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# Epidemiology of Influenza Viruses and Viruses Causing Influenza-Like Illness in Children Under 14 Years Old in the 2018-2019 Epidemic Season in Poland

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**Background:** This study aimed to investigate the epidemiology of influenza viruses and viruses that caused influenza-like disease in children under 14 years of age in the 2018-2019 epidemic season in Poland, and to identify the public health lessons that can be learned.





**Material/Methods:** Nose and throat swabs were used to obtain samples. The samples were analyzed in the National Influenza Center, Department of Influenza Research at the National Institute of Public Health-National Institute of Hygiene as well as in 16 Voivodship Sanitary Epidemiological Stations across the country. Methods of RNA isolation depended on the laboratory where the isolation was performed. In all laboratories, quantitative polymerase chain reactions were used to determine the influenza virus type as well as the subtype.

**Results:** The study group was confirmed to be infected with influenza A and B, with influenza A/H1N1/pdm09 as the dominant subtype. Among the age group of children up to 14 years of age, cases of infection with viruses that cause influenza-like disease were also reported. It was noticeable that the largest number of confirmed cases of infection was recorded in the group of the youngest children (0-4 years). In addition, several different variants of co-infection were registered.

**Conclusions:** This population study showed that in the 2018-2019 epidemic season in Poland children aged under 14 years were at risk of influenza virus infection and its complications. The presented data support increasing the percentage of children being vaccinated in Poland.

**Keywords:** **Epidemiology • Influenza, Human • Respiratory Syncytial Virus, Human • Influenza A virus • Influenza B virus**

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## Background

Influenza is an acute disease of viral etiology, characterized by very high contagiousness. It poses a threat to societies owing to its global spread, ability to change its antigenic properties, and severity of post-influenza complications, which often require hospitalization and may even lead to death.

People of all ages are at risk of contracting influenza virus. Children are particularly at risk owing to the immaturity of their immune system. For the same reason, they are also at risk of complications from influenza; this applies especially to children with impaired immunity, those with chronic diseases, and children under 2 years of age. Influenza can be severe even in a previously healthy child who does not have risk factors for a complicated course of the disease. For many years, it has been thought that only young children with comorbidities could be severely affected by influenza. However, severe cases of this disease also occur among healthy children. In a study conducted in the United States from October 2004 to September 2012, a total of 830 deaths of children and adolescents caused by influenza complications were reported, and 43% of the children did not have comorbidities [1].

Influenza infections usually occur in children because of the ease of transmission of influenza viruses within groups of children and the lack of immunological memory of influenza viruses circulating in the population in earlier epidemic seasons.

Infection in children is spread mainly by droplets through mucus aerosol containing viruses or indirectly through objects contaminated with secretions from the respiratory system of a sick person. During direct contact, the virus is transmitted by inhalation of microscopic secretions from the respiratory tract of the infected person, and it is the most infectious in the symptomatic period of infection. A child can transmit the virus for longer than 10 days, while young children can transmit the virus for up to 6 days before the symptoms appear. The onset of influenza is sudden and acute. The characteristic symptoms in children include a high fever, even above 39°C, chills, headache, muscle aches, cough, general weakness, and malaise. The course of influenza infection in children is also characterized by gastrointestinal symptoms, including abdominal pain, vomiting, and diarrhea. Otitis media may also be present [2]. It is worth emphasizing that the above-mentioned symptoms are not specific only to influenza infection, but also to infections caused by other respiratory viruses that cause influenza-like disease, including parainfluenza 1-3, human respiratory syncytial virus (RSV), human corona viruses 229E/NL63 and OC42, human rhinovirus, human adenovirus, and human metapneumovirus, which makes it difficult for a doctor to make an accurate diagnosis [3].

Influenza complications can be very serious; they include pneumonia and bronchitis, secondary bacterial pneumonia and bronchiolitis, streptococcal pharyngitis, exacerbation of chronic diseases (asthma, cystic fibrosis, diabetes, chronic kidney failure), muscle pain, myocarditis, myocarditis and pericarditis, meningitis or encephalitis, otitis media, auditory receptor dysfunction, partial hearing loss or deafness, neurological complications including Guillain-Barré syndrome, transverse myelitis, worsening of seizures, and graft rejection [2].

Various types of influenza vaccine are used to prevent influenza. In Poland, since the last epidemic season of 2019-2020, inactivated vaccines, known as split or split virion vaccines, and subunit vaccines, containing isolated surface antigens and known as subunit and virion-weakened live vaccines (intranasal), were used. Split influenza vaccines are administered intramuscularly to every child from 6 months of age, provided there are no medical contraindications. Subunit vaccines, also administered intramuscularly, are recommended for children from the age of 3 years. In November 2019, it became possible to vaccinate children and adolescents against influenza with live attenuated intranasal vaccines. The vaccine is intended for the active immunization of children and adolescents from 24 months to 17 years of age. The preparation is in the form of nasal spray [4,5]. Children play a major role in spreading influenza, so increasing the number of vaccinations among them helps to cut the transmission of the disease and reduce its spread among household members. Therefore, vaccinating children against influenza protects not only those who are vaccinated, but may also indirectly protect their relatives, such as younger siblings, parents, and grandparents. Unfortunately, flu vaccination in children in Poland is used far too rarely. Children from 2 to 5 years of age are the most vaccinated group, and infants are least frequently vaccinated, despite it being known that the youngest children are most at risk for developing complications from influenza.

Therefore, this study aimed to investigate the epidemiology of influenza viruses and viruses that caused influenza-like illness in children under 14 years of age in the 2018-2019 epidemic season in Poland, and to identify the public health lessons that can be learned.

## Material and Methods

The material used for the study were nose and throat swab samples collected during the 2018-2019 epidemic season. The samples were collected from week 40 of 2018 (October 1) to week 39 of 2019 (September 30). The samples were analyzed in the National Influenza Center, Department of Influenza Research at the National Institute of Public Health-National Institute of Hygiene (NIPH-NIH) and in 16 Voivodship Sanitary Epidemiological Stations (VSEs) across the country. The

**Table 1.** The diagnostic methods used in 16 Voivodship Sanitary Epidemiological Stations in the 2018-2019 epidemic season in Poland.

Voivodship Sanitary Epidemiological Station	Diagnostic equipment	Kits
Białystok	LightCycler 96 (Roche)	Real Time Ready Influenza A/H1N1/Detection Set, RealTime Ready RNA Virus Master, LightCycler Multiplex RNA Virus Master, Light Mix Modular EAV RNA Extraction Control (Roche)
Bydgoszcz	LightCycler 480 II (Roche)	Multiplex RNA Virus Master (Roche); sondy i startery Modular Dx Kit Inf M2, Modular Dx Kit InfA H3, InfB, Light Mix Kit CC_Hexaplex 480 II; kontrola wewn IC – Roche RNA Process Control Kit Trial Pack
Gdańsk	–	FTD Flu (Fast Track Diagnostics)
Gorzów Wlkp.	LightCycler 480 II (Roche)	FTD Flu (Fast Track Diagnostics)
Katowice	LightCycler 480 II (Roche)	PowerChek Pandemic H1N1/H3N2 Real Time RT-PCR Kit (Kogene Biotech); FTD Flu (Fast Track Diagnostics)
Kielce	–	Allplex Respiratory Panel 1 (Seegene)
Kraków	MX3005 P STRATAGENE	One tube multiplex PCR for influenza A H1N1, B, H1N1, H3, H5 and H7 (Fast Track Diagnostics)
Lublin	CFX96 Bio-Rad	FTD Flu (Fast Track Diagnostics)
Łódź	–	Bosphore H1N1Detection Kitv3 (Anatolia Geneworks)
Olsztyn	CFX96 Bio-Rad	Allplex Respiratory Panel 1 (Flu/RSV/FluA subtyping) (Seegene)
Opole	–	–
Poznań	GeneXpert (Cepheid) 7500 Real-Time PCR (Applied Biosystems)	Xpert Flu A,B, A/H1N1/pdm09
Rzeszów	Applied Biosystems 7500 Real-Time PCR System Roche Light Cycler 480 II RBC Bioscience – MagCore HF 16 Plus	Ribo-prep nucleic acid extraction kit (AmpliSens), MagCore Super/HF 16 Plus nucleic Acid Extraction Kit (RBC Bioscience), FTD Flu (Fast-Track Diagnostics), FTD Flu differentiation (Fast-Track Diagnostics)
Szczecin	Rotor-Gene (Qiagen)	PowerChek Pandemic H1N1/H3N2 Real Time RT-PCR Kit (Kogene Biotech)
Warszawa	GeneXpert (Cepheid)	Xpert Flu A,B, A/H1N1/pdm09
Wrocław	Rotor-Gene (Qiagen)	PowerChek TM Influenza A/B, Pandemic H1N1/H3N2 Real Time RT-PCR Kit (Kogene Biotech) FTD Flu (Fast Track Diagnostic)

National Influenza Center of the NIPH-NIH served as the reference laboratory. The study group consisted of children up to 14 years of age, with an additional division into 3 smaller age groups (0-4 years, 5-9 years, and 10-14 years). The study included 994 samples tested for the presence of influenza virus and reported in the sentinel and non-sentinel influenza surveillance system in Poland. The sentinel system is an integrated internet virological and epidemiological system for influenza. In 2004, after fulfilling international requirements imposed by the European Influenza Surveillance Scheme, Poland implemented their sentinel influenza surveillance system. Within the sentinel system, epidemiological and virological surveillance is conducted in cooperation with general practitioners and 16

VSEs. It is an important monitoring tool for the entire country. The sentinel system in Poland is coordinated by the National Influenza Center situated at the NIPH-NIH.

### Isolation of RNA

RNA was isolated from the nasal and pharyngeal swabs collected from the patients, which were suspended in 1 mL of saline. For RNA isolation, the Maxwell 16 Total Viral Nucleic Acid Purification kit (Promega Corporation, Madison, WI, USA) was used to isolate the genetic material at the Department of Influenza Research of the NIPH-NIH. The isolation was performed from a 200- $\mu$ L sample suspended in saline, following

the manufacturer's instructions. Elution of the RNA was performed using 50 µL of RNase-free water.

### Real-Time Polymerase Chain Reaction

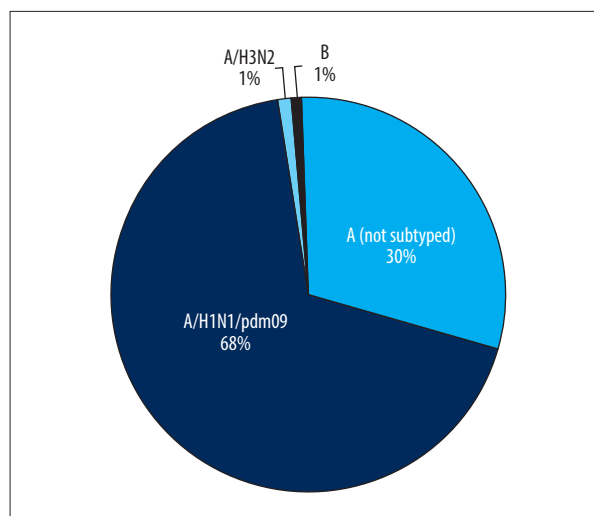
Quantitative polymerase chain reaction was used to determine the influenza virus type and subtype. At the Department of Influenza Research of the NIPH-NIH, the reaction was performed using Rotor-Gene Q (Qiagen) and the SuperScript Platinum III kit (Invitrogen). Primer and probe kits (influenza A, influenza A/H3N2/, influenza A/H1N1/pdm09, and influenza B) obtained from the International Reagent Resource (IRR) of the Centers for Disease Control and Prevention were used. The sequences of the primers and probes from IRR were not publicly available. RNA was subjected to reverse transcription (50°C for 30 min). After initiation (1 cycle at 95°C for 2 min), the DNA was subjected to 45 cycles of amplification: denaturation (95°C for 15 s), annealing (55°C for 30 s), and elongation (72°C for 20 s). The positive controls of the reactions were viruses derived from the vaccine for the 2018-2019 epidemic season (A/Michigan/45/2015, A/H1N1/pdm09, A/Singapore/INFIMH-16-0019/2016 (H3N2), and B/Phuket/3073/2013). The negative control was the RNase-free water provided in the kit. RNA of vaccine viruses selected by the World Health Organization were used as positive controls. The research was also carried out in the VSEs. The methods used are presented in **Table 1**.

### Statistical Analysis

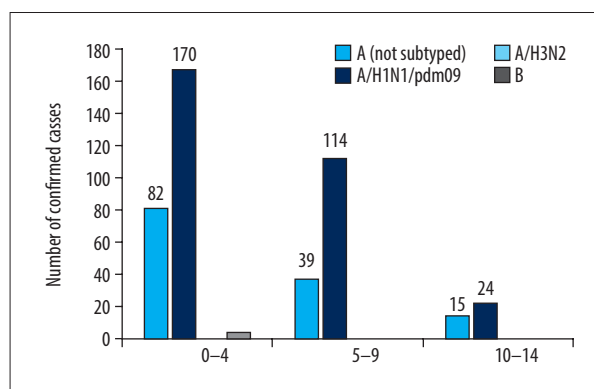
We performed statistical tests to assess whether there were differences between age groups in relation to their vulnerability to various types of viruses. Treating an influenza infection as “statistical success” and a non-influenza infection as “statistical failure”, we were able to perform 2-sample Z-tests on the data.

## Results

In the 2018-2019 epidemic season from October 2018 until the end of September 2019, a total of 994 samples from children up to 14 years of age were tested, and 230 samples (23.1%) came from the sentinel program. Confirmed cases accounted for 50.6% (503 samples) of all tested samples. There was a clear dominance of influenza A (447 cases) over influenza B (5 cases) among the infected patients. Among influenza A viruses, the majority were samples with influenza A/H1N1/pdm09 (308 samples, 68.9% of all the cases of influenza A). Only 3 cases of infection caused by influenza A/H3N2/virus (0.7%) were confirmed. This may corroborate the thesis that this virus subtype is most common in people over 65 years of age [6]. Among influenza A-positive cases, the remaining were influenza A viruses not subject to subtyping (136 samples, 30.4% of influenza A confirmations) (**Figure 1**).



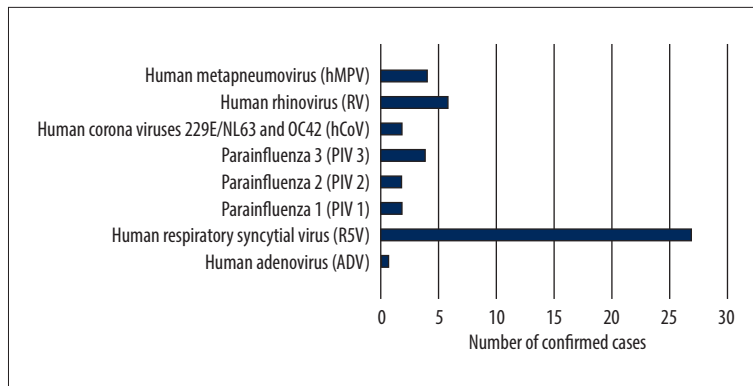
**Figure 1.** The number of confirmed cases of influenza in children aged under 14 years in the 2018-2019 influenza season in Poland.



**Figure 2.** Participation of individual types and subtypes of influenza viruses in children aged under 14 years in the 2018-2019 influenza season in Poland.

In line with the innovation in influenza surveillance introduced by the NIPH-NIH, the results were also analyzed in the 3 smaller age groups (0-4 years, 5-9 years, and 10-14 years) [7]. The highest number of infections among all 3 age groups was in the youngest children (0-4 years) for both influenza A (253 patients) and influenza B (4 patients). In children aged 5 to 9 years, 154 infections with influenza A and 1 infection with influenza B were recorded. In children aged 10 to 14 years, 40 infections were confirmed to be due to influenza A and no infections due to influenza B were found (**Figure 2**).

In the children up to 14 years of age, cases of infection with viruses that cause influenza-like disease were also reported. Additional analysis was carried out in the 3 smaller age groups (0-4 years, 5-9 years, 10-14 years). The results showed that the largest number of confirmed cases of infection with viruses that cause influenza-like disease was recorded in the group of



**Figure 3.** Number of viruses causing influenza-like illness detected in children aged under 14 years in the 2018-2019 epidemic season in Poland.

**Table 2.** Co-infections of respiratory viruses in the 2018-2019 epidemic season.

Patient's age	Influenza viruses
8 months	hCoV+PIV1-3
2 years	RSV+PIV-1
5 years	A/H1N1/pdm09+PIV-4
4 years	A+RSV+ADV
4 days	A+RSV
9 days	RSV+Enterovirus
5 years	A/H1N1/pdm09+B
5 years	A/H1N1/pdm09+B

the youngest children (0-4 years). There were 43 cases (89.6%) of confirmed infections with viruses that cause influenza-like disease in children aged 0 to 4 years, while there were 2 cases (4.2%) in children aged 5 to 9 years, and there were 3 cases (6.2%) in children aged 10 to 14 years. RSV was the most common of the viruses causing influenza-like disease (27 cases). The highest number of infections with this virus was recorded in the age group 0-4 years. In this group, single cases of infections with other viruses that cause influenza-like disease, including 6 cases of human rhinovirus and 3 cases of parainfluenza 3, were confirmed (**Figure 3**).

In children under 14 years of age, in the 2018-2019 epidemic season, co-infections, defined as simultaneous infection with 2 or more respiratory viruses, were detected. These co-infections were influenza viruses and viruses that cause influenza-like disease. The registered co-infections are presented in **Table 2**.

## Discussion

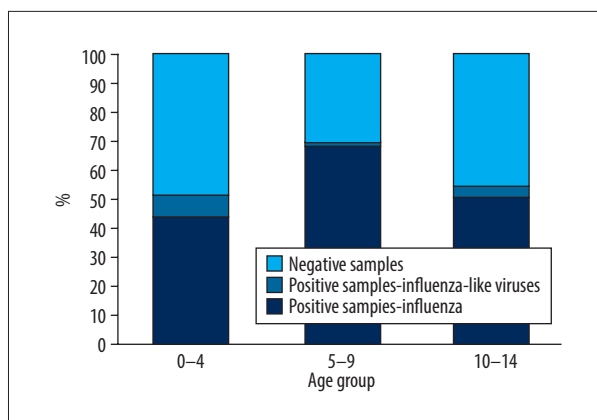
In the 2018-2019 epidemic season, influenza A was the dominant type of virus in Poland, while the dominant subtype was A/H1N1/pdm09. During this season, comparable results were obtained in other countries in Europe [8] and the world [9].

This season had different characteristics from the preceding 2017-2018 season in Poland. In contrast to the 2016-2017 influenza season, we observed a very low number of influenza B infections in 2017-2018 (10). Influenza type B in the 2016-2017 epidemic season was just as rare as it was in the 2018-2019 season [11].

Our results showed numerous infections with influenza viruses and viruses that cause influenza-like disease among children aged 0 to 14 years. The highest number of confirmed cases of influenza and viruses that cause influenza-like disease was recorded in the youngest children (aged 0 to 4 years). This may corroborate the thesis that younger children have poorly developed immune systems. The immaturity of their immune systems is conducive to more serious and longer courses of infection, as well as post-influenza complications, which often have dangerous effects on health or are life-threatening. In the 2018-2019 influenza season in Poland, 1 death of a child aged 0-4 years was recorded as due to complications from influenza. Children in large clusters of people, such as in nurseries, kindergartens, and schools, are particularly vulnerable to infections.

The high number of flu cases in the age group 0-14 years demonstrates the widespread problem of low immunization status among children in Poland [12]. According to the recommendations of the Advisory Committee on Immunization Practices, influenza vaccination is recommended for all healthy people aged 6 months and older (with no upper age limit), people from groups at a high risk of developing post-influenza complications regardless of age, pregnant women, and specific groups for epidemiological indications, including medical personnel and employees of social care homes, kindergartens, schools, trades, transport, and construction. Contraindications to influenza vaccination are the presence of acute fever, documented anaphylactic hypersensitivity to egg white or other ingredients contained in the vaccine, the occurrence of an anaphylactic or other serious allergic reaction after a previous vaccination, and Guillain-Barré syndrome [4]. In Poland, flu vaccination is included in the Protective Vaccination Program as "recommended". However,





**Figure 4.** Percentage of samples positive in relation to all tested samples in detected in children aged under 14 years in the 2018-2019 epidemic season in Poland.

despite the recommendation, only 0.85% of children were vaccinated against influenza in 2018 [13]. This number is shockingly low compared to the percentage of vaccinated children in other European countries. Children make up a very important group that is involved in the spread of the influenza virus throughout the population. Having a higher percentage of vaccinated children could reduce the number of infected patients in all age groups. Research conducted by experts from Public Health Ontario and the Institute for Clinical Evaluative Sciences in Toronto, Canada, confirmed that children vaccinated against influenza are much less likely to experience serious complications of influenza leading to hospitalization. The results of the study, published in November 2017, showed that in a group of young children vaccinated against influenza using 2 doses of the vaccine, as indicated, the risk of hospitalization caused by influenza was reduced by 60%. Additionally, it was shown that even in the group of children who received only 1 dose of the vaccine, the risk of hospitalization due to influenza was reduced by 39% [14].

From analyzing the results in the age groups of 0-4, 5-9, and 10-14 years, a certain relationship was noticed: the older the age group, the fewer cases of influenza were confirmed. In the 0-4 age group, the number of confirmed cases was definitely the highest, at 257. In the 5-9 age group, 155 influenza infections were found, while in the 10-14 age group, there were 40 cases (Figure 4).

For the age group 0-4 years, the percentage of influenza infections (with respect to all positive samples) was 85.7%; the age group 5-9 years was 98.7%; and the age group 10-14 years was 93.0%. There was a statistically significant difference between age groups 0-4 years and 5-9 years ( $P < 0.001$ ) and between age groups 5-9 and 10-14 years ( $P = 0.03$ ); however, there was no statistically significant difference between age groups 0-4 and 10-14 years ( $P = 0.19$ ). We interpret the non-significant result between age groups 0-4 and 10-14 years as

an effect of the relatively small sample size of the 10-14 age group (only 43 cases of infections).

Based on the data available on the NIZP-PZH website [13], it is known that influenza infections and suspected infections in children account for 30% to 48% of the total number of infections in the last 4 epidemic seasons, 2015-2016 through 2018/2019 [11]. Similar results were obtained in the epidemic seasons 2014-2015, 2015-2016, and 2017-2018 [10,15,16].

In children, RSV infection is a common cause of respiratory disease and is responsible for 17% to 20% of all hospitalizations of infants. The infection occurs mainly through indirect contact with bodily secretions of infected individuals. The peak incidence in the northern European climatic zone falls, as in the case of influenza, within the winter months (most cases occur in January and February) [17]. Ninety percent of children under 2 years of age are infected with RSV, with children between 6 weeks and 6 months of age accounting for most infections [18]. Cases of confirmed respiratory system infections with RSV were seen in the analyzed cases of children in the 2018-2019 season. A similar trend was observed in the epidemic seasons 2014-2015, 2015-2016, and 2017-2018 [10,15,16]. In the age group made up of the youngest children (0-4 years), there were by far the largest number of infections (34 cases). Three cases of infection with this virus were recorded among children aged 5 to 9 and 10 to 14 years.

### Limitations of the Study

This work is based solely on the analyses of samples that were reported to the sentinel influenza surveillance system. Not all tested samples are reported to the system; therefore, the number of patients studied in Poland in given seasons could be much higher.

### Conclusions

Influenza poses a severe threat to people of all age groups, including children. In every epidemic season, a large number of children and teenagers become ill or die from influenza or from post-influenza complications. We believe the most effective method of preventing this disease is vaccination. The presented data support that the rate of vaccination in Poland should be increased in children from 6 months to 14 years of age.

### Acknowledgements

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## Conflicts of Interest

None.

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