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Detection of Influenza in the Epidemic Season 2016/2017 Based on I–MOVE+ Project

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Abstract

In the influenza season 2016/2017 in Europe, the predominant virus was A/H3N2/. In Poland, the percentage of people vaccinated against influenza was 3.33%. European I–MOVE+ project shows how important it is to monitor the effectiveness of influenza vaccine. The project demonstrates that a match between the circulating vaccine strains and those included in the vaccine for the Northern Hemisphere was low-to-moderate. In the present study, there were 379 patients and 296 control subjects examined in hospitals in Poland as part of I–MOVE+ project. The real-time reverse transcription polymerase chain reaction (qRT-PCR) method was used to detect the influenza virus in all subjects. We detected the influenza subtype A/H3N2/ in 59.1% and type B virus in 2.1%. There was one co–infection of subtype A/H3N2/ with subtype A/H1N1/ and eight co–infections of type B with subtype A/H3N2/. No influenza viruses were detected in the control group. Only 19 patients and 22 control subjects were vaccinated during the epidemic season in question. A proportion of people vaccinated against influenza in Poland remains dismally low compared to other European countries.

Keywords

Epidemic season · Influenza · Laboratory testing · Subtype · Vaccine

1 Introduction

In 2016/2017 season in Poland subtype A/H3N2/ was dominant, while the major etiological agent of influenza-like infection was the respiratory syncytial virus. In the season, a three-component vaccine was in use, with inactivated split and subunit antigen of the following composition: A/California/7/2009 (H1N1) pdm09 – like virus, A/Hong Kong/4801/2014 (H3N2) – like virus, and B/Brisbane/60/2008 – like virus Victoria lineage (Grohskopf et al. 2017). In this season, the percentage of vaccinated people in Poland remained at a very low level of 3.33% general population.

The I-MOVE (Influenza – Monitoring Vaccine Effectiveness) project, aimed at measuring

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influenza vaccine effectiveness in Europe, coordinated by EpiConcept and ECDC (ECDC 2017) has been conducted in European countries since 2007. Poland has started to participate in the project since the influenza season 2010/2011 (Kissling et al. 2012). The aim of the present report is to identify and evaluate the activity of influenza virus and to determine the effectiveness of vaccination against influenza in the 2016–2017 influenza season as part of the I-MOVE project implemented in Poland.

2 Methods

This study was approved by the Review Board for Human Research at the National Influenza Center of the National Institute of Public Health – National Institute of Hygiene in Warsaw, Poland. It was conducted in accordance with the principles for human research set by the Declaration of Helsinki.

2.1 Patients and Material Sampling

To this case-control study, patients were qualified who met the EU influenza-like illness (ILI) case definition. The definition is as follows: sudden onset of symptoms, at least one of the following four systemic symptoms: fever or feverishness, malaise, headache, and myalgia, and at least one of the following three respiratory symptoms: cough, sore throat, and shortness of breath (Kissling et al. 2017). The material for the study consisted of pharyngeal and nasal swabs taken from patients whose clinical presentation met the definition of ILI. Forty six general practitioners (GP) voluntarily participated in the collection of study material in 14 voivodships of Poland. Data for laboratory qRT-PCR investigation were collected from 7 age groups: 0–4, 5–9, 10–14, 15–25, 26–44, 45–64, and 65+ years. The control group consisted of patients with ILI symptoms, but having a negative result of influenza qRT-PCR test from the pharyngeal and nasal swabs. Each sample was sent to the reference laboratory in the Department of Influenza Research – National Influenza Center of the National Institute of Public Health – National Institute of Hygiene in Warsaw with attached information regarding the date of a GP visit and swabbing, and the age and gender of a participating patient. The material sampled, submitted by GP was stored at −80 °C until laboratory virus identification.

2.2 Real–Time Reverse Transcription (qRT-PCR)

RNA Isolation Influenza virus RNA was isolated using a set of Maxwell 16 Viral Total Nucleic Acid Purification Kit (Promega Corporation, Madison, WI) with 200 µl of a clinical sample suspended in phosphate buffered saline (PBS), according to the manufacturer's instructions for low elution volume (LEV) cartridges. The RNA was suspended in 50 µl of RNase-free water.

Influenza A virus subtypes were determined by qRT-PCR with a light cycler thermocycler 2.0 (Roche Diagnostics; Rotkreuz, Switzerland). qRT-PCR analysis was performed in capillary tubes with a capacity of 20 µl using 0.5 µl (20 nM) primers and 0.5 µl (5 nM) probes for each of the reaction. Primers and probes were developed in the Influenza Reagent Resource (IRR) program run by the US Center for Disease Control (CDC). The reaction mixture containing buffer, $MgSO_4$, bovine serum albumin (BSA), RNase free water, and SuperScript® III/platinum *Taq* mix (Invitrogen by Life Technologies – Thermo Fisher Scientific, Carlsband, CA) was incubated with 5 µl of RNA in each capillary tube. As a positive control, reference strains of influenza virus in the vaccine for 2016/2017 epidemic season were used, and negative control consisted of RNase-free water. Before DNA amplification, template RNA was reverse transcribed to obtain the corresponding cDNA. Reverse transcription was performed at 50 °C for 30 min. The DNA was then subjected to the initial denaturation (1 cycle at 95 °C for 2 min), with further amplification steps comprising:

denaturation at 95 °C for 15 s, annealing at 55 °C for 30 s, and elongation at 72 °C for 20 s; repeated in 45 cycles (Hallmann-Szelińska et al. 2016).

2.3 Vaccine Effectiveness

Influenza vaccine effectiveness (IVE) is defined as the percentage of risk reduction in the vaccinated individuals relative to the unvaccinated individuals. Vaccine effectiveness was estimated according to the following formula: IVE = (1-OR) × 100. The OR is calculated by dividing the vaccination rate in the case group by the vaccination rate in the control group. It refers to the risk of influenza in the vaccinated group compared to the unvaccinated group (Weinberg and Szilagyi 2010). In addition, other factors such as age and co-morbidities can influence vaccine effectiveness.

3 Results and Discussion

In the epidemic season 2016/2017, a group of patients with positive results of influenza qRT-PCR test consisted of 379 patients: 208 females and 171 males. There were 296 subjects: 178 females and 118 males, in the control group with negative results of the test. Influenza type A was detected in 362 (95.5%) samples, in which subtype A/H3N2/ predominated in 214 (59.1%), while the remaining cases of type A were unsubtyped. One co–infection of A/H1N1/ with A/H3N2/ and eight of A/H3N2/ with influenza type B were reported. The infections caused by type B virus amounted to 2.1% (Fig. 1). Data were collected from patients in seven age groups, thanks to the voluntary participation of 46 general practitioners across 15 Polish regions. Only were 19 patients with positive PCR test results in the study group and 22 in the control group vaccinated during the reported period (Table 1).

Virological characteristics of samples within I-MOVE+ project, collected by various GPs and in various hospitals during the epidemic season 2016/2017 in Poland, distributed by the week number confirmed that the A type virus was the main circulating culprit. The collection of samples started in 45th ISO week of 2016. The pick of this season was in 3rd ISO week of the

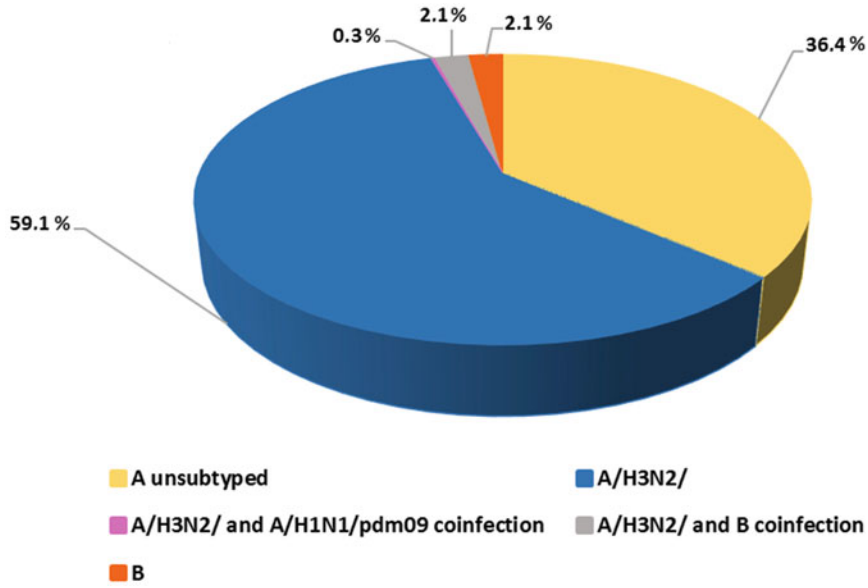

Fig. 1 Proportion of influenza viruses detected in the patient group investigated within the I-MOVE project

Table 1 Descriptive display of influenza A and B patients and control subjects evaluated in the framework of I-MOVE+ project during the epidemic season 2016–2017 in Poland (379 patients and 296 controls)

		Patients	Controls
		n (%)	n (%)
Age groups (year):	0–4	29 (7.7)	29 (9.8)
	5–9	25 (6.6)	9 (3.0)
	10–14	25 (6.6)	4 (1.4)
	15–25	35 (9.2)	37 (12.5)
	26–44	119 (31.4)	109 (36.8)
	45–64	94 (24.8)	84 (28.4)
	65+	52 (13.7)	24 (8.1)
Sex	Male	171 (45.1)	118 (39.9)
	Female	208 (54.9)	178 (60.1)
Vaccination status: 2016–2017 season	Vaccinated	19 (5.0)	22 (7.4)
	Unvaccinated	360 (95.0)	274 (92.6)
Vaccination status: 2014–2015 season	Vaccinated	13 (3.5)	14 (4.7)
	Unvaccinated	356 (96.5)	282 (95.3)
	Missing	10 (2.6)	0 (0)
Influenza type	A/H1N1/pdm09	0 (0)	–
	A/H3N2/	224 (59.1)	–
	A unsubtyped	138 (36.1)	–
	A/H3N2/ + A/H1N1/pdm09	1 (0.3)	–
	A/H3N2/ + B	8 (2.1)	–
	B	8 (2.1)	–
Chronic conditions, including obesity and pregnancy	Yes	103 (27.2)	67 (22.6)
	No	276 (72.8)	229 (77.4)

following year, which speaks for the typical course of the epidemic curve (Fig. 2).

In the epidemic season 2016/2017 in Poland, the odds ratios, calculated using logistic regression, adjusted for age, gender, date of onset, and the presence of chronic diseases were as follows: OR = 0.51 (95%CI: 0.25–1.05) across all the age groups, OR = 0.49 (95%CI: 0.16–1.51) for 15–64 years, and OR = 0.67 (95%CI: 0.16–1.99) for 65+ years The IVE was 49% (95%CI: 7–75), 51% (95%CI: 51–84), and 33% (95%CI: 9–84) in the respective age groups. Vaccination status did not have a statistically significant effect on acquiring the infection in any age group tested, which was due likely to small size of age groups. Nonetheless, the point estimate suggested a protective effect of vaccines against the infection. The IVE of 49% for the general adult population above outlined was higher than the 21% in the preceding season (Paradowska-Stankiewicz et al. 2018). For comparison, in other European studies conducted in much larger cohorts of people, consisting of several thousand people, IVE in the same 2016/2017 season was 38% (95% CI: 21–51) for all age groups, and it was 23% (95% CI: 15–49) against influenza A/H3N2/ among persons 65+ years of age (Kissling et al. 2017). Likewise, in a study conducted in several hundred people during the epidemic season 2014/2015, the overall pooled and adjusted for confounders IVE was 47.5% (95% CI: 16.4–67.0) against influenza A(H1N1) pdm2009, and it was 29.7% (95% CI: -34.4–63.2) for A(H3N2) (Valenciano et al. 2015). Our present estimate of IVE is in line with those moderate-to-low IVE found in those studies.

We conclude that in the epidemic season 2016/2017, the dominant influenza virus in Poland was A/H3N2/, which was confirmed by data obtained in the European I-MOVE project. It is hoped that this multi-national project will promote a greater awareness of influenza vaccination that despite all the efforts made so far remains at a dismally low levels in the lay public. The present formula used

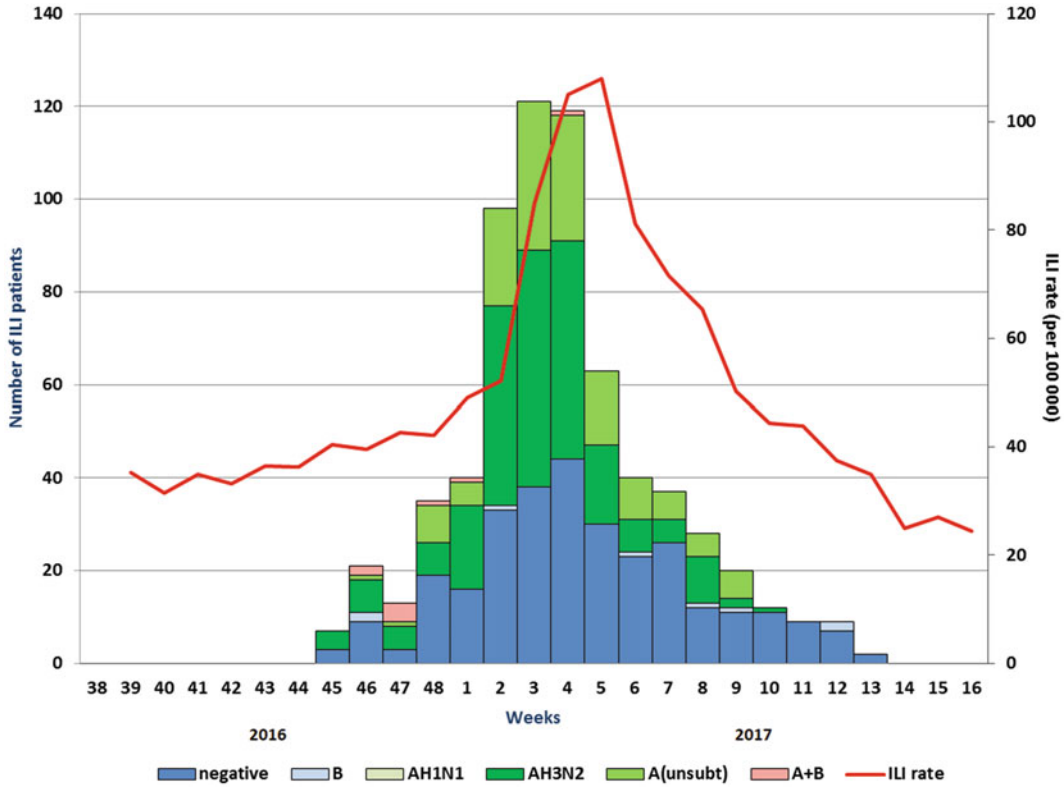

Fig. 2 Virological characteristics of samples within the I-MOVE + project distributed by the week number, in the ISO week date standard, during the epidemic season 2016–2017 in Poland. ILI, influenza-like illness

to estimate the influenza vaccine effectiveness may require modifications as it seems not to exactly mirror a drop in risk of acquiring the infection after influenza vaccination, even taking into account the studied group size and possible confounders, which may be both misleading and disenchanting in undertaking vaccination.

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Conflicts of Interest The authors declare no conflicts of interest in relation to this article.

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