

REGULAR ARTICLE

Characterization of 2009 H1N1 pandemic influenza in a population of Dutch children with influenza-like signs and symptoms

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ABSTRACT

Aim: To determine causative respiratory pathogens and describe epidemiological and clinical characteristics in a paediatric population with influenza-like illness during the 2009 H1N1-pandemic.

Methods: Observational study of 412 children visiting an outpatient clinic of a Dutch teaching hospital.

Results: From August to December 2009, 412 children were tested at the clinic; 32% proved H1N1-positive, confirmed by reverse-transcriptase-polymerase-chain-reaction (RT-PCR). Pathogens were detected in 65% of samples. Influenza A(H1N1) (n = 132), human rhinovirus (n = 55), respiratory syncytial virus (n = 45) and adenovirus (n = 34) were mostly identified. Co-infections were seen in 34 children (8.3%). Mean age was 6.8 and 4.2 years in H1N1-positive and H1N1-negative cases, respectively (p < 0.01). H1N1-positive outpatient children reported fever, cough and rhinorrhoea more frequently than their H1N1-negative counterparts. Of 72 hospitalized children, 31% proved H1N1-positive; all showed a relatively mild clinical illness. None of the children had been admitted to an intensive care unit or died. Oseltamivir treatment was initiated in 72 children and discontinued in 42 (63%) when RT-PCR results turned negative.

Conclusion: The 2009 H1N1-pandemic showed a mild clinical course in a Dutch paediatric outpatient clinic population. Respiratory pathogens were detected in the majority of children with influenza-like illness and influenza A(H1N1) virus was identified in one-third. Testing symptomatic children during an influenza pandemic has effectively limited the use of oseltamivir.

INTRODUCTION

In April 2009, officials at the Centers for Disease Control and Prevention (CDC) confirmed two cases of swine influenza infection in children living in neighbouring counties in California, after several cases had already been reported in Mexico (1,2). These observations announced the emergence of a serious global health threat caused by a new influenza A(H1N1) virus (3). The first case in the Netherlands was detected in a 3-year-old child, approximately 2 weeks after the first cases had been detected in the United States (4). By the time that the World Health Organization (WHO) declared an actual influenza pandemic in June 2009, epidemiological observations had led to the

conclusion that swine-origin influenza virus infection was associated with less favourable outcomes in children (5,6). Of 345 children hospitalized with 2009 influenza A(H1N1) in California, two-thirds had comorbidities, most commonly chronic pulmonary disease, underlying neurologic disorders and immunosuppression (7).

To our knowledge, published articles have mainly focused on the inpatient clinical experience of the H1N1-pandemic

Abbreviations

CDC, Centers for Disease Control and Prevention; ED, Emergency Department; H1N1, Influenza virus type A, subtype H1N1; PICU, Paediatric intensive care unit; RSV, Respiratory syncytial virus; RT-PCR, Reverse-transcriptase-polymerase-chain-reaction; WHO, World Health Organization.

Key notes

- This observational study describes a large paediatric outpatient clinic population during the 2009 influenza A(H1N1) pandemic in whom extensive virologic diagnosis has been performed. In two-thirds of over 400 tested children, a wide variety of respiratory pathogens had been detected, approximately half of them being pandemic influenza. Clinical illness from respiratory viral infection was mild, even among those who were hospitalized.

in children, describing merely hospitalized cases, and lacking a detailed description of the respiratory viruses involved other than the pandemic influenza A(H1N1) virus. This observational study provides a description of both the inpatient and the outpatient clinical experience of the 2009 influenza pandemic. Our aim was to characterize a population of paediatric in- and outpatients with influenza-like signs and symptoms, to determine how many children had influenza A(H1N1) infection or other specific respiratory viral infections, and to observe dual infections in this population.

PATIENTS AND METHODS

Study design and population

The design of the study was observational; data were prospectively collected. On August 12, 2009, the Slotervaart Hospital, a 410-bed teaching hospital in Amsterdam, the Netherlands, opened the doors of a special influenza outpatient clinic. The hospital serves a low- to middle-income urban population of approximately 140 000 inhabitants, of which 18% are children and 49% are ethnic minorities, mostly of Moroccan and Turkish origin. The influenza outpatient clinic operated as a separate facility for diagnosis and management of patients with suspicion of influenza virus infection. Children with any of the following influenza-like signs and symptoms were accepted to the clinic: fever $\geq 38^{\circ}\text{C}$, cough, rhinorrhoea, sore throat, headache, myalgia, malaise, chills, vomiting and/or diarrhoea. Patients were referred either directly by the responsible family physician or other healthcare provider, or by the children's parents/caregivers. From August 12, 2009, to December 31, 2009, all children aged 0–17 years presenting at the Slotervaart Hospital, with at least one of the above-mentioned signs or symptoms, were included in this observational study. Ethical approval and informed consent were not required according to Dutch law, as this study solely describes findings resulting from regular patient care provided at our hospital.

Provided care delivered at the influenza outpatient clinic

Every patient with any of the aforementioned influenza-like signs or symptoms was welcome to sign up for a consultation at the influenza outpatient clinic, even in the absence of a physician referral note (i.e. 'self-referred'). Children presenting outside of office hours and on weekends were cared for at the Emergency Department (ED) of our hospital. A complete medical history was obtained using a unified case report form, and a physical examination was performed, followed by either an oropharyngeal swab or a nasal wash, depending on the child's age and ability to cooperate. Test results were usually available within 24 h. Additional laboratory and imaging tests were performed at the discretion of the supervising paediatrician.

Appropriate medical management was determined based on clinical findings and local and (inter)national protocols. Treatment with the antiviral drug oseltamivir was initiated in suspected influenza cases in accordance with national guidelines at that time (<http://www.rivm.nl/en>): oseltamivir was prescribed only in high-risk patients, i.e. children <2 years of age or suffering from a specified chronic medical condition and in patients with a complicated course of infection. If the clinical suspicion of H1N1-infection was confirmed by a positive test result, patients were advised to finish the 5-day treatment with the antiviral drug; in the case of a negative test result, the use of oseltamivir was discontinued.

Laboratory confirmation of infection

Influenza virus ribonucleic acid was amplified and detected by real-time one-step reverse-transcriptase-polymerase-chain-reaction (RT-PCR), performed on oropharyngeal swabs or nasal washes; this method has been found to be 95% sensitive and 98% specific (8). A generic PCR (directed against the matrix gene) was used to detect influenza virus type A or B, and an H1N1-specific PCR was applied to the H1 gene (9). In addition, we also used RT-PCR to detect the following pathogens: parainfluenza-1, parainfluenza-2, parainfluenza-3, parainfluenza-4, adenovirus, respiratory syncytial virus (RSV), human rhinovirus, human metapneumovirus, human coronavirus OC43, human coronavirus 229E, human coronavirus NL63, *Chlamydia pneumoniae*, *Mycoplasma pneumoniae* and *Legionella* species. The selected pathogens were detected by using a multiplex PCR that combined the most prevalent pathogens in children as well as adults.

Statistical analysis

Statistical analysis was performed using the SPSS software package (version 17.0; SPSS Inc. Chicago, IL, USA). Continuous variables were summarized as means (and standard deviation), and for each categorical variable, the percentage of children in each group was calculated. Demographic and clinical characteristics were compared between groups using a Student's *t*-test or nonparametric test for continuous variables and Chi-square or Fisher's exact test for categorical variables, as appropriate. A *p*-value of <0.05 was considered statistically significant.

RESULTS

Study population and characteristics

From August to December 2009, a total of 423 children with one or more influenza-like signs or symptoms presented to our hospital, and valid RT-PCR test results were ultimately obtained for 412 of those. Three hundred and twenty cases were managed through our influenza outpatient clinic (78%), and 92 presented through the ED (22%). Five children had an additional visit which was more than 2 weeks apart from the first and therefore regarded as independent from the initial visit and included in the analysis. There were no differences in demographic characteristics and clinical presentation between patients presenting to the outpatient clinic as compared to the ED.

RESULTS

Study population and characteristics

H1N1-virus was detected in 132 of 412 outpatients (32%). Table 1 outlines general and clinical characteristics of both H1N1-positive and H1N1-negative children who

visited the outpatient clinic. Mean age of H1N1-positive children was significantly higher (6.8 and 4.2 years for H1N1-positives and H1N1-negatives, respectively). In total, 137 children (33%) were <2 years of age and 14 were infants younger than 3 months (3%). H1N1-positive outpatient children did report fever, cough and rhinorrhoea significantly more frequently than their H1N1-negative counterparts. There was no significant difference with regard to these clinical symptoms for H1N1-positive children who were above or below 2 years of age (data not shown). The most frequently mentioned comorbid condition was asthmatic bronchitis, which was reported in 27% and 18% of H1N1-positives and -negatives, respectively.

RT-PCR influenza and other pathogens

Pathogens were detected in 65% of 412 patient samples. The sampling method used was an oropharyngeal swab in 343 children (83%) and a nasal wash in 69 (17%); mean age of children in these groups was 4.4 and 1.4 years, respectively. Figure 1 points out the relative distribution of all detected pathogens over the entire study period and also per month. Influenza A(H1N1) showed a maximum prevalence in October, rhinovirus in August and RSV in November. Influenza B, human coronavirus, *Mycoplasma pneumoniae* and *Legionella* species were not detected at all.

In 14 H1N1-confirmed cases, the following pathogens were concurrently detected: adenovirus in 6, rhinovirus in 6, parainfluenza in 1 and *Chlamydia pneumoniae* in 1. The following co-infections were seen in 20 H1N1-negative children: rhinovirus-RSV in 6, rhinovirus-adenovirus in 5, rhinovirus-parainfluenza in 3, RSV-adenovirus in 3,

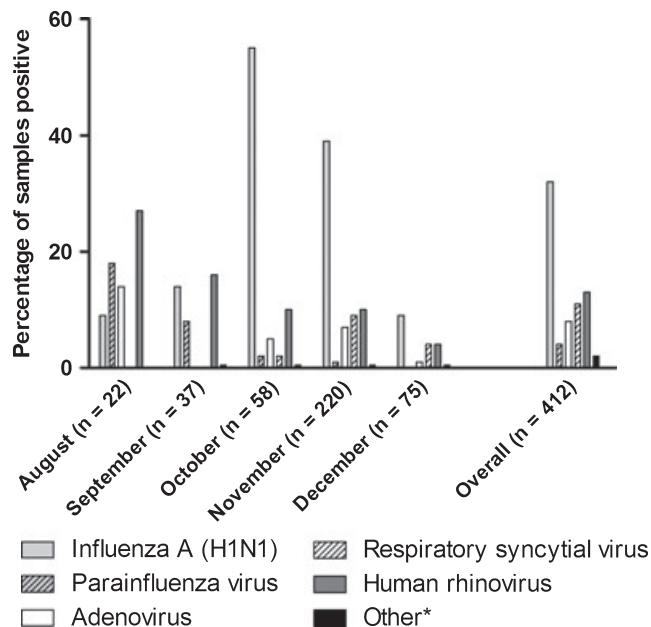


Figure 1 Detected respiratory pathogens in a symptomatic paediatric outpatient population (n = 412) during the 2009 H1N1 pandemic. *Other include human metapneumovirus and *Chlamydia pneumoniae*.

rhinovirus-metapneumovirus in 1, RSV-*Chlamydia pneumoniae* in 1 and rhinovirus-RSV-parainfluenza in 1.

Clinical management

Hospitalizations

A total of 72 children with influenza-like symptoms were hospitalized, all had been presented before to either the outpatient clinic or ED. None had to be transferred to another hospital for intensive care support. Relevant characteristics of clinically admitted patients with and without H1N1 infection are described in Table 2. Mean age of hospitalized H1N1-positive children was significantly higher (5.5 vs. 1.9). More antibiotics were initially prescribed and administered in H1N1-positive children than in H1N1-negatives (42% and 18%, respectively). Fourteen blood samples that had been collected for bacterial cultures did not result in the detection of any bacterial pathogens.

Imaging

In 57 (13 H1N1-positive and 44 H1N1-negative) of 412 children (14%) with a suspected lower airway infection, a chest radiograph was performed to exclude pulmonary abnormalities. The resulting radiographs were evaluated by the hospital's attending radiologists. Forty-three out of 57 radiographs identified abnormalities, of which enhanced peribronchial cuffing was observed in 27 (47%). Pulmonary infiltrates were seen in 16 children (10 inpatients and six outpatients), all without H1N1-infection.

Oseltamivir treatment

Oseltamivir treatment was initiated in 72, both in- and outpatient, children (17.5%), who were eligible for antiviral

Table 1 Characteristics of children visiting the outpatient influenza clinic

	RT-PCR positive for H1N1-virus infection n = 132	RT-PCR negative for H1N1-virus infection n = 280
Sex (n, %)		
Male	70 (53)	137 (49)
Female	62 (47)	143 (51)
Age (mean, SD)	6.8 (4.7)	4.2 (4.1)*
History of fever (n, %)	115 (87)	202 (72)*
Cough (n, %)	123 (93)	230 (82)*
Rhinorrhoea (n, %)	102 (77)	179 (64)*
Duration of symptoms at presentation (days; mean, SD)	3.0 (1.7)	3.2 (2.1)
Diagnosis of otitis media (n, %)	7 (5)	17 (6)
Underlying comorbidity (n, %)		
Asthmatic bronchitis	36 (27)	49 (18)
Neurological disorder	5 (4)	4 (1)
Cardiac dysfunction	2 (2)	4 (1)
Diabetes mellitus	2 (2)	3 (1)
Chronic renal condition	1 (1)	2 (1)
Laryngotracheomalacia	2 (2)	2 (1)
Other†	0 (0)	4 (1)

*p < 0.05 for difference between groups.

†Other comorbidity includes observations for: rheumatoid arthritis (n = 1), brain tumour (n = 1), 22q11 deletion (n = 1) and HIV infection (n = 1).

Table 2 Characteristics of hospitalized paediatric patients

	RT-PCR positive for H1N1-virus infection n = 22	RT-PCR negative for H1N1-virus infection n = 50
Age (years; mean, SD)	5.5 (5.2)	1.9 (2.0)*
Duration of admission (days; mean, SD)	3 (2.4)	4 (2.7)
Presence of any comorbid condition (n, %)	11 (50)	22 (44)
Temperature >38°C (n, %)	17 (77)	40 (80)
In need of oxygen suppletion (n, %)	3 (14)	18 (36)**
Clinically dehydrated (n, %)	10 (45)	21 (42)
Chest X-ray (n, %)		
Number performed	5 (23)	26 (52)*
Pulmonary infiltrate	0 (0)	10 (20)
Peribronchial cuffing	3 (14)	12 (24)
Antibiotics treatment (n, %)	4 (18)	21 (42)**
Oseltamivir treatment (n, %)		
Initiated	20 (91)	17 (34)*
Continued	10 (45)	0 (0)*

*p < 0.05 for difference between groups.

**p = 0.05 for difference between groups.

therapy according to national guidelines (data not shown for outpatient children; see Table 2 for inpatient children). No statistically significant difference in overall oseltamivir initiation rate was demonstrated with regard to H1N1 status. In accordance with the same guidelines, four infants <3 months of age were hospitalized because of the requirement to clinically monitor the administration of the antiviral drug. Only one of those infants tested H1N1-positive, and in the remaining three infants, oseltamivir administration was discontinued the next day. Overall, antiviral therapy was discontinued early in 42 out of 72 cases (63%) because RT-PCR test results had turned negative the next day.

DISCUSSION

This observational study describes the responsible respiratory pathogens and clinical characteristics of 412 Dutch children with influenza-like signs and symptoms who visited an influenza outpatient facility during the 2009 H1N1 pandemic. An extensive range of different pathogens had been detected in 65% of the tested samples. Infection with pandemic influenza A(H1N1) virus was diagnosed in 132 paediatric outpatients (32%). The majority of infected children showed a mild clinical picture.

H1N1 infected children reported fever, cough and rhinorrhoea more frequently than those who were uninfected. Despite this finding, we have continued to advocate the use of specific virologic diagnostics instead of clinical differentiation. In a recently published study that was performed among adults with symptoms of respiratory infection, it was concluded that clinical differentiation between patients with and without influenza infection based solely on influenza-like signs and symptoms is rather ineffective (10). It is very plausible that this also holds true for a paediatric

population. Furthermore, our results have shown that H1N1-positive children, both in- and outpatients, were significantly older than their H1N1-negative counterparts. The lower age of H1N1-negative children is probably best explained by a selection bias of relatively healthy infants and young children brought to the hospital by parents who were concerned by the pandemic. Finally, from our results, it seems that certain pre-existing comorbid conditions in children, particularly asthmatic bronchitis, had not been a risk factor for a more complicated course of the infection, unlike findings from other observational studies (6,11,12).

The H1N1-positivity rate started to increase from October 2009. In the course of that month, the prevalence rate exceeded 50%. The Health Council of the Netherlands had advised the vaccination of children from 'traditional' medical risk groups in that same month (13). In the course of the next month, the relative H1N1-positivity rate declined; however, the absolute number of visiting patients increased dramatically. In November 2009, the Health Council advice was changed, and it was recommended that all children from the ages of 6 months to 4 years be vaccinated, regardless of risk group. A mass vaccination program was therefore organized in November and December, after which a definite decline in the number of visiting patients was seen. In Amsterdam and surrounding area, this had resulted in a total vaccination coverage rate in children of approximately 40% (14). Based on this coverage rate, and taking into account that mass vaccination was started after the peak of the epidemic, it is estimated that maximally 10–20% of our outpatient children had received the vaccine.

In our population of symptomatic children, we detected influenza A(H1N1), human rhinovirus, RSV and adenovirus most commonly, in order of descending frequency. In a community study of considerable size, Monto et al. (15–17) observed rhinovirus as the most frequently identified viral isolate in children, with parainfluenza, RSV and influenza A being the next most common pathogens, in order of descending frequency. The differences can for most part be explained by the fact that our study period, and not Monto's, was encompassed by a true influenza pandemic. Furthermore, advanced diagnostic techniques that are used currently, such as RT-PCR, were not available during that time period. Our population did not show any influenza A(H1N1)-RSV co-infections, which is opposite to the findings from Poehling et al. (18). We also found that the consecutive outbreaks of rhinovirus, influenza and RSV one after the other followed its usual pattern (19,20). This is in contrast to the findings from a French influenza research team suggesting a delayed circulation of RSV (21).

Clinical course in 72 hospitalized children was relatively uncomplicated. There were no influenza-related deaths or transferrals to other hospitals because of paediatric intensive care unit (PICU) requirement. In the Netherlands, with over 16 million inhabitants, 56 H1N1-related PICU admissions were seen (approximately 25% of all H1N1-related intensive care admissions) and 15 children had died from influenza infection (22). For comparison, the United States total for the entire pandemic period was 344 influenza-

associated paediatric deaths (23), yet the clinical course in the majority of cases of 2009 pandemic influenza A(H1N1) in children had been mild (24). We had found that peribronchial cuffing was the most commonly described radiological abnormality, and serious imaging abnormalities were not seen, consistent with findings from a previous study (25). Oseltamivir treatment was initiated in 72 children of whom 42 discontinued the drug because RT-PCR results were negative for influenza virus the next day. In a randomized controlled trial, Heinonen et al. (26) proved that early oseltamivir treatment in children with influenza A infection decreases the incidence of acute otitis media and time to resolution of illness. Without laboratory confirmation, however, some children would have been treated for an unnecessarily long time and, as a consequence, would have been exposed to an unnecessary high risk of adverse effects (27).

A few limitations of this study should be mentioned. First, our hospital lacks a PICU facility. Severe cases of complicated influenza infection might therefore directly have been referred to one of Amsterdam's 2 PICU-containing university hospitals. A second limitation is that we restricted the RT-PCR analyses to eight viral and three 'bacterial' pathogens, and we did not routinely test for notable bacterial organisms, such as Group A *Streptococcus*. Finally, two different sampling methods had been used. Oropharyngeal swabs were performed in the majority of our children; in a minority nasal washes were performed. Comparative studies have shown that the sensitivity for the identification of respiratory viruses is lower for oropharyngeal swabs (28–30). There is therefore a possibility that our results are an underestimation of the real situation, particularly in older children in whom oropharyngeal swabs were preferably collected.

In conclusion, this is the first observational study that focuses on the paediatric outpatient clinical experience and on the circulation of other respiratory pathogens in addition to the influenza A virus during the 2009 H1N1-pandemic. Causative respiratory pathogens were detected in a majority of children visiting our clinic. In one-third of the outpatients, we identified pandemic influenza A(H1N1). Of all children, the vast majority showed a mild clinical illness. The use of oseltamivir was discontinued in high-risk children who ultimately proved H1N1-negative, which is considered a valuable advantage of testing symptomatic outpatient children.

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