Eur J Clin Microbiol Infect Dis (2012) 31:1467–1471
DOI 10.1007/s10096-011-1465-x

ARTICLE

H1N1v at a seroepidemiological glance: is the nightmare over?

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Received: 6 June 2011 / Accepted: 1 October 2011 / Published online: 9 November 2011

Abstract When the second wave of pandemic influenza A H1N1v 2009 (H1N1v) emerged in the winter of 2010/2011, public health authorities were afraid of dangerous implications and severe clinical courses again. As further H1N1v waves might appear, achievement of sufficient herd immunity is a matter of urgency. The objective of this study was to determine the seroprevalence of antibodies against H1N1v by hemagglutination-inhibition test (HI) after the second wave. We compared our recent findings with our data obtained after the first pandemic in 2009/2010. Between March and May 2011 we collected serum samples from 600 persons aged 1 to 84 years admitted to University Hospital Frankfurt/Main and analysed the titres of anti-H1N1v by HI. The overall seroprevalence of anti-H1N1v has risen from 36.9% (95% confidence interval (95%CI), 33–41) in unvaccinated persons after the first wave to 57.3% (95%CI, 53.1–61.2) in vaccinated and unvaccinated. The highest rate of seropositivity was detected in the age group of 10–19 years (66%; 95%CI, 55.8–75.2), whereas the lowest was found in the age group 40–59 years (51%; 95%CI, 40.8–61.1). Although seroprevalence has significantly increased, sufficient herd immunity is still not achieved. Therefore, general vaccination programs have to be propagated continuously by public health authorities.

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Abbreviations

anti-H1N1v	Antibodies to pandemic influenza A H1N1v 2009/2010
H1N1v	Pandemic influenza A H1N1v 2009/2010
HI	Hemagglutination-inhibition-test
95%CI	95% confidence interval

Introduction

In April 2009 a new variant of influenza A virus, subtype H1N1 (H1N1v) emerged in Mexico [1, 2] and spread all over the world resulting in the third H1N1 pandemic in mankind after 1918 and 1978/1979. Obviously, there was no herd immunity against this new virus variant in 2009 [3, 4]. Mainly, young people were affected and presented with severe and occasionally even lethal clinical courses [3–5]. After the initial outbreak, widespread and deep concern within the population was caused by a high degree of uncertainty about the transmissibility and mortality rate of this new virus infection [6], supported by an extensive and particular misleading media coverage. As previously recorded, the seroprevalence of antibodies to H1N1v (anti-H1N1v) reached nearly 37% (95% confidence interval [95% CI], 33–41) in the population of Frankfurt am Main area after the first wave in 2009/2010 [7]. Even at that time, we emphasized a relatively high number of seronegative people, for whom a new wave of H1N1v may pose a serious health risk [7]. Indeed, in winter of 2010/2011 the second wave of H1N1v swept through many countries of the northern

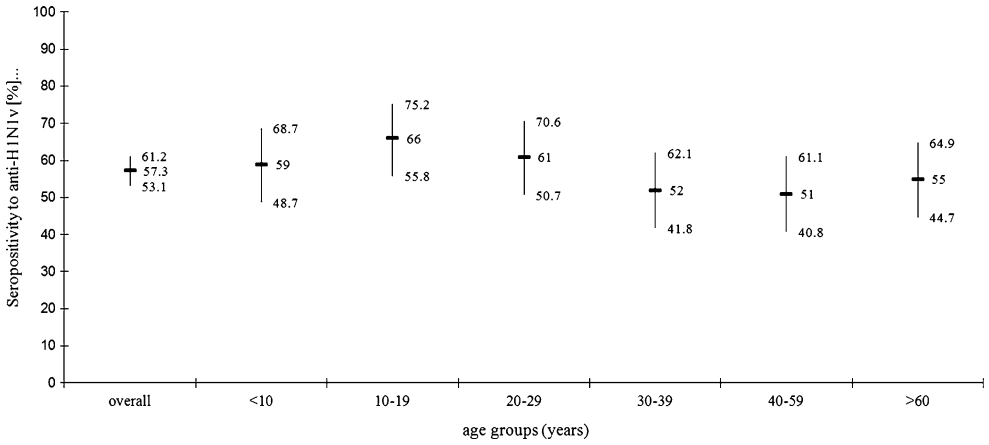

Fig. 1 Age-related distribution of anti-H1N1v seroprevalence in 600 patients (100 patients in each age group). Means are given as *horizontal bars*. 95% confidence intervals are illustrated as *verticals*

hemisphere including Germany, and threatened public health again. The objective of our seroepidemiological study was to determine the population's immunity to H1N1v after the second wave in 2010/2011. Therefore, we analysed the seroprevalence of anti-H1N1v by using an H1N1v-specific hemagglutination-inhibition test (HI) as previously described [4]. Additionally we compared our findings with our previous study concerning H1N1v seroprevalence in 2009/2010 [7].

Methods and material

We screened 600 serum samples from patients admitted to University Hospital of Frankfurt am Main, Germany, for anti-H1N1v between March and May 2011, with 300 samples taken from men and women, respectively. The H1N1v-specific HI for the determination of the H1N1v seroprevalence has been described earlier [4]. Shortly, materials used for testing were standardized fresh red blood cells (RBC) of turkeys in Alsever's solution (Bundesinstitut für Risikobewertung, Alt-Marienfelde, Berlin, Germany), and the pandemic H1N1-Virus vaccine containing highly purified H1 from the H1N1 strain A/California/7/2009 NYMC X-179A (GSK Biologicals, Dresden, Germany), which served as antigen. Serum samples were inactivated by heat incubation at 56°C followed by overnight incubation at 37°C with receptor destroying enzyme (RDE, cholera Wiltrate, Sigma–Aldrich, Seelze, Germany). Serum was added to antigen and the serum–antigen mixture was incubated for 45 min at room temperature and, lastly, RBCs (0.5%) were added. Plates were read promptly when the RBC control had completely settled. All specimens were tested in serial twofold dilutions, and respective controls were used in addition. Evaluation and analysis of the H1N1v titres obtained was adapted to the modified WHO age groups as previously described [7]. Statistical analysis was done by using the 95% confidence interval (95%CI) with a significance level of $p=0.05$ by using the program BIAS for Windows 8.3 (Epsilon Verlag, Hochheim Darmstadt 2007).

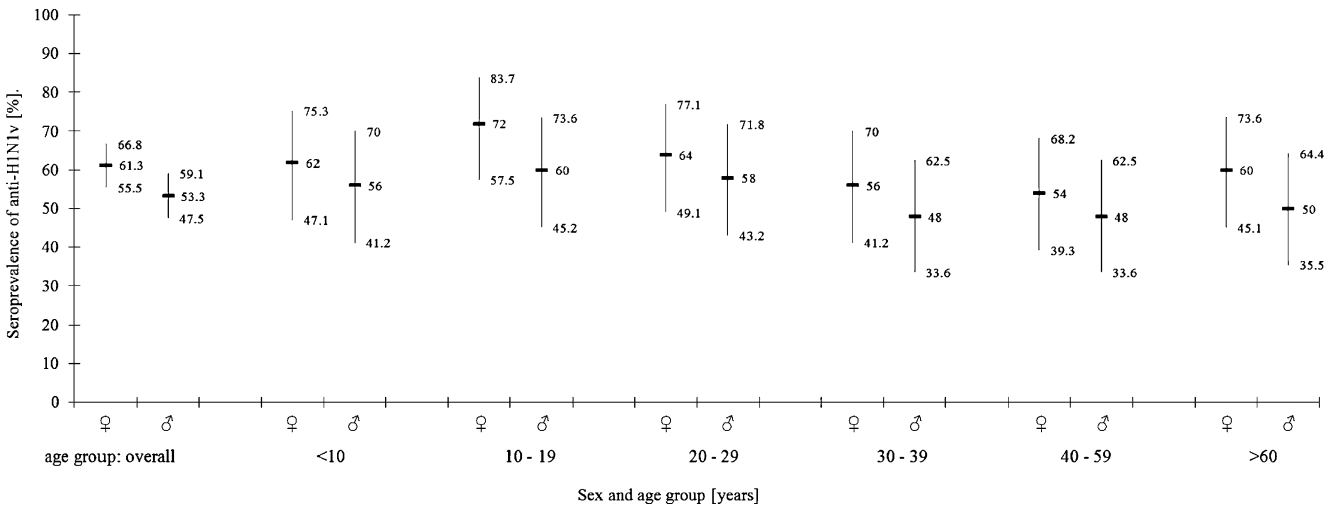

Fig. 2 Sex- and age-related distribution of anti-H1N1v seroprevalence in 600 patients (50 male and female patients in each age group, respectively). Means are given as *horizontal bars*. 95% confidence intervals are illustrated as *verticals*

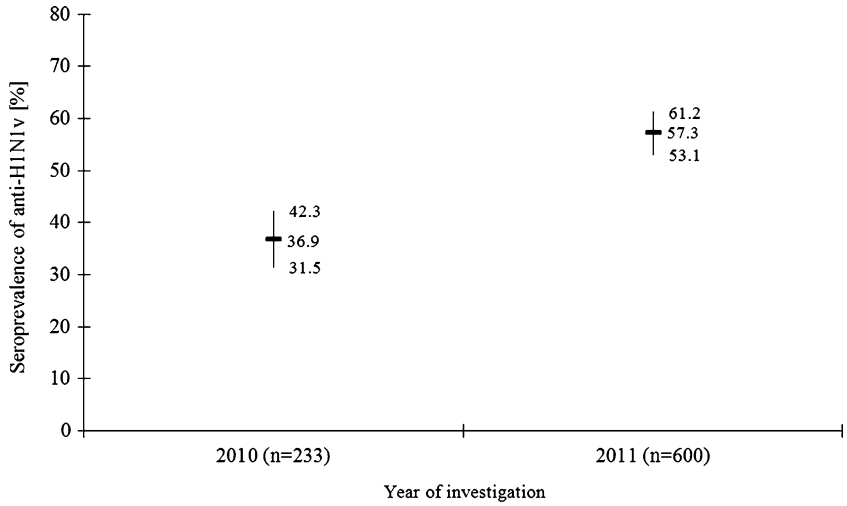

Fig. 3 Seroprevalence of anti-H1N1v in 2010/2011 (this study) compared to our historic control population of 2009/2010 in Frankfurt am Main, Germany [7]. Means are given as *horizontal bars*. 95% confidence intervals are illustrated as *verticals*

Results

We found an overall anti-H1N1v seroprevalence of 57.3% (95%CI, 53.1–61.2). The test persons' age ranged between 1 and 84 years, with the highest level of seropositivity in the group aged 10–19 years (66%; 95%CI, 55.8–75.2), whereas the lowest rate was found in the group aged 40–59 years (51%; 95%CI, 40.8–61.1) as shown in Fig. 1. No significant differences ($p>0.05$) were detectable between the age groups. The sex-related analysis (Fig. 2) showed a higher seroprevalence (61.3%; 95%CI, 55.5–66.8) in the female group; the difference to the male group (53.3%; 95%CI, 47.5–59.1) was statistically significant ($p<0.05$). Compared with our previous findings, we recorded a significant increase ($p<0.05$) of the overall seroprevalence from 36.9% (95%CI, 31.5–42.3) in 2009/2010 to 57.3% (95%CI, 53.1–61.2) in 2010/2011 (Fig. 3).

Discussion

Since the H1N1v pandemic activity slowed down in the first calendar weeks of 2010, a total of 226,140 infections and 254 cases of deaths from H1N1v had been recorded in Germany [8]. Therefore, it was not astonishing that the recurrence of H1N1v in winter 2010/2011 caused massive public anxiety and uncertainty. When in spring 2010 the second wave had declined, it became evident that this wave did not reach the size of the first one; however, serological footprints were clearly detectable in the Frankfurt population: the overall seropositivity to anti-H1N1v increased from 37% (95%CI, 33–41) at the end of the first wave [7] to 57% (95% CI, 53.1–61.2) by the end of the second wave in 2010/2011. As shown in Fig. 1, the group aged 10–19 years still had the highest level of seroprevalence (66%; 95%CI 55.8–75.2) as seen the year before—this seroepidemiological characteristic has been described previously in several studies [7, 9–12]. In contrast to our previous investigation [7], when we selected only patients without knowledge of H1N1v vaccination, we now included serum samples regardless of the H1N1v vaccination status. Therefore, the true proportion of seropositivity caused by H1N1v-infections is difficult to estimate [13]. From a global point of view, the seroprevalence rates reported elsewhere vary widely as selectively illustrated in Table 1. Concerning that aspect, three limitations should be

Table 1 Selection of seroprevalence rates to anti-H1N1v worldwide, determinated by hemagglutination inhibition assay

Country	Seroprevalence (%)	Collective	Reference
Germany	57.3	PFRA	Present study
Australia	22.0	BD	[14]
Finland	49.0	BD	[15]
Germany	36.9	uvGP	[7]
Germany	12.7	PVSVT	[16]
India	12.0	HS	[17]
Iran	58.9	GP	[18]
Japan	5.9	PVSVT	[16]
Manitoba, Canada	15.7	PW	[19]
Nigeria	46.7	HLP	[20]
Pennsylvania, USA	21.0	BD	[21]
Taiwan	57.6	HS	[22]
Taiwan	24.4	GP	[22]
Thailand	48.0	HS	[23]
Thailand	36.0	GP	[23]
United States	22.5	PVSVT	[16]

BD blood donor, *HS* hospital staff, *HLP* salesman handling with live pigs, *GP* general population, *PFRA* patients admitted to University Hospital Frankfurt am Main (Germany), *PW* pregnant women, *PVSVT* pre-vaccination serum samples from pandemic vaccine trials, *uvGP* unvaccinated general population

mentioned: (a) we investigated the seroprevalence of anti-H1N1v after the second wave, whereas the other seroprevalence rates were determined after the first course of H1N1v, and (b) the level of vaccination coverage may widely vary between the selected patient groups as illustrated in Table 1 and (c) the seropositivity rate might be test dependent. Therefore, the published data are not fully comparable.

Another interesting aspect is also illustrated in Table1, i.e. the rate of seropositivity to anti-H1N1v in Frankfurt am Main, Germany, after the first wave in 2009/2010 (37%, [7]) exceeds the value which was determined in a pre-vaccinated population by Tsai et al. (12.6%, [16]) nearly three times. A possible explanation for this observed discrepancy might be the direct proximity to the International Airport in Frankfurt am Main, Germany. The highly frequented worldwide movement of persons and freight can contribute to a rapid spread of infectious diseases, especially those which are transmitted by droplets—such as influenza. Since we evaluated a Frankfurt collective, Tsai et al. evaluated a German (among others) cross section; thus, the neighborhood to the airport has been averaged and might explain the lower seroprevalence.

For epidemiological aspects such investigations are necessary to assess the population's immunostatus. Our data and the seroprevalence rates mentioned in Table 1 are still too low to completely rule out a further H1N1v wave in future seasons. In accordance with these evaluations, useful interventions, such as vaccination campaigns, can be implemented to increase herd immunity. Thus, it is important to strengthen the population's perception and knowledge of H1N1v (and influenza disease burden in general) to take countermeasures against "vaccination-fatigue" [24]. Because the possibility of further "H1N1v flare-ups" in future seasons could not be excluded, sufficient immunity to H1N1v is important and continued vaccination against H1N1v should be strongly encouraged.

Conclusions

Although vaccination was most strongly recommended by Public Health Service, we recorded a widespread deficiency in immunoprotection to H1N1v, i.e. herd immunity was not achieved in any of our age groups. Overall, nearly 45% of patients in our Frankfurt collective are still unprotected and may be threatened by a new H1N1v wave. Such relatively low immunity levels have also been reported from several other countries [25–28]. Therefore, public health authorities should increase the population's perception of this health threatening virus by providing information on personal risk, severity of influenza illness, and efficacy of vaccination, as previously mentioned [29, 30].

Acknowledgments We would like to express our most sincere thanks to Mrs. Anita Kosic, Mrs. Safia Z. Ouazar, Mrs. Katja Prosser, Mrs. Cornelia Rühl, Mrs. Gabriele Schön and Mrs. Ingrid Winkler for their excellent technical assistance.

Competing interests The authors declare that they have no competing interests.

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