treatment of influenza A and B infections for adults and children aged ≥1 month. Peramivir is administered as a single intravenous dose (15 min infusion). During the 2009 pandemic, The FDA approved the usage of intravenous peramivir in hospitalized adults and children under the emergency use authorization application (Birnkrant and Cox 2009). Laninamivir is a long-acting multimeric compound of zanamivir, and has been approved for use in Japan since 2010 (Sugaya 2011). Laninamivir is administered in the form of a single inhalation. In children infected with the oseltamivir-resistant seasonal influenza A/H1N1 virus with H274Y mutation, laninamivir has been shown to significantly reduce the duration of influenza illness compared to those treated with oseltamivir (Sugaya and Ohashi 2010). No viruses resistant to laninamivir have been reported so far (Sugaya 2011).

2.7.3 Resistance

In general, the resistance of influenza viruses occurs readily with M2 inhibitors: 30% of treated patients shed resistant viruses within three days of treatment (Moscona 2008). In the the beginning of the 21st century, the number of adamantane-resistant

A/H3N2 strains started to increase rapidly; thus, by the year 2007 these drugs were no longer recommended as a single agent for the treatment of influenza. Interestingly, the oseltamivir-resistant seasonal A/H1N1 strain with H274Y neuraminidase mutation that emerged in 2008 stayed susceptible to adamantanes; nevertheless, it was quickly replaced by the 2009 pandemic A/H1N1 strain, which was inherently resistant to the adamantanes. By the 2010-2011 influenza season virtually all A/H1N1 and A/H3N2 strains were resistant to amantadine and rimantadine (Garg et al. 2012).

Universally, the incidence of neuraminidase inhibitor-resistant influenza viruses has been very low in untreated individuals, with little evidence of onward transmission of resistant viruses (Sheu et al. 2008). In Japan, where NIs are more widely used than in other countries, oseltamivir-resistant viruses emerged in 18% of treated Japanese children with influenza virus A (H3N2) infection, and 16% of those with A (H1N1) infection, also with no evidence that these viruses transmitted efficiently (Kiso et al. 2004). However, during the 2007-2008 A/H1N1 epidemic, oseltamivir-resistant influenza A/H1N1 strains with H274Y mutations in the neuraminidase emerged unexpectedly in many European countries, despite relatively low antiviral drug use (Meijer et al. 2009). During that season in Europe approximately 20% of the A/H1N1 viruses tested showed resistance to oseltamivir, the incidence being highest in Norway (68%) (Meijer et al. 2009). A/H3N2 and influenza B viruses, however, stayed susceptible. During the following 2008-2009 influenza season in the US, where the A/H1N1 virus became the dominant strain, nearly all H1N1 viruses were resistant to oseltamivir, but all remained sensitive to zanamivir (Van der Vries et al. 2011). The emergence of the NI-susceptible 2009 pandemic A/H1N1 strain in the subsequent year in turn entirely replaced the preceding resistant subtype, and now continues to circulate as a new seasonal A/H1N1 strain. Currently, the incidence of primary NI-resistance is low (WHO 2011). Nevertheless, sporadic NI resistance in the 2009 pandemic virus has been reported infrequently, for the most part in immunocompromised patients and children under antiviral treatment (Van der Vries et al. 2011).

3. AIMS OF THE STUDY

The specific aims of the study were:

I To estimate the burden of influenza in outpatient children in the community

II To describe the clinical manifestations of pediatric influenza in primary care settings

III To determine the average annual incidence of virologically confirmed influenza-related hospitalizations in different age groups of children

IV To assess the primary admission diagnoses of children in various age groups who are hospitalized with laboratory-proven influenza

4. MATERIALS AND METHODS

Details of the materials and methods are presented in the original publications.

4.1 Patients and study design

For **Studies I and II**, data were derived from a prospective cohort study conducted during two consecutive winter seasons of 2000-01 and 2001-02, from the beginning of October until the end of May. No exclusion criteria were used. Children younger than 13 years were enrolled in the study before the start of each season. The numbers of children followed up throughout each season were 1338 and 893, respectively **(Figure 2**). 758 children who were followed up during the first season also continued in the study during the second season. Children were regarded as active participants if they made at least one visit to the study clinic during the winter season, or if they returned at least one of the two daily symptom diaries. Because the strains of influenza A viruses circulating during the winter of 2000-01 were almost exclusively of subtype H1N1, and those during 2001-02 of subtype H3N2, all children having participated during the first season were also considered to be at risk of contracting influenza during the second season, and therefore they were included in the analysis as separate children during the two seasons. Overall, the study comprised 2231 child-seasons of follow up.

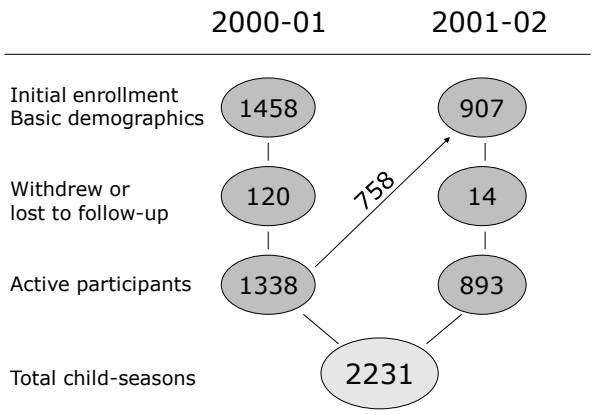

Figure 2. Flow-chart of Studies I and II.

The parents were instructed to bring their children to the study clinic whenever fever or signs of respiratory infection appeared. The clinic was open every day, including weekends and holidays. At each visit, the children were carefully examined by a study physician, and detailed clinical information including symptoms and clinical findings of the illness were obtained using a standard questionnaire. Chest and sinus radiographs were obtained in all children with a clinical suspicion of pneumonia or sinusitis. Whenever possible,

tympanometry and spectral gradient acoustic reflectometry were used together with pneumatic otoscopy to aid in diagnosing acute otitis media. During each new illness episode a nasal swab was obtained for virologic analyses. Children without complications were routinely re-examined after 5 to 7 days, and whenever parents considered it necessary. Influenza antiviral drugs or rapid diagnostic tests were not used for any children.

Studies III and IV were retrospective studies conducted at the Department of Pediatrics, Turku University Hospital, during a period of 16 consecutive years from July 1 1988, through June 30 2004. The study population consisted of all children ≤16 years of age who were hospitalized due to virologically confirmed influenza infection during the study period. To allow for reliable estimation of the population-based rates of hospitalization in different age groups (**Study III)**, we only included children who lived within the area of 38 municipalities whose acute pediatric care was provided solely by the Turku University Hospital. During the study period the average population of this area was 364,112, including 69,068 children ≤16 years of age. Within each municipality and year of study, the numbers of children in different age cohorts were determined, and the average populations in each age cohort during the study period were used in order to calculate the age-based rates of influenza admissions. The age and the place of residence of the children included in the analyses were determined according to the situation on the day of the diagnosis of influenza.

4.2 Data collection

In **Studies I and II,** data on the specific symptoms of the children were derived from a standardized case record form filled out by the study physician during each visit. The case record form consisted of detailed questions about the presence and duration of preceding signs and symptoms of the child, findings during the clinical examination, results of any laboratory tests or radiographs, and the diagnosis and treatment.

During each season, the parents were given daily symptom diaries (for the periods of October-January and February-May) that were filled in by the parents during the entire study period. The diaries consisted of daily charts inquiring about the symptoms and absences from day care or school by the child, as well as absences from work by the parents. The days of absenteeism included only actual days lost, excluding any days of absence occurring during free weekends or other days off. 85% of the parents returned both diaries.

To find all children who were hospitalized with virologically confirmed influenza for **Studies III and IV**, we searched for data from three different sources: 1) the virologic database at the Department of Virology, University of Turku, which was the only laboratory that provided routine virologic analyses for our hospital during the study period; 2) the hospital central database; and 3) the files of the pediatric infectious diseases ward at our hospital. The first search yielded 370 hospitalized children with laboratory-confirmed influenza infections. The second search was carried out to identify all children

with an International Classification of Diseases (ICD) code related to influenza (ICD-9: 4870A, 4871A, and 4878X; ICD-10: J10-J11) who were not found from the database of the Department of Virology (n = 145); the medical records of all these patients were carefully examined to confirm or rule out the viral diagnosis of influenza. This search yielded 17 additional hospitalized cases. The purpose of the third search was to find children who had tested positive for influenza by a rapid influenza test and from whom no additional viral specimens had therefore been obtained; 20 such cases were identified. Of the total of 407 children with virologically confirmed influenza, we excluded 6 hospitalized children from whom the viral specimens had been obtained more than 2 days after admission and in whom nosocomial influenza infection could not be ruled out, leaving 401 influenza virus-infected children in the final analyses. Seven children each were hospitalized twice due to influenza during the 16-year study period; these episodes were considered as separate influenza episodes.

4.3 Specimens and viral diagnosis

In **Studies I and II,** a nasal swab was obtained to determine the viral etiology of the illness during each episode of respiratory infection. The specimen was obtained from a depth of 2-3 cm in the nostril by using a sterile cotton swab, which was then inserted into a vial containing viral transport medium (Heikkinen et al. 2002). The specimens were kept in a refrigerator, and they were transported daily to the laboratory at the Department of Virology, University of Turku. Detection of influenza viruses in the specimens was based on viral culture in Madin-Darbey canine kidney cells and subsequent immunoperoxidase staining with monoclonal antibodies as previously described (Waris et al. 1990).

In **Studies III and IV**, the diagnosis of influenza was based on the detection of influenza A or B antigens in nasopharyngeal aspirates by one-incubation, monoclonal time-resolved fluoroimmunoassay (n = 364) (Nikkari et al. 1989), viral culture (n = 8), or rapid influenza testing (Directigen FluA+B, Becton Dickinson Diagnostic Systems, Sparks, MD, USA) (n = 29).

4.4 Definitions

In **Study II**, gastrointestinal symptoms included vomiting, diarrhea, and abdominal pain. Conjunctivitis was defined as distinct redness of the conjunctivae, or purulent discharge from the eyes. Impaired general condition was defined subjectively by each attending physician. In **Studies I and II,** the diagnosis of AOM was based on signs and symptoms of acute infection, together with the presence of middle ear fluid detected by pneumatic otoscopy or purulent discharge from a tympanostomy tube. The diagnoses of pneumonia and sinusitis were based on radiological confirmation of the condition in an acutely ill child. Any complications were considered to be associated with influenza if they were diagnosed within 2 weeks after the clinical visit in which the influenza-positive specimen was obtained.

In **Study III**, the length of the hospital stay was recorded as the number of nights spent in the pediatric ward. In five children who were admitted in the morning and discharged in the evening of the same day, the length of the hospital stay was recorded as 1 day. Underlying medical conditions included asthma, major neurological defects, malignancies and other immunosuppressive states, cardiovascular diseases, other clinically significant chronic illnesses, and a gestational age of <37 weeks in children <2 years of age.

In **Study IV**, the primary admission diagnoses were divided into nine groups based on the presenting signs and symptoms and any additional information found in the medical records: 1) septic symptoms; 2) respiratory symptoms; 3) acute neurologic symptoms; 4) muscular symptoms; 5) abdominal complaints; 6) general symptoms; 7) severe underlying condition; 8) social indication; and 9) other concomitant illness. All clinical data were collected by a systematic chart review and, for consistency, the main reason for admission was determined by the first author, according to all available data. The primary admission diagnosis of a child could thus be different from the official discharge diagnosis recorded in the hospital's central database. In a few cases of two or more apparent reasons for hospitalization, the children's medical charts were re-reviewed for consensus. The diagnosis of pneumonia was based on radiological confirmation of the condition at admission. The admission diagnosis was recorded as an upper respiratory tract infection if the child was hospitalized with a respiratory illness other than pneumonia, asthma/wheezing, laryngitis, tracheitis, or epiglottitis. Prolonged fever was defined as fever that had lasted ≥5 days. Vomiting was recorded as the main reason in the case of repeated vomiting without signs of dehydration. Neurologic defects included severe, mainly congenital, neurologic diseases. The category "social indication" was used for children who were admitted mainly because of social problems and not because of the severity of the illness. Most children in the category "other concomitant illness" had a febrile respiratory illness, but the respiratory symptoms were not considered as the main reason for hospitalization.

4.5 Statistical methods

In all four studies, for continuous data, the groups were compared by the *t* test or by the Mann-Whitney *U* test in the case of failed normality test. The chi-square test, Fisher´s exact test, or the sign test with binomial distribution were used for comparing the differences in proportions between the groups. Two-sided *p*-values <0.05 were considered to be statistically significant. All statistical analyses were performed with SigmaStat (version 2.0), SPSS, or StatsDirect (version 2.7.7. or 2.7.8) software.

4.6 Ethics

The study protocol was approved by the Ethics Committee of the Turku University Hospital. Written, informed consent was obtained from the parents of all participating children (**Studies I and II**).

5. RESULTS

5.1 Burden of influenza in outpatient children (I)

5.1.1 Rates of influenza illness

In this study, 372 episodes of influenza were identified in the study children (262 episodes during the first season and 110 during the second season). 133 of 372 influenza-positive children (35.8%) were <3 years of age, 148 (39.8%) were 3-6 years of age, and 91 (24.5%) were 7-13 years. In children aged <3 years, the rates of influenza illness in the first and second study year were 160 and 213 cases / 1000 children, respectively. The corresponding rates in the age group 3-6 years were 223 and 119 / 1000 children, and in children ≥7 years they were 207 and 30 cases / 1000 children. Of the 372 influenza illnesses, 81% were caused by influenza A viruses and 16% by influenza B viruses; in 3% of the cases the viruses remained untyped.

In the subgroup of 758 children who were followed up for two seasons, 223 children had 258 episodes of influenza (189 children had one episode, 33 children each had two episodes, and one child had three episodes). The average annual rate of influenza illnesses in this subgroup was 170 / 1000 children.

5.1.2 Complications and antibiotic treatments

Two children with influenza had double viral infections (one with adenovirus and one with parainfluenza virus) and were therefore excluded from further analyses. Of the 370 children with influenza, 85 (23%) had acute otitis media, which was the most frequently diagnosed complication. In children aged <3 years, AOM occurred in 52 out of 131 (39.7%) children. Other bacterial complications were clearly less frequent; pneumonia was diagnosed in 2.4% and sinusitis in 3.5% of the children. Antibiotics were prescribed significantly more often to children younger than 3 years (42.0%) than to children aged 3-6 years (27.7%) or those 7 years or older (8.8%) ($P \leq 0.01$ for all comparisons between the groups).

5.1.3 Socioeconomic impact

For determination of the children's and their parents' absences because of the child's influenza, 21 children who were cared for at home by a parent were excluded. The mean duration of a child's absence was 3.6 days in children younger than 7 years of age, and 2.8 days in those aged 7-13 years (calculated for children who were absent for at least one day). The corresponding durations of parental absence in these age groups were 2.9 and 2.1 days, respectively. For all age groups combined, a parent missed ≥1 day of work because of the child's influenza in 50% of the cases.

5.2 Clinical manifestations of influenza in outpatient children (II)

Of the 372 influenza cases documented in the study, 19 were excluded from additional analyses because of double viral infection (n = 2), or incomplete data on the degree or duration of fever (n = 17), leaving 353 episodes of influenza in the final analyses.

5.2.1 Clinical picture in different age groups

In this analysis, we determined the diverse signs and symptoms in different age groups of children during the first visit to the study clinic. The median duration of any symptom of illness before the initial visit was 3 days in children younger than 7 years, and 2 days in school-aged children. Fever was clearly the most prominent sign, occurring in 95% of all children **(Figure 3)**. Every fifth child younger than 3 years of age had a fever ≥40˚C **(Figure 3)**. Only 5% of the study children were afebrile when initially presenting to the study clinic. Rhinitis and cough were observed in 86% and 78% of children aged <3 years, respectively, and 74% and 77% of the older children. 9% of all children had gastrointestinal symptoms. 39% of school aged children complained of a headache, and 13% of muscle aches. General condition was decreased in 13% of children in this age group according to the judgment of the study physician. Expiratory wheezing was diagnosed in 1-4% of children in different age groups. Acute otitis media was the most common complication, and it was diagnosed in 19% of children younger than 3 years of age at the initial visit. None of the children were diagnosed with a febrile seizure.

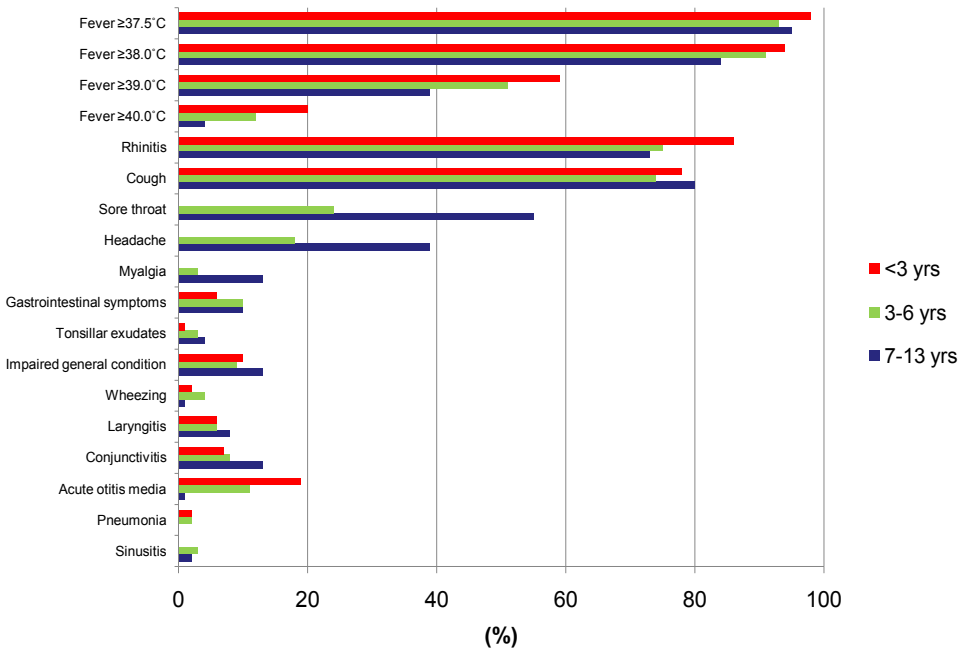

Figure 3. Signs and symptoms, and clinical diagnoses, at the initial visit in different age groups of children with influenza.

5.2.2 Comparison of clinical findings between influenza A and B

The rates of clinical signs and symptoms were further analyzed according to the type of influenza virus. Nine children with untyped influenza viruses were excluded from these analyses. No significant differences were observed in any signs or symptoms between children with influenza A or B virus infections (**table 3**).

Table 3. Signs and symptoms in 344 children with influenza A or B virus infection (influenza A, n = 286; influenza B, n = 58).

Symptom	A	B	P value**
Fever ≥37.5˚C	273 (95)	56 (97)	0.98
Fever ≥38.0˚C	260 (91)	52 (90)	0.96
Fever ≥39.0˚C	151 (53)	25 (43)	0.23
Fever ≥39.0˚C and/or impaired general condition	165 (58)	25 (43)	0.06
Rhinitis	226 (79)	41 (71)	0.22
Cough	223 (78)	43 (74)	0.64
Sore throat	74 (37)*	14 (30)*	0.49
Headache	54 (27)*	10 (22)*	0.57
Myalgia	14 (7)*	3 (7)*	0.99

*For sore throat, headache, and myalgia, only children 3 years of age or older were included (influenza A, n=199; B, n=46).
** P values calculated with Chi-square test using Yates´ correction.

5.3 Incidence of virologically confirmed influenza-related hospitalizations in children (III)

5.3.1 Hospitalizations in different age groups

During the 16-year observation period, a total of 401 children were hospitalized with virologically confirmed influenza. The median age of the children was 1.6 years (mean, 3.5 years). The mean duration of symptoms before admission to hospital was 3.8 days (median, 2 days; range, 0.5-35 days). Underlying conditions were diagnosed in 10.2% of the infants younger than 6 months of age and 45.2% of the school-aged children (P<0.0001 for trend). The proportions of different underlying conditions in different age groups are presented in **table 4.**

Table 4. Underlying medical conditions in 401 children hospitalized with influenza.

Underlying condition	Age, years				
	<0.5 n=88 (21.9%)	0.5-2.9 n=169 (42.1%)	3.0-6.9 n=71 (17.7%)	7.0-16.9 n=73 (18.2%)	Total n=401
			n (%)		
Asthma	0 (0)	9 (5.3)	9 (12.7)	11 (15.1)	29 (7.2)
Neurologic condition	2 (2.3)	11 (6.5)	7 (9.9)	7 (9.6)	27 (6.7)
Prematurity	6 (6.8)	9 (5.3)	0 (0)	0 (0)	15 (3.7)
Cardiovascular disease	1 (1.1)	2 (1.2)	0 (0)	0 (0)	3 (0.7)
Malignancy or immunosuppression	0 (0)	2 (1.2)	7 (9.9)	8 (11.0)	17 (4.2)
Other	0 (0)	4 (2.4)	3 (4.2)	7 (9.6)	14 (3.5)
Any	**9 (10.2)**	**37 (21.9)**	**26 (36.6)**	**33 (45.2)**	**105 (26.2)**

Influenza A was identified in 330 (82.3%), and influenza B in 70 (17.5%), of the children; one child (0.2%) was infected with both influenza A and B simultaneously. Influenza B caused 37.0% of all hospitalizations among children aged 7-16 years, compared with 11.4% among infants aged <6 months.

The annual population-based influenza hospitalizations attributable to influenza A and B are presented in **Figure 4**. The average annual incidence of influenza-attributable hospitalization in children <1 year of age was 225 / 100,000 (CI, 188-262), and in all children younger than 17 years of age it was 36 / 100,000 children (CI, 33-49). In children aged 0-3 years and 0-5 years of age, the average annual hospitalization rates were 134 / 100,000 and 93 / 100,000, respectively.

When influenza A and influenza B related hospitalizations were analyzed separately, the annual incidences were highest in the youngest children with both types of influenza (influenza A, 245 / 100,000 children and influenza B, 31 / 100,000 in children <6 months of age). However, the relative differences between different age groups were smaller with influenza B than with influenza A.

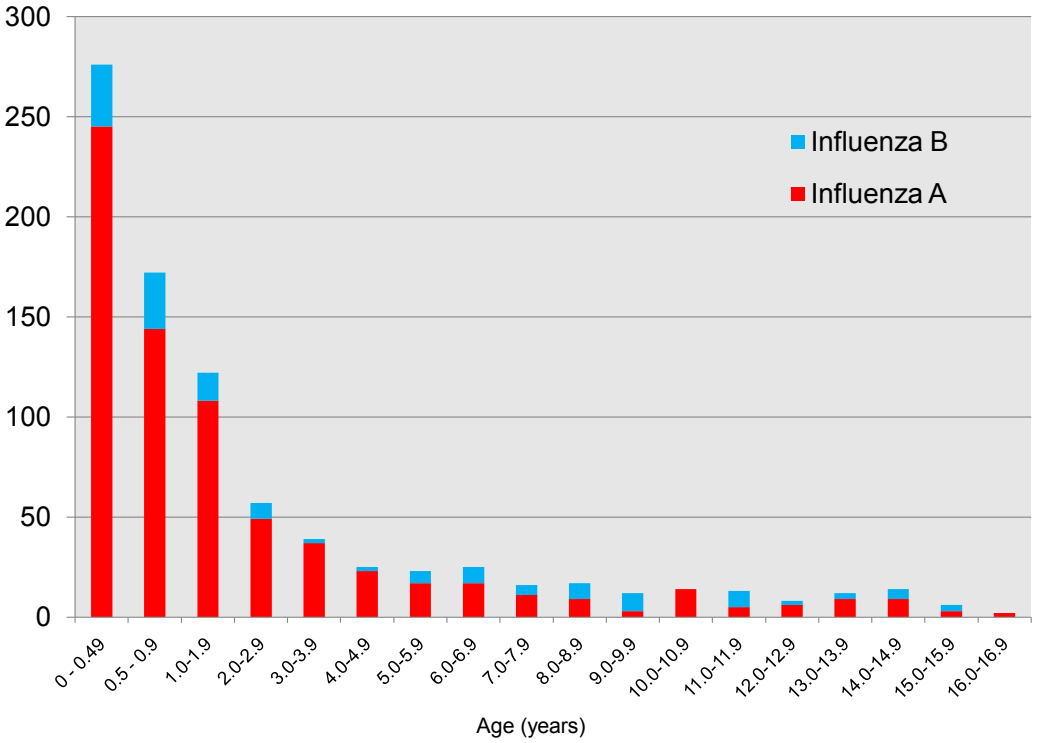

Figure 4. Average annual incidence (per 100,000 children) of influenza A and B hospitalizations in different age groups of children.

5.3.2 Seasonal variation in hospitalizations

The total numbers of children hospitalized with influenza varied markedly between different seasons during the 16-year study period. The numbers were highest during the last observation season (2003-2004) with 53 admissions, and lowest during the season of 1990-1991 (n=6), with a 9-fold difference between the seasons (**Figure 5**). Influenza A prevailed over influenza B during 12 of 16 seasons (75%), and in 7 of them (44%) influenza A accounted for >90% of all hospitalizations. Influenza B viruses predominated in 4 seasons (25%), when their relative proportion among all influenza hospitalizations ranged from 57% to 85%.

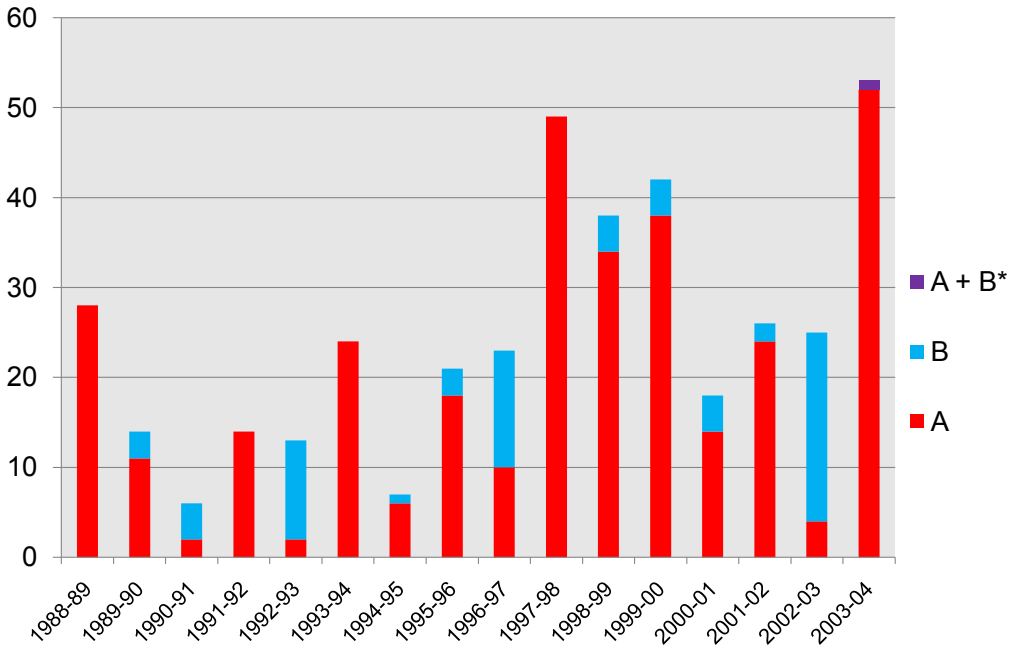

Figure 5. Annual numbers of children hospitalized with influenza A or influenza B during 1988-2004.

*One child had both influenza A and B simultaneously.

5.3.3 Admission to the intensive care unit

40 children (10.0%) hospitalized with influenza-attributable illnesses required admission to the pediatric intensive care unit (**Table 5**). Six children (1.5%) needed mechanical ventilation. The proportion of children in intensive care was lowest among infants <6 months of age and highest among school-aged children. There were two deaths during the 16-year study period, which corresponds to an average annual death rate of 0.2 per 100,000 children. 14 of 40 children (35%) admitted to the PICU had a chronic underlying illness, mostly a neurologic condition.

Table 5. Hospitalized influenza-positive children admitted to intensive care unit.

	Age group				
	<6 months n=88	**0.5-2.9 years** n=169	**3.0-6.9 years** n=71	**7.0-16.9 years** n=73	**Total** n=401
Intensive care	5 (5.7)	16 (9.5)	6 (8.5)	13 (17.8)	40 (10.0)
Mechanical ventilation	0	5 (3.0)	1 (1.4)	0	6 (1.5)

5.4 Admission diagnoses of children hospitalized with influenza-attributable illnesses (IV)

5.4.1 Admission diagnoses in different age groups

The primary admission diagnoses of children in the four different age groups are presented in **Figure 6**. Respiratory illness was the most common admission diagnosis in the entire group of children, accounting for 37.7% of all influenza-related admissions. Every fifth child was admitted due to a sepsis-like illness. Among infants <6 months of age, suspected sepsis was the principal reason for admission in 52.3% of the children. Acute neurologic conditions accounted for 15.2% of all hospitalizations; 77.0% of these were febrile convulsions.

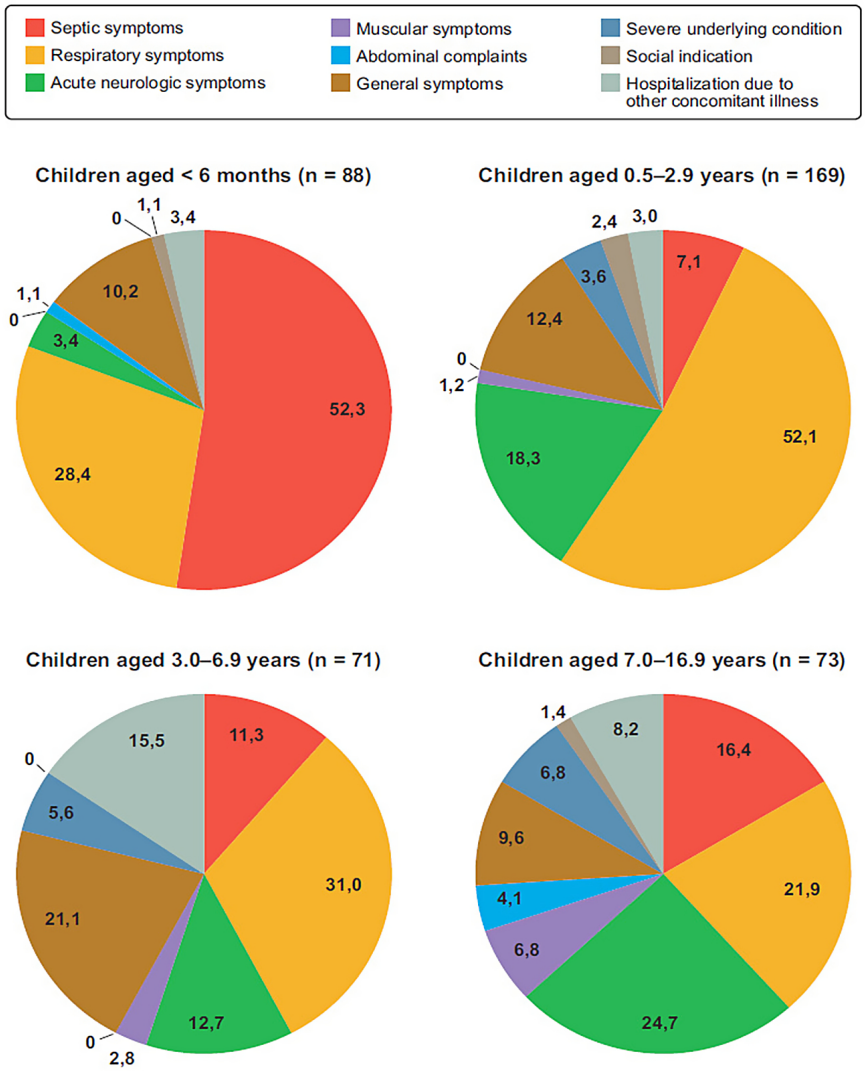

Figure 6. Main admission diagnoses of 401 children with virologically confirmed influenza in different age groups (all numbers are percentages).

5.4.2 Comparison of admission diagnoses in children with influenza A and B

The rates of different admission diagnoses were further analyzed according to the influenza type; one child was excluded from this analysis because of double infection with both influenza A and B. Due to the significant difference in median ages of children with different influenza types (influenza A, 1.5 years; influenza B, 5.5. years), the relative proportions of different admission categories were compared within each of the different age groups **(Figure 7**). There were no significant differences in any of the admission diagnosis categories between children with influenza A and B in any age group.

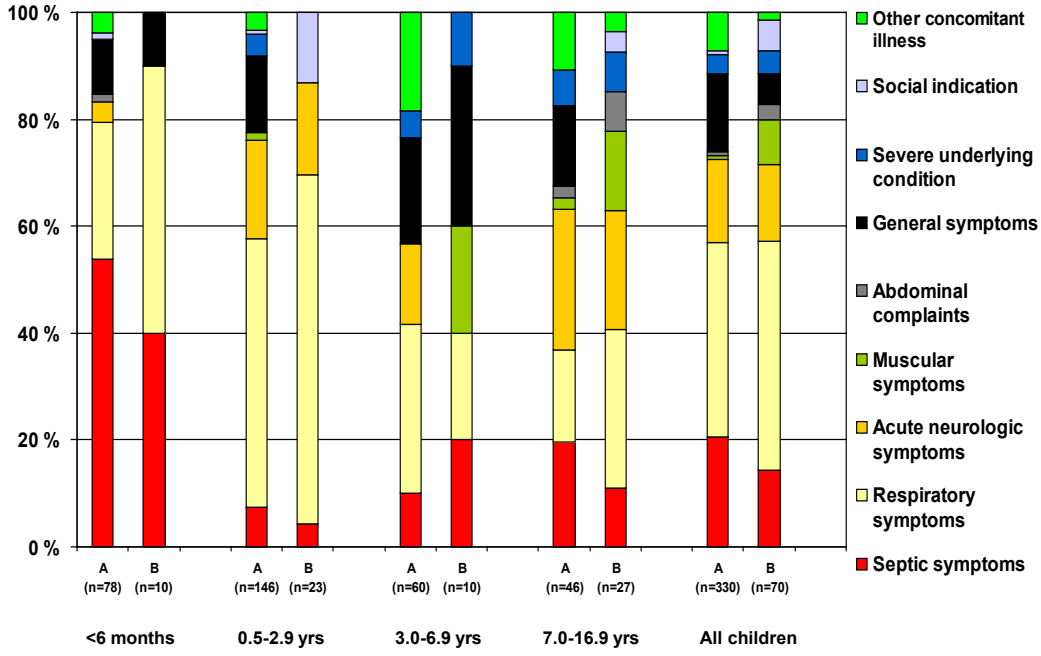

Figure 7. Relative proportions of main admission diagnoses in different age groups of children hospitalized with influenza A or B infections. There were no significant differences in any of the admission diagnosis categories between children with influenza A and B, in any age group.

6 DISCUSSION

6.1 Epidemiology of pediatric influenza (I, III)

Earlier, epidemiologic studies estimating the impact of influenza relied merely on surveillance data, predominantly concerning, for example, excess rates of mortality or hospitalizations, or outpatient visits, compared to peri-influenza seasons when influenza viruses are not circulating (Izurieta et al. 2000, Neuzil et al. 2000a). However, it is nowadays acknowledged that, especially in children, this method is susceptible to bias. During an average influenza epidemic there is a wide range of other respiratory viruses circulating along with influenza viruses (Sugaya et al. 2000, Zambon et al. 2001, Heikkinen et al. 2003), making accurate influenza diagnosis in young children based solely on clinical signs practically impossible (Peltola et al. 2005). Our studies were based on virologic confirmation of influenza illness on an individual level, which avoids its confounding with other concurrently circulating respiratory viruses. On the other hand, it should be noted that due to the limitations of viral isolation techniques – for instance the time of specimen collection in relation to the onset of illness, and those regarding adequate specimen collection and processing – results such as attack rates and admission incidences need to be considered as conservative estimates, as there is always a proportion of true influenza-positive children who are not caught by the available virologic tests. In **Study I**, we chose to obtain nasal swabs instead of nasopharyngeal aspirates for viral detection in order to increase compliance and feasibility of the study for children, at the potential expense of an approximate 10% decline in the true incidence of influenza-positive children (Heikkinen et al. 2002). In **Study III**, the mean duration of symptoms before admission to hospital of the study children was nearly four days, and since not all the children were tested for influenza on the day of arrival (especially those with non-respiratory symptoms), there may have been a substantial number of false negative test results among hospitalized children during the 16-year observation period. For these reasons, it can be assumed that the actual burden of influenza in children in terms of attack rates as well as hospitalization incidences demonstrated in the present studies is a slight underestimation.

The severity of influenza epidemics varies markedly from season to season, depending on the intrinsic virulence of the circulating strain(s), the extent of antigenic variation of the virus, as well as on the susceptibility of the population (Cox and Subbarao, 2000). Therefore, to be able to accurately evaluate the overall burden of influenza, multiple seasons need to be explored. Our outpatient study covered two influenza seasons, with demonstrable differences in influenza attack rates between the different age groups of children during these two seasons. In the first study year (2000-01), the epidemic was caused almost entirely by type A/H1N1 influenza viruses, which had not been circulating in Finland in four years, whereas in the second year the circulating viruses were primarily of type A/H3N2, which had been prevalent for several years before 2000.

The attack rates during the first winter were rather similar in all age groups, while in the second winter there was a 7-fold difference in the attack rates between children aged <3 years, compared to those ≥7 years, which could be explained by the pre-existing immunity against the circulating strain in the older children. The average annual attack rate of 16.7% from our two mild study seasons is in accordance with earlier studies of laboratory-confirmed influenza in children, with attack rates ranging from 16% to nearly 40% (Glezen and Couch 1978, Monto and Sullivan 1993, Neuzil et al. 2002a, Poehling et al. 2006b, Tsolia et al. 2006).

Although the great majority of influenza-infected children are treated as outpatients, young children are frequently hospitalized with influenza-attributable illnesses (Izurieta et al. 2000, Neuzil et al. 2000a). Nonetheless, it is only during the last ten years or so that there has been an increasing interest in studying the population-based incidence of laboratory-confirmed influenza-related hospitalizations in children. In earlier studies, however, the observation periods have been fairly short, ranging from one to seven influenza seasons (Iwane et al. 2004, Montes et al. 2005, Poehling et al. 2006b, Ampofo et al. 2006, Rojo et al. 2007, Ajayi-Obe et al. 2008, Chiu et al. 2009, Dawood et al. 2010a, Sakkou et al. 2011). To our knowledge, our study is the only study of laboratory-confirmed, pediatric, influenza-attributable hospitalizations that covers >10 influenza seasons. The long observation period of our study makes the results more generalizable by balancing the year-to-year variation of influenza epidemics.

In **Study III**, the incidence of hospitalization due to influenza was distinctly highest in infants under 6 months of age, with an annual admission rate of 276 / 100,000 children. This result is in agreement with the six population-based studies published earlier in which the incidence in this age group had been estimated, with annual rates ranging from 153 to 450 per 100,000 children (Iwane et al. 2004, Montes et al. 2005, Ampofo et al. 2006, Poehling et al. 2006b, Ajayi-Obe et al. 2008, Dawood et al. 2010a). The reason for the high admission rates of young infants undoubtedly lies in the unspecific nature of the early symptoms of influenza, with fever as a prominent feature, which easily raises the suspicion of bacterial sepsis in the youngest infants (Dagan and Hall, 1984, Bender et al. 2010). In a recent retrospective 4-year study from the US, 50% and 70% of influenza-positive children <6 months and <3 months of age, respectively, that were referred to the ED of a tertiary hospital were subsequently hospitalized (Bender et al. 2010). Unfortunately, due to the unstructured nature of viral sampling of the non-hospitalized patients at the ED of our hospital during the study period, we were unable to reliably estimate the corresponding proportions of children in different age groups.

Hospitalization rates decreased with increasing age. A clear drop in rates was observed between the age categories of 1.0-1.9 years and 2.0-2.9 years (from 122 per 100,000 to 56 per 100,000). Similar patterns have also been demonstrated in some earlier population-based studies using administrative data of excess hospitalizations (Izurieta et al. 2000, Chiu et al. 2002). This phenomenon, together with the fact that there were three times fewer chronic underlying conditions among children <2 years of age than among

school-aged children, emphasize the greater overall susceptibility of healthy children <2 years of age to the severe illness attributable to influenza, compared to older age-groups. Despite the decreasing tendency of influenza-related admissions with increasing age, influenza virus infections still dominate among all hospitalizations for acute respiratory tract illnesses, even in school-aged children (Glezen et al. 1987a, Glezen et al. 2000).

Generally, influenza B is contracted a little later in life than influenza A. This was also shown in our studies. Among inpatient children 7-16 years of age, influenza B caused 37% of all hospitalizations, compared to 13% among children <7 years of age. However, we found that population-based hospitalization incidence attributable to influenza B was highest in the youngest children, as with influenza A, even though the differences between the different age groups were smaller than those for influenza A-related admissions. There are two other population-based studies that have compared the hospitalization incidences of children with virologically confirmed influenza A and B: one from Germany (Weigl et al. 2002), and the other from Hong Kong (Chiu et al. 2009). However, in the latter there were no influenza B-related hospitalizations of children <2 years of age during the observation period. In the 4-year German study (Weigl et al. 2002), the incidences of influenza B-associated admissions of infants <1 year of age as well as those <5 years of age (49 and 30 per 100,000, respectively) were slightly higher than those observed in our study (30 and 11 per 100,000). From these results it can be concluded that the total effect of influenza B infections in young children is far from negligible.

In our study, roughly half of all virologically confirmed cases of influenza were found by a computerized search, based on influenza-related ICD codes, demonstrating a surprisingly low accuracy of the diagnoses of influenza-positive children discharged from a tertiary care hospital. This finding is, nonetheless, in line with that of a large, year-round population-based study by Poehling et al. (2006b), in which only 28% of children with laboratory-proven influenza infections had a discharge diagnosis of influenza, despite the usefulness of rapid influenza tests. Together, these findings strongly suggest that studies relying on ICD codes only may critically underestimate the real rates of influenza-related hospitalizations.

6.2 Socioeconomic impact of influenza (I, III)

With regard to pediatric influenza, a remarkable proportion of the total economic burden arises from indirect costs like parental work absenteeism due to a child's illness. In a recent study from the US, 27% of all productivity days lost because of influenza could be attributed to influenza in children (Molinari et al. 2007). In our study, the frequency and duration of parental work loss was greatest among children <3 years of age, with 195 days of work lost by parents for every 100 influenza-infected children. This number is substantial considering that approximately 65% of Finnish children <5 years are cared for at home by their mothers or fathers (Kartovaara and Sauli 2000), and thus do not need any extra arrangements with day care in case of illness. However, as the

majority of our study children attended a daycare center or family day care, the rates of parental absenteeism presented in our study may be somewhat higher than in real life. Nonetheless, we included in our calculations only the real work days lost, and excluded any days of illness occurring during free weekends, holidays, etc. By using an average gross daily income of ~167 € of Finnish employees, Salo et al. (2006) estimated that among 0.5-<3 year-old children, influenza-related productivity costs (caused by parental work absenteeism) alone are over three million euros yearly.

In older children, school absenteeism constitutes an essential part of the overall burden of influenza. In addition to missing important educational time in school they require, at least those in primary school, an adult caregiver at home. We found that for every school-aged child with influenza, approximately 2.2 school days were missed yearly. Among those who were absent for at least one day, the number of missed school days was nearly three. This is, however, clearly less than what was observed in two other European outpatient studies of children younger than 14 and 15 years of age (Tsolia et al 2006, Esposito et al. 2011a), with mean school absence ranging from 5.2 to 7.6 days in influenza-infected children. Possible explanations for the observed difference may be the above mentioned strict calculation criteria we used, as well as differing recommendations for re-entering school after febrile illness in different communities. In any case, even though 2-3 days absence from school a year because of influenza may sound of minor importance, prevention of clinical influenza illness by vaccination in this age group can produce substantial economic savings at the population level; in the Finnish cost-effectiveness analysis of pediatric influenza vaccination, indirect costs among school-aged children accounted for nearly 75% of all influenza-attributable costs (>16 million €) in this age group (Salo et al. 2006).

Besides fewer days off from day care, school, or work, prevention of influenza in children would inevitably result in reduced visits to the doctor as well as reduced antibiotic prescriptions, both contributing towards the direct costs of influenza. The resulting reduction of prescriptions for antibiotics would be an essential benefit, since excessive use of antibiotics leads to the emergence of resistant bacteria. In our **Study I**, almost half of the children <3 years of age had antibiotics prescribed for their illness, 95% of which for acute otitis media. The situation was much worse in a recent multicenter study from Italy, where more than 70% of children <15 years of age with influenza had antibiotic treatment, despite the fact that only 6-9% had acute otitis media and 5.5-6.5% had pneumonia – the two most common bacterial complications of influenza – diagnosed (Esposito et al. 2011a). Consistently, in a Greek outpatient study comprising two influenza seasons, influenza accounted for 37% of all antibiotic courses given to the children with febrile respiratory infection during the study period (Tsolia et al. 2006). Even when preserved only for correctly diagnosed bacterial complications of influenza, excessive courses of antibiotics are an important element in the socioeconomic burden of outpatient influenza, especially among children under 3 years of age. Considering that a single episode of AOM attributable to influenza infection costs approximately 208 € in

Finland (Salo et al. 2006), it is clear that the reduction of the frequency of this common complication either by vaccination or by antiviral treatment would result in significant savings at a community level.

Hospitalization for influenza has significant consequences, even for previously healthy children. A child ill enough to be hospitalized is commonly subjected to invasive examinations like blood tests, and sometimes also a lumbar puncture, and is subsequently treated with intravenous antibiotics. All these procedures cause notable stress on the child and the family, let alone the costs for the society. In 2006 in Finland, an average pediatric influenza-attributable hospitalization was estimated to cost 1555 € (Salo et al. 2006). This is, nevertheless, considerably less than the estimated average cost of a hospitalization of an American child <5 years of age with influenza (Fairbrother et al. 2010). However, it is clear that the price of a hospital stay rises exponentially when intensive care treatment is needed. In our study 10% of the children required admission to the intensive care unit, and 15% of those needed mechanical ventilation. These figures are largely accordant with earlier studies, with PICU admissions ranging from 0.6 to 19% in different settings (Quach et al. 2003, Moore et al. 2006, Poehling et al. 2006b, Rojo et al. 2006, Coffin et al. 2007, Dawood et al. 2010a). Since it is acknowledged that children with chronic underlying conditions run the highest risk for severe complications related to influenza (and thus have the longest hospitalizations), in most countries annual influenza vaccination of these children has been recommended for many years. However, given the fact that previously healthy infants aged <6 months have the highest rates of influenza-related hospitalization across all seasons, yet they cannot be vaccinated against influenza so far, alternative ways for the prevention of influenza illness in this age group of children is needed in order to effectively reduce the overall burden associated with pediatric influenza-related hospitalizations.

6.3 Clinical features of influenza in different age groups of children (II, IV)

6.3.1 Outpatients

Previously published articles of the clinical picture of pediatric influenza have mainly focused on hospitalized children, lacking a detailed description of symptoms attributable to influenza in an average, healthy child at the primary care level. The particular strength of our prospective outpatient study was that we obtained viral samples from a large number of normal children during every episode of illness seen at the study clinic, regardless of the severity of the symptoms. Thus we could avoid possible bias by testing only a selected population of children.

Unlike adults (Monto et al. 2000), most children in the outpatient setting present with rhinitis during the early phase of the illness (Friedman and Attia 2004, Poehling et al. 2006b): a fact that is likely to further complicate the distinction between influenza and other common viral respiratory infections. This was also observed in our study: even

among school-aged children, almost three out of four had rhinitis in the first two days of the illness. In addition, underdiagnosis of influenza may in some cases be attributed to the less dramatic onset of illness accompanied by nonspecific symptoms; rather than abrupt clinical malaise, children may develop symptoms over a longer period before contact to healthcare (Poehling et al. 2006b). In **Study II**, among children younger than 7 years of age, the median duration of illness symptoms before presenting to the study clinic was 3 days. This is, however, likely to be somewhat shorter than what is seen in real life, because the parents in our study were advised to contact the study clinic as soon as their child developed symptoms of respiratory infection, regardless of the severity of symptoms.

Conversely, rapid onset of high fever, which is considered almost pathognomonic in adults during an influenza outbreak, can also be part of the initial presentation of influenza in children. In our outpatient study, every fifth child <3 years of age with influenza had a fever ≥40ºC, and 10% of all children had decreased general condition at presentation. Even though only three children out of 370 with influenza were eventually referred to the emergency department from our study clinic, it is understandable that the decreased general condition of a child in a primary care usually calls for further evaluation in a hospital. The prominence of fever in the symptomatology of pediatric influenza was outlined in a recent case-control analysis of Heinonen et al. (2012), in which fever was shown to be the only sign that independently predicted influenza virus infections in outpatient children, and the predictive capability of fever increased with incremental elevations in the child's temperature.

In our outpatient study, headache and myalgia, the symptoms frequently associated with adult influenza (Boivin et al. 2000, van Elden et al. 2001), occurred in only 39% and 13% of school-aged children, respectively, who could be expected to be able to verbally describe their subjective symptoms. In contrast, in the (only) other outpatient pediatric study in which these symptoms have been previously reported (Friedman and Attia 2004) headache was recorded in 44% and muscle aches in 33% of the children. However, it should be noted that in that one-season study, the patients were selected from an emergency department of a tertiary pediatric center according to predetermined criteria implying influenza infection, thus the results may not be generalizable to the average outpatient population. Taken together, it appears that neither headache nor muscle aches, which are considered pathognomonic of early influenza in adults, are elemental features of outpatient pediatric influenza.

Consistent with previous reports (Jartti et al. 2004, Tsolia et al. 2006), acute expiratory wheezing was rarely associated with influenza in our outpatient study. However, it should be noted that the percentage of children <3 years of age with wheezing on admission to hospital (**Study IV**) was 7-fold compared to that of outpatients with wheezing on their first visit (**Study II**). Even though rhinoviruses and respiratory syncytial virus have been demonstrated to be the most common etiologic agents in acute wheezing and exacerbation of asthma in young children (Jartti et al. 2004), our hospital study shows that the role

of influenza viruses in provoking acute wheezing attacks cannot be completely ignored. Like wheezing, acute laryngitis associated with influenza was a fairly infrequent finding in our outpatient study. However, the clinical picture of influenza-attributable laryngitis has been demonstrated to be more severe than that caused by parainfluenza viruses (Peltola et al. 2002).

Acute otitis media is by far the most common bacterial complication of influenza in young children (Ruuskanen et al. 1989, Heikkinen et al. 1991, Poehling et al. 2006b, Tsolia et al. 2006). In our outpatient study, AOM was diagnosed in 19% of the children <3 years of age, and in 10% of all study children with influenza at the first visit to the study clinic. However, in line with a study by Heikkinen and Ruuskanen (1994), in which it was shown that half of AOM episodes develop after 3 to 4 days of the onset of upper respiratory tract infection, in two weeks' time from the initial visit, the total number of AOM in all three age groups had doubled. In a study by Poehling et al. (2006b), the rate of AOM was 28% in outpatient children <5 years of age with influenza, when diagnosed in an average of 3.9 days from the onset of illness symptoms. Consistent with our results (23%), Tsolia et al. (2006) reported a total rate of 18.5% in the occurrence of AOM in outpatient influenza-positive children aged 0.5-<14 years.

Evaluation of the signs of AOM is unavoidably somewhat subjective, and susceptible to interobserver bias (Karma et al. 1989). Furthermore, careful examination of a young child with otoscopy can be challenging, especially if cerumen has to be removed. The limitations of our outpatient study included this potential risk for subjective interpretation of otoscopic signs, since a total of 18 study physicians participated in the clinical examination of the patients during the 2-year study period. However, to minimize the risk of interobserver bias we routinely used tympanometry as well as acoustic reflectometry, in addition to pneumatic otoscopy, to aid in diagnosing AOM. Furthermore, the detailed clinical description of the otoscopic findings as well as the print of tympanometry results were collected in a standardized case record form, to reduce possible discrepancies between otoscopic findings and the clinical diagnosis. Moreover, in case of diagnostic uncertainty the children could easily be re-examined after one or two days. For these reasons, despite the relatively high number of investigators in the study, we assume that the rates of AOM in our study reflect the situation in real life.

In conclusion, our findings of the clinical picture of outpatient pediatric influenza differ in many aspects from the traditional concept of influenza symptomatology. Our results also show that signs and symptoms are most severe in children younger than three years of age; thus, vaccination of these children against influenza would be most beneficial.

6.3.2 Inpatients

Age is an important confounder for assessing the clinical profile of influenza in children. This fact was underlined in our inpatient study with striking differences in admission diagnoses among different age groups of children. It was surprising that more than half

of all infants <6 months of age were primarily hospitalized due to a sepsis-like illness. As demonstrated earlier (Glezen et al. 1980, Dagan et al. 1984, Quach et al. 2003, Bender et al. 2010), in this age group the constitutional respiratory symptoms of early influenza are often absent, and the only manifestation of the illness may be high fever mimicking bacterial sepsis. This, in turn, often results in so called "sepsis work-up" and the initiation of an empirical antibiotic treatment. It should be noted, however, that suspicion of septic illness was also relatively common among admitted school-aged children (16.4%), half of whom had malignancy or immunosuppression as an underlying condition.

It has been demonstrated that quickly available test results can affect the diagnostic evaluation and treatment of children with influenza infection in terms of reduced number of blood tests, urinalyses, and chest x-rays, thus saving time and money (Bonner et al. 2003). But a relevant question is, how should febrile infants younger than three months of age with a positive rapid test result be managed? Should a positive test for influenza impose any deviation from standard practice of evaluation when it comes to the youngest babies? Although influenza-associated bacterial infections have been well described in children with chronic underlying illnesses (Coffin et al. 2007, Streng et al. 2011), there are fewer data on concomitant serious bacterial infections (SBIs) – like bacteremia, bacterial meningitis, pneumonia or pyelonephritis – in healthy neonates and infants with influenza. Generally, SBIs are detected in 9.5% to 13% of infants < 3 months of age (Byington et al. 2004, Mintegi et al. 2009). However, it has been demonstrated that febrile infants with a confirmed viral infection are at a lower risk of SBI than those without (Titus and Wright 2003, Byington et al. 2004). In a recent study of infants under 3 months of age with influenza, only 2.3% of children (5/218) had SBI (Bender et al. 2010). Bacteremia occurred in 2 (0.9%) of these infants. In another study, only one SBI was detected in a one-month-old infant out of 79 hospitalized influenza-positive children <6 months of age (Poehling et al. 2006b). Even though the risk of serious bacterial co-infection in influenza-positive infants is low, it is not zero – a recent prospective 5-year study from Spain showed that in infants younger than 3 months of age, urine culture was positive in 4% (3/72) of those with laboratory-proven influenza (Mintegi et al 2009), while none of them had a positive blood culture. In our study, there was no laboratory-confirmed bacteremia among influenza-positive infants <3 months of age either, although in 8 out of 38 cases the data of blood culture was not available. In their article (Mintegi et al. 2009), the Spanish authors suggest that routine blood culture may no longer be necessary in infants <3 months of age with a positive rapid influenza test; however, the examination of urine culture should be kept in mind regardless of the result of a rapid test. Still, it could very well be argued that the suspicion for SBI should not be completely abandoned in infants with confirmed influenza, at least when standard-of-care parameters suggest other testing.

Another significant finding from our hospital study was that, even though influenza is predominantly a respiratory infection, less than 40% of all hospital admissions were primarily related to respiratory symptoms. Much of the same kind of finding was noted

in a Canadian 3-year retrospective study of children <18 years, with admission diagnoses related to respiratory tract observed in only 46% of the patients (Quach et al. 2003). Earlier, Glezen et al. found that in almost half of the children hospitalized with a proven influenza infection, this infection had a major involvement with an organ system other than the respiratory tract (Glezen et al. 1980). These findings are clinically important, as it can be assumed that children with primarily non-respiratory clinical manifestations are prone to remain undiagnosed and, consequently, may not receive adequate treatment with antivirals. Recently it was demonstrated that oseltamivir treatment, when initiated within 24 hours of the onset of symptoms, shortened the median time to the resolution of the illness by 4.0 days in unvaccinated children with influenza A: a difference that the majority of pediatricians, let alone the parents of the sick children, would consider clinically noteworthy (Heinonen et al. 2010). Our study, compatible with others (Glezen et al. 1980, Quach et al. 2003), demonstrated that limiting the consideration of serious morbidity attributable to influenza only to pulmonary conditions underestimates the role of seasonal influenza as a cause of hospitalization of children.

Among respiratory-related admissions, pneumonia was the most frequent diagnosis in all age groups except for infants <6 months of age. Similarly to the recent study of influenza-associated pneumonia by Dawood et al. (2010b), the pneumonia cases in our study clearly concentrated in the age group 0.5-<3 years (58% of all pneumonia-related admissions), the majority (3/4) being previously healthy. In a Finnish study by Lahti et al. (2006), most children with influenza-associated pneumonia were considered to have primary viral pneumonia, thus the drug of choice for treatment would be a neuraminidase inhibitor; however, as the exclusion of bacterial co-infection with pneumonia is difficult (Lahti et al. 2006), it is commonly recommended to treat all radiologically verified pneumonia cases with antibiotics. Even though influenza-related pneumonia in children is usually a relatively benign disease with low mortality (Lahti et al. 2006), severe, and in some cases fatal, bacterial pneumonias have been observed during seasonal epidemics (Finelli et al. 2008, Johnson et al. 2009). In a 3-year US study of influenza-attributable pediatric mortality, 35% of the children who died had pneumonia (Finelli et al. 2008).

In **Study IV**, acute neurologic symptoms accounted for 15% of all influenza-related hospitalizations, which can be considered an unusually high percentage for a respiratory infection. Among school-aged children, neurologic symptoms were the most important primary reason for admission (25%); however, the relative proportions of different admission diagnoses were more evenly distributed than in other age groups. As in earlier reports (Quach et al. 2003, Moore et al. 2006), febrile seizure was the most frequent neurologic complication in all age groups. Previously, it has been demonstrated that influenza is associated with an incidence of febrile seizures nearly two times higher than observed with adenovirus or parainfluenza virus infection (Chiu et al. 2001). Similarly to Newland et al. (2007), we found that the rate of febrile convulsions was particularly high among children aged 6 months to <3 years. Apart from febrile seizures, other neurologic complications were rarely encountered during our 16-year study period. The incidence

of encephalitis in our study (1%) correlates to the rates reported from other countries and settings (Quach et al. 2003, Moore et al. 2006, Coffin et al. 2007, Sakkou et al. 2011). For comparison, during the 2009 A/H1N1 pandemic, the rate of serious neurologic complications attributable to influenza has been reported to be approximately 2.5-3% (Farooq et al. 2012, Launes et al. 2011).

Some of our study children had a severe chronic neurologic disease as the primary reason for hospitalization. Most of these children were febrile, with increased amounts of respiratory secretions, but without evidence of pneumonia, seizures, or other serious complications on admission. Keren et al. (2005) have shown that underlying neurologic disease is associated with the highest risk of respiratory failure in children with influenza infections. Due to the small number of children in this admission category, as well as among those needing mechanical ventilation in our study, we were unable to draw any such conclusions. Notwithstanding, the two children who died with influenza during the study period both had a serious pre-existing neurologic condition.

As our hospitalization studies were retrospective, one shortcoming that must be taken into account is that the clinical data were obtained from medical records in which the clinical history had been recorded by several pediatric residents over the years. Choosing the principal admission diagnosis was in some cases challenging, especially in children with underlying conditions, as some of the children potentially had two or more admission diagnoses available. A limitation related to the collection of the virologic data should also be acknowledged. Even though viral sampling of admitted children during influenza epidemics had been a routine procedure within our department, it is possible that some influenza-positive children lacking respiratory symptoms may have remained untested. Second, children admitted with late complications of influenza may have had false negative test results due to a decline in antigen titers in nasopharynx. Furthermore, due to the moderate sensitivity of rapid tests in children, some of the tests may have remained false negative. However, we believe that, regardless of the viral diagnostic method used, all of the children included in the analyses had true influenza, which is the most important point in the study.

To conclude, our hospital study reveals the broad spectrum of illness due to influenza in the pediatric population, and thereby strengthens the arguments for effective prevention of influenza illness in children. Our findings also show that the impact of influenza is greatly underestimated if examined exclusively in the context of respiratory disease.

6.3.3 Comparison of the clinical picture between influenza A and B

There are limited data on the differences of clinical features between influenza A and B in children, and there are virtually no previous studies in which the signs and symptoms have been adjusted for the age of the child. It is possible that the traditional concept of a more severe illness of influenza A viruses compared to influenza B is seriously confounded by age. On average, children with influenza B tend to be older than those

with influenza A, and in previous studies (Peltola et al. 2003, Hite et al. 2007, Esposito et al. 2011a), the significant difference in the median ages of these groups interferes with reliable comparisons between the viral types. In our studies, we could not find any significant differences in the clinical picture, either in outpatients or inpatients, between influenza A and B, when signs and symptoms were analyzed within different age groups. However, we acknowledge that we had quite small numbers of influenza B cases in some age groups, and therefore we cannot rule out any statistically significant differences between the groups that could be seen with higher numbers of patients. Furthermore, the observation of similar clinical pictures of influenza A and B in our studies does not challenge the fact that in different seasons the overall virulence of different influenza strains may differ remarkably, which naturally influences the clinical features of the illness in a given year.

6.4 Future challenges

Despite extensive research into pediatric hospitalizations attributable to influenza in recent years, there are still relatively scant data on the burden of influenza in an outpatient setting. In Europe especially, further population-based studies on virologically confirmed influenza are needed to establish the health toll of outpatient influenza in normal, healthy children. Due to the substantial differences in the health care systems between different countries, these kinds of studies are especially important when developing vaccination policies against influenza in a given country.

Influenza is the only viral respiratory disease currently preventable by vaccination, and it is widely accepted that vaccination is the elementary approach in the prevention of influenza in all age groups. However, in the current situation vaccination of children is not without problems. First, for the most vulnerable group in terms of hospitalization, infants younger than 6 months of age, there is no licensed vaccine available at all. Even though there are a few studies showing initial evidence for the safety and immunogenicity of TIV in infants <6 months of age, further studies in this area are undoubtedly needed. In the meantime, reducing the burden of influenza in young infants requires maximizing every opportunity to provide influenza vaccines for their family members, including older siblings and other close contacts, as well as for pregnant women.

Second, as the effectiveness of the currently available influenza vaccines is far from perfect, and varies from year to year depending on the match between the circulating strains and the vaccine composition, research into the development of more effective vaccines is still needed. An ideal influenza vaccine would provide a broader than just strain-specific immunity. A slight improvement in the present state of affairs is coming soon, as a new nasal-spray influenza vaccine (LAIV) is expected to enter the Finnish market in the autumn of 2012. However, because the vaccine is only licensed for children aged 2-<18 years of age, it will not, unfortunately, improve the situation among the youngest children.

Even with the currently licensed vaccines, however, the annual burden of pediatric influenza could be substantially decreased if the rate of influenza immunization in children (>6 months of age) could be increased. The reasons for the relatively poor vaccination coverage among children include, among other things, a lack of belief among the general public that influenza is responsible for significant illness in the community. Our results indicate that influenza is worth preventing because of the misery it causes, and the risks for complications associated with it, as well as the remarkable economic burden it causes for the society. An appreciation of the extensive consequences of influenza in children should prompt health care workers toward more active attempts at prevention and treatment of this illness.

SUMMARY AND CONCLUSIONS

The objective of this study was to assess the burden of influenza in children by evaluating the epidemiology (in terms of morbidity and hospitalization rates), socioeconomic consequences, and complications of this illness, and by describing the clinical features of influenza in both outpatient and hospitalized children.

In Study I, we demonstrated that an average annual influenza epidemic causes a substantial heath toll on outpatient children and their families. The burden of illness was greatest in children <3 years of age: in that age group the attack rates, as well as children's absences from daycare and parents' absences from work, were highest. Acute otitis media was the most frequent complication of influenza, and it occurred in 40% of influenza-positive children aged <3 years. The results indicate that vaccination of children <3 years of age might be beneficial in reducing the total burden of pediatric influenza on society.

Study II showed that the symptoms of early influenza in outpatient children differ in many aspects from the traditional concept of influenza symptomatology. Most children with influenza had rhinitis already during the early phase of the illness, whereas headache and muscle aches were found to not be essential features of pediatric influenza. High fever was the most remarkable sign of influenza in all age groups, and the clinical picture of the illness was most severe in children <3 years of age. The clinical diagnosis of influenza is very difficult, especially in young children, and the findings underscore the importance of microbiologic diagnosis of influenza for optimal treatment of the illness.

In Study III, it was demonstrated that the average annual incidence of influenza-related admissions was clearly highest in infants <6 months of age (276 / 100,000). The population-based rates of influenza B hospitalizations were also highest in the youngest age groups, although the differences between age groups were smaller than those for influenza A-attributable admissions. In total, influenza B infections accounted for 18% of all influenza-related hospitalizations. The high incidence of influenza-associated hospitalizations among infants younger than 6 months of age emphasizes the need to find effective ways to prevent influenza illness in this age group, in which influenza vaccines are not currently licensed for use.

In Study IV, a wide spectrum of clinical conditions was observed in children with influenza at the time of hospitalization. The primary admission diagnoses varied greatly between different age groups, with more than half of infants <6 months of age being admitted due to suspected sepsis. Interestingly, respiratory symptoms accounted for only 38% of the admissions. No significant differences in the primary admission diagnoses could be demonstrated between children with influenza A and B infections. The leading role of sepsis-like illness in young infants is clinically important because in addition to hospitalization it often leads to invasive examinations and initiation of empirical antibiotic treatment, all of which cause a remarkable burden on infants and their families, as well as high costs to the society.

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REFERENCES

Agoritsas K, Mack K, Bonsu BK, Goodman D, Salamon D, Marcon MJ. Evaluation of the Quidel QuickVue test for detection of influenza A and B viruses in the pediatric emergency medicine setting by use of three specimen collection methods. J Clin Microbiol 2006;44:2638-41.

Air GM. Sequence relationships among the hemagglutinin genes of 12 subtypes of influenza A virus. Proc Natl Acad Sci U S A 1981;78:7639-43.

Ajayi-Obe EK, Coen PG, Handa R, et al. Influenza A and respiratory syncytial virus hospital burden in young children in East London. Epidemiol Infect 2008;136:1046-58.

Ampofo K, Gesteland PH, Bender J, et al. Epidemiology, complications, and cost of hospitalization in children with laboratory-confirmed influenza infection. Pediatrics 2006;118:2409-17.

Ashkenazi S, Vertruyen A, Aristegui J, et al. Superior relative efficacy of live attenuated influenza vaccine compared with inactivated influenza vaccine in young children with recurrent respiratory tract infections. Pediatr Infect Dis J 2006;25:870-9.

Barker WH. Excess pneumonia and influenza associated hospitalization during influenza epidemics in the United States, 1970-78. Am J Public Health 1986;76:761-5.

Barker WH, Mullooly JP. Impact of epidemic type A influenza in a defined adult population. Am J Epidemiol 1980;112:798-811.

Barr CE, Schulman K, Iacuzio D, Bradley JS. Effect of oseltamivir on the risk of pneumonia and use of health care services in children with clinically diagnosed influenza. Curr Med Res Opin 2007;23:523-31.

Bean B, Moore BM, Sterner B, Peterson LR, Gerding DN, Balfour HH,Jr. Survival of influenza viruses on environmental surfaces. J Infect Dis 1982;146:47-51.

Beare AS, Hobson D, Reed SE, Tyrrell DA. Antibody responses to and efficacy of an inactivated spray vaccine. Bull World Health Organ 1969;41:549-51.

Beigel JH, Farrar J, Han AM, et al. Avian influenza A (H5N1) infection in humans. N Engl J Med 2005;353:1374-85.

Belshe RB, Mendelman PM, Treanor J, et al. The efficacy of live attenuated, cold-adapted, trivalent, intranasal influenzavirus vaccine in children. N Engl J Med 1998;338:1405-12.

Belshe RB, Edwards KM, Vesikari T, et al. Live attenuated versus inactivated influenza vaccine in infants and young children. N Engl J Med 2007;356:685-96.

Belshe RB. The need for quadrivalent vaccine against seasonal influenza. Vaccine 2010;28 Suppl 4:D45-53.

Belshe RB, Coelingh K, Ambrose CS, Woo JC, Wu X. Efficacy of live attenuated influenza vaccine in children against influenza B viruses by lineage and antigenic similarity. Vaccine 2010;28:2149-56.

Bender JM, Ampofo K, Gesteland P, et al. Influenza virus infection in infants less than three months of age. Pediatr Infect Dis J 2010;29:6-9.

Bettinger JA, Sauve LJ, Scheifele DW, et al. Pandemic influenza in Canadian children: a summary of hospitalized pediatric cases. Vaccine 2010;28:3180-4.

Beveridge WI. The chronicle of influenza epidemics. Hist Philos Life Sci 1991;13:223-34.

Bhat N, Wright JG, Broder KR, et al. Influenza-associated deaths among children in the United States, 2003-2004. N Engl J Med 2005;353:2559-67.

Birnkrant D, Cox E. The Emergency Use Authorization of peramivir for treatment of 2009 H1N1 influenza. N Engl J Med 2009;361:2204-7.

Block SL, Heikkinen T, Toback SL, Zheng W, Ambrose CS. The efficacy of live attenuated influenza vaccine against influenza-associated acute otitis media in children. Pediatr Infect Dis J 2011;30:203-7.

Boivin G, Hardy I, Tellier G, Maziade J. Predicting influenza infections during epidemics with use of a clinical case definition. Clin Infect Dis 2000;31:1166-9.

Bonner AB, Monroe KW, Talley LI, Klasner AE, Kimberlin DW. Impact of the rapid diagnosis of influenza on physician decision-making and patient management in the pediatric emergency department: results of a randomized, prospective, controlled trial. Pediatrics 2003;112:363-7.

Boone SA, Gerba CP. The occurrence of influenza A virus on household and day care center fomites. J Infect 2005;51:103-9.

Bootsma MC, Ferguson NM. The effect of public health measures on the 1918 influenza pandemic in U.S. cities. Proc Natl Acad Sci U S A 2007;104:7588-93.

Bourgeois FT, Valim C, McAdam AJ, Mandl KD. Relative impact of influenza and respiratory syncytial virus in young children. Pediatrics 2009;124:e1072-80.

Bracco Neto H, Farhat CK, Tregnaghi MW, et al. Efficacy and safety of 1 and 2 doses of live attenuated influenza vaccine in vaccine-naive children. Pediatr Infect Dis J 2009;28:365-71.

Brankston G, Gitterman L, Hirji Z, Lemieux C, Gardam M. Transmission of influenza A in human beings. Lancet Infect Dis 2007;7:257-65.

Brokstad KA, Cox RJ, Olofsson J, Jonsson R, Haaheim LR. Parenteral influenza vaccination induces a rapid systemic and local immune response. J Infect Dis 1995;171:198-203.

Bullough PA, Hughson FM, Skehel JJ, Wiley DC. Structure of influenza haemagglutinin at the pH of membrane fusion. Nature 1994;371:37-43.

Bush RM, Bender CA, Subbarao K, Cox NJ, Fitch WM. Predicting the evolution of human influenza A. Science 1999;286:1921-5.

Butt KM, Smith GJ, Chen H, et al. Human infection with an avian H9N2 influenza A virus in Hong Kong in 2003. J Clin Microbiol 2005;43:5760-7.

Byington CL, Enriquez FR, Hoff C, et al. Serious bacterial infections in febrile infants 1 to 90 days old with and without viral infections. Pediatrics 2004;113:1662-6.

Call SA, Vollenweider MA, Hornung CA, Simel DL, McKinney WP. Does this patient have influenza? JAMA 2005;293:987-97.

Cao B, Li XW, Mao Y, et al. Clinical features of the initial cases of 2009 pandemic influenza A (H1N1) virus infection in China. N Engl J Med 2009;361:2507-17.

Capua I, Alexander D. Perspectives on the global threat: the challenge of avian influenza viruses for the world's veterinary community. Avian Dis 2010;54:176-8.

Carrat F, Vergu E, Ferguson NM, et al. Time lines of infection and disease in human influenza: a review of volunteer challenge studies. Am J Epidemiol 2008;167:775-85.

Cauchemez S, Valleron AJ, Boelle PY, Flahault A, Ferguson NM. Estimating the impact of school closure on influenza transmission from Sentinel data. Nature 2008;452:750-4.

CDC (Centers for Disease Control and Prevention). Outbreak of swine-origin influenza A (H1N1) virus infection - Mexico, March-April 2009. MMWR Morb Mortal Wkly Rep 2009a;58:467-70.

CDC (Centers for Disease Control and Prevention). Swine influenza A (H1N1) infection in two children--Southern California, March-April 2009. MMWR Morb Mortal Wkly Rep 2009b;58:400-2.

CDC (Centers for Disease Control and Prevention). Neurologic complications associated with novel influenza A (H1N1) virus infection in children - Dallas, Texas, May 2009. MMWR Morb Mortal Wkly Rep 2009c;58:773-8.

CDC (Centers for Disease Control and Prevention). Surveillance for pediatric deaths associated with 2009 pandemic influenza A (H1N1) virus infection - United States, April-August 2009. MMWR Morb Mortal Wkly Rep 2009d;58:941-7.

Ceyhan M, Karadag Oncel E, Badur S, et al. Effectiveness of a new bioequivalent formulation of oseltamivir (Enfluvir(R)) on 2010-2011 seasonal influenza viruses: an open phase IV study. Int J Infect Dis 2012;16:e273-8.

Chan MC, Lee N, Chan PK, Leung TF, Sung JJ. Fecal detection of influenza A virus in patients with concurrent respiratory and gastrointestinal symptoms. J Clin Virol 2009;45:208-11.

Chi CY, Wang SM, Lin CC, et al. Clinical features of children infected with different strains of influenza B in southern Taiwan. Pediatr Infect Dis J 2008;27:640-5.

Chiappini E, Galli L, Azzi A, Resti M, Bonsignori F, de Martino M. Lymphocytopenia as a marker for pandemic influenza A/H1N1 2009 virus infection in children. J Med Virol 2011;83:1-4.

Chiu SS, Chan KH, Chen H, et al. Virologically confirmed population-based burden of hospitalization caused by influenza A and B among children in Hong Kong. Clin Infect Dis 2009;49:1016-21.

Chiu SS, Lau YL, Chan KH, Wong WH, Peiris JS. Influenza-related hospitalizations among children in Hong Kong. N Engl J Med 2002;347:2097-103.

Chiu SS, Tse CY, Lau YL, Peiris M. Influenza A infection is an important cause of febrile seizures. Pediatrics 2001;108:E63.

Chowell G, Viboud C, Simonsen L, Miller MA. Measuring the benefits of school closure interventions to mitigate influenza. Expert Rev Respir Med 2011;5:597-9.

Claas EC, Osterhaus AD, van Beek R, et al. Human influenza A H5N1 virus related to a highly pathogenic avian influenza virus. Lancet 1998;351:472-7.

Coffin SE, Zaoutis TE, Rosenquist AB, et al. Incidence, complications, and risk factors for prolonged stay in children hospitalized with community-acquired influenza. Pediatrics 2007;119:740-8.

Compans RW, Dimmock NJ, Meier-Ewert H. Effect of antibody to neuraminidase on the maturation and hemagglutinating activity of an influenza A2 virus. J Virol 1969;4:528-34.

Couch RB, Douglas RG,Jr, Fedson DS, Kasel JA. Correlated studies of a recombinant influenza-virus vaccine. 3. Protection against experimental influenza in man. J Infect Dis 1971;124:473-80.

Cowling BJ, Chan KH, Fang VJ, et al. Facemasks and hand hygiene to prevent influenza transmission in households: a cluster randomized trial. Ann Intern Med 2009;151:437-46.

Cowling BJ, Chan KH, Fang VJ, et al. Comparative epidemiology of pandemic and seasonal influenza A in households. N Engl J Med 2010;362:2175-84.

Cox NJ, Subbarao K. Global epidemiology of influenza: past and present. Annu Rev Med 2000;51:407-21.

Cunha BA, Pherez FM, Schoch P. Diagnostic importance of relative lymphopenia as a marker of swine influenza (H1N1) in adults. Clin Infect Dis 2009;49:1454-6.

Dagan R, Hall CB. Influenza A virus infection imitating bacterial sepsis in early infancy. Pediatr Infect Dis 1984;3:218-21.

Daley AJ, Nallusamy R, Isaacs D. Comparison of influenza A and influenza B virus infection in hospitalized children. J Paediatr Child Health 2000;36:332-5.

Dauvilliers Y, Montplaisir J, Cochen V, et al. Post-H1N1 narcolepsy-cataplexy. Sleep 2010;33:1428-30.

Dawood FS, Fiore A, Kamimoto L, et al. Burden of seasonal influenza hospitalization in children, United States, 2003 to 2008. J Pediatr 2010a;157:808-14.

Dawood FS, Fiore A, Kamimoto L, et al. Influenza-associated pneumonia in children hospitalized with laboratory-confirmed influenza, 2003-2008. Pediatr Infect Dis J 2010b;29:585-90.

Dawood FS, Jain S, Finelli L, et al. Emergence of a novel swine-origin influenza A (H1N1) virus in humans. N Engl J Med 2009;360:2605-15.

Demicheli V, Jefferson T, Rivetti D, Deeks J. Prevention and early treatment of influenza in healthy adults. Vaccine 2000;18:957-1030.

Dodds L, McNeil SA, Fell DB, et al. Impact of influenza exposure on rates of hospital admissions and physician visits because of respiratory illness among pregnant women. CMAJ 2007;176:463-8.

Doing KM, Jerkofsky MA, Dow EG, Jellison JA. Use of fluorescent-antibody staining of cytocentrifuge-prepared smears in combination with cell culture for direct detection of respiratory viruses. J Clin Microbiol 1998;36:2112-4.

Dowell SF. Low attack rate of summertime influenza: could it be the host? Clin Infect Dis 2001;33:1951-2.

Drake JW. Rates of spontaneous mutation among RNA viruses. Proc Natl Acad Sci U S A 1993;90:4171-5.

Duin N, Sutcliffe J. A History of Medicine From Prehistory to the Year 2020. London: Simon & Schuster Ltd., 1992.

ECDC (European Centre for Disease Prevention and Control). Seasonal Human Influenza and Vaccination - The Facts. 2009. Available at: http://www.edcd.europa. eu/en/healthtopics/Documents/0712_seasonal_human_influenza_vaccination.pdf2009.

ECDC (European Centre for Disease Prevention and Control). Announced number of new and cumulative confirmed fatal 2009 pandemic influenza A (H1N1) cases in EU and EFTA countries, as of week 17–2010. Available at: http://www.edcd.europa. eu/en /healthtopics/H1N1/Pages/Reported_number_of_new_and_cumulative_confirmed 2010.

Echevarria-Zuno S, Mejia-Arangure JM, Mar-Obeso AJ, et al. Infection and death from influenza A H1N1 virus in Mexico: a retrospective analysis. Lancet 2009;374:2072-9.

Eisenberg KW, Szilagyi PG, Fairbrother G, et al. Vaccine effectiveness against laboratory-confirmed influenza in children 6 to 59 months of age during the 2003-2004 and 2004-2005 influenza seasons. Pediatrics 2008;122:911-9.

EMA. European Medicines Agency starts review of Pandemrix; 2010a; Available at: http://www.ema.europa.eu/docs/en_GB/document_library/Press_release/2010/08/WC500096005.pdf. (Accessed 8/27, 2010).

EMA. European Medicines Agency. CHMP summary of positive opinion for Fluenz. 2010b; Available at: http://www.ema. europa.eu/ema /index.jsp?curl=pages//medicines /human/medicines/001101/smops/Positive/human_smop_000145.jsp&mid=WC0b01ac058001d127. (Assessed 10/22, 2010).

EMA. European Medicines Agency. Summary on compassionate use of oseltamivir. 2010c; available at: http://www.ema.europa.eu/docs/en _GB/document_library/Other/2010/05/WC500090250.pdf. (Assessed 9/29, 2011).

EMA. European Medicines Agency: Conditions of use, conditions for distribution and patients targeted and conditions for safety monitoring addressed to member states for IV Zanamivir available for compassionate use. 2011; Available at:http://www.ema.europa.

eu/docs/en_GB/document_library/Other/2010/02/WC500074124.pdf. (Assessed 9/28, 2011).

Englund JA. Maternal immunization with inactivated influenza vaccine: rationale and experience. Vaccine 2003;21:3460-4.

Englund JA, Walter EB, Fairchok MP, Monto AS, Neuzil KM. A comparison of 2 influenza vaccine schedules in 6- to 23-month-old children. Pediatrics 2005;115:1039-47.

Englund JA, Walter E, Black S, et al. Safety and immunogenicity of trivalent inactivated influenza vaccine in infants: a randomized double-blind placebo-controlled study. Pediatr Infect Dis J 2010;29:105-10.

Esposito S, Molteni CG, Daleno C, et al. Clinical and socioeconomic impact of different types and subtypes of seasonal influenza viruses in children during influenza seasons 2007/2008 and 2008/2009. BMC Infect Dis 2011a;11:271.

Esposito S, Pugni L, Daleno C, et al. Influenza A/H1N1 MF59-adjuvanted vaccine in preterm and term children aged 6 to 23 months. Pediatrics 2011b;127:e1161-8.

Espy MJ, Smith TF, Harmon MW, Kendal AP. Rapid detection of influenza virus by shell vial assay with monoclonal antibodies. J Clin Microbiol 1986;24:677-9.

Evans KD, Kline MW. Prolonged influenza A infection responsive to rimantadine therapy in a human immunodeficiency virus-infected child. Pediatr Infect Dis J 1995;14:332-4.

Fairbrother G, Cassedy A, Ortega-Sanchez IR, et al. High costs of influenza: Direct medical costs of influenza disease in young children. Vaccine 2010;28:4913-9.

Farias JA, Fernandez A, Monteverde E, et al. Critically ill infants and children with influenza A (H1N1) in pediatric intensive care units in Argentina. Intensive Care Med 2010;36:1015-22.

Farooq O, Faden HS, Cohen ME, et al. Neurologic complications of 2009 influenza-a H1N1 infection in children. J Child Neurol 2012;27:431-8.

FDA (U.S. Food and Drug Administration). Emergency Use of Tamiflu in Infants Less than 1 year of Age. 2009; Available at: http://www.fda.gov /Drugs/DrugSafety/PostmarketDrugSafetyInformationforPatientsandProviders/ucm183870.htm (Accessed 09/25, 2009).

FDA (U.S. Food and Drug Administration). FDA approves first quadrivalent vaccine to prevent seasonal influenza. 2012; Available at: http://www.fda. gov/NewsEvents/Newsroom/PressAnnouncements/ucm294057.htm 2012. 2012. (Accessed 02/29, 2012).

Ferguson NM, Cummings DA, Fraser C, Cajka JC, Cooley PC, Burke DS. Strategies for mitigating an influenza pandemic. Nature 2006;442:448-52.

Finelli L, Fiore A, Dhara R, et al. Influenza-associated pediatric mortality in the United States: increase of Staphylococcus aureus coinfection. Pediatrics 2008;122:805-11.

Fiore AE, Shay DK, Broder K, et al. Prevention and control of influenza: recommendations of the Advisory Committee on Immunization Practices (ACIP), 2008. MMWR Recomm Rep 2008;57:1-60.

Fiore AE, Bridges CB, Cox NJ. Seasonal influenza vaccines. Curr Top Microbiol Immunol 2009;333:43-82.

Fiore AE, Fry A, Shay D, et al. Antiviral agents for the treatment and chemoprophylaxis of influenza --- recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR Recomm Rep 2011;60:1-24.

Fisher RG, Gruber WC, Edwards KM, et al. Twenty years of outpatient respiratory syncytial virus infection: a framework for vaccine efficacy trials. Pediatrics 1997;99:E7.

Fouchier RA, Schneeberger PM, Rozendaal FW, et al. Avian influenza A virus (H7N7) associated with human conjunctivitis and a fatal case of acute respiratory distress syndrome. Proc Natl Acad Sci U S A 2004;101:1356-61.

Fouchier RA, Munster V, Wallensten A, et al. Characterization of a novel influenza A virus hemagglutinin subtype (H16) obtained from black-headed gulls. J Virol 2005;79:2814-22.

Fox JP, Hall CE, Cooney MK, Foy HM. Influenzavirus infections in Seattle families, 1975-1979. I. Study design, methods and the occurrence of infections by time and age. Am J Epidemiol 1982a;116:212-27.

Fox JP, Cooney MK, Hall CE, Foy HM. Influenzavirus infections in Seattle families, 1975-1979. II. Pattern of infection in invaded households and relation of age and prior antibody to occurrence of infection and related illness. Am J Epidemiol 1982b;116:228-42.

Frank AL, Taber LH, Wells CR, Wells JM, Glezen WP, Paredes A. Patterns of shedding of myxoviruses and paramyxoviruses in children. J Infect Dis 1981;144:433-41.

Frank AL, Taber LH, Wells JM. Comparison of infection rats and severity of illness for influenza A subtypes H1N1 and H3N2. J Infect Dis 1985;151:73-80.

Friedman MJ, Attia MW. Clinical predictors of influenza in children. Arch Pediatr Adolesc Med 2004;158:391-4.

Frobert E, Sarret C, Billaud G, et al. Pediatric neurological complications associated with the A(H1N1)pdm09 influenza infection. J Clin Virol 2011;52:307-13.

Gamblin SJ, Haire LF, Russell RJ, et al. The structure and receptor binding properties of the 1918 influenza hemagglutinin. Science 2004;303:1838-42.

Garg S, Fry AM, Patton M, Fiore AE, Finelli L. Antiviral treatment of influenza in children. Pediatr Infect Dis J 2012;31:e43-51.

Garten RJ, Davis CT, Russell CA, et al. Antigenic and genetic characteristics of swine-origin 2009 A(H1N1) influenza viruses circulating in humans. Science 2009;325:197-201.

Gasparini R, Durando P, Ansaldi F, et al. Influenza and respiratory syncytial virus in infants and children: relationship with attendance at a paediatric emergency unit and characteristics of the circulating strains. Eur J Clin Microbiol Infect Dis 2007;26:619-28.

Gerhard W. The role of the antibody response in influenza virus infection. Curr Top Microbiol Immunol 2001;260:171-90.

Glezen WP, Couch RB. Interpandemic influenza in the Houston area, 1974-76. N Engl J Med 1978;298:587-92.

Glezen WP, Paredes A, Taber LH. Influenza in children. Relationship to other respiratory agents. JAMA 1980;243:1345-9.

Glezen WP. Serious morbidity and mortality associated with influenza epidemics. Epidemiol Rev 1982;4:25-44.

Glezen WP, Decker M, Joseph SW, Mercready RG,Jr. Acute respiratory disease associated with influenza epidemics in Houston, 1981-1983. J Infect Dis 1987a;155:1119-26.

Glezen WP, Decker M, Perrotta DM. Survey of underlying conditions of persons hospitalized with acute respiratory disease during influenza epidemics in Houston, 1978-1981. Am Rev Respir Dis 1987b;136:550-5.

Glezen WP. Emerging infections: pandemic influenza. Epidemiol Rev 1996;18:64-76.

Glezen WP, Taber LH, Frank AL, Gruber WC, Piedra PA. Influenza virus infections in infants. Pediatr Infect Dis J 1997;16:1065-8.

Glezen WP, Gaglani MJ, Kozinetz CA, Piedra PA. Direct and indirect effectiveness of influenza vaccination delivered to children at school preceding an epidemic caused by 3 new influenza virus variants. J Infect Dis 2010;202:1626-33.

Grayson ML, Melvani S, Druce J, et al. Efficacy of soap and water and alcohol-based hand-rub preparations against live H1N1 influenza virus on the hands of human volunteers. Clin Infect Dis 2009;48:285-91.

Griffin MR, Neuzil KM. The global implications of influenza in Hong Kong. N Engl J Med 2002;347:2159-62.

Grijalva CG, Poehling KA, Edwards KM, et al. Accuracy and interpretation of rapid influenza tests in children. Pediatrics 2007;119:e6-11.

Groothuis JR, Levin MJ, Rabalais GP, Meiklejohn G, Lauer BA. Immunization of high-risk infants younger than 18 months of age with split-product influenza vaccine. Pediatrics 1991;87:823-8.

Gross ER, Gander JW, Reichstein A, Cowles RA, Stolar CJ, Middlesworth W. Fulminant pH1N1-09 influenza-associated myocarditis in pediatric patients. Pediatr Crit Care Med 2011;12:e99-e101.

Hackett S, Hill L, Patel J, et al. Clinical characteristics of paediatric H1N1 admissions in Birmingham, UK. Lancet 2009;374:605.

Halasa NB, Gerber MA, Chen Q, Wright PF, Edwards KM. Safety and immunogenicity of trivalent inactivated influenza vaccine in infants. J Infect Dis 2008;197:1448-54.

Hall CE, Cooney MK, Fox JP. The Seattle virus watch. IV. Comparative epidemiologic observations of infections with influenza A and B viruses, 1965-1969, in families with young children. Am J Epidemiol 1973;98:365-80.

Hall CB, Douglas RG,Jr, Geiman JM, Meagher MP. Viral shedding patterns of children with influenza B infection. J Infect Dis 1979;140:610-3.

Halloran ME, Piedra PA, Longini IM,Jr, et al. Efficacy of trivalent, cold-adapted, influenza virus vaccine against influenza A (Fujian), a drift variant, during 2003-2004. Vaccine 2007;25:4038-45.

Han SN, Meydani SN. Antioxidants, cytokines, and influenza infection in aged mice and elderly humans. J Infect Dis 2000;182 Suppl 1:S74-80.

Han F, Lin L, Warby SC, et al. Narcolepsy onset is seasonal and increased following the 2009 H1N1 pandemic in china. Ann Neurol 2011;70:410-7.

Hancock K, Veguilla V, Lu X, et al. Cross-reactive antibody responses to the 2009 pandemic H1N1 influenza virus. N Engl J Med 2009;361:1945-52.

Harper SA, Bradley JS, Englund JA, et al. Seasonal influenza in adults and children--diagnosis, treatment, chemoprophylaxis, and institutional outbreak management: clinical practice guidelines

of the Infectious Diseases Society of America. Clin Infect Dis 2009;48:1003-32.

Hatakeyama S, Sugaya N, Ito M, et al. Emergence of influenza B viruses with reduced sensitivity to neuraminidase inhibitors. JAMA 2007;297:1435-42.

Hayden FG, Atmar RL, Schilling M, et al. Use of the selective oral neuraminidase inhibitor oseltamivir to prevent influenza. N Engl J Med 1999;341:1336-43.

Hayden FG, Gubareva LV, Monto AS, et al. Inhaled zanamivir for the prevention of influenza in families. Zanamivir Family Study Group. N Engl J Med 2000;343:1282-9.

Hayden FG, Belshe R, Villanueva C, et al. Management of influenza in households: a prospective, randomized comparison of oseltamivir treatment with or without postexposure prophylaxis. J Infect Dis 2004;189:440-9.

Hedrick JA, Barzilai A, Behre U, et al. Zanamivir for treatment of symptomatic influenza A and B infection in children five to twelve years of age: a randomized controlled trial. Pediatr Infect Dis J 2000;19:410-7.

Heikkinen T, Ruuskanen O, Waris M, Ziegler T, Arola M, Halonen P. Influenza vaccination in the prevention of acute otitis media in children. Am J Dis Child 1991;145:445-8.

Heikkinen T, Ruuskanen O. Temporal development of acute otitis media during upper respiratory tract infection. Pediatr Infect Dis J 1994;13:659-61.

Heikkinen T, Thint M, Chonmaitree T. Prevalence of various respiratory viruses in the middle ear during acute otitis media. N Engl J Med 1999;340:260-4.

Heikkinen T, Salmi AA, Ruuskanen O. Comparative study of nasopharyngeal aspirate and nasal swab specimens for detection of influenza. BMJ 2001;322:138.

Heikkinen T, Marttila J, Salmi AA, Ruuskanen O. Nasal swab versus nasopharyngeal aspirate for isolation of respiratory viruses. J Clin Microbiol 2002;40:4337-9.

Heikkinen T, Chonmaitree T. Importance of respiratory viruses in acute otitis media. Clin Microbiol Rev 2003;16:230-41.

Heikkinen T, Ziegler T, Peltola V, et al. Incidence of influenza in Finnish children. Pediatr Infect Dis J 2003;22:S204-6.

Heikkinen T. Influenza in children. Acta Paediatr 2006;95:778-84.

Heikkinen T, Renko M, Rosenberg L, et al. Lasten influenssarokotustyöryhmän raportti 14.6.2006: KTL, 2006.

Heikkinen T, Heinonen S. Effectiveness and safety of influenza vaccination in children: European perspective. Vaccine 2011;29:7529-34.

Heinonen S, Silvennoinen H, Lehtinen P, et al. Early oseltamivir treatment of influenza in children 1-3 years of age: a randomized controlled trial. Clin Infect Dis 2010;51:887-94.

Heinonen S, Silvennoinen H, Lehtinen P, Vainionpaa R, Ziegler T, Heikkinen T. Effectiveness of inactivated influenza vaccine in children aged 9 months to 3 years: an observational cohort study. Lancet Infect Dis 2011a;11:23-9.

Heinonen S, Silvennoinen H, Lehtinen P, Vainionpaa R, Heikkinen T. Feasibility of diagnosing influenza within 24 hours of symptom onset in children 1-3 years of age. Eur J Clin Microbiol Infect Dis 2011b;30:387-92.

Heinonen S, Peltola V, Silvennoinen H, Vahlberg T, Heikkinen T. Signs and symptoms predicting influenza in children: a matched case-control analysis of prospectively collected clinical data. Eur J Clin Microbiol Infect Dis 2012;31:1569-74.

Hemmes JH, Winkler KC, Kool SM. Virus survival as a seasonal factor in influenza and polimyelitis. Nature 1960;188:430-1.

Hilleman MR. Realities and enigmas of human viral influenza: pathogenesis, epidemiology and control. Vaccine 2002;20:3068-87.

Hirst GK, Rickard ER, Friedewald WF. Studies in Human Immunization Against Influenza : Duration of Immunity Induced by Inactive Virus. J Exp Med 1944;80:265-73.

Hite LK, Glezen WP, Demmler GJ, Munoz FM. Medically attended pediatric influenza during the resurgence of the Victoria lineage of influenza B virus. Int J Infect Dis 2007;11:40-7.

Hoberman A, Greenberg DP, Paradise JL, et al. Effectiveness of inactivated influenza vaccine in preventing acute otitis media in young children: a randomized controlled trial. JAMA 2003;290:1608-16.

Horimoto T, Kawaoka Y. Pandemic threat posed by avian influenza A viruses. Clin Microbiol Rev 2001;14:129-49.

Hu JJ, Kao CL, Lee PI, et al. Clinical features of influenza A and B in children and association with myositis. J Microbiol Immunol Infect 2004;37:95-8.

Hurt AC, Alexander R, Hibbert J, Deed N, Barr IG. Performance of six influenza rapid tests in detecting human influenza in clinical specimens. J Clin Virol 2007;39:132-5.

Hurwitz ES, Haber M, Chang A, et al. Studies of the 1996-1997 inactivated influenza vaccine among children attending day care: immunologic response, protection against infection, and clinical effectiveness. J Infect Dis 2000;182:1218-21.

Ikonen N, Strengell M, Kinnunen L, et al. High frequency of cross-reacting antibodies against 2009 pandemic influenza A(H1N1) virus among the elderly in Finland. Euro Surveill 2010;15:19478.

Ito T, Couceiro JN, Kelm S, et al. Molecular basis for the generation in pigs of influenza A viruses with pandemic potential. J Virol 1998;72:7367-73.

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;113:1758-64.

Izurieta HS, Thompson WW, Kramarz P, et al. Influenza and the rates of hospitalization for respiratory disease among infants and young children. N Engl J Med 2000;342:232-9.

Jain S, Kamimoto L, Bramley AM, et al. Hospitalized patients with 2009 H1N1 influenza in the United States, April-June 2009. N Engl J Med 2009;361:1935-44.

Jartti T, Lehtinen P, Vuorinen T, et al. Respiratory picornaviruses and respiratory syncytial virus as causative agents of acute expiratory wheezing in children. Emerg Infect Dis 2004;10:1095-101.

Jefferson T, Rivetti A, Harnden A, Di Pietrantonj C, Demicheli V. Vaccines for preventing influenza in healthy children. Cochrane Database Syst Rev 2008;(2):CD004879.

Jefferson T, Del Mar CB, Dooley L, et al. Physical interventions to interrupt or reduce the spread of respiratory viruses. Cochrane Database Syst Rev 2011;(7):CD006207.

Johnson PR, Feldman S, Thompson JM, Mahoney JD, Wright PF. Immunity to influenza A virus infection in young children: a comparison of natural infection, live cold-adapted vaccine, and inactivated vaccine. J Infect Dis 1986;154:121-7

Johnson NP, Mueller J. Updating the accounts: global mortality of the 1918-1920 "Spanish" influenza pandemic. Bull Hist Med 2002;76:105-15.

Johnson BF, Wilson LE, Ellis J, et al. Fatal cases of influenza a in childhood. PLoS One 2009;4:e7671..

Joshi AY, Iyer VN, St Sauver JL, Jacobson RM, Boyce TG. Effectiveness of inactivated influenza vaccine in children less than 5 years of age over multiple influenza seasons: a case-control study. Vaccine 2009;27:4457-61.

Juven T, Mertsola J, Waris M, et al. Etiology of community-acquired pneumonia in 254 hospitalized children. Pediatr Infect Dis J 2000;19:293-8.

Karma PH, Penttila MA, Sipila MM, Kataja MJ. Otoscopic diagnosis of middle ear effusion in acute and non-acute otitis media. I. The value of different otoscopic findings. Int J Pediatr Otorhinolaryngol 1989;17:37-49.

Kartovaara L, Sauli H. Children in Finland. Helsinki, Finland: Statistics Finland, 2000.

Kash JC, Tumpey TM, Proll SC, et al. Genomic analysis of increased host immune and cell death responses induced by 1918 influenza virus. Nature 2006;443:578-81.

Kendal AP. Cold-adapted live attenuated influenza vaccines developed in Russia: can they contribute to meeting the needs for influenza control in other countries? Eur J Epidemiol 1997;13:591-609.

Keren R, Zaoutis TE, Bridges CB, et al. Neurological and neuromuscular disease as a risk factor for respiratory failure in children hospitalized with influenza infection. JAMA 2005;294:2188-94.

Kilbourne ED. Influenza pandemics of the 20th century. Emerg Infect Dis 2006;12:9-14.

Kimberlin DW, Shalabi M, Abzug MJ, et al. Safety of oseltamivir compared with the adamantanes in children less than 12 months of age. Pediatr Infect Dis J 2010;29:195-8.

King JC,Jr, Stoddard JJ, Gaglani MJ, et al. Effectiveness of school-based influenza vaccination. N Engl J Med 2006;355:2523-32.

Kiso M, Mitamura K, Sakai-Tagawa Y, et al. Resistant influenza A viruses in children treated with oseltamivir: descriptive study. Lancet 2004;364:759-65.

Koopmans M, Wilbrink B, Conyn M, et al. Transmission of H7N7 avian influenza A virus to human beings during a large outbreak in commercial poultry farms in the Netherlands. Lancet 2004;363:587-93.

Kuiken T, Taubenberger JK. Pathology of human influenza revisited. Vaccine 2008;26 Suppl 4:D59-66.

Kumar A, Zarychanski R, Pinto R, et al. Critically ill patients with 2009 influenza A(H1N1) infection in Canada. JAMA 2009;302:1872-9.

Kurz X, Domergue F, Slattery J, Segec A, Szmigiel A, Hidalgo-Simon A. Safety monitoring of Influenza A/H1N1 pandemic vaccines in EudraVigilance. Vaccine 2011;29:4378-87.

Kwong JC, Stukel TA, Lim J, et al. The effect of universal influenza immunization on mortality and health care use. PLoS Med 2008;5:e211.

Lahti E, Peltola V, Virkki R, Ruuskanen O. Influenza pneumonia. Pediatr Infect Dis J 2006;25:160-4.

Lambert SB, Allen KM, Carter RC, Nolan TM. The cost of community-managed viral respiratory illnesses in a cohort of healthy preschool-aged children. Respir Res 2008;9:11.

Lau LL, Cowling BJ, Fang VJ, et al. Viral shedding and clinical illness in naturally acquired influenza virus infections. J Infect Dis 2010;201:1509-16.

Launes C, Garcia-Garcia JJ, Jordan I, Martinez-Planas A, Selva L, Munoz-Almagro C. 2009 Influenza A H1N1 infections: delays in starting treatment with oseltamivir were associated with a more severe disease. Pediatr Infect Dis J 2011;30:622-5.

Li IW, Hung IF, To KK, et al. The natural viral load profile of patients with pandemic 2009 influenza A(H1N1) and the effect of oseltamivir treatment. Chest 2010;137:759-68.

Libster R, Bugna J, Coviello S, et al. Pediatric hospitalizations associated with 2009 pandemic influenza A (H1N1) in Argentina. N Engl J Med 2010;362:45-55.

Liou YS, Barbour SD, Bell LM, Plotkin SA. Children hospitalized with influenza B infection. Pediatr Infect Dis J 1987;6:541-3.

Lipsitch M, Viboud C. Influenza seasonality: lifting the fog. Proc Natl Acad Sci U S A 2009;106:3645-6.

Loeb M, Russell ML, Moss L, et al. Effect of influenza vaccination of children on infection rates in Hutterite communities: a randomized trial. JAMA 2010;303:943-50.

Longini IM,Jr, Koopman JS, Monto AS, Fox JP. Estimating household and community transmission parameters for influenza. Am J Epidemiol 1982;115:736-51.

Louie JK, Schechter R, Honarmand S, et al. Severe pediatric influenza in California, 2003-2005: implications for immunization recommendations. Pediatrics 2006;117:e610-8.

Lowen AC, Mubareka S, Steel J, Palese P. Influenza virus transmission is dependent on relative humidity and temperature. PLoS Pathog 2007;3:1470-6.

Lupovitch A. White cell differential count and influenza A. Am J Med 2005;118:1306,7; author reply 1307-9.

Lyytikäinen O, Kuusi M, Snellman M, et al. Influenssa A(H1N1)v -epidemian valtakunnalliset seurantatulokset. Suomen Lääkärilehti 2010;65:1996.

Läkemedelverket (The Swedish Medical products Agency). Occurrence of narcolepsy with cataplexy among children and adolescents in relation to the H1N1 pandemic and Pandemrix vaccinations – Results of a case inventory study by the MPA in Sweden during 2009–2010**.** 2011; Available at: http://www.lakemedelsverket.se/upload/nyheter/2011/PandemrixRegReport110328.pdf.

Matsuzaki Y, Katsushima N, Nagai Y, et al. Clinical features of influenza C virus infection in children. J Infect Dis 2006;193:1229-35.

Mazick A, Gergonne B, Wuillaume F, et al. Higher all-cause mortality in children during autumn 2009 compared with the three previous years: pooled results from eight European countries. Euro Surveill 2010;15:19480.

McCaughey C. Influenza: a virus of our times. Ulster Med J 2010;79:46-51.

McGeer AJ. Diagnostic testing or empirical therapy for patients hospitalized with suspected influenza: what to do? Clin Infect Dis 2009;48 Suppl 1:S14-9.

McIntosh K, Lieu T. Is it time to give influenza vaccine to healthy infants? N Engl J Med 2000;342:275-6.

McLean E, Pebody RG, Campbell C, et al. Pandemic (H1N1) 2009 influenza in the UK: clinical and epidemiological findings from the first few hundred (FF100) cases. Epidemiol Infect 2010;138:1531-41.

Meijer A, Lackenby A, Hungnes O, et al. Oseltamivir-resistant influenza virus A (H1N1), Europe, 2007-08 season. Emerg Infect Dis 2009;15:552-60.

Meissner HC. Influenza vaccines: a pediatric perspective. Curr Opin Pediatr 2007;19:58-63.

Mereckiene J, Cotter S, D'Ancona F, et al. Differences in national influenza vaccination policies across the European Union, Norway and Iceland 2008-2009. Euro Surveill 2010;15:19700.

Mintegi S, Garcia-Garcia JJ, Benito J, et al. Rapid influenza test in young febrile infants for the identification of low-risk patients. Pediatr Infect Dis J 2009;28:1026-8.

Molinari NA, Ortega-Sanchez IR, Messonnier ML, et al. The annual impact of seasonal influenza in the US: measuring disease burden and costs. Vaccine 2007;25:5086-96.

Montes M, Vicente D, Perez-Yarza EG, Cilla G, Perez-Trallero E. Influenza-related hospitalisations among children aged less than 5 years old in the Basque Country, Spain: a 3-year study (July 2001-June 2004). Vaccine 2005;23:4302-6.

Monto AS, Davenport FM, Napier JA, Francis T,Jr. Modification of an outbreak of influenza in Tecumseh, Michigan by vaccination of schoolchildren. J Infect Dis 1970;122:16-25.

Monto AS, Cavallaro JJ. The Tecumseh study of respiratory illness. II. Patterns of occurrence of infection with respiratory pathogens, 1965-1969. Am J Epidemiol 1971;94:280-9.

Monto AS, Sullivan KM. Acute respiratory illness in the community. Frequency of illness and the agents involved. Epidemiol Infect 1993;110:145-60.

Monto AS, Robinson DP, Herlocher ML, Hinson JM,Jr, Elliott MJ, Crisp A. Zanamivir in the prevention of influenza among healthy adults: a randomized controlled trial. JAMA 1999;282:31-5.

Monto AS, Gravenstein S, Elliott M, Colopy M, Schweinle J. Clinical signs and symptoms predicting influenza infection. Arch Intern Med 2000;160:3243-7.

Monto AS. Epidemiology of influenza. Vaccine 2008;26 Suppl 4:D45-8.

Moore DL, Vaudry W, Scheifele DW, et al. Surveillance for influenza admissions among children hospitalized in Canadian immunization monitoring program active centers, 2003-2004. Pediatrics 2006;118:e610-9.

Morgan CI, Hobson MJ, Seger B, Rice MA, Staat MA, Wheeler DS. 2009 Pandemic influenza A (H1N1) in critically ill children in Cincinnati, Ohio. Pediatr Crit Care Med 2011;.

Morishima T, Togashi T, Yokota S, et al. Encephalitis and encephalopathy associated with an influenza epidemic in Japan. Clin Infect Dis 2002;35:512-7.

Moriuchi H, Katsushima N, Nishimura H, Nakamura K, Numazaki Y. Community-acquired influenza C virus infection in children. J Pediatr 1991;118:235-8.

Moro PL, Broder K, Zheteyeva Y, et al. Adverse events in pregnant women following administration of trivalent inactivated influenza vaccine and live attenuated influenza vaccine in the Vaccine Adverse Event Reporting System, 1990-2009. Am J Obstet Gynecol 2011;204:146.e1,146.e7.

Moscona A. Neuraminidase inhibitors for influenza. N Engl J Med 2005;353:1363-73.

Moscona A. Medical management of influenza infection. Annu Rev Med 2008;59:397-413.

Muhammad RD, Haber P, Broder KR, et al. Adverse Events Following Trivalent Inactivated Influenza Vaccination in Children: Analysis of the Vaccine Adverse Event Reporting System. Pediatr Infect Dis J 2010;.

Mullooly JP, Barker WH. Impact of type A influenza on children: a retrospective study. Am J Public Health 1982;72:1008-16.

Munoz FM. Influenza virus infection in infancy and early childhood. Paediatr Respir Rev 2003;4:99-104.

Munoz FM, Campbell JR, Atmar RL, et al. Influenza A virus outbreak in a neonatal intensive care unit. Pediatr Infect Dis J 1999;18:811-5.

Muscatello DJ, Cretikos MA, Macintyre CR. All-cause mortality during first wave of pandemic (H1N1) 2009, New South Wales, Australia, 2009. Emerg Infect Dis 2010;16:1396-402.

Nakajima K, Desselberger U, Palese P. Recent human influenza A (H1N1) viruses are closely related genetically to strains isolated in 1950. Nature 1978;274:334-9.

Nelson MI, Simonsen L, Viboud C, et al. Stochastic processes are key determinants of short-term evolution in influenza a virus. PLoS Pathog 2006;2:e125.

Neuzil KM, Reed GW, Mitchel EF, Simonsen L, Griffin MR. Impact of influenza on acute cardiopulmonary hospitalizations in pregnant women. Am J Epidemiol 1998;148:1094-102.

Neuzil KM, Mellen BG, Wright PF, Mitchel EF,Jr, Griffin MR. The effect of influenza on hospitalizations, outpatient visits, and courses of antibiotics in children. N Engl J Med 2000a;342:225-31

Neuzil KM, Wright PF, Mitchel EF,Jr, Griffin MR. The burden of influenza illness in children with asthma and other chronic medical conditions. J Pediatr 2000b;137:856-64.

Neuzil KM, Zhu Y, Griffin MR, et al. Burden of interpandemic influenza in children younger than 5 years: a 25-year prospective study. J Infect Dis 2002a;185:147-52.

Neuzil KM, Hohlbein C, Zhu Y. Illness among schoolchildren during influenza season: effect on school absenteeism, parental absenteeism from work, and secondary illness in families. Arch Pediatr Adolesc Med 2002b;156:986-91.

Newland JG, Laurich VM, Rosenquist AW, et al. Neurologic complications in children hospitalized with influenza: characteristics, incidence, and risk factors. J Pediatr 2007;150:306-10.

Nicas M, Nazaroff WW, Hubbard A. Toward understanding the risk of secondary airborne infection: emission of respirable pathogens. J Occup Environ Hyg 2005;2:143-54.

Nichol KL. Cost-effectiveness and socio-economic aspects of childhood influenza vaccination. Vaccine 2011;29:7554-8.

Nicholls JM, Chan MC, Chan WY, et al. Tropism of avian influenza A (H5N1) in the upper and lower respiratory tract. Nat Med 2007;13:147-9.

Nikkari S, Halonen P, Kharitonenkov I, et al. One-incubation time-resolved fluoroimmunoassay based on monoclonal antibodies in detection of influenza A and B viruses directly in clinical specimens. J Virol Methods 1989;23:29-40.

Nishiura H. Case fatality ratio of pandemic influenza. Lancet Infect Dis 2010;10:443-4.

Nobusawa E, Sato K. Comparison of the mutation rates of human influenza A and B viruses. J Virol 2006;80:3675-8.

Nohynek H, Jokinen J, Partinen M, et al. AS03 adjuvanted AH1N1 vaccine associated with an abrupt increase in the incidence of childhood narcolepsy in Finland. PLoS One 2012;7:e33536.

Nunes B, Viboud C, Machado A, et al. Excess mortality associated with influenza epidemics in portugal, 1980 to 2004. PLoS One 2011;6:e20661.

O'Brien KL, Walters MI, Sellman J, et al. Severe pneumococcal pneumonia in previously healthy children: the role of preceding influenza infection. Clin Infect Dis 2000;30:784-9.

O'Brien MA, Uyeki TM, Shay DK, et al. Incidence of outpatient visits and hospitalizations related to influenza in infants and young children. Pediatrics 2004;113:585-93.

Ohmit SE, Monto AS. Symptomatic predictors of influenza virus positivity in children during the influenza season. Clin Infect Dis 2006;43:564-8.

Olson DR, Heffernan RT, Paladini M, Konty K, Weiss D, Mostashari F. Monitoring the impact of influenza by age: emergency department fever and respiratory complaint surveillance in New York City. PLoS Med 2007;4:e247.

O'Riordan S, Barton M, Yau Y, Read SE, Allen U, Tran D. Risk factors and outcomes among children admitted to hospital with pandemic H1N1 influenza. CMAJ 2010;182:39-44.

Pada S, Tambyah PA. Overview/reflections on the 2009 H1N1 pandemic. Microbes Infect 2011;13:470-8.

Paget J, Marquet R, Meijer A, van der Velden K. Influenza activity in Europe during eight seasons (1999-2007): an evaluation of the indicators used to measure activity and an assessment of the timing, length and course of peak activity (spread) across Europe. BMC Infect Dis 2007;7:141.

Patrozou E, Mermel LA. Does influenza transmission occur from asymptomatic infection or prior to symptom onset? Public Health Rep 2009;124:193-6.

Peiris M, Yuen KY, Leung CW, et al. Human infection with influenza H9N2. Lancet 1999;354:916-7.

Peiris JS, Yu WC, Leung CW, et al. Re-emergence of fatal human influenza A subtype H5N1 disease. Lancet 2004;363:617-9.

Peltola V, Heikkinen T, Ruuskanen O. Clinical courses of croup caused by influenza and parainfluenza viruses. Pediatr Infect Dis J 2002;21:76-8.

Peltola V, Ziegler T, Ruuskanen O. Influenza A and B virus infections in children. Clin Infect Dis 2003;36:299-305.

Peltola V, Reunanen T, Ziegler T, Silvennoinen H, Heikkinen T. Accuracy of clinical diagnosis of influenza in outpatient children. Clin Infect Dis 2005;41:1198-200.

Peltola V, Mertsola J, Ruuskanen O. Comparison of total white blood cell count and serum C-reactive protein levels in confirmed bacterial and viral infections. J Pediatr 2006;149:721-4.

Perez-Padilla R, de la Rosa-Zamboni D, Ponce de Leon S, et al. Pneumonia and respiratory failure from swine-origin influenza A (H1N1) in Mexico. N Engl J Med 2009;361:680-9.

Perrotta DM, Decker M, Glezen WP. Acute respiratory disease hospitalizations as a measure of impact of epidemic influenza. Am J Epidemiol 1985;122:468-76.

Piedra PA, Schulman KL, Blumentals WA. Effects of oseltamivir on influenza-related complications in children with chronic medical conditions. Pediatrics 2009;124:170-8.

Ploin D, Liberas S, Thouvenot D, et al. Influenza burden in children newborn to eleven months of age in a pediatric emergency department during the peak of an influenza epidemic. Pediatr Infect Dis J 2003;22:S218-22.

Ploin D, Gillet Y, Morfin F, et al. Influenza burden in febrile infants and young children in a pediatric emergency department. Pediatr Infect Dis J 2007;26:142-7.

Poehling KA, Zhu Y, Tang YW, Edwards K. Accuracy and impact of a point-of-care rapid influenza test in young children with respiratory illnesses. Arch Pediatr Adolesc Med 2006a;160:713-8.

Poehling KA, Edwards KM, Weinberg GA, et al. The underrecognized burden of influenza in young children. N Engl J Med 2006b;355:31-40.

Poehling KA, Szilagyi PG, Staat MA, et al. Impact of maternal immunization on influenza hospitalizations in infants. Am J Obstet Gynecol 2011;204:S141-8.

Principi N, Esposito S, Marchisio P, Gasparini R, Crovari P. Socioeconomic impact of influenza on healthy children and their families. Pediatr Infect Dis J 2003;22:S207-10.

Quach C, Piche-Walker L, Platt R, Moore D. Risk factors associated with severe influenza infections in childhood: implication for vaccine strategy. Pediatrics 2003;112:e197-201.

Reichert TA, Sugaya N, Fedson DS, Glezen WP, Simonsen L, Tashiro M. The Japanese experience with vaccinating schoolchildren against influenza. N Engl J Med 2001;344:889-96.

Reid AH, Fanning TG, Hultin JV, Taubenberger JK. Origin and evolution of the 1918 "Spanish" influenza virus hemagglutinin gene. Proc Natl Acad Sci U S A 1999;96:1651-6.

Rhorer J, Ambrose CS, Dickinson S, et al. Efficacy of live attenuated influenza vaccine in children: A meta-analysis of nine randomized clinical trials. Vaccine 2009;27:1101-10.

Rice J, Resar LM. Hematologic abnormalities associated with influenza A infection: a report of 3 cases. Am J Med Sci 1998;316:401-3.

Rojo JC, Ruiz-Contreras J, Fernandez MB, Marin MA, Folgueira L. Influenza-related hospitalizations in children younger than three years of age. Pediatr Infect Dis J 2006;25:596-601.

Rouleau I, Charest H, Douville-Fradet M, Skowronski DM, De Serres G. Field performance of a rapid diagnostic test for influenza in an ambulatory setting. J Clin Microbiol 2009;47:2699-703.

Ruben FL. Inactivated influenza virus vaccines in children. Clin Infect Dis 2004;38:678-88.

Rudenko LG, Slepushkin AN, Monto AS, et al. Efficacy of live attenuated and inactivated influenza vaccines in schoolchildren and their unvaccinated contacts in Novgorod, Russia. J Infect Dis 1993;168:881-7.

Russell CA, Jones TC, Barr IG, et al. Influenza vaccine strain selection and recent studies on the global migration of seasonal influenza viruses. Vaccine 2008;26 Suppl 4:D31-4.

Ruuskanen O, Putto A, Sarkkinen H, Meurman O, Irjala K. C-reactive protein in respiratory virus infections. J Pediatr 1985;107:97-100.

Ruuskanen O, Arola M, Putto-Laurila A, et al. Acute otitis media and respiratory virus infections. Pediatr Infect Dis J 1989;8:94-9.

Sagrera X, Ginovart G, Raspall F, et al. Outbreaks of influenza A virus infection in neonatal intensive care units. Pediatr Infect Dis J 2002;21:196-200.

Sakkou Z, Stripeli F, Papadopoulos NG, et al. Impact of influenza infection on children's hospital admissions during two seasons in Athens, Greece. Vaccine 2011;29:1167-72.

Salk JE, Pearson HE. Immunization against influenza with observations during an epidemic of influenza A one year after vaccination. Am J Hyg 1945;42:307-22.

Salo H, Kilpi T, Sintonen H, Linna M, Peltola V, Heikkinen T. Cost-effectiveness of influenza vaccination of healthy children. Vaccine 2006;24:4934-41.

Sato M, Hosoya M, Kato K, Suzuki H. Viral shedding in children with influenza virus infections treated with neuraminidase inhibitors. Pediatr Infect Dis J 2005;24:931-2.

Schultze V, D'Agosto V, Wack A, Novicki D, Zorn J, Hennig R. Safety of MF59 adjuvant. Vaccine 2008;26:3209-22.

Shaman J, Pitzer VE, Viboud C, Grenfell BT, Lipsitch M. Absolute humidity and the seasonal onset of influenza in the continental United States. PLoS Biol 2010;8:e1000316.

Shephard RJ, Shek PN. Cold exposure and immune function. Can J Physiol Pharmacol 1998;76:828-36.

Sheu TG, Deyde VM, Okomo-Adhiambo M, et al. Surveillance for neuraminidase inhibitor resistance among human influenza A and B viruses circulating worldwide from 2004 to 2008. Antimicrob Agents Chemother 2008;52:3284-92.

Shope RE. Swine Influenza : Iii. Filtration Experiments and Etiology. J Exp Med 1931;54:373-85.

Shuler CM, Iwamoto M, Bridges CB, et al. Vaccine effectiveness against medically attended, laboratory-confirmed influenza among children aged 6 to 59 months, 2003-2004. Pediatrics 2007;119:e587-95.

Simmerman JM, Suntarattiwong P, Levy J, et al. Influenza virus contamination of common household surfaces during the 2009 influenza A (H1N1) pandemic in Bangkok, Thailand: implications for contact transmission. Clin Infect Dis 2010;51:1053-61.

Simonsen L, Clarke MJ, Williamson GD, Stroup DF, Arden NH, Schonberger LB. The impact of influenza epidemics on mortality: introducing a severity index. Am J Public Health 1997;87:1944-50.

Simonsen L, Clarke MJ, Schonberger LB, Arden NH, Cox NJ, Fukuda K. Pandemic versus epidemic influenza mortality: a pattern of changing age distribution. J Infect Dis 1998;178:53-60.

Skowronski DM, Hottes TS, Chong M, et al. Randomized controlled trial of dose response to influenza vaccine in children aged 6 to 23 months. Pediatrics 2011;128:e276-89.

Smit PM, Bongers KM, Kuiper RJ, von Rosenstiel IA, Smits PH, Brandjes DP. Characterization of 2009

H1N1 pandemic influenza in a population of Dutch children with influenza-like signs and symptoms. Acta Paediatr 2012;101:67-72.

Smith W, Andrewes C, Laidlaw P. A virus obtained from influenza patients. 1933;222:66.

Spada B, Biehler K, Chegas P, Kaye J, Riepenhoff-Talty M. Comparison of rapid immunofluorescence assay to cell culture isolation for the detection of influenza A and B viruses in nasopharyngeal secretions from infants and children. J Virol Methods 1991;33:305-10.

Streng A, Grote V, Liese JG. Severe influenza cases in paediatric intensive care units in Germany during the pre-pandemic seasons 2005 to 2008. BMC Infect Dis 2011;11:233.

Studahl M. Influenza virus and CNS manifestations. J Clin Virol 2003;28:225-32.

Su WJ, Shao PL, Liu MT, et al. Low seroprotection against preseasonal influenza local strains in children might predict the upcoming epidemic influenza strains. Clin Infect Dis 2010;51:171-6.

Subbarao K, Klimov A, Katz J, et al. Characterization of an avian influenza A (H5N1) virus isolated from a child with a fatal respiratory illness. Science 1998;279:393-6.

Suess T, Remschmidt C, Schink SB, et al. The role of facemasks and hand hygiene in the prevention of influenza transmission in households: results from a cluster randomised trial; Berlin, Germany, 2009-2011. BMC Infect Dis 2012;12:26.

Sugaya N, Mitamura K, Nirasawa M, Takahashi K. The impact of winter epidemics of influenza and respiratory syncytial virus on paediatric admissions to an urban general hospital. J Med Virol 2000;60:102-6.

Sugaya N, Mitamura K, Yamazaki M, et al. Lower clinical effectiveness of oseltamivir against influenza B contrasted with influenza A infection in children. Clin Infect Dis 2007;44:197-202.

Sugaya N, Ohashi Y. Long-acting neuraminidase inhibitor laninamivir octanoate (CS-8958) versus oseltamivir as treatment for children with influenza virus infection. Antimicrob Agents Chemother 2010;54:2575-82.

Sugaya N. Widespread use of neuraminidase inhibitors in Japan. J Infect Chemother 2011;17:595-601.

Szilagyi PG, Fairbrother G, Griffin MR, et al. Influenza vaccine effectiveness among children 6 to 59 months of age during 2 influenza seasons: a case-cohort study. Arch Pediatr Adolesc Med 2008;162:943-51.

Taber LH, Paredes A, Glezen WP, Couch RB. Infection with influenza A/Victoria virus in Houston families, 1976. J Hyg (Lond) 1981;86:303-13.

Takahashi H, Otsuka Y, Patterson BK. Diagnostic tests for influenza and other respiratory viruses: determining performance specifications based on clinical setting. J Infect Chemother 2010;16:155-61.

Tamma PD, Ault KA, del Rio C, Steinhoff MC, Halsey NA, Omer SB. Safety of influenza vaccination during pregnancy. Am J Obstet Gynecol 2009;201:547-52.

Tamura D, Miura T, Kikuchi Y. Oseltamivir phosphate in infants under 1 year of age with influenza infection. Pediatr Int 2005;47:484.

Tamura D, Fujino M, Ozawa M, et al. Significance of seasonal influenza viruses in the stool of pediatric patients. Pediatr Infect Dis J 2010;29:578-9.

Taubenberger JK, Reid AH, Krafft AE, Bijwaard KE, Fanning TG. Initial genetic characterization of the 1918 "Spanish" influenza virus. Science 1997;275:1793-6.

Taylor RM. Studies on survival of influenza virus between epidemics and antigenic variants of the virus. Am J Public Health Nations Health 1949;39:171-8.

Tellier R. Review of aerosol transmission of influenza A virus. Emerg Infect Dis 2006;12:1657-62.

THL. Kausi-influenssarokotukset.2011; Available at: http://www.ktl.fi/portal/suomi/terveyden_ammattilaisille/rokottaminen/influenssarokotukset . 2011.

Thomas Y, Vogel G, Wunderli W, et al. Survival of influenza virus on banknotes. Appl Environ Microbiol 2008;74:3002-7.

Thompson WW, Shay DK, Weintraub E, et al. Mortality associated with influenza and respiratory syncytial virus in the United States. JAMA 2003;289:179-86.

Titus MO, Wright SW. Prevalence of serious bacterial infections in febrile infants with respiratory syncytial virus infection. Pediatrics 2003;112:282-4.

To KK, Chan KH, Li IW, et al. Viral load in patients infected with pandemic H1N1 2009 influenza A virus. J Med Virol 2010;82:1-7.

Toovey S, Rayner C, Prinssen E, et al. Assessment of neuropsychiatric adverse events in influenza patients treated with oseltamivir: a comprehensive review. Drug Saf 2008;31:1097-114.

Tsolia MN, Logotheti I, Papadopoulos NG, et al. Impact of influenza infection in healthy children examined as outpatients and their families. Vaccine 2006;24:5970-6.

Tumpey TM, Basler CF, Aguilar PV, et al. Characterization of the reconstructed 1918 Spanish influenza pandemic virus. Science 2005;310:77-80.

Tweed SA, Skowronski DM, David ST, et al. Human illness from avian influenza H7N3, British Columbia. Emerg Infect Dis 2004;10:2196-9.

Uyeki TM. Influenza diagnosis and treatment in children: a review of studies on clinically useful tests and antiviral treatment for influenza. Pediatr Infect Dis J 2003;22:164-77.

Uyeki TM, Prasad R, Vukotich C, et al. Low sensitivity of rapid diagnostic test for influenza. Clin Infect Dis 2009;48:e89-92.

van der Vries E, Schutten M, Boucher CA. The potential for multidrug-resistant influenza. Curr Opin Infect Dis 2011;24:599-604.

van Elden LJ, van Essen GA, Boucher CA, et al. Clinical diagnosis of influenza virus infection: evaluation of diagnostic tools in general practice. Br J Gen Pract 2001;51:630-4.

Vesikari T, Fleming DM, Aristegui JF, et al. Safety, efficacy, and effectiveness of cold-adapted influenza vaccine-trivalent against community-acquired, culture-confirmed influenza in young children attending day care. Pediatrics 2006;118:2298-312.

Vesikari T, Pellegrini M, Karvonen A, et al. Enhanced immunogenicity of seasonal influenza vaccines in young children using MF59 adjuvant. Pediatr Infect Dis J 2009;28:563-71.

Vesikari T, Knuf M, Wutzler P, et al. Oil-in-Water Emulsion Adjuvant with Influenza Vaccine in Young Children. N Engl J Med 2011;365:1406-16.

Viboud C, Miller M, Olson D, Osterholm M, Simonsen L. Preliminary Estimates of Mortality and Years of Life Lost Associated with the 2009 A/H1N1 Pandemic in the US and Comparison with Past Influenza Seasons. PLoS Curr 2010;:RRN1153.

Waddington CS, Walker WT, Oeser C, et al. Safety and immunogenicity of AS03B adjuvanted split virion versus non-adjuvanted whole virion H1N1 influenza vaccine in UK children aged 6 months-12 years: open label, randomised, parallel group, multicentre study. BMJ 2010;340:c2649.

Wagner R, Matrosovich M, Klenk HD. Functional balance between haemagglutinin and neuraminidase in influenza virus infections. Rev Med Virol 2002;12:159-66.

Walter EB, Englund JA, Blatter M, et al. Trivalent inactivated influenza virus vaccine given to two-month-old children: an off-season pilot study. Pediatr Infect Dis J 2009;28:1099-104.

Waris M, Ziegler T, Kivivirta M, Ruuskanen O. Rapid detection of respiratory syncytial virus and influenza A virus in cell cultures by immunoperoxidase staining with monoclonal antibodies. J Clin Microbiol 1990;28:1159-62.

Weber TP, Stilianakis NI. Inactivation of influenza A viruses in the environment and modes of transmission: a critical review. J Infect 2008;57:361-73.

Webster RG, Bean WJ, Gorman OT, Chambers TM, Kawaoka Y. Evolution and ecology of influenza A viruses. Microbiol Rev 1992;56:152-79.

Wei CJ, Boyington JC, Dai K, et al. Cross-neutralization of 1918 and 2009 influenza viruses: role of glycans in viral evolution and vaccine design. Sci Transl Med 2010;2:24ra21.

Weigl JA, Puppe W, Schmitt HJ. The incidence of influenza-associated hospitalizations in children in Germany. Epidemiol Infect 2002;129:525-33.

Weinberg GA, Erdman DD, Edwards KM, et al. Superiority of reverse-transcription polymerase chain reaction to conventional viral culture in the diagnosis of acute respiratory tract infections in children. J Infect Dis 2004;189:706-10.

Weinberger DM, Simonsen L, Jordan R, Steiner C, Miller M, Viboud C. Impact of the 2009 influenza pandemic on pneumococcal pneumonia hospitalizations in the United States. J Infect Dis 2012;205:458-65.

Weis W, Brown JH, Cusack S, Paulson JC, Skehel JJ, Wiley DC. Structure of the influenza virus haemagglutinin complexed with its receptor, sialic acid. Nature 1988;333:426-31.

Whitley RJ, Hayden FG, Reisinger KS, et al. Oral oseltamivir treatment of influenza in children. Pediatr Infect Dis J 2001;20:127-33.

WHO. Influenza(Seasonal)Factsheet211. 2009; Available at: http://www.who.int/ mediacentre /factsheets/fs211/en/index.html 2009.

WHO. Pandemic (H1N1) 2009 - update 103. 2010a; Available at: http://www.who.int /csr /don /2010_06_04/en/index.html. 2010 (Accessed 4/6, 2010).

WHO. Director-General's Opening Statement at Virtual Press Conference (10 August 2010). 2010b; Available at: http://www.who.int/mediacentre /news/statements/2010/h1n1_vpc_20100810/en/index.html. 2010.

WHO. Summary of influenza antiviral susceptibility surveillance findings, September 2010 - March 2011. 2011; Available at: http://www.who.int /influenza/gisrs_laboratory/updates/antiviral_susceptibility/en/index.html. (Assessed 6 June 2011).

WHO. Cumulative Number of Confirmed Human Cases of Avian Influenza A(H5N1) Reported to WHO. 2012; Available at: http://www.who.int /influenza/human_animal_interface/H5N1_cumulative_table_archives/en/index.html. (Accessed 05/02, 2012).

Wilkes JJ, Leckerman KH, Coffin SE, et al. Use of antibiotics in children hospitalized with community-acquired, laboratory-confirmed influenza. J Pediatr 2009;154:447-9.

Wilschut JC, McElhaney JE, Palache AM. Rapid Reference to Influenza. 2006;1-240.

Wolf DG, Rekhtman D, Kerem E, et al. A summer outbreak of influenza A virus infection among young children. Clin Infect Dis 2004;39:595-7.

Wong CM, Yang L, Chan KP, et al. Influenza-associated hospitalization in a subtropical city. PLoS Med 2006;3:e121.

Woo PC, Chiu SS, Seto WH, Peiris M. Cost-effectiveness of rapid diagnosis of viral respiratory tract infections in pediatric patients. J Clin Microbiol 1997;35:1579-81.

Wootton SH, Scheifele DW, Mak A, Petric M, Skowronski DM. Detection of human influenza virus in the stool of children. Pediatr Infect Dis J 2006;25:1194-5.

Wright PF, Thompson J, Karzon DT. Differing virulence of H1N1 and H3N2 influenza strains. Am J Epidemiol 1980;112:814-9.

Zaman K, Roy E, Arifeen SE, et al. Effectiveness of maternal influenza immunization in mothers and infants. N Engl J Med 2008;359:1555-64.

Zambon MC, Stockton JD, Clewley JP, Fleming DM. Contribution of influenza and respiratory syncytial virus to community cases of influenza-like illness: an observational study. Lancet 2001;358:1410-6.

Zitterkopf NL, Leekha S, Espy MJ, Wood CM, Sampathkumar P, Smith TF. Relevance of influenza a virus detection by PCR, shell vial assay, and tube cell culture to rapid reporting procedures. J Clin Microbiol 2006;44:3366-7.

Zuccotti G, Pogliani L, Pariani E, Amendola A, Zanetti A. Transplacental antibody transfer following maternal immunization with a pandemic 2009 influenza A(H1N1) MF59-adjuvanted vaccine. JAMA 2010;304:2360-1.