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Original Article

Influenza virus infections from 0 to 2 years of age: A birth cohort study

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Abstract *Background/purpose:* Influenza vaccine has been recommended in Finland since 2007 for all children of 6–35 months of age and in 2009 for those ≥6 months against pandemic influenza. We investigated the incidence of influenza and vaccine effectiveness in a birth cohort of children in 2008–2011.
Methods: We followed 923 children from birth to 2 years of age for respiratory tract infections. A nasal swab sample for PCR for influenza A and B viruses was taken at the onset of acute respiratory infections. Samples were collected either at the study clinic or at home by parents. Vaccination data was retrieved from the health registries.
Results: Vaccination coverage of children aged 6–23 months was 22–47% against seasonal influenza and 80% against the A(H1N1)pdm09 virus in the pandemic season 2009–2010. During 3 influenza seasons, 1607 nasal swab samples were collected. Influenza was confirmed in 56 (6.1%) of 923 children (16 A(H1N1), 14 A(H3N2), and 26 B viruses). The incidence of influenza was 5.1% in 2008–2009, 2.7% in 2009–2010, and 5.0% in 2010–2011. Effectiveness of the adjuvanted vaccine against the pandemic influenza A(H1N1)pdm09 was 97% (95% confidence interval, 76–100%). Three children with influenza were hospitalized.
Conclusion: The yearly incidence of seasonal influenza was 5% in this cohort of very young children with variable influenza vaccine coverage. Adjuvanted vaccine against the pandemic influenza was highly effective. Both seasonal and pandemic influenza cases were mostly non-severe.

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Introduction

Annual influenza epidemics strongly affect children.[1–4] The rates of influenza infection and hospitalizations are especially high among young children. Children are also the main disseminators of influenza in the community. The burden of influenza infections in both children themselves and wider in the society can be substantially decreased through vaccination of children.[5–8] Good effectiveness of inactivated influenza vaccines in young children has been documented, when there is a good match between the vaccine virus strains and the circulating virus strains.[9]

Only few countries outside North America recommend influenza vaccines for healthy children, even if WHO recommends that all children from 6 months to 59 months of age should be vaccinated.[10] So far, European Centre for Disease Prevention and Control (ECDC) has not recommended universal vaccination of young children. In 2013 the United Kingdom issued a recommendation for annual influenza vaccinations first for school-aged children, gradually extending the program to children 2–17 years of age, by mostly using live attenuated intranasal vaccine.[11]

Finland has since 2007 offered seasonal influenza vaccine for all children 6–35 months of age as part of the fully reimbursed national immunization program. However, the annual vaccination rates of young children against seasonal influenza in Finland have been low, varying from 13 to 24% in years 2012–2016.[12] In 2009 influenza A(H1N1) pandemic, a monovalent, adjuvanted influenza vaccine (Pandemrix) was used in Finland for children and adults, with a very high coverage in children. The aim of this study was to examine the incidence and severity of influenza infection, and influenza vaccine effectiveness in a birth cohort of children in the era of national influenza immunization program. The study period extended from 2008 to 2011 and covered the pandemic season in 2009.

Methods

Study population and design

This study was conducted within the prospective, observational birth-cohort study Steps to the Healthy Development and Well-being of Children (the STEPS study).[13] Of 9936 children born in the Hospital District of Southwest Finland between January 2008 and April 2010, 1827 were recruited to the STEPS study before or soon after birth without selection criteria. An intensive follow-up of respiratory tract infections from birth to two years of age of the child (until January 2010–April 2012) was offered to these families, and 923 children were enrolled. Of the study children, 805 (87%) were actively followed up at 12 months and 555 (60%) at 24 months of age.[14,15] The mean duration of the follow-up was 1.69 years (standard deviation, 0.47). For background information, parents completed detailed questionnaires before the child's birth and on specific ages of the child.

Parents of the participating children were encouraged to visit the study clinic every time the child experienced symptoms of an acute respiratory tract infection. The study clinic was open on weekdays. All visits were free of charge to the families. At the clinic, the existence and duration of symptoms (rhinorrhea, cough, wheezing, fever, and other symptoms) and the findings of clinical examination were uniformly documented and nasal swab samples for quantitative reverse transcription polymerase chain reaction (RT-qPCR) assays for investigation of virus etiology were taken by use of flocked swabs (Copan, Brescia, Italy). Alternatively, trained parents collected nasal swabs from the child at home at the onset of symptoms of an acute respiratory tract infection and sent the swabs in dry sterile tubes to the laboratory by mail as described earlier.[16,17] Study children had personal study diaries, where symptoms of acute respiratory tract infections were recorded on daily basis by children's parents. Physician-reported information on visits for respiratory tract infection elsewhere, the diagnoses made, and antibiotic treatments given were also documented in the diary. Fever was defined as body temperature above 38 °C per rectum or 37.5 °C from axilla or tympanic membrane. Data on all pediatric hospitalizations in the study area were collected from the electronic register of the Hospital District of Southwest Finland.

The study protocol was approved by the Ethics Committee of the Hospital District of Southwest Finland (reference number 16/180/2008 §62). Parents of participating children gave their written, informed consent.

Vaccinations

Individual influenza vaccination data were collected from the electronic registers of regional well baby clinics, where the children received their vaccines as part of the national immunization program. A trivalent, inactivated subunit influenza vaccine (Vaxigrip, Sanofi Pasteur MSD, Diegem, Belgium; Influvac, Abbott Biologicals, Olst, Netherlands; or Fluarix, GSK Biologicals, Dresden, Germany) with an intramuscular dose of 0.5 ml was used prior to all influenza seasons studied. Two doses with a 4-week interval were given to children who received their first influenza vaccinations. Prior to or during the influenza pandemic in 2009, children were also vaccinated with a half of an adult dose (ca. 1.9 μg of hemagglutinin) of a monovalent, AS03 adjuvanted vaccine against the influenza A(H1N1)pdm09 virus (Pandemrix, GSK Biologicals, Dresden, Germany). Children who had received two doses of seasonal influenza vaccine as their first influenza vaccinations, or one dose of pandemic vaccine in 2009, at least 14 days prior to the onset of illness were classified as "fully vaccinated"; children who had received only one dose of seasonal vaccine at least 14 days prior to the onset of illness as "partially vaccinated"; and children not vaccinated in the current season or vaccinated less than 14 days prior to the onset of illness as "unvaccinated".

Influenza seasons

The study covered three influenza seasons in the years 2008–2009, 2009–2010, and 2010–2011. During season 2011–2012 only a small number of the cohort children were less than 2 years of age and this season was not included in the study. The start and end dates of each influenza season were determined by data from the National Infectious

Diseases Register kept up by the National Institute for Health and Welfare in Finland (Fig. 1). In 2008–2009, the circulating influenza A(H3N2) virus strain had a good match with the H3N2 type vaccine virus A/Brisbane/10/2007.[12] In the pandemic season 2009–2010, the influenza A(H1N1) pdm09 strain had a good antigenic similarity to the vaccine virus A/California/07/2009. A(H1N1)pdm09 was the dominating influenza A virus also in the 2010–2011 epidemic season, when A/California/07/2009 virus was retained as the H1N1 strain in the seasonal vaccine. During 2010–2011,

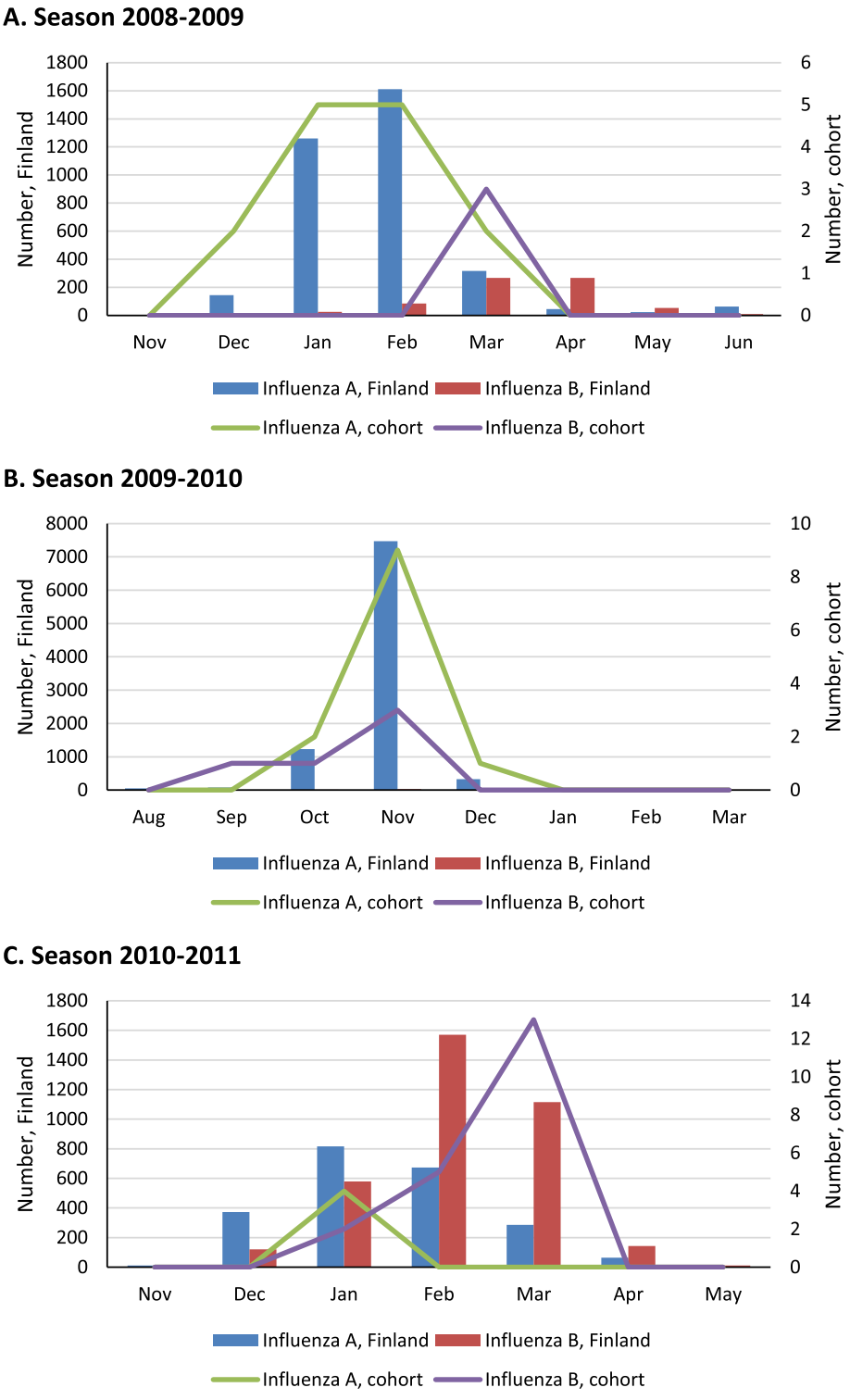

Figure 1. Monthly numbers of all reported influenza cases in Finland according to the National Infectious Diseases Register (bars, left axis) and monthly numbers of influenza cases in the study cohort (lines, right axis). A) Season 2008–2009; B) Season 2009–2010; and C) Season 2010–2011.

circulating influenza A(H3N2) virus strains differed antigenically from the vaccine strain A/Perth/16/2009, but these viruses were infrequently found during the season. In 2008–2009, influenza B vaccine virus was Yamagata strain B/Florida/4/2007, which gave a poor protection against circulating influenza B Yamagata viruses in Finland because of a genetic drift. In both seasons 2009–2010 and 2010–2011, influenza B vaccine virus was B/Brisbane/60/2008 with a good match with circulating influenza B viruses of Victoria lineage. In 2010–2011 influenza B Victoria lineage viruses dominated but there was also circulation of Yamagata lineage viruses not matching the vaccine virus.

Influenza virus detection

All nasal swab samples collected during the three influenza seasons were analyzed for influenza viruses using RT-qPCR. Nasal swabs were suspended in phosphate-buffered saline, and nucleic acids were extracted from the specimens by NucliSense easyMag (BioMerieux, Boxtel, the Netherlands) or MagnaPure 96 (Roche, Penzberg, Germany) automated extractor. Nucleic acid extracts were reverse transcribed and the complementary deoxyribonucleic acids amplified using PCR for influenza A(H3N2), influenza A(H1N1) and influenza B viruses as described earlier.[18] Amplifications were performed in a Rotor-Gene (Qiagen, Hilden, Germany) cycler and amplification curves crossing the cycle threshold at <40 cycles were considered as a positive reaction. Negative controls for the extraction, reverse transcription and PCR steps, and positive controls for the influenza A(H1N1), influenza A(H3N2) and influenza B RT-qPCR were included in each run.

Statistical analysis

The incidence of PCR-confirmed influenza was calculated for each influenza season. The effectiveness of influenza vaccination was calculated by comparing the proportions of subjects with an influenza infection, confirmed by PCR, between fully vaccinated and unvaccinated children aged $\geq$6 months at the beginning of influenza season by using formula (1 − risk ratio). Children with missing vaccination data were excluded from the analysis. Statistical analyses were performed using SAS version 9.1 (SAS, Cary, NC, USA).

Results

Incidence of influenza

Background characteristics of the study children are shown in Table 1. Altogether 1607 nasal swab samples were analyzed for influenza viruses from the cohort of 923 children during three influenza seasons (Fig. 2). Because of the 2-year recruitment, the end of the follow-up at 2 years of age, and drop-outs during the follow-up, the number of children at the start of each influenza season varied from 335 to 637. The mean (range) age of study children was 3.7 (0.03–8.0), 8.1 (0.1–17.4), and 15.6 (7.3–23.8) months at the start of the first, second, and third influenza season, respectively. During the first influenza season (2008–2009), the incidence of laboratory-confirmed influenza infection was 5.1% (17 of 335) and most cases were caused by influenza A(H3N2) viruses (Table 2). Pre-pandemic A(H1N1) viruses were not detected. During the second season (2009–2010), the incidence was 2.7% with the influenza A(H1N1)pdm09 virus being detected in 12 (1.9%) and influenza B virus in 5 (0.8%) of 637 children. During the third influenza season (2010–2011), influenza B viruses dominated but A(H1N1)pdm09 viruses were also detected, with a 5.0% total incidence of laboratory-confirmed influenza. Altogether 56 (6.1%) of 923 children had a symptomatic, laboratory-confirmed influenza infection during the first 2 years of life. Taking account of the mean duration of follow-up (1.69 years), the yearly incidence of PCR-confirmed influenza virus infection was 3.6%.

Of all acute respiratory tract infections that occurred during influenza seasons and were analyzed for virus etiology, influenza viruses were detected in 3.5%. Thirty-one (2.8%) of 1110 home collected samples and 25 (5.0%) of 497 study clinic collected samples were positive for influenza A or B virus.

Table 1 Background characteristics of study children.

Characteristic	Number of characteristic/number of children (%)
Male	488/923 (53)
Older siblings	376/923 (41)
Maternal education level at least professional	574/892 (64)
Mother smoked during pregnancy	44/801 (5)
Breast-fed for at least 6 months	432/716 (60)
Attendance at outside-home daycare at the age of 13 months	185/784 (24)
Attendance at outside-home daycare at the age of 24 months	370/681 (54)
Diagnosed asthma by the age of 24 months	38/676 (6)

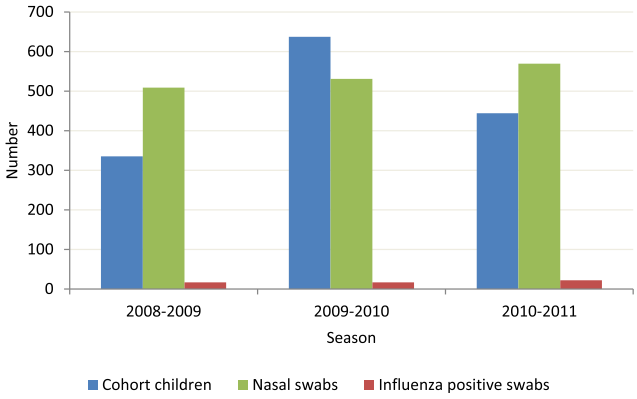

Figure 2. The number of study children, nasal swab samples collected, and swabs positive for influenza A or B virus during each season.

Table 2 Incidence of influenza virus infections detected by RT-qPCR during three consecutive influenza seasons in study children.

Virus	Season		
	2008–2009, n = 335	2009–2010, n = 637	2010–2011, n = 444
Influenza A(H1N1)[a]	0 (0)	12 (1.9)	4 (0.9)
Influenza A(H3N2)	14 (4.2)	0 (0)	0 (0)
Influenza B	3 (0.9)	5 (0.8)	18 (4.1)
Influenza A or B, total	17 (5.1)	17 (2.7)	22 (5.0)

[a] All influenza A(H1N1) infections were caused by A(H1N1) pdm09 viruses.
Data is presented as number of cases (%).

To ensure that we did not miss any severe influenza cases, we searched the diagnoses and virologic results regarding all hospital stays of the study children during the three influenza seasons from the electronic register of the Hospital District of Southwest Finland. We found three hospitalizations because of influenza, which were all identified also by our active follow-up.

Clinical findings

As all cohort children were very young at the time of the first influenza season, which was dominated by influenza A(H3N2) viruses, the mean age of children with influenza A(H3N2) infection was only 6.1 months (Table 3). The mean ages of children with influenza A(H1N1)pdm09 and influenza B virus infections were 11.9 and 13.1 months, respectively. Physician visit either at the study clinic or elsewhere was documented for 30 (54%) of 56 children with laboratory-confirmed influenza. Three children (5%) were hospitalized because of a sepsis-like illness with high fever and one of them was diagnosed with pneumonia. They were discharged home after few days of inpatient care and they fully recovered. Acute otitis media was diagnosed in 11 (23%) children with influenza infection. Fever was documented in all of the 47 children with detailed symptom data available.

Influenza vaccine effectiveness

Vaccination data was available for 758 (82%) children. Influenza vaccination coverage and vaccine effectiveness were calculated among children 6 months of age or older at the beginning of the season (Table 4). Because of the early phase of the study in the beginning of the first influenza season (2008–2009), there were only 60 such children; 13 (22%) of them were vaccinated against influenza. Among these children, we documented 4 influenza A(H3N2) cases, all in unvaccinated children, and no influenza A(H1N1) or influenza B virus infections. Vaccine effectiveness could not be reliably estimated for 2008–2009 season because of the low number of subjects. Prior to the pandemic season 2009–2010, 291 (80%) of 363 children received a monovalent, adjuvanted vaccine against pandemic influenza (Pandemrix) and 169 (47%) children received a trivalent seasonal influenza vaccine. Eight of 9 influenza A(H1N1) pdm09 cases occurred in unvaccinated children, resulting in vaccine effectiveness of 97% (95% confidence interval [CI], 76–100). In addition, 1 unvaccinated child had an influenza B infection. Prior to the third season (2010–2011), 117 (33%) of 358 children were fully vaccinated by a seasonal influenza vaccine. Vaccine effectiveness was 41% (95% CI, −75–80) during this season, which was dominated by influenza B viruses.

Discussion

Our prospective study was designed and started before the 2009 influenza pandemic, which by chance occurred during the study. We documented a 5% incidence of influenza in this cohort of young children for both 2008–2009 and 2010–2011 seasons. During 2009 pandemic season, only 2% of study children had a confirmed A(H1N1)pdm09 infection. A high proportion of study children between 6 months and 2 years of age were vaccinated against the pandemic virus with an adjuvanted vaccine, which appeared to be highly effective. Clinical manifestations of both pandemic and

Table 3 Clinical characteristics of study children with influenza.

Characteristic	Influenza A(H1N1)[a],n = 16	Influenza A(H3N2), n = 14	Influenza B, n = 26	Influenza A or B, total, n = 56
Age, months	11.9 (0.2–20.1)	6.1 (1.1–8.5)	13.1 (2.3–22.8)	10.4 (0.2–22.8)
Male	11 (69)	8 (57)	11 (42)	30 (54)
Physician visit	10 (63)	9 (64)	11 (42)	30 (54)
Upper respiratory tract infection	16 (100)	14 (100)	26 (100)	56 (100)
Hospitalization	2 (13)	0 (0)	1 (4)	3 (5)
Pneumonia or bronchiolitis	0 (0)	0 (0)	1 (4)	1 (2)
Acute otitis media	5 (31)	3 (21)	3 (12)	11 (20)
Antibiotics	5 (31)	3 (21)	3 (12)	11 (20)
Oseltamivir	2 (13)	0 (0)	1 (4)	3 (5)

[a] All influenza A(H1N1) infections were caused by A(H1N1)pdm09 viruses.
Data is presented as the mean (range) for age and number of characteristic (%) for other variables.

Table 4 Influenza vaccine effectiveness for monovalent adjuvanted vaccine (Pandemrix) in 2009–2010 (n = 363) and for seasonal trivalent inactivated vaccine in 2010–2011 (n = 358).[a]

Epidemic season and virus type	N of cases/total n (%)		RR (95% CI)	VE, % (95% CI)	P
	Unvaccinated	Vaccinated			
2009–2010, influenza A(H1N1)pdm09	8/72 (11)	1/291 (0.3)	0.03 (0.004–0.24)	97 (76–100)	<0.0001
2010–2011, influenza A or B	14/241 (5.8)	4/117 (3.4)	0.59 (0.20–1.75)	41 (−75–80)	0.33
2010–2011, influenza B	11/241 (4.6)	4/117 (3.4)	0.75 (0.24–2.30)	25 (−130–76)	0.42

[a] Calculated among fully vaccinated children ≥6 months of age at the beginning of influenza season.
CI: confidence interval; RR: rate ratio; VE: vaccine effectiveness.

seasonal influenza were relatively mild in the majority of the cases.

In studies done before the 2009 pandemic, the yearly incidence of influenza that leads to health care visit has varied from 8 to 20%.[1,19,20] In our study, only the first season (2008–2009) occurred before the pandemic. Five percent incidence of influenza in our study during this season should be related to the fact that the children were very young at the onset of season, with the mean age of 3.7 months. Vaccination rate was relatively low in those at or above 6 months of age, and most children could not be vaccinated because they were younger than 6 months of age. At this age, children in Finland are mostly cared for at home and thus have less contacts with other people than older children who attend daycare centers. Notably, pregnant women were not vaccinated against influenza before this season; their vaccinations were started at the occurrence of 2009 pandemic and continued thereafter.

In the USA, the incidence of medical visits due to influenza virus infection during the 2009 pandemic was reported to be highest in children and adolescents aged 2–17 years and considerably high also in children younger than 2 years of age.[21] Serologic studies from 19 countries have shown similar age distribution.[22] Our study children were at the time of 2009 pandemic between 0 and 17 months of age. Influenza A(H1N1)pdm09 infection was documented in 1.9%. Such a low attack rate was very likely a result of an effective vaccination campaign. The effectiveness of the monovalent, adjuvanted pandemic influenza vaccine was 97%, even though we were able to capture also mild influenza cases by our active follow-up. In earlier studies, done by using register data mostly from hospitalized patients, the effectiveness of AS03 adjuvanted pandemic vaccine (Pandemrix) in children has varied from 77% to 92%.[23,24] Earlier effectiveness data for children younger than 2 years of age is very limited. Similar to our results, an excellent effectiveness (96–97%) was reported for children 6–35 months of age in Canada.[25]

In 2010–2011, influenza A(H1N1)pdm09 virus and influenza B viruses cocirculated. Less than 1% of the study children were diagnosed with influenza A(H1N1)pdm09 infection, and 4% of children suffered from influenza B infection. The fact that only 36% of children received seasonal influenza vaccine in 2010, whereas 80% received pandemic influenza vaccine and 47% seasonal influenza vaccine in the preceding season, strongly suggests that the pandemic vaccine administered in 2009 provided protection also during the 2010–2011 season, as reported earlier.[26] The seasonal, trivalent influenza vaccine was not effective against influenza B in this epidemic season when both Victoria and Yamagata lineage B viruses circulated.

The possibility of underestimation in our incidence figures should be considered. Clearly, all influenza infections during the first 2 years of life cannot be identified by an attempt of direct detection at the onset of symptoms. The follow-up in our study was intensive and included sample collection at home and an easy access to physician at the study clinic. We collected 1607 nasal swab specimens during 1416 child-seasons, which equals on average 1.1 samples per child during each influenza season. The low percentage of influenza virus positive samples (3.5%) of all samples supports our incidence estimates. The low frequency of influenza viruses is in contrast with the detection frequencies of 59% for rhinovirus and 6% for respiratory syncytial virus in nasal swabs collected during the entire follow-up from 0 to 2 years of age.[15] Despite the high number of samples collected, they represent not more than 53% of all symptomatic respiratory tract infections. This is due to the detailed diary documentation of even mild respiratory symptoms, which are highly frequent in this age group of children. If influenza viruses would have caused a similar percentage of respiratory tract infections that were missing a diagnostic sample compared to those with a sample, we would have missed about 40 cases, and the extrapolated incidence of influenza virus infection during the study would have been about 10%. Notably, respiratory tract infections without a sample were milder and more often afebrile compared to those during which a diagnostic sample was taken.[15] Scrutiny of hospital records did not reveal any hospitalized influenza cases apart from those identified by our follow-up, which further supports that we did not miss substantial numbers of severe influenza cases.

We were able to study the etiology of even mild respiratory infections that did not necessitate a physician visit by using nasal swab collection at home by parents. We have reported previously that home sampling is a reliable and efficient method for respiratory virus detection.[27] The possible decrease in viral copy numbers caused by home sampling and transfer of samples to laboratory is presumably compensated by the fact that the collection of samples at home is possible at the first onset of symptoms, when the viral load is the highest.

The clinical manifestations of influenza in our study children were mostly non-severe, even though very young children are a risk group for severe influenza and hospitalization.[28–30] This can be explained by our study design

that captured also mild cases. Nevertheless, some of our youngest study children with influenza needed hospitalization. Acute otitis media was a common reason for antibiotic treatment. These clinical findings are in accordance with previous literature.[1]

As a limitation, our cohort included more families with only one child compared to the general population in Southwest Finland.[13] Part of the families dropped out of the study but we calculated our results only from the duration of active follow-up. Vaccination coverage in our study children was higher than on average in Finland. Reasons for that can include families living in an urban area and the parents being more interested in child health promoting issues than parents in general. According to the National Institute for Health and Welfare in Finland, the influenza vaccination coverage of children 6–35 months of age was 30% in 2008–2009, 32% against seasonal influenza and 74% against the pandemic influenza in 2009–2010 (in the age group of 6 months to 4 years concerning the pandemic vaccine), and about 20% in 2010–2011.

Conclusions

In this actively followed birth cohort of children less than 2 years of age with variable influenza vaccine coverage, the yearly incidence of laboratory-confirmed influenza was 2.7% in the 2009 pandemic season and 5% in other seasons in 2008–2011. In most cases the clinical manifestations of influenza were relatively mild. During the 2009 pandemic, the adjuvanted vaccine (Pandemrix) was used with a high coverage and it was highly effective in children 6–23 months of age. Collection of nasal swab samples at home and transfer to laboratory by regular mail is an efficient method to study the epidemiology of influenza in children.

Conflicts of interest

VP has received consultancy fees from GSK and Novartis, and a travel to a meeting from Sanofi Pasteur. All other authors: no conflicts of interest.

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