Seroepidemiology of pandemic influenza A (H1N1) 2009 virus infections in northern Greece

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On June 11, 2009, WHO declared that the rapidly spreading swine-origin influenza A (H1N1) influenza virus constituted a global pandemic. Rapid identification of the virus indicated that it was a novel H1N1-reassortant influenza A virus that originated from a triple reassortant North American swine influenza A virus that had acquired two virus genes (NA and M) from a Eurasian swine influenza A virus; the virus thus contains genetic material from avian (PA and PB2), human (PB1) and two lineages of swine influenza A viruses (1). Compared with seasonal influenza A(H1N1) viruses, the novel virus is genetically and antigenically very different from human H1N1 viruses that have been circulating during the last 60 to 70 years (2). Since the majority of the world's population is lacking immunity against this new virus, it has spread throughout the world with an unprecedented speed. Reports from the US and the United Kingdom have suggested that pre-existing antibodies and thus cross-protection against the pandemic virus exist in some individuals, especially those that are currently over 65 years old (3,4). Limited data is available on this from Europe. But without a direct measure of the baseline age-specific immunity profile of the population and the changes that result from 2009 H1N1 infection as the pandemic progresses, predictions about future incidence are necessarily subject to substantial uncertainty. In response to this health emergency, Greece set up an enhanced surveillance system for pandemic H1N1 2009 by the 30th of April 2009. Specimens were sent to one of the two reference laboratories, in Athens (Hellenic Pasteur Institute) covering southern Greece and in Thessaloniki (Aristotle University of Thessaloniki, Medical School, Second Microbiology Department) covering northern Greece. In the present study, we report the results of a seroepidemiological survey in Northern Greece to document the age specific prevalence of antibodies to 2009 pandemic virus before and after the pandemic and to provide a direct measure of the incidence of infection in the population. A total of 485 residual serum samples were included in the study. To establish the baseline prevalence of antibodies to 2009 pandemic H1N1 virus, we used 85 serum samples (40 females and 45 males) obtained in 2007-2008 from individuals aged 4 years to 85 years (mean age 43 years old) in northern Greece. The

Table 1. *Age distribution of seropositivity rate among the pre and post-pandemic period*

Samples	n	Sex Male/Female	Origin Thessaloniki/other cities	Seropositivity (%)
Pre-pandemic population	85	45/40	60/25	3,5
Post-pandemic (age groups)	400	200/200	320/80	34,5
0-10	80	40/40	70/10	37,56
11-25	80	40/40	60/20	45
26-45	80	40/40	60/20	33,75
46-65	80	40/40	60/20	25
>65	80	40/40	70/10	30,9
Total	485	245/240	380/105	-

specimens belonged to 60 people living in Thessaloniki and 25 inhabitants of other parts of northern Greece. Additionally, 400 serum samples were collected from April to December 2010, in order to provide a direct measure of the incidence of infection in the population. The specimens belonged to 200 females and 200 males. The age range of the patients was 1-79 years (mean age 41 years old) and the samples were divided into five age groups, according to their age (Table1).

Of the 400 samples, 320 were collected from inhabitants of Thessaloniki, 80 from people living in other parts of northern Greece. None of the sera belonged to people suffering from infectious diseases or any known immunodeficiency syndromes. The questionnaire included the subject's age, gender, place of residence, history of influenza infection during the pandemic or any other respiratory tract infection, presence/absence of influenza-like symptoms. It is important to pinpoint that all specimens belonged to unvaccinated individuals, of both seasonal and pandemic H1N1 influenza virus. Blood samples were collected in evacuated tubes by venipuncture, kept at room temperature for 30-45 minutes for allowing clot reaction and transported in ice within 4-6 hours of sampling. Sera were separated by centrifugation. Aliquots were made and stored at -20°C until testing. Immunity to influenza A(H1N1) 2009 virus was determined by ELISA (Genzyme, Virotech), according to the manufacturers' instuctions.

Statistical analysis of the results was performed using the SPSS 13.0 software. The seropositive rates between the different age groups were compared by the x2 test. Any value of p<0,05 was considered statistically significant. The multivariate logistic regression model was used to investigate the potential correlative factors that influenced the frequency and distribution of pandemic H1N1 antibodies in different groups. Since late March 2009, the novel genetically reassorted swine-origin H1N1 influenza A virus is spreading worldwide. Its high transmissibility and rapid spread led to the declaration of the first influenza pandemic in 40 years by the WHO.

We conducted the present study in order to estimate the antibody levels and associated factors in various study groups in northern Greece. Age and sex distribution of the study subjects sampled during the pre-pandemic period and those sampled from different age groups during the post- pandemic period is presented in Table 1. Our results demonstrated that seropositivity in the pre-pandemic period was very low (3,5%). Only 3 out of the 85 individuals were found to be seropositive for the influenza pandemic H1N1 virus. Those 3 individuals were 75, 78 and 83 years old and had acquired immunity probably due to cross-reactivity with other circulating influenza subtypes. It is also worth to remark that none of them had been vaccinated with the seasonal influenza vaccine during the period of the sample collection (2007-2008). A study in India has reported a negligible pre-pandemic seropositivity rate (0,9%), while other studies from Japan and the UK also describe the presence of cross-reactive antibodies to the 2009 pandemic influenza virus among the oldest age groups (born in 1930 or earlier) (5,6,7). However, a similar study from Singapore observed no or minimal cross-reactivity (8).

In case cross-reactivity against the 2009 pandemic influenza virus is indeed due to infections caused by the Spanish influenza and /or its immediate descendant viruses in the late 1910s and the 1920s, this would seem to suggest that specific anti-influenza immunity can last for an extremely long time, even a lifetime. A recent study showing the existence of B cells clones specific for the Spanish influenza HA in the elderly, indicate that immunological memory may last a whole lifetime (9). Among the 400 post-pandemic specimens tested, the seroprevalence was 34,5% (n=138), while 53,6% of them were females. The mean age of the seropositive individuals was 39,4 years. The inhabitants of Thessaloniki were seropositive at a rate of 38,75% (120 out of 320), while the seropositivity of people living in other parts of N. Greece was 26,25% (21 out of 80) (Table 2). Additionally, the 400 samples were divided into five groups, according to their age (Table 1). Among the five post-pandemic age groups involved in the study, the highest seropositivity rate was observed in the 11-25 years old age group (45%). The mean age of the above seropositive people was 21,16 years, 50% of them were females and the majority (83,3%) were living in Thessaloniki. This can be explained by the fact that A(H1N1) influenza virus mostly affected young adults during the pandemic. On the other hand, the lowest seropositivity rate (25%) was found at the 46-65 years old age group, indicating low infection rates. Half of them were females and most of them were living in Thessaloniki as well (85%). Their mean age was 57,8 years.

Table 2. *Sex and origin distribution of seropositivity rate*

Sex	Seropositivity (%)
Males	46.4
Females	53.6
Thessaloniki	38.75
Other cities of N. Greece	26.25

Statistical analysis of the results demonstrated that that there was a statistical significant difference between seroprevalence during the pre and post-pandemic period (p=0.0000), and also between seropositivity and origin of the study samples (p=0,035), with inhabitants of Thessaloniki, which is the northern Greek capital city, having a bigger possibility of being seropositive than the ones living in other parts of N. Greece. This can be explained by the fact that people in larger cities are living more closely to one another than people living in small cities in northern Greece. As expected, no statistically significant difference was found between seroprevalence and sex (p=0,065). In a seroepidemiological study conducted in India, higher seropositivity was noted in 15-19 years age group, while the lower seropositivity was observed in elderly population (5). The similar lower incidence of infection was reported in elderly in New Zealand (10). Seropositivity among elderly in China has been reported to be 9,4% in pre-pandemic and 42,5% in post-pandemic sera (11).

This serological study shows the extent of H1N1 infection during the 2009 influenza pandemic in N. Greece. Further serological studies of seasonal influenza that will document the age-specific subtytpes, could enhance understanding of the epidemiology of influenza and the role of natural infection in the induction of natural immunity to A(H1N1) influenza virus.

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