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# Subtypes of Influenza Virus Infection and Outcomes in Individuals Older than 65 Years of Age in Poland in the 2016/2017 to 2019/2020 Epidemic Seasons

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**Background:** Influenza is a viral disease causing many deaths each season. With aging, the human immune system becomes weaker, so people over the age of 65 years are at higher risk of complications after influenza infections. This population study, conducted in Poland, aimed to identify the subtypes of influenza virus infection and outcomes in individuals more than 65 years of age in the 2016/2017 to 2019/2020 epidemic seasons.

**Material/Methods:** The research materials were nose and throat swabs. Research was conducted in 16 Voivodship Sanitary and Epidemiological Stations and in the Department of Influenza Research, National Influenza Centre, NIPH-NIH. Methods of RNA isolation depended on the laboratory where the isolation was performed. In all laboratories, quantitative polymerase chain reaction (qRT-PCR) was used to determine the influenza virus type and subtype.

**Results:** The analysis of the incidence of influenza among people over the age of 65 included the 2016/2017, 2017/2018, 2018/2019, and 2019/2020 influenza epidemic seasons. We analyzed the percentage of positive samples, the dynamics of epidemic seasons, and the percentage share of influenza viruses in the 65+ age group, according to the epidemic season and percentage of deaths.

**Conclusions:** This population study showed that, in Poland, between the 2016/2017 and 2019/2020 epidemic seasons, people who were more than 65 years of age were at higher risk of influenza virus infection and its complications. The findings support the importance of seasonal influenza vaccination in the population over age 65 years.

**MeSH Keywords:** **Influenza A virus • Influenza B virus • Vaccination • Virology**

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## Background

Influenza is a viral disease that affects as many as 3–5 million people worldwide each year, causing 290 000 to 650 000 deaths [1]. The majority of deaths occur among people over the age of 65–70 years [2,3]. Contracting influenza at this age can lead not only to death, but also to numerous complications, including cardiac complication because such patients often have chronic diseases than can exacerbate the consequences of influenza infection [4]. The only effective method of protection against the influenza virus is seasonal vaccination. People over the age of 65 are included in the high-risk group by the WHO, and influenza vaccination is therefore particularly recommended for them [1].

With age, the human immune system becomes increasingly weaker, which debilitates the response to vaccination. Therefore, in the USA in 2009, the Fluzone High-Dose vaccine was introduced to the market, which contains a 4 times higher dose of 3 antigens and is reserved for people over 65 years of age [5]. Studies have shown that the effectiveness of such vaccinations in the elderly is significantly higher [6]. Since the 2018/2019 season, this vaccine has also been available in a 4-component version. This type of vaccine is only available in the US market.

The second vaccine intended only for people over the age of 65 is the FLUAD vaccine. It is an inactivated ternary vaccine adjuvanted with MF59. The addition of the adjuvant is intended to enhance the immune response to vaccination [5]. This type of vaccine was introduced to the Italian market in 1997. It is currently registered in 38 countries, including 15 European countries. In Poland, this type of vaccine is not available [7].

In Poland, there are 2 types of vaccines suitable for people over 65 years of age. These include 4-component split and subunit vaccines [8]. Polish local governments have decided to support vaccination of the elderly, and a free vaccination program for people over the age of 65 has been introduced in many regions of the country or a 50% discount of the purchase has been offered. Such measures are aimed to encourage elderly people to become vaccinated against influenza and achieve the 75% vaccination coverage rate of the population recommended by ECDC and WHO [9].

The population studies conducted earlier in Poland show that the groups most exposed to influenza are children (0–4 years of age) and people over 65 years of age [10]. Influenza infections caused by the A/H3N2/ subtype are most common in people over the age of 65 [11]; they lead to outbreaks in long-term nursing homes and cause high mortality in this age group [12].

Therefore, this population study, conducted in Poland, aimed to identify the subtypes of influenza virus infection and outcomes

in individuals more than 65 years of age in the 2016/2017 to 2019/2020 epidemic seasons.

## Material and Methods

The research material consisted of nose and throat swabs collected as part of the participation of general practitioners in the SENTINEL and NON-SENTINEL influenza surveillance systems. The research was conducted by the laboratories of Voivodship Sanitary and Epidemiological Stations, and hospital laboratories, as well as the laboratory of the Department of Influenza Research, National Influenza Center. The results were analyzed in patients over 65 years of age.

### Viral RNA isolation

A previously collected swab was suspended in 1 ml of physiological saline. The Maxwell 16 Viral Total Nucleic Acid Purification Kit (Promega Corporation, Madison, WI, USA) was used to isolate the genetic material at the Department of Influenza Research, National Influenza Center. According to the recommendations of the manufacturer, 200 µL of the suspension was taken. An inventory of isolation reagents used in other laboratories was not prepared.

### Typing and subtyping influenza viruses

Quantitative polymerase chain reaction (qRT-PCR) was used to determine the influenza virus type and subtype. At the Department of Influenza Research, National Influenza Center, the reaction was performed using Rotor-Gene Q (Qiagen) and the SuperScript Platinum III kit (Invitrogen). We used primers and probes kits (Influenza A, Influenza A/H3N2/, Influenza A/H1N1/pdm09, Influenza B) obtained from the International Reagent Resource run by the Centers for Disease Control and Prevention. The sequences of the primers and probes from IRR were not publicly available. RNA was subjected to reverse transcription (50°C, 30 min). The obtained DNA was subjected to initial denaturation (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. RNA of vaccine viruses selected by the WHO were used as positive controls. The research was also carried out in the Voivodship Sanitary and Epidemiological Stations. The methods used are presented in the **Table 1**.

## Results

### Percentage of positive samples

The analysis of the incidence of influenza among people over the age of 65 in the epidemic seasons 2016/2017, 2017/2018,

**Table 1.** The diagnostic methods used in 16 Voivodship Sanitary Epidemiological Stations 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); probes and starters Modular Dx Kit Inf M2, Modular Dx Kit InfA H3, InfB, Light Mix Kit CC_Hexaplex 480 II; internal control 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)

2018/2019, and 2019/2020 was carried out based on virological data from all over Poland. An increase in the number of tests from season to season was observed. At the same time, the percentage of positive samples decreased (Figure 1). In the 2016/2017 epidemic season, the percentage of positive samples was 50.68%, in the 2017/2018 season it was 43.18%, in the 2018/2019 season it was 35.32%, and in the 2019/2020 season it was 27.34%.

### Dynamics of influenza

The dynamics of epidemic seasons was also analyzed. The epidemic peak recorded was the earliest in the 2016/2017

season (week 3 of 2017). In the 2017/2018 epidemic season, the epidemic peak was observed much later, in the 8th week. In the 2018/2019 epidemic season, the peak of the season was in week 7, while in the 2019/2020 season it occurred in week 10 (Figure 2).

### Percentage of influenza viruses depending on the epidemic season

The percentage share of influenza viruses in the 65+ age group, depending on the epidemic season, was also examined. In the 2016/2017, 2018/2019, and 2019/2020 seasons,

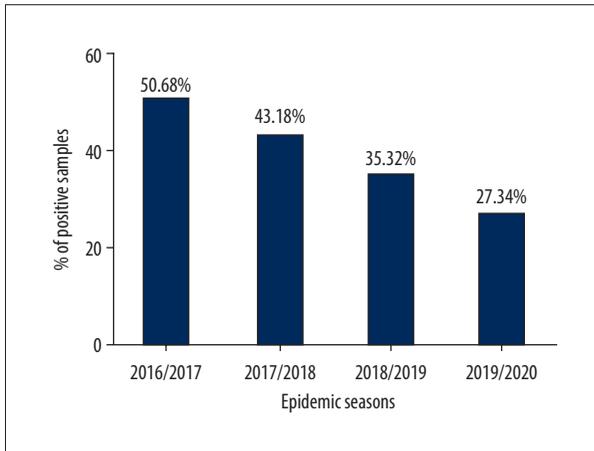


Figure 1. Percentage of positive samples in the group of people aged 65+ over several epidemic seasons in Poland.

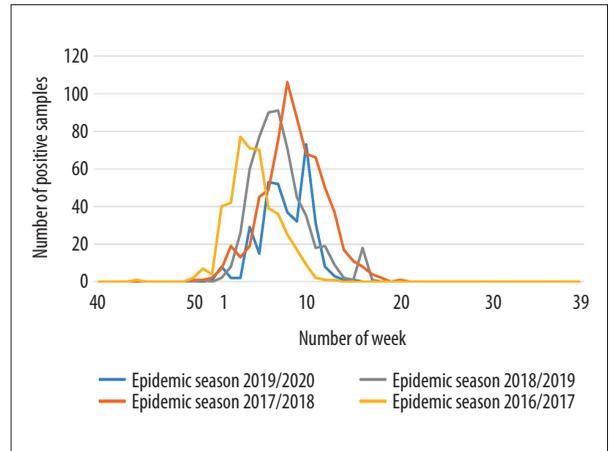


Figure 2. Dynamics of influenza in the last epidemic seasons among elderly in Poland.

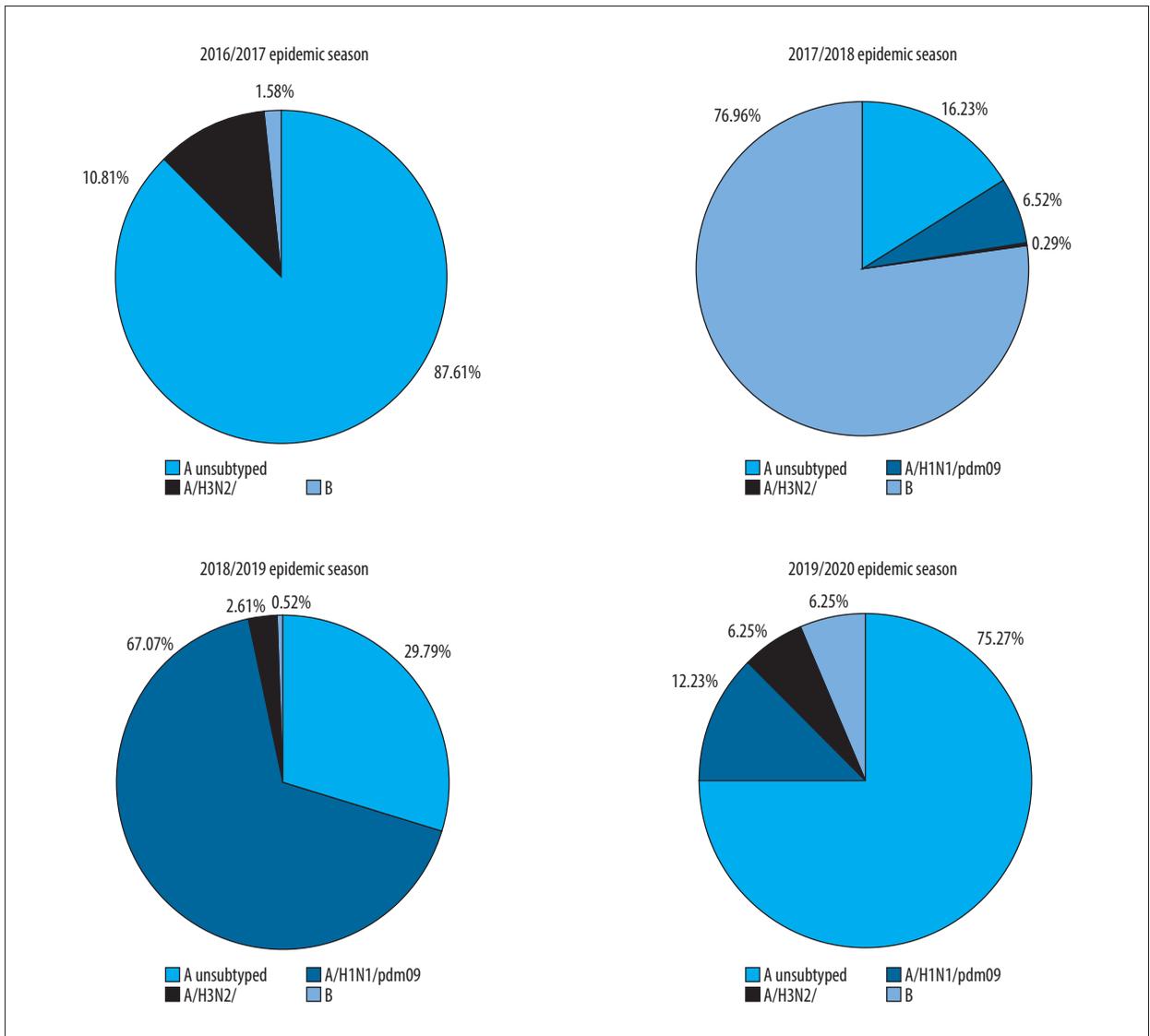
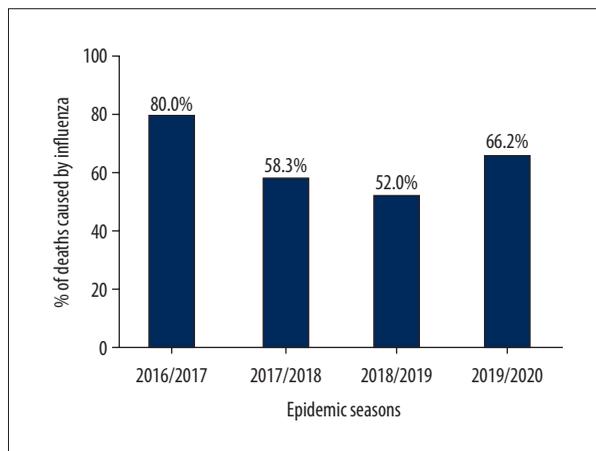


Figure 3. Percentage of influenza viruses in the 65+ age group in the different epidemic seasons.



**Figure 4.** Percentage of deaths caused by the influenza virus in the age group 65+ in relation to the total number of deaths caused by influenza virus in Poland.

type A influenza was dominant, while in the 2017/2018 season type B influenza was dominant. In the 2016/2017 epidemic season, the A/H3N2/ subtype was dominant, but subtype A/H1N1/pdm09 was dominant in the 2018/2019 season. The 2019/2020 epidemic season, the codomination of the subtypes A/H1N1/pdm09 and A/H3N2/ was recorded. Interestingly, in the 2016/2017 epidemic season, no circulation of the A/H1N1/pdm09 subtype in Poland was confirmed (Figure 3).

#### The percentage of deaths caused by the influenza virus

The percentage of deaths caused by the influenza virus in the 65+ age group in relation to the total number of deaths caused by the influenza virus in Poland in different epidemic seasons was also assessed. It was determined that in the 2016/2017-2019/2020 epidemic seasons, this percentage was always above 50%, which means that each season half of deaths caused by the influenza virus were recorded in the 65+ age group. In the 2016/2017 epidemic season, this value reached 80%, in the 2017/2018 epidemic season it was 58.3%, in the 2018/2019 epidemic season it was 52%, and in the 2019/2020 epidemic season it was 66.2% (Figure 4).

#### Discussion

The types of influenza virus that predominated among patients over the age of 65 coincided with the general trend in other European countries during the respective epidemic seasons [13]. Only in the 2018/2019 season, there was a codomination of the A/H1N1/pdm09 and A/H3N2/ subtypes in Europe, while in Poland the dominance of the A/H1N1/pdm09 subtype was clearly evident.

Comparison of the dynamics of the epidemic seasons shows that the type of virus circulating in a given season is an important aspect. We can see that, at the time of type A dominance, the peak of the epidemic season is recorded earlier, while during the dominance of type B, the peak occurs later. Data from the 2019/2020 epidemic season do not support this trend, but it may be due to the COVID19 pandemic in Poland, the outbreak of which coincided with the influenza epidemic peak.

People over the age of 65 are at a particular risk of influenza, especially due to its complications. Because the percentage of the vaccinated population in this group of people has not exceeded 10% for 10 years [14], local governments have made an attempt to encourage the elderly to become vaccinated by providing free vaccines or offering a 50% discount on the purchase of a vaccine. These efforts are expected to result in a higher proportion of the vaccinated elderly population, and thus in reduced economic costs. Reducing the number of cases among the elderly will result in reduction of medical appointments, hospitalization, and deaths [15]. Research carried out in Brazil confirms that seasonal immunization could help avoid nearly one-quarter of deaths, 90% of which would be in people over the age of 65 [16]. This analysis was conducted over several epidemic seasons, so the number of deaths that could have been avoided differed depending on the epidemic season.

#### Limitation of the study

The work is based solely on the results of analyses of samples reported to the Influenza Surveillance System *SENTINEL*. Not all tested samples are reported to the system; therefore, the number of patients studied in Poland in given seasons could actually be much higher.

#### Conclusions

Influenza is a huge threat to the elderly and causes a large number of deaths every epidemic season. The best way to prevent influenza is by influenza vaccination every flu season.

This population study showed that, in Poland, between 2016/2017 and 2019/2020 epidemic seasons, people who were more than 65 years of age were at risk of influenza virus infection and its complications. These findings support the importance of seasonal influenza vaccination in the population over 65 years.

In connection with the COVID-19 pandemic, elderly people should especially be sure to receive regular vaccinations because influenza virus, upon entering the body, damages the epithelium of the respiratory tract and opens the door to other viruses or bacteria, including SARS-CoV-2.

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