



## Pandemic influenza H1N1 outbreak in the Military School

### Epidemija pandemijskog gripa H1N1 u Vojnoj gimnaziji

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#### Abstract

**Background/Aim.** The first cases of the pandemic pH1N1 influenza virus infection was observed in the United States and Mexico in April 2009 and the first laboratory confirmed case in Serbia was registered in June 2009. The aim of this paper was to report on the investigation of the first confirmed outbreak of the 2009 pandemic H1N1 influenza in Serbia and to describe the clinical and epidemiologic findings from this investigation. **Methods.** Descriptive and analytical epidemiological methods were used. Data were collected from medical records of the Military School students and epidemiological questionnaire. Pandemic H1N1 infection was initially confirmed by the RT-PCR assay in nasopharyngeal and oropharyngeal swabs and subsequently by the complement fixation test in serum samples. **Results.** The attack rate of acute respiratory illness was 70.8% (204/288). Pandemic H1N1 virus infection was confirmed in 44 of 82 tested cases of acute respiratory illness (53.7%). The most common clinical manifestations of pandemic influenza H1N1 were fever (88.6%), cough (61.4%), malaise (38.6%), runny nose (36.4%), headache (29.6%), sore throat (20.5%) and muscle pain (15.9%). **Conclusion.** The findings from this investigation suggest that pandemic H1N1 influenza in a high military school was widespread but did not cause severe illness.

#### Key words:

influenza a virus, h1n1 subtype; disease outbreaks; schools; military personnel; serbia.

#### Apstrakt

**Uvod/Cilj.** Prvi slučajevi pandemijske infekcije virusom influenza pH1N1 ustanovljeni su u Americi i Meksiku u aprilu 2009. a u Srbiji prvi laboratorijski potvrđen slučaj registrovan je u junu 2009. godine. Cilj rada bio je da se prikaže istraživanje prve dokazane epidemije pandemijske influenza H1N1 u Srbiji i opišu klinički i epidemiološki nalazi iz ovog istraživanja. **Metode.** Primenjen je deskriptivni i analitički epidemiološki metod. Izvor podataka bila je medicinska dokumentacija učenika Vojne gimnazije i epidemiološki upitnik. Infekcija pandemijskim virusom H1N1 prvo je dokazana pomoću RT-PCR u nazofaringealnim i orofaringealnim brisevima, a zatim i reakcijom vezivanja komplementa u uzorcima seruma. **Rezultati.** Stopa javljanja akutnog respiratornog oboljenja iznosila je 70.8% (204/288). Infekcija pandemijskim H1N1 virusom potvrđena je kod 44 od 82 testiranih slučajeva akutnog respiratornog oboljenja (53,7%). Najčešće kliničke manifestacije pandemijske influenza H1N1 bile su povišena temperatura (88,6%), kašalj (61,4%), malaksalost (38,6%), rinitis (36,4%), glavobolja (29,6%), gušobolja (20,5%) i bol u mišićima (15,9%). **Zaključak.** Rezultati ovog istraživanja ukazuju da je pandemijska influenza H1N1 bila raširena u Vojnoj gimnaziji, ali da nije izazvala teške forme oboljenja.

#### Ključne reči:

grip a virus, podtip h1n1; epidemije; škole, vojne; srbija.

#### Introduction

The first cases of the pandemic H1N1 (pH1N1) influenza virus infection were observed in the United States<sup>1</sup> and Mexico<sup>2</sup> in April 2009. Since then virus has spread worldwide to other continents. The first laboratory confirmed case was registered in Serbia on June 24, 2009<sup>3</sup>. On October 23, 2009 the outbreak of acute respiratory infection (ARI) was declared in a

high military school. Based on weekly and monthly reports of the Institute of Public Health of Serbia "Dr Milan Jovanovic Batut", we had information that there was no occurrence of pH1N1 influenza in the epidemic form in Serbia at that time. During the outbreak investigation, pandemic H1N1 virus was confirmed in taken nasopharyngeal and oropharyngeal swabs. This was the first recognized outbreak of the pandemic strain in a boarding school in the Republic of Serbia.

The aim of this paper was to report on the first recognized outbreak of pH1N1 influenza in a boarding school in Serbia and to describe the clinical and epidemiologic findings from this investigation.

## Methods

The Military School (MS) is a high boarding school. The MS is a semi-closed type of school, and the students are allowed to leave the dormitory in the afternoon, after classes. During the outbreak in MS there were 288 students. The students of the 1st grade ( $n = 87$ ) were located on the ground, 1st and 2nd floors, the 2nd grade students ( $n = 72$ ) on the 2nd and 3rd floors, the 3rd grade students ( $n = 81$ ) on the 3rd and 4th floors and the 4th grade students ( $n = 48$ ) on the 5th floor. Classes were organized so that the students of the 1st and 3rd grades attended school in one shift, while the students of the 2nd and 4th grades in another shift. No one of the students had received vaccine against pH1N1 influenza because vaccine was not available before and during the outbreak.

Descriptive and analytical epidemiological methods were used in this study. The students were divided into groups for comparison based on case definitions, vaccination status, grade and dormitory floor.

Vaccination status was determined for immunization with the inactivated trivalent influenza vaccine for the 2008–2009 season from the medical records.

The frequency and duration of signs and symptoms, were presented for the groups of cases: ARI cases that had not been laboratory tested, ARI of cases unknown cause (ARI cases that had been laboratory tested but etiologic agent was not found), pH1N1 cases and total ARI cases.

The students health status data ( $n = 288$ ) were obtained from their medical records and epidemiological questionnaire. Data about symptoms were available for all the affected students ( $n = 204$ ). Data about the duration of illness were available for 157 students. Vaccination status data was available for 224 of the students. The personnel in the MS was not included in the study.

A clinical case of ARI was defined as a person reporting anyone of the following respiratory symptoms: runny nose, sore throat, or cough.

A case of pH1N1 influenza included the presence of symptoms of ARI with laboratory confirmation by RT-PCR assay or serologic confirmation of a four-fold rise in antibodies to influenza A. Serological confirmation of influenza type A was considered a sufficient evidence for pH1N1 influenza since this strain was predominant at the time of the pandemic and that other strains of influenza type A were present in a very small percentage<sup>3–5</sup>.

Nose and throat swabs were taken from 8 ARI cases and tested at the Institute of Vaccines, Virology and Sera, Torlak, Belgrade, Serbia, by the RT-PCR assay for the presence of pH1N1 influenza, seasonal influenza H1N1, influenza H3N2, influenza B and Respiratory syncytial virus (RSV).

Serological testing included 94 students divided into two groups: 82 students with symptoms of ARI (including 8 cases tested by RT-PCR assay) and a group of 12 students

without ARI symptoms. We tested a group of students without ARI symptoms in order to quantify rates of asymptomatic infection. Paired serum samples were taken at intervals of 4 weeks and tested by complement fixation test (CFT) for the presence of antibodies against influenza virus A and B and adenoviruses. Serological testing of paired sera was carried out at the Military Medical Academy. The results of CFT were considered as positive if there was at least fourfold increase in antibody titers.

After the outbreak we proposed and implemented the following control measures: symptomatic students were placed on the top dormitory floors and asked to stay isolated from the rest of the students and those having high-grade fever were admitted to the school hospital; all the students were advised to strictly follow personal hygiene measures (eg, frequent hands and face washing, using a handkerchief while coughing and sneezing); and to avoid group activities and clustering.

To analyze data we used the  $\chi^2$  test, Fisher's exact test, calculation of the relative risk and Student's *t*-test.  $p < 0.05$  was considered significant. Statistical tests were performed by using Epi Info 6.0 and Statistical Package for the Social Sciences (SPSS) for Windows version 16.0.

## Results

The outbreak of this infection in the MS was declared after collecting epidemiological data for 8 students, with symptoms and signs of ARI, who were referred to the Military Medical Academy for medical examination on October 23, 2009. The RT-PCR assay showed the presence of pH1N1 virus. Therefore, epidemiological survey was carried out and control measures proposed.

The collective of the MS were males, aged from 14 to 18 years ( $16.3 \pm 1.1$ ). Body mass index (BMI) of all the students was in normal range ( $BMI = 21.9 \pm 2.3 \text{ kg/m}^2$ ), with no chronic disease.

This epidemiological investigation showed that the outbreak of ARI began among the students of the 3rd grade on October 12, 2009. Figure 1 presents the number of ARI cases by grades in this outbreak. The number of ARI cases reached the peak among the students of the 3rd grade on October 22, in the students of the 1st grade on October 23, in the students of the 4th grade on October 26 and in the students of the 2nd grade on November 1. The first wave of the epidemic spread among the 1st and 3rd grade students and the 2nd wave involved the students of the 2nd and 4th grades. A total of 204 students were affected by the end of the outbreak.

Table 1 shows that acute respiratory infections spread to all grades of the students even though they were stationed at different floors of the dormitory. The ARI attack rate among the students depending on the grades ranged from 63.9% to 77.1%, and by the dormitory floors from 64% to 83% as shown in Table 1. The total attack rate of ARI was 70.8% (204/288), and the attack rate of pH1N1 influenza was not found because we did not test all the ARI cases for pH1N1.

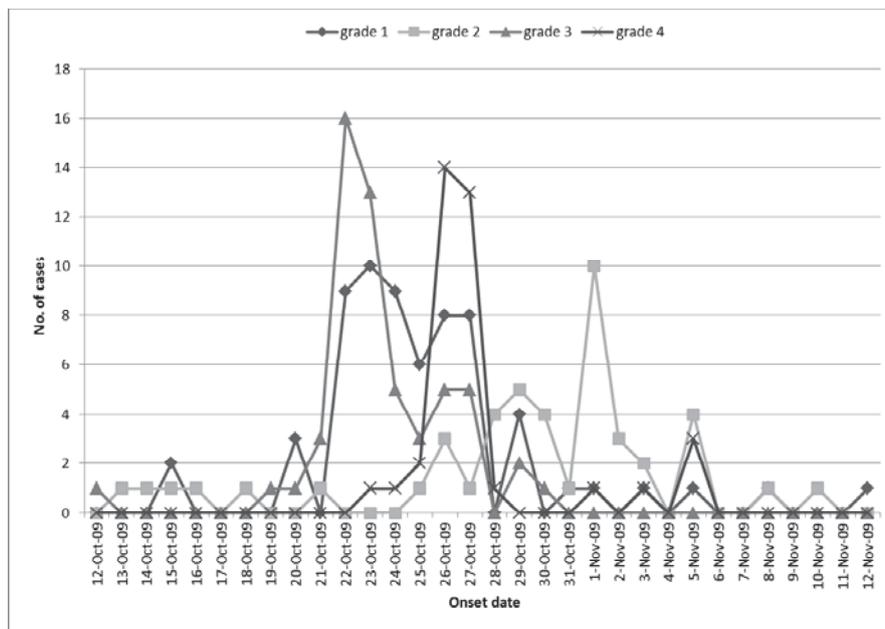


Fig. 1 – The cases of acute respiratory infection (ARI) in the Military School, presented by the school grades.

Table 1

Attack rate of acute respiratory illness (ARI) in the Military School students by floors and grades

Grade	Floor						Total
	0	I	II	III	IV	V	
I	18/22 (82)	33/44 (75)	14/21 (67)	0	0	0	65/87 (75)
II	0	0	24/35 (69)	22/37 (65)	0	0	46/72 (64)
III	0	0	0	15/21 (71)	41/60 (68)	0	56/81 (69)
IV	0	0	0	0	0	37/48 (77)	37/48 (77)
Total	18/22 (82)	33/44 (75)	38/56 (68)	37/58 (64)	41/60 (68)	37/48 (77)	204/288 (71)

Note: Data are presented as the number of ARI cases/students (%).

The results of swabs tested by the RT-PCR assay confirmed the presence of a pH1N1 virus in 4/8 (50%) samples. Nasopharyngeal swabs were also tested for seasonal influenza H1N1, influenza H3N2, influenza type B and respiratory syncytial virus (RSV) and results were negative. The results of serological tests for the presence of influenza type B and adenoviruses were negative in all tested samples. However, in paired sera of 44 persons (including 4 cases that were positive for pH1N1 by RT-PCR assay) a fourfold increase in titer to influenza A was registered. CFT to influenza type A was found positive in 44/82 (53.7%) of the affected individuals. In the remaining 38/82 (46.3%) ARI cases (including 4 cases that remained negative by RT-PCR assay testing) the etiologic agent was not established. In the group of students without ARI symptoms seroconversion was not detected.

The cases of ARI and pH1N1 influenza in the Military School, during October and November 2009, are presented in Figure 2. The first confirmed case of pH1N1 influenza at the Military School developed symptoms on October 12, 2009. Two large peaks can be observed in this outbreak. The first peak occurred on October 22 and the second, higher one on October 26.

In order to determine whether pH1N1 influenza was clinically different from other ARI, the frequencies of signs

and symptoms were examined separately (Table 2). Comparison of the representation of certain symptoms and signs of disease revealed that the pH1N1 cases were significantly more frequently malaise ( $p = 0.021$ ) and had dry cough ( $p = 0.029$ ) compared to the cases of ARI of unknown cause. Other signs and symptoms showed no statistically significant differences between the investigated groups of patients.

The most common clinical manifestations of pH1N1 influenza were fever (88.6%), cough (61.4%), dry cough (50%), malaise (38.6%), runny nose (36.4%), headache (29.6%), sore throat (20.5%), muscle pain (15.9%), while diarrhea and vomiting were rarely registered. Fever over 39°C was observed in 7 (15.9%) of the patients, while 5 patients had no fever.

Illness duration was determined in 157 patients, and it was in the range from 1 to 6 days. The average illness duration in the ARI outbreak was  $2.71 \pm 1.35$  days (Table 3). By comparing the values of the average duration of illness in patients with pH1N1 influenza ( $3.00 \pm 1.49$  days) and in patients with ARI of unknown cause ( $2.50 \pm 1.13$ ), there was no statistically significant difference [Student's  $t$ -test,  $t = 1.636$ ;  $df = 74$ ;  $p = 0.106$ ; 95% Confidence interval (CI), -0,109–1,109].

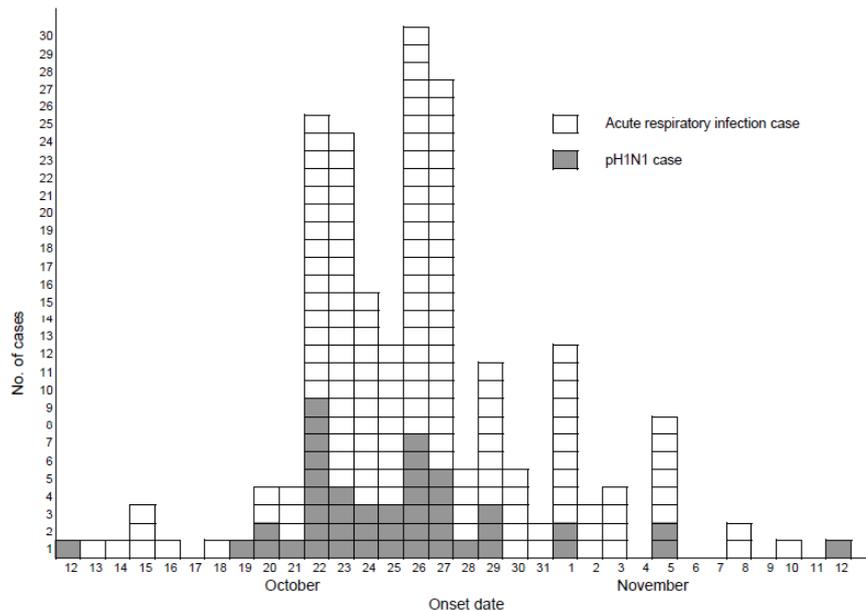


Fig. 2 – Clinical acute respiratory infection and pandemic H1N1 influenza in the Military School, October– November 2009 (n = 204).

Table 2

Symptoms and signs of acute respiratory infection (ARI) in the affected students (n = 204)

Symptoms and signs of ARI	ARI (n = 204) n (%)	ARI of unknown cause (n = 38) n (%)	pH1N1* (n = 44) n (%)	pH1N1 vs ARI unknown cause RR (95% CI); p
Headache	44 (21.57)	9 (23.68)	13 (29.55)	1.25 (0.60–2.29); 0.550 ‡
Malaise	45 (22.06)	6 (15.79)	17 (38.64)	2.45 (1.07–5.57); 0.021 ‡
Runny nose	75 (36.76)	10 (26.32)	16 (36.36)	1.38 (0.71–2.67); 0.330 ‡
Sore throat	37(18.14)	5 (13.16)	9 (20.45)	1.55 (0.57–4.24); 0.381 ‡
Sneezing	31(15.20)	4 (10.53)	6 (13.64)	1.30 (0.39–4.25); 0.668 †
Dry cough	67 (32.84)	10 (28.95)	22 (50.00)	1.90 (1.03–3.49); 0.029 ‡
Productive cough	19 (9.31)	4 (10.53)	5 (13.64)	1.08 (0.31–3.73); 1.000 †
Shortness of breath	6 (2.94)	1 (2.63)	3 (6.82)	2.59 (0.28–23.88); 0.620 †
Conjunctivitis	4 (1.96)	1 (2.63)	3 (6.82)	2.59 (0.28–23.88); 0.620 †
Diarrhea	9 (4.41)	1 (2.63)	3 (6.82)	2.59 (0.28–23.88); 0.620 †
Nausea	5 (2.45)	0 (0.00)	3 (6.82)	NA; 0.245 †
Vomiting	5 (2.45)	1 (2.63)	2 (4.55)	1.73 (0.16–18.31); 1.000 †
Muscle ache	21 (10.29)	4 (10.53)	7 (15.91)	1.51 (0.48–4.77); 0.476 ‡
Joint pain	7 (3.43)	0 (0.00)	2 (4.55)	NA; 0.497 †
Temp. 37–37.9°C	103 (50.49)	20 (52.63)	18 (40.91)	0.78 (0.49–1.24); 0.288 ‡
Temp. 38–38.9°C	54 (26.47)	6 (15.79)	14 (31.82)	2.02 (0.86–4.73); 0.092 ‡
Temp. > 39°C	20 (9.80)	7 (18.42)	7 (15.91)	0.86 (0.33–2.24); 0.763 ‡

\* – pandemic H1N1 influenza; † – Fisher’s exact test; ‡ –  $\chi^2$  test; RR – relative risk; CI – confidence interval.

Table 3

Duration of illness in the cases of acute respiratory infections (ARI; n = 157), including the pandemic H1N1 influenza cases (n = 40)

Duration of clinical illness (days)	No laboratory tested ARI (n = 81) n (%)	ARI unknown cause (n = 36) n (%)	pH1N1 (n=40) n (%)	Total ARI (n = 157) n (%)
1	17 (20.99)	7(19.44)	9 (22.50)	33 (21.02)
2	25 (30.86)	11 (30.56)	6 (15.00)	42 (26.75)
3	19 (23.46)	14 (38.89)	11 (27.50)	44 (28.03)
4	11 (13.58)	2 (5.56)	4 (10.00)	17 (10.83)
5	6 (7.41)	1 (2.78)	10 (25.00)	17 (10.83)
6	3 (3.70)	1 (2.78)	0 (0.00)	4 (2.55)
$\bar{x} \pm SD$	2.67 ± 1.351	2.50 ± 1.134	3.00 ± 1.485	2.71 ± 1.345

Clinical manifestations in the majority of the patients could be described as mild. Severe complications were not observed during the outbreak. Only 9 cases of bronchitis were registered, but they required no hospitalization.

We found that the inactivated trivalent influenza vaccine for the 2008–2009 season received more than half of the students, 167/288 (58%), and 57/288 (20%) received no vaccine. For the remaining students, 64/288 (22%), vaccination status was unknown.

Among not vaccinated, 47/57 (82.5%) were those affected by ARI, while among vaccinated that number was significantly lower, 107/167 (64.1%) (RR, 1.29; 95% CI, 1.09–1.52,  $\chi^2 = 6.69$ ;  $p = 0.010$ ).

For 67 patients there were vaccination status data and the results of serological tests. Of 13 persons who did not receive trivalent influenza vaccine 2008–2009, 9 (69.2%) showed seroconversion for influenza A vs 21/54 (38.9%) of those that received vaccine (RR 1.78; 95% CI, 1.09–2.91;  $\chi^2 = 3.90$ ;  $p = 0.048$ ; Yates corrected:  $\chi^2 = 2.77$ ;  $p = 0.096$ ).

## Discussion

Influenza virus was easily and rapidly spreading among the MS students. It is likely that the widespread susceptibility of persons under 60 years allowed such a rapid dissemination of the virus<sup>4–6</sup>. The descriptive analysis suggests that household contacts who were under 18 years of age were at significantly higher risk for ARI and influenza-like illness (ILI) than contacts who were 19 to 50 years of age<sup>7</sup>. The first epidemiological reports referring to the pH1N1 influenza in 2009 indicated that the cases of infections and deaths were mostly among adults aged 20–40 years<sup>8</sup>. People born before 1957 were in the lower risk of infection with pH1N1 influenza in 2009. Lower number of infections was not only a reflection of lower testing in this age group. The mechanism that explains this association is not entirely clear but is consistent with the findings of its age related increase in the prevalence of titer of neutralizing antibodies against the pH1N1 virus<sup>9</sup> and may be a reflection of immunity resulting from exposure to a similar virus in life. Maximally effective host immune response to influenza can be arisen by earlier infections throughout life<sup>10</sup>. This is consistent with a high incidence of pH1N1 influenza outbreak in 2009 at schools<sup>11</sup> and reduced the frequency of outbreaks in nursing homes.

Two waves of outbreak were recorded MS. The first wave affected students of 1st and 3rd grades, and the 2nd one the students of the 2nd and 4th grades. This is understandable because the students of the 1st and 3rd grades and the students of the 2nd and 4th grades attended school in the same shift.

A fourfold increase of antibodies against influenza A titer was found in 44 of 82 ARI cases. Based on RTPCR assay findings and epidemiological situation we concluded that influenza A infection was confirmed due to pandemic pH1N1 virus.

The attack rate of ARI was 70.8% in this outbreak. Since the influenza type A was determined by serological test in 53.7% (44/82) of the tested ARI cases it can be esti-

mated that the attack rate of pH1N1 influenza was approximately 38%. During an outbreak at a residential school at Panchgani, Maharashtra, India, the clinical attack rate for ILI in students was 76.4% and the attack rate for pH1N1 influenza cases was 42.4%<sup>12</sup>. In April 2009 the first recorded school outbreak of pH1N1 influenza occurred in a New York City high school and infected nearly 30% of the student's population<sup>13</sup>. Epidemiological investigation of an outbreak of pH1N1 influenza at a boarding school in China showed a 22.2% attack rate and a 32% infection rate in students aged 15–21 years old. The incidence rate of boarders was higher than day-boarders<sup>14</sup>. On the basis of published data it can be concluded that the incidence of pH1N1 influenza at schools ranged from 8% in England<sup>15</sup> to 42% in India<sup>12</sup>. The higher attack rate of ARI and pH1N1 influenza in this outbreak in comparison to most other described outbreaks at schools can be explained by the specifics of the school where besides traditional classes, students have other additional common activities (sharing dormitory space, sport activities, learning in reading room, meals in the school restaurant etc.)

In the group of students without ARI symptoms seroconversion for influenza was not detected. Our findings suggested no asymptomatic seroconversion. In the outbreak that occurred at a vocational boarding school in Guangzhou, P.R. China, 156 asymptomatic patients (9.9%) of the 1570 participating students were confirmed to be infected by pH1N1 virus<sup>14</sup>.

Clinical manifestations in patients during the outbreak in the MS were: fever (88.6%), cough (61%) and malaise (38.6%), runny nose (36.4%), headache (29.6%), sore throat (20.5%) and muscle ache (15.9%). Fever and cough were the most common symptoms also in investigations of other researchers, and malaise was not taken into consideration by other researchers. Overall, the symptoms were mild and there were no hospitalizations.

In pH1N1 influenza outbreak in a New York City high school, clinical manifestation among confirmed cases were fever 93%, cough 90%, headache 79%, sore throat 76%, muscle ache 76%, runny nose 69%, nausea 46%, diarrhea 26% and vomiting 17%<sup>13</sup>.

During the outbreak of pH1N1 influenza in a residential school in India, clinical presentations in ILI cases were fever (100%), cough (100%), nasal discharge (30%), headache (16.9%), sore throat (15.6%), body-ache (9.8%), fatigue (4.4%), vomiting (3.4%) and diarrhea (1.4%). There was no significant difference between clinical presentation of ILI and confirmed pH1N1 influenza cases. Analysis of the pH1N1 influenza in Serbia in 2009/2010 season in general population showed that the leading symptoms were fever (83.0%), dry cough (75.2%) and muscle aches (51.8%)<sup>3</sup>. Clinical manifestations in pandemic flu patients hospitalized in the Clinic for Infectious and Tropical Diseases, Military Medical Academy, were fever (100%), fatigue (95.9%), cough (82.6%), headache (66.3%), while dyspnea and diarrhea were registered in 1/4 of the patients<sup>16</sup>.

The mean duration of the illness among pH1N1 cases during the outbreak in the MS was 3 days. In an outbreak at a school in New York the mean duration of the illness in the confirmed cases of pH1N1 influenza was 6 days, and 75%

had recovered by 9 days after the onset of symptoms<sup>13</sup>. The mean duration of illness (*ie*, symptomatic period) was 4 days during the outbreak of pH1N1 influenza at a residential school in India<sup>12</sup>. Reasons for a shorter duration of the illness in our patients were probably a good physical fitness of students and the absence of risk factors such as chronic diseases and obesity.

Based on our results we can say that the seasonal influenza vaccine 2008–2009 provided a certain level of protection against pH1N1 virus. The results of the effect of inactivated seasonal influenza vaccination on risk of pH1N1 influenza in a cohort of nurses in Canada who participated in a recent randomized controlled trial suggest a possible positive effect of 2008–2009 trivalent inactivated seasonal influenza vaccine reducing the risk of infection with pH1N1 influenza<sup>17</sup>. In a study of seasonal influenza vaccine and protection against pandemic (H1N1) 2009-associated illness among US military personnel was found moderate association with protection against clinically apparent, laboratory-confirmed pandemic (H1N1) 2009-associated illness for immunization with either TIV or LAIV 2008–2009 seasonal influenza vac-

cines. This association with protection was found to be especially apparent for severe disease as compared to milder outcome, as well as in the youngest and older populations. Prior vaccination with seasonal influenza vaccines in 2004–2008 was also independently associated with protection<sup>18</sup>.

### Conclusion

School population was very favorable for pandemic influenza H1N1 virus spreading. In high school population, pandemic influenza had milder clinical manifestations. Clinical manifestations of the 2009 H1N1 influenza virus appear to be similar to those of previously observed in seasonal influenza. Our finding suggests that the seasonal influenza vaccine 2008–2009 provided a certain level of protection against pandemic influenza A (H1N1) virus.

This study illustrates the significance of field epidemiologic investigations for understanding an emergent threat. This was particularly relevant during the emergence of pandemic (H1N1) virus when its pathogenicity was uncertain.

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