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Surveillance of influenza in the post-pandemic period in the Vojvodina, Serbia, October 2010 – May 2015

Надзор над инфлуенцом током пост-пандемијског периода у Војводини, Србија, од октобра 2010 до маја 2015

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SUMMARY

Introduction/Objective In August 2010, World Health Organization declared the beginning of the post-pandemic phase.

The aim of this study was to evaluate the epidemiological and virological characteristics of influenza and correlation between the influenza occurrence and weather conditions.

Methods We used surveillance reports of influenza and laboratory data from October 2010 to May 2015. Data for the analysis were collected through sentinel surveillance of influenza-like illness (ILI), severe acute respiratory illness (SARI), acute respiratory distress syndrome (ARDS), and by virological surveillance. The nasal and throat swabs from all influenza cases were performed by PCR laboratory method.

Results During the observed period, the highest rates of ILI were registered during the seasons 2010/11 and 2012/13, with influenza A (H1N1)pdm09 and influenza B being predominant, respectively. The highest weekly age-specific rates of ILI were registered in school-age children (ages 5 to 14). Out of 1466 samples collected, 720 (49.1%) were laboratory confirmed as influenza, and influenza A virus was more frequently detected than influenza B. Among confirmed cases of influenza, participation of patients with SARI or ILI was nearly equal (46.0%, vs. 44.1%, respectively). There was a weak correlation observed between the decrease in temperature and rainfall and the increase in influenza detection (ρ=-0.04214 vs. ρ=-0.01545, respectively, p>0.05).

Conclusions There is a need for continuous surveillance in order to predict seasonal trends and prepare for a timely response to influenza outbreak.

Keywords: influenza virus; epidemiology; virology; sentinel surveillance

САЖЕТАК

Увод/Циљ У августу 2010. године, Светска здравствена организација је прогласила почетак пост-пандемијске фазе у надзору над грипом.

Циљ рада био је да се процене епидемиолошке и вирусолошке карактеристике грипа и однос између климатских услова и преваленције грипа у постпандемијском периоду.

Методе Коришћени су подаци из надзора над грипом и резултати лабораторијских испитивања у пе-риоду од октобра 2010. до маја 2015. године. Пода-ци су прикупљени кроз сентинелни надзор за обољења слична грипу (ОСГ), тешку акутну респираторну болест (ТАРБ), акутни респираторни дистрес синдром (АРДС) и вирусолошким надзором. Сви случајеви грипа потврђени су из назофарингеалног бриса *PCR* лабораторијском методом.

Резултати У посматраном периоду, највише стопе ОСГ регистроване су током сезоне 2010/11 и 2012/13, када су доминирали вирус грипа типа А (H1N1)pdm09, односно вирус грипа типа Б. Највише вредности узрасно специфичних стопа ОСГ ре-гистроване су у школском узрасту (5–14 година). Од 1466 тестираних узорака, лабораторијска потврда вируса грипа добијена је код 720 (49,1%), а вирус грипа типа А чешће је детектован од вируса грипа типа Б. Међу потврђеним случајевима грипа, учешће оболелих са дијагно-зама ТАРБ или ОСГ је било скоро подједнако (46.0% тј. 44.1%). Није утврђена статистички значајна разлика између смањења температуре и падавина у односу на пораст броја потврђених случајева грипа (ρ=-0,04214 наспрам ρ=-0,01545, p>0,05).

Закључак Наставак надзора над грипом кроз процену сезонских трендова омогућава спремност на одговор у случају појаве епидемије грипа.

Кључне речи: вирус грипа; епидемиологија; вирусологија; сентинелни надзор

INTRODUCTION

Increased influenza's viral activity causes a high incidence, exceeding incidence of other infectious diseases [1]. The results of epidemiological surveillance of influenza are important as they provide the information which serve as the early warnings of epidemics, and give us an insight into the type and/or subtype of influenza virus, so we can prepare the adequate influenza vaccines and antiviral drugs. They are also significant for implementing appropriate measures for reducing the number of deaths caused by influenza, hospitalization rates due to complications, and the costs of the treatment of influenza [1-3].

The main factors which determine the seasonality of influenza are still unclear, especially in the tropical areas of the world. Probably, the combined action of environmental factors (humidity, temperature), factors related to immunity of the population, and demographic factors (population density, population flows, school calendar, and traveling or migration) interfere with viral circulation in different parts of the world [4, 5].

Surveillance of influenza at the European level has been conducted since 1996 [6]. In order to improve the monitoring of the epidemiological situation, to ensure efficient response and to reduce the negative consequences on the health of the population, sentinel surveillance of ILI (influenza like illness) and ARI (acute respiratory infection) was introduced in the Autonomous Province (AP) of Vojvodina (the northern region of Serbia) in the 2004/05 season, as a pilot study, according to the model of surveillance conducted in Slovenia [7]. In the preparation for an influenza pandemic, sentinel surveillance in Vojvodina became the standard model and also a part of a newly established national influenza surveillance, throughout the Republic of Serbia, since 2009. Due to the quality of the results of the surveillance of influenza in Vojvodina, special Public Health Program was introduced for surveillance of ILI/ARI, severe acute respiratory illness (SARI) and acute respiratory distress syndrome (ARDS) [8]. Similar to other countries in Europe, Vojvodina, as part of Serbia, has a reference laboratory for influenza. In this way, virological surveillance as integral part of epidemiological surveillance, provide the useful information about circulation of an actual influenza virus [9].

In August 2010, the World Health Organization (WHO) declared the post-pandemic period [10]. However, the influenza A (H1N1) pdm09, A (H3N2) and B viruses have continued to circulate in the population after the pandemic period [11].

The main goal of this study was to observe seasonal influenza activity along with influenza occurrence and weather conditions in Vojvodina based on data collected between October 2010 to May 2015, in the post-pandemic period (five surveillance seasons).

METHODS

Data for this observational study were obtained from the sentinel surveillance of ILI, surveillance of all hospitalized patients with SARI and/or ARDS, and virological surveillance of influenza in Vojvodina through the whole season (from calendar week 40 of each year to calendar week 20 of the next year). All information about patients was anonymised and de-identified. Data on temperature and average rainfall were provided by the Republic Hydrometeorological Service of Serbia.

Sentinel surveillance of ILI

During the seasons of surveillance of ILI and ARI, from 2010 to 2015, sentinel surveillance has been conducted in all seven districts of Vojvodina. In the surveillance of ILI and ARI, only outpatients in Health Centers of Vojvodina were covered. In accordance with the WHO and national recommendations, the surveillance of influenza was conducted within four age groups (0-4, 5-14, 15-64 and ≥65 year) during the 2010/11 and 2011/12. Since the 2012/13 influenza season, the surveillance has included five age groups (0-4, 5-14, 15-29, 30-64 and ≥65 year). Between the 2010 and 2013 seasons, sentinel surveillance has been implemented in 19 municipalities with more than 30,000 inhabitants, encompassing between 5.2 and 7.1 percent of the total population. During the last two seasons (2013-2015) sentinel surveillance of influenza has been extended to the municipalities with less than 30,000 inhabitants and covered all of 45 municipalities of the province (and has included 135 sentinel physicians). Although the number of sentinel physicians during the two last season's was the same, because we included different sentinel physicians, the observed populations were not equal (Table 1).

Table 1. Number of observed population during sentinel surveillance of influenza-like illness (ILI) by five seasons in the AP of Vojvodina

Age groups / Seasons	0-4	5-14	15-29	15-64	30-64	≥65	Total	Number of included sentinel physicians
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	
2010/11	9281 (10.5)	15563 (8.2)	-	47301 (3.5)	-	27413 (8.7)	99558 (5.2)	93
2011/12	9843 (11.1)	17857 (9.5)	-	60001 (4.5)	-	25565 (8.1)	113266 (5.9)	94
2012/13	10388 (11.7)	19837 (10.5)	23000 (6.3)	-	55418 (5.7)	27651 (8.7)	136294 (7.1)	89
2013/14	23664 (26.7)	61157 (32.4)	56303 (15.5)	-	102271 (10.5)	48352 (15.3)	304719 (15.8)	135
2014/15	(19.2)	(17.6)	(9.5)	-	(6.8)	(10.6)	(9.6)	135

n- Surveillance population by age groups and seasons, (**%**) percent of population in certain age group according to the census.

The surveillance system used the network of sentinel physicians (general practitioners and pediatricians) who have reported the number of new cases of ILI in their reference populations weekly, and collected respiratory samples for virological evaluations. In addition, sentinel physicians have electronically entered the data of new cases of ILI and ARI by week, and have regularly sent samples for virological confirmation to the WHO National Influenza Center, the Center of Virology of the Institute of Public Health of Vojvodina in Novi Sad. Each week, a global population adjusted with ILI and ARI rate for Vojvodina which allows to following the indicators of influenza activity [7]. Data of the surveillance of influenza were collected for the whole season (from calendar week 40 of each year to calendar week 20 of the next year), [12].

SARI and ARDS surveillance

Since the 2010/11 influenza season, in accordance with the WHO recommendations, SARI surveillance has been carried out during the winter season only, from the week 40 to the week 20 [13].

As described in detail previously [7], the hospital coordinators for SARI and ARDS surveillance from all 15 acute care hospitals in the Vojvodina have sent daily reports on every hospitalized SARI and/or ARDS case to the district coordinators of influenza in the local departments of Public Health. The study included patients from the intensive care units and high dependency units (severe form of infections), general/internal medicine, paediatric medicine, infectious disease ward and respiratory disease ward where patients of all ages were registered [8].

Case Definitions

This study included all the patients with ILI and any flu-associated infections who required hospitalization in accordance with the SARI and ARDS definitions. The WHO criteria for ILI and SARI were used for screening in the primary care/outpatient settings and inpatient hospital settings: ILI cases were defined as those with a sudden onset of fever (>38°C) and cough/sore throat within seven days of the onset, while patients with an acute respiratory illness with onset during the previous seven days requiring overnight hospitalization that includes: history of fever or measured fever of 38°C, and cough, and shortness of breath or difficulty in breathing were termed as SARI [14].

ARDS cases were defined as acute onset of bilateral infiltrates on the chest radiograph; arterial oxygen tension [PaO2]/fraction of inspired oxygen [FiO2] ratio <27kPa, and absence of a cardiac failure or left atrial hypertension [assessed clinically, echocardiographically**,** or with invasive monitoring] and required invasive ventilation [15-17].

Virological surveillance

The reference laboratory for virological surveillance of influenza in Vojvodina is WHO National Influenza Center at the Institute of Public Health of Vojvodina, Novi Sad [18].

The nasal and throat swabs from patients with suspected influenza were placed in the same vial with transport medium and kept at -20°C. The transport of the samples to the laboratory was organized on a daily basis by the local departments of Public Health. Viral RNA was extracted using the commercially available QIAamp Viral RNA Mini Kit (Qiagen, Germany) according to the manufacturer's instructions. The reverse transcription and amplification were performed using the AgPath-IDTM One-Step RT-PCR Reagents (Applied Biosystems, USA) on an Applied Biosystems 7500 real-time thermocycler (Applied Biosystems, USA). Influenza A and B virus detection (without further determination of B/Yamagata-like and B/Victoria-like) viruses was done by singleplex real- time RT-PCR assays. Testing was done according to the protocol developed by the CDC, enclosed with the reagents. The results were analysed using the Applied Biosystems 7500

Software version 2.0.6, and the interpretation of the data was done according to WHO guidelines [19]. Immediately after the testing was finished, the results of the laboratory tested samples were sent to the Institute of Public Health of Serbia in Belgrade, local departments of Public Health, the sentinel/hospital physician, and to the patients [7].

Statistical Analysis

Similar to the previously used methodology [7], the population under surveillance was used as a denominator for calculations of the weekly incidence of ILI and the numerator was the number of the clinical cases of ILI in the total population and in the age groups. Also, the weekly age-specific incidences of ILI for monitored age groups were measured per 100,000 of population.

The epidemic threshold of incidence of 246.3/100,000 was determined in the previous five pre-pandemic sentinel seasons on the basis of the weekly incidence rate of ILI value [7].

A correlation analysis was performed using the Spearman correlation coefficient between the detected monthly influenza cases and the average monthly temperature and rainfall using the data from the Republic Hydrometeorological Service of Serbia (RHMS), for the provincial capital of the AP of Vojvodina, city of Novi Sad [20]. Because the Meteorological annual data from RHMS contains the average monthly values only for six different meteorological stations -regions in Serbia (Beograd, Novi Sad, Vranje, Zlatibor, Loznica and Niš), we used values of average monthly temperature and rainfall only for city of Novi Sad (Vojvodina), which presents of weather conditions in this region of Serbia. Average temperature and rainfall were measured in degrees Celsius (°C) and millimeters (mm), respectively.

Two tailed *p*-values less than 0.05 were considered statistically significant.

RESULTS

Surveillance of ILI

During the study period (2010-2015), the highest weekly incidence rates were between 474.5/100,000 (2010/11 season, with influenza A (H1N1)pdm09 being predominant) and 712.3/100,000 (2012/13 season, with influenza B being predominant). The lowest weekly incidence rate was registered during the 2013/14 season, predominated by influenza A (H3N2). In the 2010/11 influenza season, with predominance influenza A (H1N1)pdm09 (ILI incidence above the epidemic threshold of 246.3 cases per 100,000 population) the duration of the epidemic period was even 8 weeks. Only during the 2013/14 season, values of the weekly ILI incidence were below the epidemic threshold (Figure 1).

Figure 2 shows the trend of ILI weekly incidence rate by age group during the five post-pandemic seasons. During the observed period, it was evident that the highest weekly age-specific incidence of ILI was registered in school-age children (5 to 14 years old), with the highest incidence

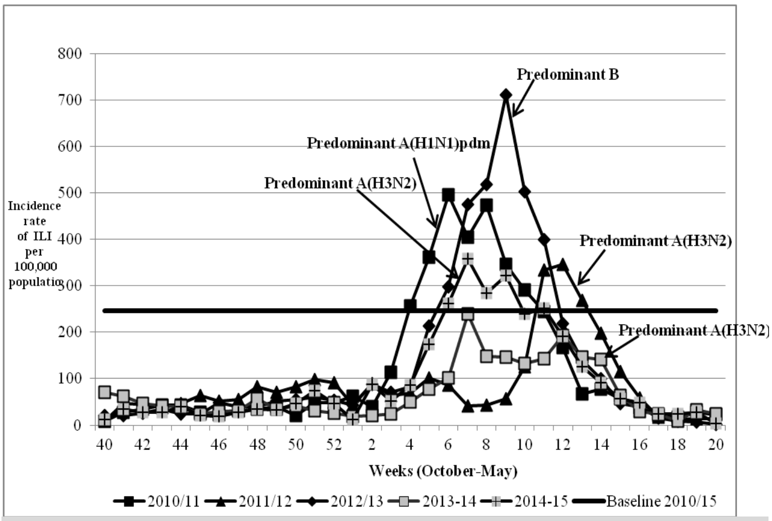

Figure 1. Influenza-like illness (ILI) in the 2010-15 seasons according to the influenza sentinel surveillance system in the AP of Vojvodina.

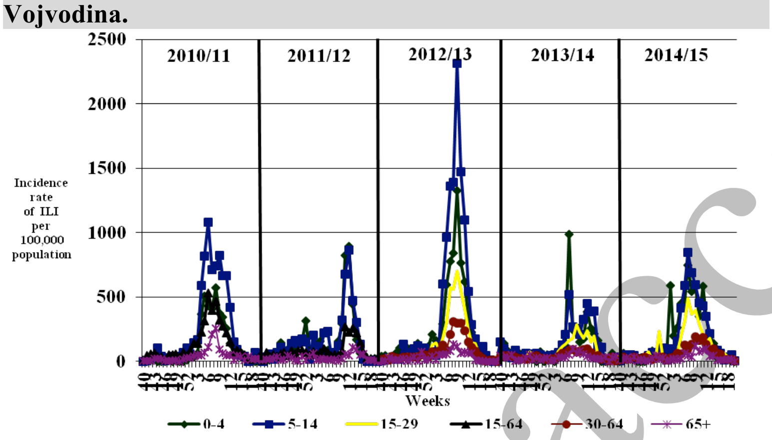

Figure 2. Age-specific incidence of ILI per 100,000 population in the AP of Vojvodina during 5 influenza seasons. The black vertical lines indicate the separation between different influenza seasons (2010–15).

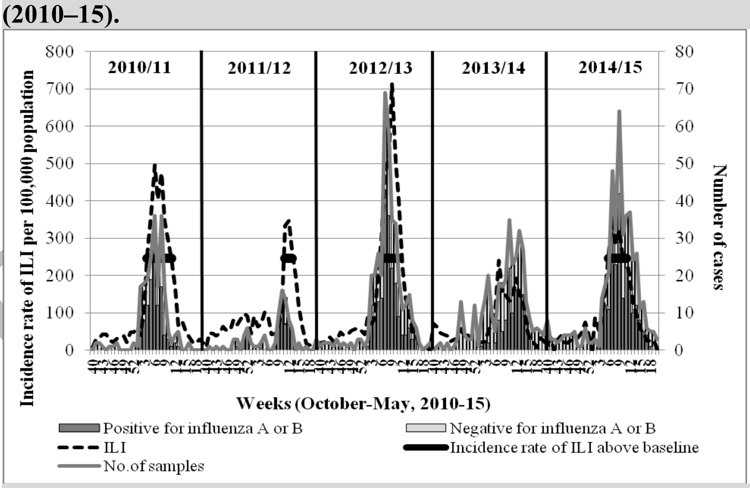

Figure 3. Incidence rate of ILI consultations per week by case status for the sentinel surveillance network, in the AP of Vojvodina, during 5 influenza seasonsThe black vertical lines indicate the separation between different influenza seasons (2010-15).

rate of 2315.2/100,000 in the 9/2013 week. In all five seasons, the lowest weekly age-specific incidence of ILI was registered in the oldest population.

In all five influenza seasons, the highest value of weekly ILI incidence rate was accompanied by the highest number of suspected and confirmed influenza cases (Figure 3).

Influenza by case definitions and prevalence distribution of influenza viruses

In the observed period, 1466 specimens from patients with ILI, SARI or ARDS, were tested, and 720 samples were identified as influenza A or B positive (49.1%). There were a total of 562 influenza A and 158 influenza B virus infections confirmed during the five influenza seasons. During all five seasons, the influenza A (H1N1)pdm09 was predominant (40.0%, 288/720). Participation of patients with SARI or ILI was nearly equal (46.0%, 331/720 vs. 44.1%, 318/720, respectively). Patients with ARDS were not registered only during 2011/12 influenza season (Table 2).

Table 2. Distribution of influenza infection by case definitions and by type/subtype in the AP of Vojvodina, 2010-15 seasons.

Variable		2010/11		2011/12		2012/13		2013/14		2014/15		Total	
		n	%	n	%	n	%	n	%	n	%	n	%
Clinical form	ILI	29	24.6	49	92.5	72	36.2	63	53.8	105	45.1	318	44.1
	SARI	66	55.9	4	7.5	110	55.3	47	40.2	104	44.6	331	46.0
	ARDS	23	19.5	0	-	17	8.5	7	6.0	24	10.3	71	9.9
Influenza virus type/ subtype	A (H1N1)pdm09	101	85.6	0	-	68	34.2	33	28.2	86	36.9	288	40.0
	A (H3N2)	6	5.1	52	98.1	28	14.0	77	65.8	90	38.6	253	35.1
	A (non-subtype)	3	2.5	0	-	2	1.0	7	6.0	9	3.9	21	3.0
	B	8	6.8	1	1.9	101	50.8	0	-	48	20.6	158	21.9
Total		118	100	53	100	199	100	117	100	233	100	720	100

ILI – influenza-like illness, SARI – severe acute respiratory infection;
ARDS – acute respiratory distress syndrome.

Weather conditions and prevalence of influenza

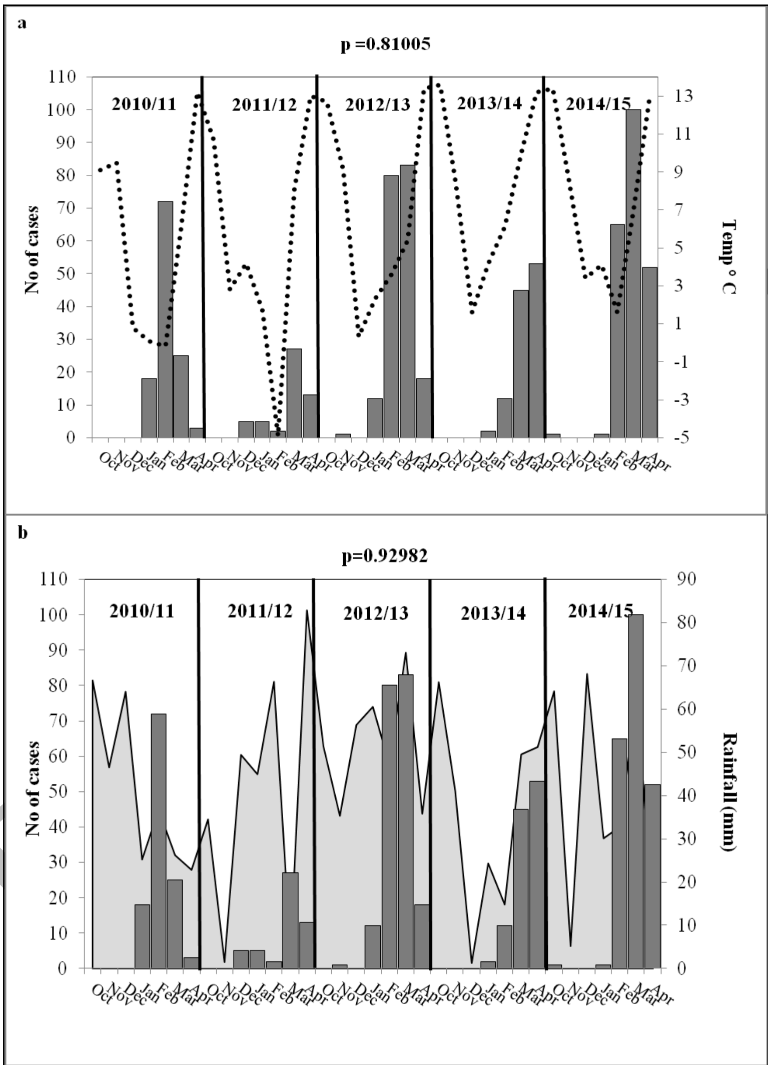

Figure 4. Correlation of the number of influenza cases in the AP of Vojvodina in the investigation period with: (a) the average temperature (°C); (b) the average rainfall (mm).
The black vertical lines indicate the separation between different influenza seasons (2010–15).

The dependence of the influenza activities on weather conditions in the Province (meteorological station city of Novi Sad) during the investigation period is shown in Figure 4a and 4b. During three influenza seasons (2011/12, 2012/13 and 2014/15), the highest number of confirmed influenza infections was registered during March. The average monthly temperature (October-April, 2010-15) was between -4.9 °C and 13.6 °C, and average values of rainfall in the same period were between 1.3 and 82.8 mm. These data shows that the average monthly temperature and average rainfall do not have any significant correlation with the number of influenza-positive cases (ρ=0.04214 vs ρ=-0.01545; p>0.05, respectively).

DISCUSSION

We presented the first description of the epidemiological and virological characteristics of influenza and correlation between the influenza occurrence and weather conditions after the pandemic 2009/10 season in the Vojvodina. As it comes out from the obtained data, all influenza viruses were detected across Vojvodina and affected all age groups during the post-pandemic seasons.

During 2010/11 season, in Western Europe, transmission peaked during late January and early February, and in Eastern Europe 2–3 weeks later, with the influenza A (H1N1) virus as predominant in 2009. Opposite the Europe, where the influenza A (H3N2) was rare, it was predominant in North America. During this season, in a few countries, a higher number of severe cases of influenza was registered compared to the previous season, but the reasons for this were not clear [21]. As it was expected, the influenza A(H1N1)pdm09 virus predominated among the viruses detected during the first post-pandemic season, but the results of the phylogenetic analysis of HA sequences of the A(H1N1)pdm09 viruses circulating in Lombardy showed that viruses isolated during the 2010/11 season segregated in a different genetic group with respect to those identified during the 2009 pandemic [22].

In Vojvodina, during the 2010/11 season, similar to the previous season, the influenza A (H1N1)pdm09 was predominant (85.6%, 101/118), with peaked transmission during late January and throughout all of February. The highest incidence rate of ILI was registered in the 5-14 age group. The epidemic wave (incidence rate of ILI above 246.3/100,000 population) lasted for 8 weeks, and even 75.4% (89/118) of all confirmed cases of influenza were hospitalized for SARI or ARDS.

Similar to the results of WHO review [23], in Vojvodina, during the 2011/12 season, influenza transmission peaked during late March. The influenza A (H3N2) was predominant 98.1% (52/53), and the highest incidence rate of ILI was registered among the youngest patients and in the school-age children (0-4 and 5-14 years old). The epidemic wave has only lasted for 3 weeks.

In North America, the 2012/13 influenza season started earlier than in the other parts of the northern hemisphere. In Europe, the influenza season peaked two weeks later than in North America, and it was also unusually long, associated with a late rise in influenza B cases in many countries. Peaking of influenza activity was registered around week 5/2013 and lasted until week 16/2013. In North America and Europe, later in the season, the influenza B type was more commonly detected than either of the influenza A subtypes [24, 25].

In Vojvodina, the highest incidence rate of ILI was registered in the 9/2013 week (712.3/100,000 population), and the epidemic wave lasted 6 weeks (between weeks 6 and 11). Among confirmed cases of influenza, the predominant type was influenza B (50.8%, 101/199).

In the 2013/14 season in Europe, influenza activity started later than usual, with a very small increase during the final weeks of December, followed by more marked increase throughout January. Overall activity continued to increase through February. Influenza activity during the 2013/14 season was less intense, with fewer positive samples detected than in the previous post-pandemic seasons. Overall, the influenza A (H1N1)pdm09 was predominant in most of the northern European countries and A (H3N2) was predominant in the most of the eastern European countries. Unlike North America, where influenza B was detected in the late season, the detection of the influenza B in Europe remained low during the whole season [26].

In our territory, during the 2013/14 influenza season, the first influenza case was registered in the 2/2014 week, and the incidence rate of ILI was below the baseline for all season. Similarly to the 2011/12 influenza season, the highest incidence rate of ILI was registered among the youngest and in the school-age children. We think that the reasons for the highest incidence rates of ILI among mentioned age groups, perhaps are in the fact that young people are tested more often [7]. Further, as is known, children may shed virus more profusely and for longer periods than adults [19].

Similarly to the results for Eastern Europe [26], the predominant subtype of influenza in Vojvodina was A (H3N2), with 65.8% (77/117) of all confirmed cases. Unlike the previous season, when influenza B was the predominant type, it was not detected among the tested samples during the 2013/14 season.

Considering the European countries, the timing of influenza detections during the 2014/15 season was similar to the previous years. The influenza activity started increasing in the last few weeks of 2014, similar to the situations in the 2013/14 and 2011/12 seasons. The peak of influenza activity in 2015 has varied between countries, but it was most often between weeks 6 and 9. However, the percentage of positive influenza cases was still above the threshold in many countries in April. During the 2014/15 season, the influenza A (H3N2) virus has been predominant in most regions, and an increased proportion of the influenza B virus has been registered after the activity peak. During this influenza season, most of the circulating influenza A (H3N2) viruses were different from the virus used in vaccines in the northern hemisphere [27, 28].

The data obtained from this study showed that during the 2014/15 season, epidemic influenza activity started from the 6/2015 week and lasted to the 11/2015 weeks. Like in the previous seasons, the lowest weekly age-specific incidence of ILI was registered in the oldest population. This phenomenon can be interpreted by the fact that the older patients are more likely to be registered in the hospital institutions than at the primary care level (sentinel surveillance of influenza). During the last influenza season, the predominant virus was A (H3N2), with 38.6% (90/233), whereas the registered proportion of influenza B was 20.6% (48/233) of all confirmed cases. The last cases of influenza were registered on April 30th, 2015.

In terms of seasonal pattern, in the northern hemisphere, influenza viruses are more frequently detected in the autumn, winter and spring, with the peak of influenza activity occurring typically between December and March and lasting for 6–8 weeks. In temperate regions of the southern hemisphere, influenza activity typically peaks between May and September. Besides, influenza viruses can be isolated sporadically throughout the year, too [19].

In the countries with tropical climate, influenza activity has been reported to peak in the rainy season, between June and August [29].

The climate in our country can be described as moderate-continental, with intermediate temperatures and relatively low humidity. June is the rainiest, with the average of 12% to 13% of total annual precipitation sum. February and October have the least of precipitation [20]. During the study period (from the beginning of October to the end of April, 2010-2015) there was no significant correlation between the number of influenza detections by months and average monthly temperature and rainfall.

Although almost 55% of all confirmed cases of influenza were notificated in the city of Novi Sad, because the absence data of monthly average temperature and rainfall in other parts of the Province and their comparison with the influenza cases, this may be considered as a limitation of this study.

CONCLUSION

The findings of the presented sentinel ILI and SARI surveillance, along with the virological surveillance, which are comparable with the results of surveillance of influenza in other countries across Europe, offer the possibility to determine the epidemiology of influenza during the post-pandemic seasons and predict future epidemics occurrence of influenza in Vojvodina. Likewise, the increasing number of samples among the age groups with the highest value of weekly ILI incidence rate (0-4 and 5-14 year olds) would lead to a more precise view on the distribution of influenza in Vojvodina in the younger population.

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