Seroprevalence Study in Vojvodina (Serbia) Following 2009 Pandemic Influenza A(H1N1)V

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INTRODUCTION
First cases of 2009 pandemic influenza A(H1N1) were imported in Vojvodina on 24th June, 2009 [1]. During the summer of 2009, a total of 123 cases were registered, most of whom were associated with the EXIT International Music Festival [1, 2].

The winter wave of pandemic influenza began in late October 2009 among high school students returning from school organized visits to Prague, Vienna and Bratislava. Epidemiological investigation showed that majority of students had a mild acute respiratory illness and did not seek medical attention. Only a few cases visited a physician and were classified as having an acute viral respiratory infection. The epidemic peaked in mid-December 2009 and was over by the end of 2009.

Immunization campaign with a monovalent vaccine started on 17th December, after the peak of epidemic, and lasted two months. Immunization coverage in Vojvodina was low, below 3% of the general population and around 10% of the target population [3].

OBJECTIVE
The seroprevalence study was performed in order to assess effects of the 2009 pandemic influenza A(H1N1)v epidemic on herd immunity. It was a part of the Serbian Ministry of Health funded nationwide study. Prevalence of antibodies against the 2009 pandemic influenza A(H1N1)v was determined in a 1% sample of the population that was monitored for Influenza Like Illness (ILI) and Acute Respiratory Infections (ARI) through the sentinel surveillance system in Vojvodina.

METHODS
Sentinel Surveillance on Influenza Like Illness and Acute Respiratory Infections

Sentinel surveillance in Vojvodina first started in the season 2004/05 and closely resembled the Slovenian surveillance program [4]. During the season 2009/10 it was conducted from September 2009 to September 2010. There were 103 sentinel physicians, either general practitioners or pediatricians from 19 health centers covering municipalities with more than 30,000 inhabitants. From the total population of Vojvodina of 2,031,992 according to the 2002 census, 5.1% or 102,723 were monitored.

Size and selection of the sample and control group

The study comprised all seven Vojvodina districts and was conducted by the Centre for

Proportional age stratified sampling was applied to cover around 1% of the population under sentinel surveillance. Age groups tracked by sentinel physicians for cases of ILI and ARI were as follows: 0–4, 5–14, 15–64, and 65+ years.

Dates of sampling for each sentinel physician were determined by investigators. On that day sentinel physicians were requested to offer their patients to participate in the research. Those who opted to comply were offered more detailed explanations and signed an informed consent. Patients were approached as they showed up till the number of subjects previously determined in each stratum was reached. All sera were coded and laboratory personnel analyzed them blindly.

Samples were collected in May and June 2010. Total of 1004 samples were obtained (0.05% of the total population of Vojvodina), representing 0.98% of the population monitored by sentinel surveillance.

Control samples originated from the sera bank in the Institute “Torlak” and consisted of randomly selected and age-adjusted 1054 sera collected in the pre-pandemic period, during 2008 and early 2009. Formally, matching was not ideal, since control sera did not come only from Vojvodina, but from all over Serbia. However, apart from mere convenience, our approach was based on two assumptions: a) pre-pandemic rates were low, and b) at that time, the seroprevalence was similar in the whole region (Table 1).

Methods were approved by the Ethical Board of the Institute of Public Health of Vojvodina.

Serologic testing

Sera were tested in the Institute Torlak by the hemagglutination inhibition (HI) reaction using influenza A/California/7/2009 (H1N1) antigen. All sera were processed before the testing in order to remove non-specific inhibitors (RDE(II) "SEIKEN") and non-specific hemagglutinins (turkey red cells). They were tested in dilution from 1:8 to 1:256. Antibody titres ≥1:32 and ≥1:8 were considered protective and diagnostic, respectively [5].

Statistical analysis

The results are presented as frequencies with 95% confidence intervals (CI). Overall, as well as within districts and age groups differences were tested by χ² test. Statistical package SPSS14 for Windows was used. Since the study was conducted three months after the immunization campaign, seroprevalence was also analyzed by immunization status of the subjects.

RESULTS

Both diagnostic and protective titres were several times higher in the study group, as compared to the control group. The differences between control (pre-pandemic) and study (post-pandemic) sera for all age groups were significant for both HI titres ≥1/8 and ≥1/32 (chi square test, p<0.001) (Table 2).

The highest percentages of subjects with diagnostic and protective titres were registered in the age group 15–19 years for both study and control sera. Seroprevalence of diagnostic and protective titres was decreasing towards youngest and oldest age groups with the lowest values registered in the age group 65 and over.

### Table 1. Population under sentinel surveillance, study and control group by age

<table>
<thead>
<tr>
<th>Age group (years)</th>
<th>Number of patients (%)</th>
<th>Population under sentinel surveillance</th>
<th>Study group</th>
<th>Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–4</td>
<td>9800 (9.5)</td>
<td>80 (8.0)</td>
<td>104 (9.9)</td>
<td></td>
</tr>
<tr>
<td>5–14</td>
<td>17525 (17.1)</td>
<td>175 (17.4)</td>
<td>175 (16.6)</td>
<td></td>
</tr>
<tr>
<td>15–19</td>
<td>5114 (5.0)</td>
<td>57 (5.7)</td>
<td>55 (5.2)</td>
<td></td>
</tr>
<tr>
<td>20–64</td>
<td>42879 (41.7)</td>
<td>426 (42.4)</td>
<td>604 (57.3)</td>
<td></td>
</tr>
<tr>
<td>65+</td>
<td>27405 (26.7)</td>
<td>266 (26.5)</td>
<td>116 (11.0)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>102723 (100.0)</td>
<td>1004 (100.0)</td>
<td>1054 (100.0)</td>
<td></td>
</tr>
</tbody>
</table>

### Table 2. Antibody titres against 2009 pandemic influenza A(H1N1)v in sera of the study and control group by age

<table>
<thead>
<tr>
<th>Titre HI</th>
<th>Age group (years)</th>
<th>Control group</th>
<th>Study group</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number of subjects</td>
<td>Number of positives (%)</td>
<td>95% CI</td>
<td>Number of subjects</td>
</tr>
<tr>
<td>≥1/8</td>
<td>0–4</td>
<td>104 (1.9)</td>
<td>-0.7–4.6</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td>5–14</td>
<td>175 (10.5)</td>
<td>2.3–9.2</td>
<td>175</td>
</tr>
<tr>
<td></td>
<td>15–19</td>
<td>55 (24.3)</td>
<td>30.5–5.9</td>
<td>57</td>
</tr>
<tr>
<td></td>
<td>20–64</td>
<td>604 (12.9)</td>
<td>10.2–15.6</td>
<td>426</td>
</tr>
<tr>
<td></td>
<td>65+</td>
<td>116 (9.5)</td>
<td>4.2–14.8</td>
<td>266</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>1054 (11.9)</td>
<td>9.9–13.8</td>
<td>1004</td>
</tr>
<tr>
<td>≥32</td>
<td>0–4</td>
<td>104 (1.0)</td>
<td>-0.9–2.8</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td>5–14</td>
<td>175 (0.0)</td>
<td>0.0–0.0</td>
<td>175</td>
</tr>
<tr>
<td></td>
<td>15–19</td>
<td>55 (20.0)</td>
<td>9.4–30.6</td>
<td>57</td>
</tr>
<tr>
<td></td>
<td>20–64</td>
<td>604 (7.2)</td>
<td>0.3–2.0</td>
<td>426</td>
</tr>
<tr>
<td></td>
<td>65+</td>
<td>116 (0.9)</td>
<td>-0.8–2.5</td>
<td>266</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>1054 (20.1)</td>
<td>1.1–2.7</td>
<td>1004</td>
</tr>
</tbody>
</table>

HI – hemagglutination inhibition; CI – confidence interval; p – probability
Table 3. Antibody titres against 2009 pandemic influenza A(H1N1)v in sera samples of vaccinated and unvaccinated subjects

<table>
<thead>
<tr>
<th>Vaccination status of subjects</th>
<th>Number of subjects</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Titre HI ≥1/8 (%)</td>
<td></td>
</tr>
<tr>
<td>Vaccinated</td>
<td>85 (76.6)</td>
<td></td>
</tr>
<tr>
<td>Unvaccinated</td>
<td>315 (35.9)</td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td>4 (26.7)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>404 (40.2)</td>
<td>259 (25.8)</td>
</tr>
</tbody>
</table>

The differences between groups were significant for both fractions with HI titres ≥1/8 and ≥1/32 (chi square test, p<0.001).

When immunization status was checked, there were 111 participants who received monovalent vaccine 5 to 6 months before sampling. Both diagnostic (46.9% vs. 23.4%) and protective titres (76.6% vs. 35.9%) were about twice higher in the vaccinated, as compared to the non-vaccinated group with highly statistically significant differences (Table 3).

There were no statistically significant differences in seroprevalence between seven districts in Vojvodina. The highest protective titres were registered in North Bačka and South Bačka districts, and the highest diagnostic titres in West Bačka and South Bačka districts (Table 4).

DISCUSSION

Six months after the epidemic influenza A(H1N1)v in Vojvodina, every 4th subject had protective antibody titre. The data for 1054 pre-pandemic serum samples showed that there was a low frequency (1.9%) of pre-existing protective antibodies to the pandemic influenza virus A(H1N1)v with the highest frequency (20.0%) in adolescents (15-19 years). The number of subjects in this age group was several times lower than in other age groups and it might have influenced the result. In general, the nature of pre-existing antibodies reactive to the pandemic virus in all age groups, including the youngest, is unclear and needs further investigation.

It is complicated to compare the results with other seroepidemiological studies of influenza A(H1N1) virus. Studies were implemented during different phase of the pandemic, before or after immunization campaign and with different immunization coverage. Different tests (microneutralization test or hemagglutination inhibition test) and different cut-off values to represent positive results also make comparisons difficult.

A study in Finland on 1000 samples collected in the pre-pandemic period from persons born between 1909 and 2005 confirmed the presence of antibodies and cross-protective immunity especially in elderly. It showed that 96% of the 1909-1919 cohort had antibodies in diagnostic titre and 55.6% had protective antibody titre. The percentage of positive samples decreased in the 1920-1929 and 1930-1939 cohorts, while in younger individuals protective antibodies were almost not detected at all [6]. A study in Norway on 689 samples collected in August 2008 showed that the highest frequency pre-existing protective antibodies to the pandemic influenza virus was seen in people of over 80 years of age (4.8%) but higher titre was also seen in ages 10-19 years (1.8%) and 20-29 years (3.9%) [7]. Another study concluded that cross reacting antibodies were not a result of earlier vaccinations since vaccines do not induce cross reacting antibodies [8]. The pre-existing antibodies at titres correlating with protection in adolescents and young adults suggest further investigation [7].

Initial studies from Mexico [9], USA [10], and later from Europe [11], as well as countries in the Southern Hemisphere [12, 13] showed that the disease affected children, young adults and population younger than 65 years of age. The seroprevalence study in Vojvodina confirms that most intensive spread of the epidemic occurred in school age children probably due to the fact that school collectives contribute to easier spread of respiratory infections including influenza [14]. Similar findings were determined in Norway, England and USA where the highest percentage of samples with protective titre was detected in the younger age groups 5-14 years or in the age group 10-19 years [7, 15, 16]. In Vojvodina the lowest seroprevalence of protective antibodies was registered in the oldest cohort and it showed that elderly need to be vaccinated for the following influenza seasons.

Serologic study in November and December 2009, after the second epidemic wave, showed that 21% of population
in Pittsburgh was infected and developed immunity to the 2009 pandemic influenza A(H1N1)v [15]. The serological study in Norway on samples collected before the epidemic started, during the early phase and after the main epidemic wave and vaccination campaign showed rise in prevalence of antibodies at protective titres from 3.2 (August 2009) to 44.9% (January 2010) [7].

The serologic study in Vojvodina showed that after the pandemic season and immunization campaign 25.8% of subjects had protective antibody titre, with the largest portion as the consequence of the epidemic. Due to the fact that immunization campaign started after the peak of the epidemic it is not possible to exclude the previous existence of naturally acquired immunity in the group of vaccinated subjects. However, in a group of unvaccinated subjects, antibody prevalence followed the findings for the whole study group regarding the detectable (40.2% vs. 35.9%) and protective antibody titre (25.8% vs. 23.4%).

Limitation of our study was four times higher percentage of vaccinated subjects in the sample than officially recognized. Immunization coverage in Vojvodina as in the rest of Serbia was low (3% of general population or 10% of the total population of 1,500,000). The majority of immunized individuals were those with a chronic illness who usually seek medical attention more frequently [3]. That may be the reason why vaccinated subjects were more represented in the sample.

A seroprevalence study in England on 1954 samples collected in August and September 2009, after the first epidemic wave showed geographic differences in the epidemic spread within the country [16]. These differences were explained as the consequences of population density and structure and not as differences in the transmission of influenza virus. However, in four towns in Scotland geographical differences were observed after the second wave of the epidemic [17]. Our study showed that there were no significant differences between the districts. Similarly, there were no significant differences in seroprevalence observed between Australian states in a study on blood donors, although the ability to detect minor variations was limited by the sample size [18].

CONCLUSION

Seroprevalence study enables a more complete picture on epidemic occurrence of the influenza A (H1N1) virus and provides additional valuable information for national influenza prevention program.

The 2009 pandemic influenza A(H1N1)v epidemic significantly influenced the herd immunity in our population leading to the highest immunity levels in adolescents 15-19 years of age six months after the outbreak regardless of low immunization coverage.

Epidemic of 2009 pandemic influenza A(H1N1)v affected all the regions in the province with similar herd immunity levels.

Results of this seroprevalence study as well as studies performed in other countries represent additional scientific evidence confirming occurrence of an influenza A(H1N1)v pandemic.

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NOTE

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REFERENCES

КРАТАК САДРЖАЈ
Увод
Студија преваленције антитела у Војводини (Србија) након пандемије грипа A (H1N1)v 2009. године
Методе рада
Резултати
Закључак
Кључне речи: епидемиологија; грип; преваленција антитела

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