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A comparative analysis of influenza virus infections in the 2013/2014 and 2014/2015 epidemic seasons in the reporting system, for different age groups in Poland

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A – Study Design, **B** – Data Collection, **C** – Statistical Analysis, **D** – Data Interpretation, **E** – Manuscript Preparation, **F** – Literature Search, **G** – Funds Collection

Summary **Background.** Influenza remains the cause of many seasonal infections, leading even to death, in all age groups, for all patient health states, under all health policies, and in all latitudes. Influenza infection needs to be thought of not only in terms of the loss of health and the exacerbation of existing diseases, but also in terms of the quantifiable financial consequences borne by the state.
Objectives. The aim of the study was to compare two influenza seasons: 2013/14 and 2014/15 through an analysis of the number of samples in different age groups, collected on a weekly basis for 52 weeks, and to interpret the results in terms of type and subtype.
Material and methods. Virological and epidemiological data were obtained from the SENTINEL and NON-SENTINEL programs. Virological tests were performed using RT-PCR and multiplex RT-PCR biological molecular methods.
Results. The maximum number of confirmed cases of influenza coincides in time with the maximum number declared cases and suspected cases of influenza and influenza-like viruses. The peak occurrence of influenza-like virus detection was earlier than the peak detection of influenza virus. In the 2014/15 influenza season, significant differences in the percentage of positive samples were observed between the 5–9 and 10–14 age groups. During the 2013/14 influenza season, there was no statistically significant difference in the percentage of positive samples between the 15–25, 26–44, and 45–64 year old age groups.
Conclusions. A new division of age groups allows more accurate assessment of the incidence of influenza and influenza-like illnesses and can assist health workers in preventing multiorgan influenza-related complications and deaths.
Key words: flu, type A, type B, epidemic season.

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Background

Influenza has been and remains the cause of many seasonal infections, often leading to irreversible complications and even to death, in all age groups, all patient heath profiles, and all latitudes [1, 2]. The variability of the influenza virus means that the flu vaccine needs to be adjusted prior to each influenza season. A Global Influenza Surveillance Network (GISRS) has been coordinated by the World Health Organization (WHO) since 1947. Since the 2004/2005 epidemic season, the SENTINEL epidemiological and virological influenza surveillance system has been in effect in Poland. This program is realized through the cooperation of physicians and the laboratories of the 16 Voivodeship Sanitary-Epidemiological Stations (VSESs) [3].

The efficient functioning of influenza surveillance during the influenza season allows a virological and epidemiological assessment of both newly emerging subtypes of the type A influenza virus and the arrival of flu epidemics and pandemics [4].

Objectives

The aim of the study was to perform a comparative analysis and verification of the epidemiological and virological data on viruses that caused influenza and influenza-like illnesses in Poland in the two consecutive epidemic seasons: 2013/14 and 2014/15. Appropriate laboratory tests confirming the cause of the illness in four and seven age groups, along with viral typing, was also carried out.

Material and methods

Doctors involved in the surveillance system sent patient samples to the VSESs, which transmitted the results of the tests (or sent the samples) to the National Influenza Centre (NIC). The NIC is the coordinator of the surveillance project. In the investigated seasons, a total of 5152 samples were tested (2620 in 2013/14 and 2532 in 2014/15), originating from with symptoms of influenza or suspected influenza infection. The epidemiological and virological surveillance project covers a fixed population that represents the entire country. The data were collected over the 52 weeks of each influenza season for the weekly cycles of both the SENTINEL and NON-SENTINEL influenza surveillance programs [5].

From week 22 in 2014 (epidemic season 2013/14), there was a change in the report format affecting the age groups. The 5–14 age group was divided into a 5–9 groups and a 10–14 group; the 15–64 year old age group was split into

15–25, 25–44, and 45–64 year-old groups. The diagnostic materials consisted of nasal and throat swabs, as well as bronchus-associated lymphoid fluid (BALF).

The samples were tested by extraction of viral RNA using a Maxwell 16 Total Viral Nucleic Acid Purification Kit (Promega Corporation, Madison, WI), according to the manufacturer's instructions for low elution volume (LEV). The samples were analyzed using two molecular biological methods: First, RT-PCR was used with an RV12 ACE Detection Kit (Seegene, Seoul, South Korea). For the cDNA synthesis, a reagent called The First Strand cDNA Synthesis Kit (Fermentas, York, UK) was used, in compliance with the manufacturer's instructions. The amplicons were then detected by gel electrophoresis. The second method was one-step RT-PCR analysis conducted using the Roche Light Cycler System 2.0. The primers were used in accordance with WHO recommendations (WHO, 2014). The vaccine viruses used as positive controls were A/California/7/2009 (H1N1) pdm09 and A/Victoria/361/2011 (H3N2); RNase-free water [6] was used for the negative control. Statistical analysis was mainly performed using descriptive statistics methods. The percentage of positive samples between the epidemic seasons was compared using the chi-square test.

Results

In the 2013/14 epidemic season, 555 of the samples (21.2%) were positive; in the 2014/15 season, there were 697 (27.5%) positive samples (Fig. 1). The number of samples received for the collated periods of the 2013/14 flu season is similar, whereas the maximum number of cases was noted in the early 2014/15 season. In Figures 2 and 3, the percentage of samples positive for the influenza virus is shown, as is the percentage of samples in which influenza-like viruses were detected. The influenza virus was detected in 233 of the positive samples (41.2%) from 2013/14 and 470 positive samples (67.4%) from 2014/15. The percentage of samples in which the influenza virus was detected in the weeks following shows high variability (due to the small numbers in the these weeks), but the maximum of confirmed influenza cases is seen in the same period as the maximum of reported cases and of suspected cases of influenza and influenza-like illnesses (Fig. 2).

It is worth noting that the maximum detection of influenza-like viruses occurs earlier than the maximum detection of the influenza virus (Fig. 3).

The distribution of type A and type B influenza viruses involved in the total number of positive samples in the two

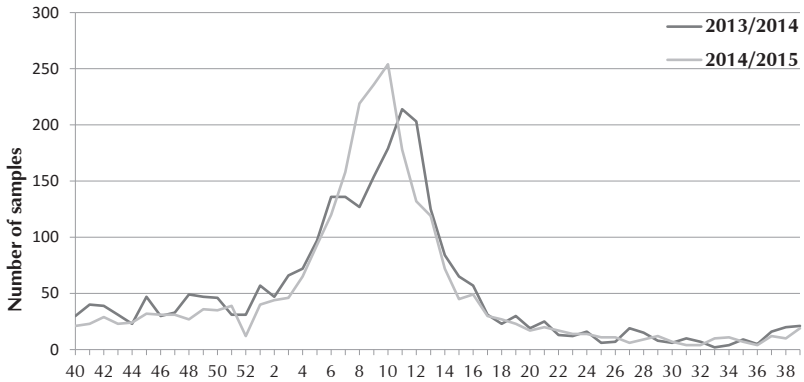

Figure 1. The number of samples tested in epidemic seasons 2013/2014 and 2014/2015

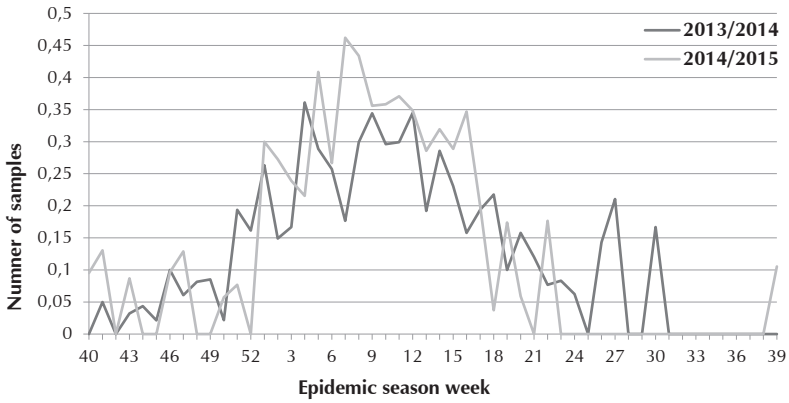

Figure 2. The percentage of samples in which the influenza virus was detected in successive weeks of the influenza seasons 2013/2014 and 2014/2015

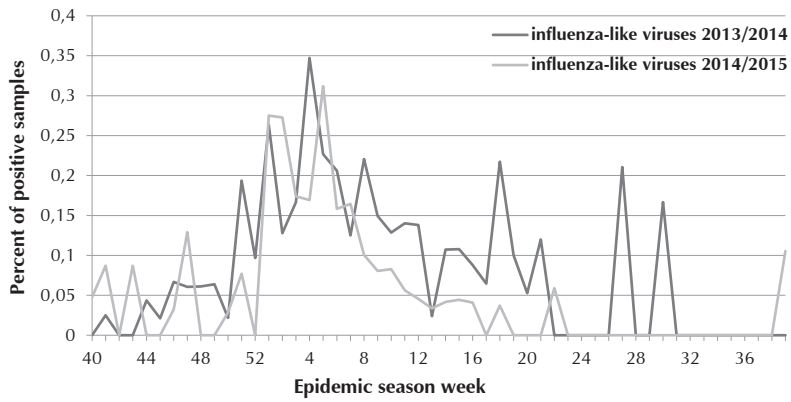

Figure 3. Percentage of samples in which an influenza-like illness was detected earlier than the influenza virus in successive weeks of influenza seasons 2013/2014 and 2014/2015

epidemic seasons is presented in Figure 4. In the 2013/14 season, influenza type B constituted an insignificant value (of 233 typed samples, only 3 were type B).

Table 1 presents the participation and distribution of the types of influenza virus among the positive samples in the examined age groups. In the 2014/15 season, no clear link is seen between the percentage of identified influenza viruses of type A or B and the age group (Tab. 1).

In the 2013/14 and 2014/15 influenza seasons, 29.6% and 27.2%, respectively, of the samples were subtyped (Tab. 2).

In the epidemic season 2014/15, there was a significant difference in the percentage of positive samples between the 5–9 and 10–14 year-old groups (Chi-square test, $p < 0.05$), while in the 2013/14 season, there were no significant differences. In the 2013/14 epidemic season, there were no

Table 1. Comparison of the type of confirmation in age groups in the 2013/2014 and 2014/2015 influenza seasons

Age group (years)	Epidemic season 2013/2014		Epidemic season 2014/2015	
	A	B	A	B
0–4	*n* = 40	*n* = 1	*n* = 77	*n* = 20
	97.6%	2.4%	79.4%	20.6%
5–9	*n* = 21	*n* = 0	*n* = 32	*n* = 32
	100.0%	0.0%	50.0%	50.0%
10–14	*n* = 12	*n* = 0	*n* = 12	*n* = 21
	100.0%	0.0%	36.4%	63.6%
15–25	*n* = 15	*n* = 1	*n* = 18	*n* = 14
	93.8%	6.3%	56.3%	43.8%
26–44	*n* = 59	*n* = 0	*n* = 61	*n* = 28
	100.0%	0.0%	68.5%	31.5%
45–64	*n* = 51	*n* = 0	*n* = 57	*n* = 35
	100.0%	0.0%	62.0%	38.0%
65+	*n* = 32	*n* = 1	*n* = 41	*n* = 21
	97.0%	3.0%	66.1%	33.9%
Unknown	*n* = 0	*n* = 0	*n* = 0	*n* = 1
	–	–	0%	100.0%
Total	***n* = 230**	***n* = 3**	***n* = 298**	***n* = 172**
	98.7%	**1.3%**	**63.4%**	**36.6%**

Table 2. Comparison of the results of subtyping of influenza type A in the 2013/2014 and 2014/2015 seasons

Age group (years)	2013/2014 epidemic season				2014/2015 epidemic season			
	Type A	Subtyped	A/H1N1/pdm09	A/H3N2/	Type A	Subtyped	A/H1N1/pdm09	A/H3N2/
0–4	40	*n* = 7	*n* = 4	*n* = 3	77	*n* = 25	*n* = 24	*n* = 1
		17.5%	57.1%	42.9%		32.5%	96.0%	4.0%
5–9	21	*n* = 8	*n* = 0	*n* = 8	32	*n* = 12	*n* = 8	*n* = 4
		38.1%	0.0%	100.0%		37.5%	66.7%	33.3%
10–14	12	*n* = 2	*n* = 0	*n* = 2	12	*n* = 3	*n* = 1	*n* = 2
		16.7%	0.0%	100.0%		25.0%	33.3%	66.7%
15–25	15	*n* = 1	*n* = 0	*n* = 1	18	*n* = 3	*n* = 2	*n* = 1
		6.7%	0.0%	100.0%		16.7%	66.7%	33.3%
26–44	59	*n* = 20	*n* = 12	*n* = 8	61	*n* = 17	*n* = 10	*n* = 7
		33.9%	60.0%	40.0%		27.9%	58.8%	41.2%
45–64	51	*n* = 21	*n* = 19	*n* = 2	57	*n* = 17	*n* = 14	*n* = 3
		41.2%	90.5%	9.5%		29.8%	82.4%	17.6%
65+	32	*n* = 9	*n* = 7	*n* = 2	41	*n* = 4	*n* = 3	*n* = 1
		28.1%	77.8%	22.2%		9.8%	75.0%	25.0%
Total	**230**	***n* = 68**	***n* = 42**	***n* = 26**	**298**	***n* = 81**	***n* = 62**	***n* = 19**
		29.6%	**61.8%**	**38.2%**		**27.2%**	**76.5%**	**23.5%**

The effect of the introduction of new age groups

Table 3. Comparison of the percentage of positive samples and the results of typing in the 5–9, 10–14 and 5–14 year old age groups in the 2013/2014 and 2014/2015 epidemic seasons

Age group (years)	2013/2014 epidemic season					2014/2015 epidemic season				
	No. of samples	Positive samples	Type A	Type B	Other*	No. of samples	Positive samples	Type A	Type B	Other*
5–9	121	*n* = 23	*n* = 21	*n* = 0	*n* = 2	180	*n* = 65	*n* = 32	*n* = 31	*n* = 2
		19.0%	91.3%	0.0%	8.7%		36.1%	49.2%	47.7%	3.1%
10–14	75	*n* = 13	*n* = 12	*n* = 0	*n* = 1	100	*n* = 47	*n* = 13	*n* = 21	*n* = 13
		17.3%	92.3%	0.0%	7.7%		47.0%	27.7%	44.7%	27.7%
5–14	196	*n* = 36	*n* = 33	*n* = 0	*n* = 3	280	*n* = 112	*n* = 45	*n* = 52	*n* = 15
		18.4%	91.7%	0.0%	8.3%		40.0%	40.2%	46.4%	13.4%

* – influenza-like viruses.

Table 4. Comparison of the percentage of positive samples and the results of typing in the 15–25, 26–44, 45–64, and 15–64 year old age groups in the 2013/2014 and 2014/2015 epidemic seasons

Age group (years)	2013/2014 epidemic season					2014/2015 epidemic season				
	No. of samples	Positive samples	Type A	Type B	Other*	No. of samples	Positive samples	Type A	Type B	Other*
15–25	151	*n* = 22	*n* = 15	*n* = 1	*n* = 6	164	*n* = 35	*n* = 20	*n* = 14	*n* = 1
		14.6%	68.2%	4.5%	27.3%		21.3%	57.1%	40.0%	2.9%
26–44	286	*n* = 60	*n* = 59	*n* = 0	*n* = 1	325	*n* = 94	*n* = 61	*n* = 28	*n* = 5
		21.0%	98.3%	0.0%	1.7%		28.9%	64.9%	29.8%	5.3%
45–64	262	*n* = 58	*n* = 51	*n* = 0	*n* = 7	277	*n* = 95	*n* = 62	*n* = 30	*n* = 3
		22.1%	87.9%	0.0%	12.1%		34.3%	65.3%	31.6%	3.2%
15–64	699	*n* = 140	*n* = 125	*n* = 1	*n* = 14	766	*n* = 224	*n* = 143	*n* = 72	*n* = 9
		20.0%	89.3%	0.7%	10.0%		29.2%	63.8%	32.1%	4.0%

* – influenza-like viruses.

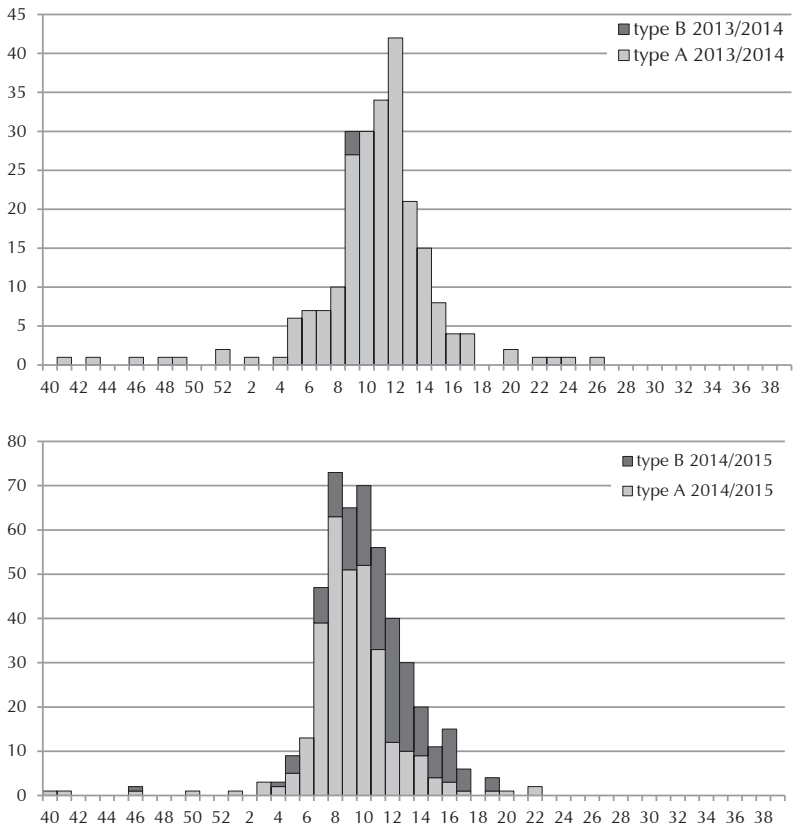

Figure 4. Proportion of influenza viruses of type A and type B from all samples in consecutive weeks of influenza seasons 2013/2014 and 2014/2015

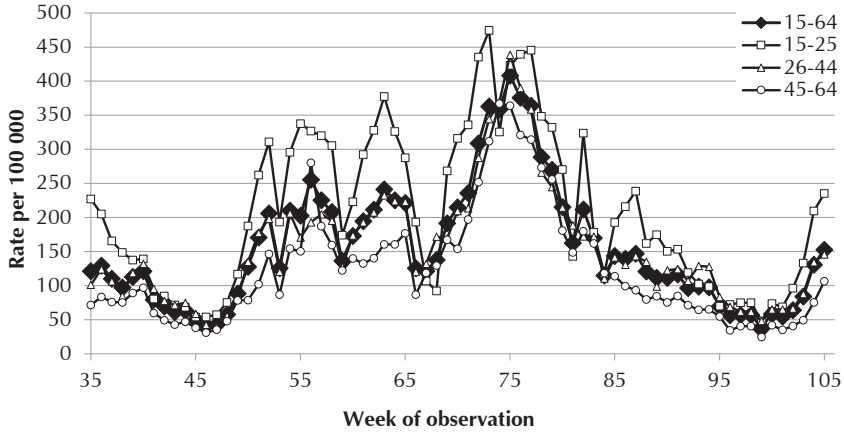

Figure 5. Comparison of changes in the incidence of influenza on the 15–64, 15–25, 26–44, and 45–64 year old age groups, over a period of time sufficient to take into account the observation of 7 age groups (2014–2015)

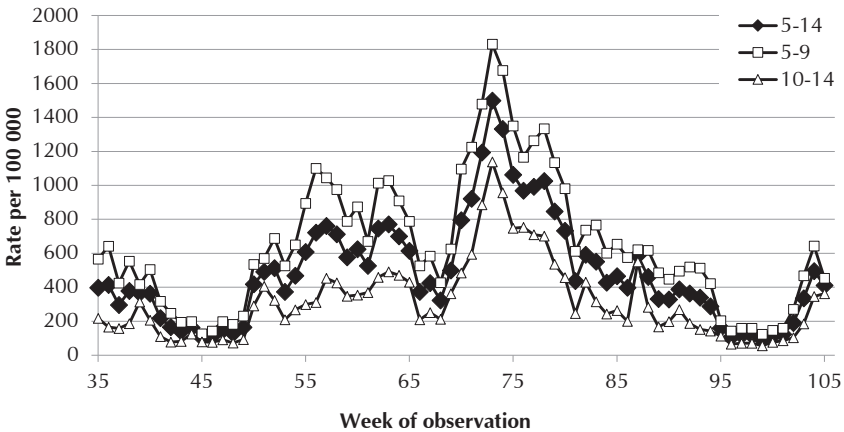

Figure 6. Comparison of changes in the incidence of influenza in the 5–14, 5–9, and 10–14 year-old age groups, over a period of time sufficient to take into account the observation of 7 age groups (2014–2015)

statistically significant differences in the percentage of positive samples between the age groups of 15–25, 26–44, and 45–64 years old, while these differences were statistically significant in the epidemic season 2014/15 ($p = 0.015$). The number of subtyped samples does not allow for a formal assessment of the differences between the age groups, but a substantial difference is noted between the new groups (15–25, 26–44, and 45–64 years old), contributing to the samples identified as subtype A/H1N1/pdm09.

The number of type A and type B influenza viruses found in all the positive samples from the two epidemic seasons is presented in Figure 4.

There was a significant difference between the 15–25, 26–44, and 45–64 year old age groups, as well as in the 15–64 year-old group, not only in the level, but also in the length of the period of high incidence (Fig. 5), which is clearly highest in the 15–25 year-old group.

The 5–9 and 10–14 year-old groups differ considerably in the level of maturity, which is much higher in the younger age group (Fig. 6).

Discussion

The incidence of influenza and influenza-like illnesses in the newly introduced age groups during the epidemic seasons of 2013/14 and 2014/15 clearly underlines the vales of such observations. The percentage of positive results for influenza virus in consecutive weeks of observation is highly variable. However, the peak number of confirmed positive cases of influenza virus and the peak number of reported and suspected cases of influenza and influenza-like illnesses fall in at the same period of time. It should be remembered that, when influenza viruses are spreading, there are at the same time more than 200 types of other respiratory viruses. However, infections caused by influenza virus can be significant, leading to multiorgan complications and, in high-risk groups, death [7].

For both seasons, it should be noted that the peak of laboratory-confirmed influenza-like virus detection is earlier than the peak detection of the influenza virus. There was no clear relationship between the percentage of type A and type B influenza virus confirmed in different age groups. Comparing the results obtained in the former age groups with those obtained with the new grouping, a better assessment of the incidence and duration of flu can be made, and significant differences can be seen to indicate a higher incidence in the younger age groups (5–9 and 10–14 years old).

The WHO report shows that the dominant influenza virus in the 2014/15 influenza season in the northern hemisphere was A/H3N2/, with the exception of Slovenia (where A/H1N1/pdm09 prevailed) and Georgia and Ukraine, where the dominant virus was a type B influenza strain [8–11].

Earlier reports from the Department of Influenza Research, National Influenza Center, as well as the data for 27 consecutive weeks, show that in children up to 14 years old, the dominant strain in Poland was A/H1N1/pdm09; over the entire population, the A/H3N2/ strain dominated. In

the 2014/15 season, the percentage of the A/H1N1/pdm09 subtype was higher than in the 2013/14 influenza season (76.5% *vs.* 61.8%). There were no significant differences between age groups, with the only exception being the 0–4 group; in the 2014/15 season, there was a significantly higher percentage of the A/H1N1/pdm09subtype. However, the data for the whole season points to the dominance of the A/H1N1/pdm09 strain among both children and adults [12, 13]. Puig-Barbera et al., during the Northern hemisphere 2013/2014 influenza season, observed three patterns of circulation: A/H3N2/-dominant, A/H1N1/-dominant, and mixed, with more A/H3N2/ than A/H1N1/ [14]. On the other hand, DeMarcus et al. identified the A/H1N1/ virus in 79.2% of all influenza-positive specimens [15]. These differences may result from the different number of physicians participating in the surveillance project and the different number of samples tested in each country.

The data for the epidemiological seasons indicates a small percentage of vaccinated population in each age group. The total percent of vaccinated population in Poland during the 2014/15 influenza season amounted to only 3.55%.

Conclusions

1. The peak incidence of influenza and of influenza-like illnesses was observed in the first quarter of the year.
2. The peak incidence coincided with the laboratory confirmations of isolated influenza strains.
3. The new, finer division of age groups allows more accurate assessment of the incidence rate and isolated viruses.
4. There is a need to increase vaccination against flu in all age groups.

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Conflict of interest: The authors declare no conflict of interests.

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