Influenza is an infectious disease that is a threat to both children and adults. The most effective way to prevent infections among children is seasonal vaccination in every epidemic season, which is recommended from the age of 6 months onward. This study is a report of the prevalence of influenza infection in the population of children up to the age of 14 years and of the type of influenza virus involved during the 2017/18 epidemic season in Poland. We found that influenza A and B viruses co-dominated in the season. Among the influenza A viruses, A/H1N1/ pdm09 subtype was a more frequent source of infection than A/H3N2/ subtype. In addition, the prevalence of infection was re-analyzed in children stratified into the age groups of 0–4, 5–9, and 10–14 years old. We found a relation between the age of a child and the type of influenza virus causing infection. The youngest children under 4 years were the most vulnerable to both influenza and influenza-like infections; the former caused mostly by influenza A and the latter by RSV. In contradistinction, influenza B dominated in the oldest children aged 10–14 and RSV infections were not present in this age group. The characteristics of influenza viruses may however vary on the seasonal basis.

Keywords
Children · Infection · Influenza · Molecular biology · Respiratory tract · Virology

1 Introduction

The most characteristic symptom of influenza is a sudden onset of fever (≥38 °C), sore throat, muscle pain, cough, and general weakness (Brydak 2008). The diagnosis is particularly difficult among children in whom verbal communication is not yet developed (Wrotek et al. 2018).

The most effective way to prevent the infections among children is seasonal flu vaccination administered every season (Committee on Infectious Diseases 2017). According to the Advisory Committee on Immunization Practices in the USA, a trivalent vaccine is recommended from the age of 6 months for the 2017/18 epidemic season (Grohskopf et al. 2017). Unfortunately, the highest percentage of hospitalizations and deaths is recorded in infants <6 months of age.
(Zawłocka et al. 2016). To protect newborns who are at high risk of complications after influenza infection, a cocoon vaccination strategy is recommended. People from the immediate infant environment, such as parents, grandparents, or siblings, are vaccinated against influenza, which protects the child against the illness in a secondary manner (Nitsch-Osuch 2017). The quadrivalent vaccines, which have been already developed, are recommended from the age of 3 years due to the lack of available research in younger children. This study seeks to define the prevalence of influenza infection in the population of children up to the age of 14 years and of the type of prevailing influenza virus causing infection among children during the 2017/18 epidemic season in Poland.

2 Methods

The study group included children up to 14 years of age, with an additional division into three successive age groups of 0–4, 5–9, and 10–14 years old. The material for the study were nasal and throat swabs taken during the 2017/18 epidemic season and analyzed in 16 Voivodship Sanitary and Epidemiological Stations and in the Department of Influenza Research, National Influenza Center in the National Institute of Public Health – National Institute of Hygiene (NIPH-NIH) in Warsaw, Poland. During the epidemic season, 1286 samples were tested for influenza and reported in the Sentinel and Non-Sentinel Influenza Surveillance System.

The ribonucleic acid (RNA) was isolated from the nasal and pharyngeal swabs. A Maxwell 16 Viral Total Nucleic Acid Purification Kit was used (Promega Corporation; Madison, WI), according to the instructions provided by the manufacturer. From 200 μl of clinical samples, suspended in 1 ml of physiological saline, 50 μl of RNA resuspended in RNase-free water was obtained. Molecular tests were performed to confirm the presence of influenza A and B viruses and to determine the viral subtypes. The Light Cycler 2.0 System was used (Roche Diagnostics; Rotkreuz, Switzerland). The primers and probes were obtained from the International Reagent Resource (IRR) run by the Centers for Disease Control and Prevention (CDC). The reaction was carried out according to the manufacturer’s instructions. RNA was subjected to reverse transcription (at 50 °C for 30 min). The obtained DNA was subjected to the initiating denaturation process (1 cycle at 95 °C for 2 min), followed by 45 cycles of amplification: denaturation at 95 °C for 15 s, annealing at 55 °C for 10 s, and elongation at 72 °C for 20 s. Positive control was the viral RNA obtained from the strains used for the 2017/2018 vaccine (A/Michigan/45/2015 (H1N1)pdm09, A/HongKong/4801/2014 (H3N2), and B/Brisbane/60/2008/). Negative control was the water free from RNase.

Using the RT-PCR reactions, the presence of the following respiratory viruses was confirmed: influenza A virus, influenza B virus, human respiratory syncytial virus A and B, human adenovirus, human metapneumovirus, human coronavirus 229E/NL63, human coronavirus OC43, human parainfluenza 1, 2, 3, and 4 viruses, human rhinovirus A/B/C, human enterovirus, and human bocavirus 1/2/3/4. The RV15 OneStep ACE Detection Kit (Seeplex; Seoul, South Korea) was used for these reactions, according to the manufacturer’s instructions. After the reaction, the product was separated on a 2% agarose gel using electrophoresis.

Discrete data were presented as counts and proportions. Differences between the proportions of infected people were compared with a t-test. A value of zero was assumed for the noninfected person and a value of one for the infected one, and the arithmetic mean was used to calculate the percentage of the infected persons. A p-value <0.01 defined statistically significant differences. The prevalence of influenza in the age groups was determined with 95% confidence intervals (95%CI).

3 Results

In the 2017/18 epidemic season, there were 1286 samples tested from children up to 14 years of age. We noticed the co-dominance of influenza A and B viruses in the whole of children’s population; 53.9% and 46.1%, respectively. Among the
influenza A viruses, subtype A/H1N1/pdm09 was confirmed in 18.5% of all positive samples and it prevailed over subtype A/H3N2/ (Fig. 1).

In the children’s population stratified into the age groups of 0–4, 5–9, and 10–14 years, influenza A virus dominated in 0–4 year olds, accounting for 28.8%. The infections caused by influenza B virus were noticed in 14.4% in this group. A different situation was noticed in the age groups of 5–9 and 10–14 years, where influenza B virus dominate, accounting for 34.1% and 31.5% of infections, respectively. In all three age groups, the untyped infections of influenza A dominated, accounting for 17.8%, 17.2%, and 10.7% of infections, respectively, whereas subtype A/H3N2/was present in the lowest proportions; 0.4%, 1.2%, and 0.7%, respectively (Fig. 2).

The results were also analyzed taking into account only the type of influenza virus and the patient’s age. There were a significantly lower percentage of children infected with influenza A virus in the age group 10–14 years than in the groups 0–4 and 5–9 years ($p < 0.001$ and $p < 0.01$, respectively). The difference in the number of influenza A virus infections between the age groups 0–4 and 5–9 years was insignificant ($p = 0.21$). In addition, there was a significantly higher percentage of children infected with influenza B virus in the age groups 5–9 and 10–14 years than in the group 0–4 years ($p < 0.001$). The difference in the number of influenza B virus infections between the age groups 5–9 and 10–14 was insignificant ($p = 0.28$).

A comparative analysis of the 2017/18 and 2016/17 epidemic seasons was also performed for the age group 0–4 years. In the former season, there was a clear dominance of influenza A (111 infections) and subtype A/H3N2/ (74 infections). In contradistinction, in the latter season, co-dominance of influenza A and B viruses (209 and 104 infections, respectively) was recorded. There was a clear difference in the dominant subtype of the influenza A virus. In the former season, it was subtype A/H3N2/, while in the latter season it was A/H1N1/pdm09. In the 2016/17 season, there were only two infections of influenza B virus and none of A/H1N1/pdm09 virus infection (Fig. 3).

The influenza-like infections were reported in the 0–4 and 5–9 age groups and accounted for 81.2% and 18.8%, respectively (Fig. 4). Among children aged 10–14 years, no influenza-like virus was reported. Influenza-like viruses were dominated by RSV (30 infections). The highest number of infections with this virus was recorded in the age group 0–4 years. In this group, there also were four PIV-3 infections and single infections with other influenza-like viruses.
Fig. 2 Proportions of influenza virus infections in children stratified into three successive age groups in the 2017/18 epidemic season. Data are means ±SD.

Fig. 3 Number of confirmed influenza infections in the age group 0–4 years in the 2016/17 and 2017/18 epidemic seasons.

Fig. 4 Proportions of confirmations of influenza-like infections in children stratified into three successive age groups in the 2017/18 epidemic season.
4 Discussion

In the 2017/18 epidemic season, influenza A and B viruses co-dominated in the population of children under the age of 14 years, with a margin of influenza B over A. These results are akin to those noticed in other European countries, e.g., Denmark or Spain (Flu News Europe 2018). Among the influenza A viruses, infections with subtype A/H1N1/pdm09 dominated over A/H3N2/. This season had entirely different characteristics from the preceding 2016/17 season in Poland when no A/H1N1/pdm09 infections had been reported among children (Cieślak et al. 2018).

An interesting tendency appeared in the analysis of the prevailing viral contagion by children’s age. In the older children of 5–9 and 10–14 years, there was a significantly higher incidence of influenza B infection than in the youngest children of 0–4 years. That is in line with the past results from other European research centers. In one of them, results from 12 European countries have been analyzed with reference to the 2012/13 season and the dominance of influenza B virus is reported in children aged 5–14 years (Beauté et al. 2015). In another study, the relation between influenza virus type and subtypes and the age of children was determined. That analysis covers 29 countries around the world in the years 1999–2014. It has been confirmed that infections with influenza B virus most often occur in the 5–17 age bracket (Caini et al. 2018).

The present report demonstrates the lowest percentage of influenza A in the oldest children aged 10–14. Further, infection with subtype A/H3N2/ constituted the lowest percentage of influenza A in each age group. This last trend seems to have been in line with the notion put forward by Adlhoch et al. (2018) suggesting that subtype A/H3N2/ may be the most common in older persons, particularly aged over 65 rather than in children. This report also demonstrates that unsubtyped influenza A was the most prevalent across all the age groups of children. This finding, however, may contain a spurious component due to possibly ineffective subtyping of influenza virus or occasional lack of subtyping it at all. Despite these limitations, we believe we have demonstrated in this report that there is a relation between the child’s age and the type of influenza virus causing infection. In the 2017/18 epidemic season, the youngest children under 4 years were the most vulnerable to both influenza and influenza-like infections, the former caused mostly by influenza A and the latter by RSV. In contradistinction, influenza B dominated in the oldest children aged 10–14 and RSV infections were not present in this age group. However, characteristics of influenza viruses are highly changeable season to season. These data may thus not exactly apply to each and every epidemic season.

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Conflicts of Interest The authors declare no conflict of interests in relation to this article.

Ethical Approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The study was approved by an institutional Ethics Committee.

Informed Consent Informed consent was obtained from all individual participants included in the study before collection of nasopharyngeal samples.

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