Eurosurveillance

Volume 14, Issue 41 - 15 October 2009

## Rapid communications



## Perspectives



## Surveillance and outbreak reports



## Letters



ecdc
EUROPEAN CENTRE FOR DISEASE PREVENTION AND CONTROL

# Use of an inactivated vaccine in mitigating pandemic influenza A(H1N1) spread: a modelling study to assess the impact of vaccination timing and prioritisation strategies

**V Sypsa[1], I Pavlopoulou[2], A Hatzakis (ahatzak@med.uoa.gr)[1]**
1. Department of Hygiene, Epidemiology and Medical Statistics, Athens University Medical School, Athens, Greece
2. Paediatric Research Laboratory, Faculty of Nursing, Athens University, Athens, Greece

This article was published on 15 October 2009.
Citation style for this article: Sypsa V, Pavlopoulou I, Hatzakis A. Use of an inactivated vaccine in mitigating pandemic influenza A(H1N1) spread: a modelling study to assess the impact of vaccination timing and prioritisation strategies. Euro Surveill. 2009;14(41):pii=19356. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19356

The impact of prioritisation and of timing of vaccination strategies on reducing transmission of pandemic influenza A(H1N1) was evaluated in a community with the structure of the Greek population using a stochastic simulation model. Prioritisation scenarios were based on the recommendations of the United States Centers' for Disease Control and Prevention Advisory Committee on Immunization Practices and vaccination was assumed to initiate either before or during the ongoing epidemic. In the absence of intervention, an illness attack rate (AR) of 34.5% is anticipated. Vaccinating the priority groups before the epidemic (pregnant women, people who live with or care for children <6 months of age, healthcare/emergency services personnel, children 6 months–4 years old and high-risk children 5-18 years old) will have a negligible impact on the overall AR. Vaccinating the recommended groups before the epidemic (priority groups as well as all persons 6 months–24 years old and high-risk individuals 25-64 years old) is anticipated to result in overall and age-specific ARs within the range of seasonal influenza (5%-15%). Initiating vaccination early during the epidemic (AR≤1% of the population) is predicted to result in overall ARs up to 15.2%-19.9% depending on daily vaccination coverage rates. When vaccination is initiated at a later stage (AR: 5%), only coverage of 80% of the whole population at intensive daily vaccination rates would be able to reduce ARs to approximately 15%.

## Introduction

On 11 June 2009, the World Health Organization (WHO) raised the pandemic alert level to phase 6 and declared A(H1N1) influenza the first global pandemic of the 21st century. Delays in the development, production and licensure of a vaccine for the current pandemic as well as restrictions in the global manufacturing capacity dictate careful planning of strategies concerning prioritisation and distribution policies. Another important issue to be considered is the timing of vaccination during an ongoing pandemic. Previous modelling studies investigating the impact of various strategies for mitigating a potential pandemic have shown that the benefit of vaccination depends closely on the time it is initiated [1,2].

In the current study we employ a simulation model to investigate the impact of vaccination strategies and of vaccination timing on the overall illness attack rate (AR) of pandemic influenza A(H1N1) in a small community.

## Methods

### The simulation model

We have used a discrete-time stochastic individual-based simulation model employed previously to simulate A(H1N1) spread [3]. Model parameters were chosen such as to yield age-specific attack rates, in the absence of intervention, similar to that observed in the A(H1N1) outbreak in the community of La Gloria in Mexico [3]. A structured model community of approximately 2,000 people was generated to match the age-distribution, household size and number and size of schools of the Greek population. The model community of 2,000 people was divided into four neighbourhoods of approximately equal size that share one kindergarten, one primary school and one high school. Influenza was introduced at day 0 by randomly assigning a number of initial infective individuals, and person-to-person transmission probabilities were used to simulate influenza spread over time. As the population was assumed to be structured (households, schools, neighbourhoods and community), different transmission probabilities applied to different mixing groups. They were highest for contacts within households and lower for contacts within schools, followed by neighbourhoods and, finally, the entire community [3]. In the absence of intervention, a proportion of symptomatic individuals (80%, 75% and 50% of preschool children, school-age children and adults, respectively) were assumed to stay at home and withdraw from the remaining mixing groups (schools, neighbourhoods, community).

### Vaccine efficacy

We have modelled key vaccine efficacy parameters defined previously, i.e efficacy for infection-confirmed symptomatic illness ($VE_{SP}$), efficacy for susceptibility ($VE_S$) and, given infection, efficacy for illness ($VE_P$) and efficacy for infectiousness ($VE_I$) [4]. Based on estimates from previous trials on the efficacy of homologous inactivated vaccines [5-14], we have assumed a $VE_{SP}$ of 80% for individuals 2-64 years old and of 60% for children 6-24 months and adults >= 65 years old. Estimates for $VE_S$ and $VE_P$

for individuals 2-64 years old were obtained from Basta *et al.* [15] (40% and 67%, respectively) with a modification in the case of children 0-24 months old and elderly to yield a lower $VE_{SP}$ ($VE_S$=20% and $VE_P$=50%).

### Vaccination strategies

Four vaccination scenarios, based on the United States Centers' for Disease Control and Prevention Advisory Committee on Immunization Practices (CDC's ACIP) recommendations [16], were evaluated (Table 1). In all scenarios, 80% vaccination coverage was assumed (total coverage). High-risk groups included individuals with chronic respiratory diseases (including asthma), chronic cardiovascular diseases, chronic metabolic disorders (including diabetes mellitus), chronic renal and hepatic diseases and immunosuppression.

### Timing of vaccination

All scenarios were evaluated under the assumption that vaccination takes place early enough so that the vaccinated persons have developed immunity before the introduction of pandemic influenza A(H1N1) in the community. Selected scenarios were further explored assuming that 2%, 6% and 10% of the 2,000-persons community are vaccinated daily (daily coverage) and the first vaccinated individuals develop an immune response when the AR reaches 1%, 5%, 10% or 15% of the population.

## Results

### Effectiveness of vaccination strategies

In the absence of intervention, an AR of 34.5% is anticipated [3]. Vaccinating the priority groups would reduce the AR to 28.0% (Table 2). Under the scenario of vaccinating the recommended groups, the estimated AR is anticipated to be reduced below 10% (AR: 9.6%). When vaccination is extended to all individuals aged between 25 and 64 years, the AR is estimated to be reduced to 2.7%. Offering vaccination additionally to individuals >= 65 years of age is not anticipated to further lower the AR (AR: 2.5%).

The age-specific attack rates under these vaccination strategies are depicted in the Figure. Vaccinating the recommended groups results in low attack rates in all age groups (9.4%, 10.2%, and 8.1% for 0-24, 25-64 and 65+ years, respectively). When vaccination is extended to include also all individuals aged between 25 and 64 years, low attack rates are predicted for all age groups (5.0%, 1.5% and 2.7% for 0-24, 25-64 and 65+ years, respectively). Offering

**Table 1**

**Evaluated vaccination strategies proposed by the Centers' for Disease Control and Prevention Advisory Committee on Immunization Practices [16] in a community of 2,000 people representative of the Greek population**

| 1. Priority groups | | 2. Recommended groups | | 3. Recommended groups + 25-64 years | | 4. Whole population | |
|---|---|---|---|---|---|---|---|
| Target groups | % of the whole population | Target groups | % of the whole population | Target groups | % of the whole population | Target groups | % of the whole population |
| Pregnant women | 1.0% | Pregnant women | 1.0% | Pregnant women | 1.0% | Pregnant women | 1.0% |
| Household contacts of children younger than 6 months of age | 1.7% | Household contacts of children younger than 6 months of age | 1.7% | Household contacts of children younger than 6 months of age | 1.7% | Household contacts of children younger than 6 months of age | 1.7% |
| Health care and emergency services personnel | 0.9% | Health care and emergency services personnel | 0.9% | Health care and emergency services personnel | 0.9% | Health care and emergency services personnel | 0.9% |
| Children 6 months-4 years | 4.3% | Persons 6 months-24 years | 28.9% | Persons 6 months-24 years | 28.9% | Persons 6 months-24 years | 28.9% |
| High-risk children 5-18 years | 0.9% | High-risk individuals 25-64 years | 4.9% | Individuals 25-64 years | 53.8% | Individuals ≥25 years | 70.5% |
| **Total*** | **6.6%** | **Total*** | **28.5%** | **Total*** | **66.7%** | **Total*** | **80.3%** |

*Estimated in 200 simulations assuming vaccination coverage of 80% within each target group

**Table 2**

**Simulated illness attack rates and effectiveness of different vaccination strategies based on the Centers' for Disease Control and Prevention Advisory Committee on Immunization Practices [16] in a community of 2,000 people representative of the Greek population**

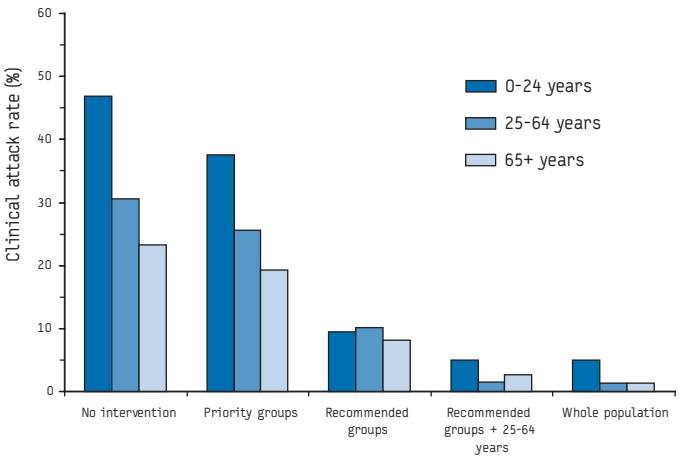
| Target population | Attack rate (AR) | (% decrease)* | Number of vaccinations /1,000 persons | Number of cases prevented/person vaccinated |
|---|---|---|---|---|
| Priority groups | 28.0% | (18.8%) | 66 | 0.96 |
| Recommended groups | 9.6% | (72.2%) | 285 | 0.86 |
| Recommended groups + 25-64 years old | 2.7% | (92.2%) | 667 | 0.47 |
| Whole population | 2.5% | (92.8%) | 803 | 0.40 |

Note: The model assumes 80% vaccination coverage of the target populations and that vaccinated persons become immune before the start of the epidemic
*Compared to an AR of 34.5% in the absence of intervention

vaccination to individuals ≥65 years of age is not anticipated to offer a notable additional benefit for this age group (Figure).

**Figure**

**Age-specific clinical attack rates according to the implemented vaccination strategy, pandemic influenza A(H1N1) 2009**




Note: The model assumes 80% coverage of the target groups and that vaccination takes place early enough so that the vaccinated persons have developed immunity before the introduction of influenza A(H1N1) in the community.

**Impact of timing and daily rate of vaccination**

Under the scenario where vaccination of the recommended groups starts early so that the first vaccinated persons develop an immune response when the cumulative AR is 1%, the AR at the end of the epidemic is predicted to be 15.2%-19.9% for 2%-10% daily vaccination rates (Table 3). Initiating vaccination at a later stage of the epidemic (cumulative AR of 5%) would lead to moderate decreases in the total number of symptomatic cases that is not expected to decrease below 21% of the population, even with intensive daily vaccination rates (100 persons vaccinated daily/1,000 population). When the first vaccinated persons develop immunity near or at the peak of the epidemic (AR: 10% or 15%, respectively), the effectiveness of the intervention in reducing the number of symptomatic infections is estimated to be low (AR: 24.8%-28.5% and 27.8%-29.8%, respectively, for 2%-10% daily vaccination rates). Under the scenario of staged vaccination of the whole population, overall attack rates below 10% are anticipated only in the case where vaccination is initiated early in the epidemic (AR 1%) with intensive daily vaccination coverage (6%-10% of the population vaccinated/day) (Table 3).

## Discussion

In the present study, mathematical modelling was used to evaluate the impact of vaccination strategies recommended by CDC's ACIP for pandemic influenza A(H1N1) as well as the impact of the timing of vaccination in a community typical of the European setting [3]. Vaccinating only the priority groups will have a negligible impact on the overall clinical attack rate. Vaccinating the groups recommended by CDC (i.e. priority groups and all children and young adults up to 24 years old) is predicted to be successful

**Table 3**

**Impact of vaccination according to the timing of vaccination and to daily coverage during an ongoing epidemic (assuming up to 80% vaccination coverage of the target populations): A. Vaccination of recommended groups; B. Vaccination of the whole population.**

| | | A. Vaccination of recommended groups | | | B. Staged vaccination of the whole population (first recommended groups, then individuals 25-64 years, then ≥65 years) | | |
|---|---|---|---|---|---|---|---|
| | | Attack rate (AR) | (% decrease)* | Number of cases prevented/ person vaccinated | Attack rate (AR) | (% decrease)* | Number of cases prevented/ person vaccinated |
| **Before the epidemic** (vaccinated individuals already immune when the epidemic starts) | | 9.6% | (72.2%) | 0.86 | 2.5% | (92.8%) | 0.40 |
| During the epidemic | | | | | | | |
| The first vaccinated persons develop an immune response when the AR is: | Proportion of population vaccinated/day (%) | | | | | | |
| 1% | 2% | 19.9% | (42.3%) | 0.57 | 17.0% | (50.7%) | 0.26 |
| | 6% | 15.7% | (54.5%) | 0.70 | 8.8% | (74.5%) | 0.34 |
| | 10% | 15.2% | (55.9%) | 0.72 | 7.3% | (78.8%) | 0.36 |
| 5% | 2% | 26.2% | (24.1%) | 0.38 | 25.5% | (26.1%) | 0.16 |
| | 6% | 22.8% | (33.9%) | 0.47 | 16.9% | (51.0%) | 0.25 |
| | 10% | 21.7% | (37.1%) | 0.50 | 15.3% | (55.7%) | 0.26 |
| 10% | 2% | 28.5% | (17.4%) | 0.31 | 28.2% | (18.3%) | 0.12 |
| | 6% | 26.2% | (24.1%) | 0.36 | 23.2% | (32.8%) | 0.17 |
| | 10% | 24.8% | (28.1%) | 0.42 | 20.6% | (40.3%) | 0.20 |
| 15% | 2% | 29.8% | (13.6%) | 0.27 | 29.2% | (15.4%) | 0.11 |
| | 6% | 28.3% | (18.0%) | 0.30 | 26.2% | (24.1%) | 0.14 |
| | 10% | 27.8% | (19.4%) | 0.32 | 24.6% | (28.7%) | 0.15 |

*Compared to an AR of 34.5% in the absence of intervention

in mitigating the pandemic as it results in clinical attack rates below 10%, i.e. within the range of regular seasonal influenza (5%-15%). An additional advantage of this strategy is that it has significant indirect effects in the age groups that are not included in the target populations (i.e. individuals aged 25-64 and ≥65 years). Extending vaccination to include also individuals 25-64 years old is anticipated to result in very low attack rates of approximately 3%. However, once the demand for vaccine for these prioritised groups as well as for individuals 25-64 years old is met, offering vaccination to people over the age of 65 will not offer a notable additional benefit for this age group.

The above findings refer to the best-case scenario where vaccines are available before the onset of the epidemic in the population, such as e.g. in the case of countries of the northern hemisphere with still a small number of influenza A(H1N1) cases. When vaccination is implemented during the epidemic, its impact on the attack rate is predicted to be lower. Under intensive daily coverage, clinical attack rates of approximately 15% may be achieved by initiating vaccination either of the recommended groups early in the epidemic (AR 1%) or of the whole population somewhat later (AR 5%).

In the current analysis, we assumed that the pandemic evolves in a single wave whereas 2-3 waves have been observed in the majority of past pandemics [17,18]. As a result, although the model predicts modest to negligible reductions in the overall attack rate when vaccination is not introduced early during the ongoing epidemic, it might be used to abort the second and third waves [17]. Vaccination strategies were evaluated in a community with the structure of the Greek population (age and sex distribution, number and size of households etc). As a result, the quantitative results reported here are valid for Greece alone. However, due to the similarity in the age structure and household size of the Greek and the European population, results may apply qualitatively to other communities in the European region. A further point that requires caution is that the model was set up such as to simulate the age-specific attack rates of the pandemic influenza A(H1N1) outbreak in the community of La Gloria in Mexico. This particular outbreak provided very useful information as it evolved in the absence of intervention. However, the age-specific attack rates observed in the community of La Gloria might be considered as a worst-case assumption and the proportion of symptomatic infections that will be observed in European countries is likely to be smaller. A final point is that we did not deal explicitly with the time lag between vaccination and effectiveness and the partial efficacy between doses, in case multiple doses are required, but rather combined this delay time with that of production and distribution and refer only to the date at which vaccination becomes effective. Similarly, we have not estimated the number of doses needed to implement the various strategies but rather the number of vaccinated persons.

In conclusion, vaccinating the groups recommended by CDC's ACIP in countries with still a small number of pandemic influenza A(H1N1) cases is anticipated to reduce illness attack rates within the range of seasonal influenza (approximately 10%) with significant indirect effects among individuals older than 24 years who are not included in the target groups. For countries experiencing an ongoing epidemic, initiating vaccination of the recommended groups early might result in attack rates near the upper limit estimates of seasonal influenza.

Funding

The study was funded by the Special Account for Research Grants of the National and Kapodistrian University of Athens (program code number: 70/4/9101).

## References

1. Ferguson NM, Cummings DA, Fraser C, Cajka JC, Cooley PC, Burke DS. Strategies for mitigating an influenza pandemic. Nature. 2006;442(7101):448-52.
2. Carrat F, Luong J, Lao H, Sallé AV, Lajaunie C, Wackernagel H. A 'small-world-like' model for comparing interventions aimed at preventing and controlling influenza pandemics. BMC Med. 2006;4:26.
3. Sypsa V, Hatzakis A. School closure is currently the main strategy to mitigate influenza A(H1N1)v: a modeling study. Euro Surveill. 2009;14(24):pii=19240. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19240
4. Halloran ME, Longini IM Jr, Struchiner CJ. Design and interpretation of vaccine field studies. Epidemiol Rev. 1999;21(1):73-88.
5. Allison MA, Daley MF, Crane LA, Barrow J, Beaty BL, Allred N, et al. Influenza vaccine effectiveness in healthy 6- to 21-month-old children during the 2003-2004 season. J Pediatr. 2006;149(6):755-62.
6. Hoberman A, Greenberg DP, Paradise JL, Rockette HE, Lave JR, Kearney DH, et al. Effectiveness of inactivated influenza vaccine in preventing acute otitis media in young children: a randomized controlled trial. JAMA. 2003;290(12):1608-16.
7. Clover RD, Crawford S, Glezen WP, Taber LH, Matson CC, Couch RB. Comparison of heterotypic protection against influenza A/Taiwan/86 (H1N1) by attenuated and inactivated vaccines to A/Chile/83-like viruses. J Infect Dis. 1991;163(2):300-4.
8. Neuzil KM, Dupont WD, Wright PF, Edwards KM. Efficacy of inactivated and cold-adapted vaccines against influenza A infection, 1985 to 1990: the pediatric experience. Pediatr Infect Dis J. 2001;20(8):733–40.
9. Zangwill KM, Belshe RB. Safety and efficacy of trivalent inactivated influenza vaccine in young children: a summary of the new era of routine vaccination. Pediatr Infect Dis J. 2004;23(3):189–97.
10. Bridges CB, Thompson WW, Meltzer MI, Reeve GR, Talamonti WJ, Cox NJ, et al. Effectiveness and cost-benefit of influenza vaccination of healthy working adults: A randomized controlled trial. JAMA. 2000;284(13):1655-63.
11. Jefferson TO, Rivetti D, Di Pietrantonj C, Rivetti A, Demicheli V. Vaccines for preventing influenza in healthy adults. Cochrane Database Syst Rev. 2007 Apr 18;(2):CD001269.
12. Nichol KL, Lind A, Margolis KL, Murdoch M, McFadden R, Hauge M, et al. The effectiveness of vaccination against influenza in healthy, working adults. N Engl J Med. 1995;333(14):889-93.
13. Campbell DS, Rumley MH. Cost-effectiveness of the influenza vaccine in a healthy, working-age population. J Occup Environ Med. 1997;39(5):408–14.
14. Govaert TM, Thijs CT, Masurel N, Sprenger MJ, Dinant GJ, Knottnerus JA. The efficacy of influenza vaccination in elderly individuals. A randomized double-blind placebo-controlled trial. JAMA. 1994;272(21):1661-5.
15. Basta NE, Halloran ME, Matrajt L, Longini IM Jr. Estimating influenza vaccine efficacy from challenge and community-based study data. Am J Epidemiol. 2008;168(12):1343-52.
16. CDC Advisory Committee on Immunization Practices. CDC Advisors Make Recommendations for Use of Vaccine Against Novel H1N1. 29 July 2009 [cited 28 August 2009]. Available from: http://www.cdc.gov/media/pressrel/2009/r090729b.htm.
17. Glezen WP. Emerging infections: pandemic influenza. Epidemiol Rev. 1996;18(1):64-76.
18. Taubenberger JK, Morens DM. Pandemic influenza--including a risk assessment of H5N1. Rev Sci Tech. 2009;28(1):187-202

# Pandemic H1N1 influenza: predicting the course of a pandemic and assessing the efficacy of the planned vaccination programme in the United States

**S Towers (stowers@purdue.edu)[1], Z Feng[2]**
1. Department of Statistics, Purdue University, West Lafayette, Indiana, United States
2. Department of Mathematics, Purdue University, West Lafayette, Indiana, United States

This article was published on 15 October 2009.
Citation style for this article: Towers S, Feng Z. Pandemic H1N1 influenza: predicting the course of a pandemic and assessing the efficacy of the planned vaccination programme in the United States. Euro Surveill. 2009;14(41):pii=19358. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19358

We use data on confirmed cases of pandemic influenza A(H1N1), disseminated by the United States Centers for Disease Control and Prevention(US CDC), to fit the parameters of a seasonally forced Susceptible, Infective, Recovered (SIR) model. We use the resulting model to predict the course of the H1N1 influenza pandemic in autumn 2009, and we assess the efficacy of the planned CDC H1N1 vaccination campaign. The model predicts that there will be a significant wave in autumn, with 63% of the population being infected, and that this wave will peak so early that the planned CDC vaccination campaign will likely not have a large effect on the total number of people ultimately infected by the pandemic H1N1 influenza virus.

## Introduction

For several years the United States (US) Centers for Disease Control and Prevention (CDC) have had an established protocol for laboratory influenza testing and collection, and dissemination of associated statistics [1]. These statistics are published and regularly updated online [2].

With the recognition of a new, potentially pandemic strain of influenza A(H1N1) in April 2009, the laboratories at the US CDC and the World Health Organization (WHO) dramatically increased their testing activity from week 17 onwards (week ending 2 May 2009), as can be seen in Figure 1. In this analysis, we use the extrapolation of a model fitted to the confirmed influenza A(H1N1)v case counts during summer 2009 to predict the behaviour of the pandemic during autumn 2009.

## Methods

The CDC/WHO influenza count data used in these studies were obtained from the weekly online surveillance reports [2]. At the time of writing, the data up to week 38 (week ending 26 September 2009) were the most recent. However, we observed that in each weekly update the data significantly change for at least five weeks prior to the week of the update, likely due to a large backlog in testing. In this analysis we thus used data only up to week 33 (week ending 22 August).

The pandemic potential of influenza A(H1N1)v was recognised during week 16 (week ending 25 April) [3]. We assumed that there was no time bias in the CDC/WHO seasonal influenza count data prior to that date. Based on the extrapolation of the exponential decline behaviour of regular seasonal influenza prior to week 16 into the temporal region of heightened testing activity, we found that the data after week 20 (ending 23 May) contain no significant time bias. We thus used the data from week 21 to 33 (from 24 May to 22 August 2009).

The behaviour of the H1N1 influenza pandemic over time was modelled using a seasonally forced deterministic Susceptible, Infective, Recovered (SIR) model [4]:

$$dS/dt=-\beta(t)\, SI/N \quad (1)$$
$$dI/dt=\beta(t)\, SI/N - \gamma I, \quad (2)$$

where N=305,000,000*.

We assumed that $\gamma=1/3$ days-1 [5], and that the contact rate, $\beta(t)$, was periodically forced via

$$\beta(t)=\beta_0+\beta_1 \cos(2\pi t) \quad (3)$$

The reproduction number was given by $R_0=\beta(t)/\gamma$.

To simulate the time evolution of the influenza H1N1 pandemic, we assumed an initial number of infective individuals and susceptibles, $I_0=1$* and $S_0=N$, respectively, at an initial time $t_0$. Given particular values of $\beta_0$, $\beta_1$, and $t_0$, we numerically solved equations (1) and (2) to estimate the fraction of the population infected with pandemic H1N1 influenza each week.

We compared the shape of the results of the deterministic model to the shape of the actual pandemic influenza data, and found the parameters $\{\beta_0,\beta_1,t_0\}$ that provided the best Pearson chi-square statistics.

The grid search for the parameters that minimised the chi-square value was performed with parameter ranges:

$\beta_0$ between $0.92\gamma$ to $2.52\gamma$ in increments of $0.02\gamma$,*
$\beta_1$ between $0.05\gamma$ to $0.80\gamma$ in increments of $0.01\gamma$, and*
$t_0$ between weeks -8 to 10 (relative to the beginning of 2009),

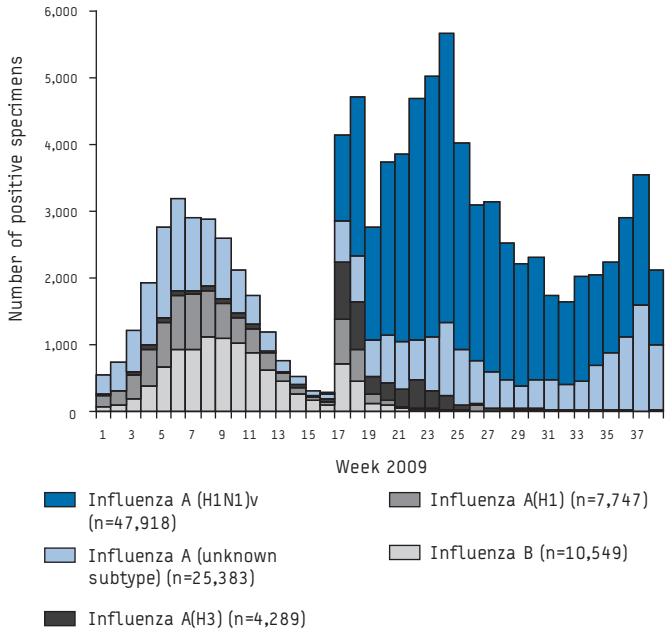
in increments of one week.

The planned CDC vaccination programme against pandemic H1N1 influenza will begin with six to seven million doses being delivered by the end of the first full week in October (week 40), with 10 to 20 million doses being delivered weekly thereafter [6]. We included the effects of this vaccination campaign into

our seasonally forced SIR model by decreasing the number of susceptibles in the population by the corresponding amounts. For healthy adults, full immunity to H1N1 influenza is achieved about two weeks after vaccination with one dose of the vaccine [7,8], and we took this into account in the model by beginning the reduction in susceptibles in week 42 instead of in week 40. We optimistically assumed the higher-end estimate of the planned vaccine roll-out, and we also optimistically assumed that 100% of vaccinated people would achieve full immunity within two weeks.

**Figure 1**

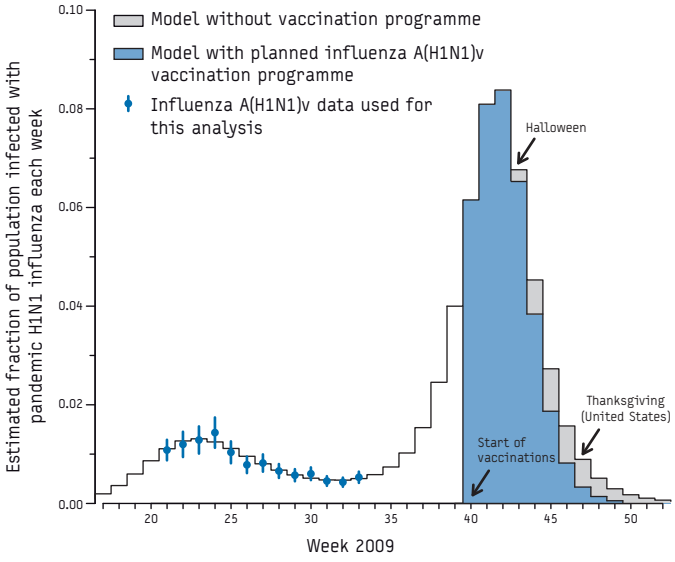
**Influenza-positive tests reported to the US CDC by US WHO/ NREVSS-collaborating laboratories, national summary, United States, 2009 until 26 September**




US CDC: United States Centers for Disease Control and Prevention; NREVSS: National Respiratory and Enteric Virus Surveillance System; WHO: World Health Organization.

**Figure 2**

**Model of the H1N1 influenza pandemic in the United States and prediction for autumn 2009**




## Results

When the seasonally forced SIR model was compared to the influenza H1N1 data, the parameters $\{\beta_0,\beta_1,t_0\}$ that yielded the minimum chi-square value were {1.56, 0.54, 24 Feb 2009}, with 95% confidence intervals (CI) of {1.43,1.77, 0.39,0.54, 8 Feb 2009,7 Mar 2009}.

The best-fit model is shown in Figure 2, with the influenza H1N1 data overlaid. The model predicts that the peak wave of infection will occur near the end of October in week 42 (95% CI: week 39,43), with 8% of the population being infected during that week (95% CI: 6%,13%). By the end of 2009, the model predicts that a total of 63% of the population will have been infected (95% CI: 57%,70%).

When the model was modified to include the effect of the planned vaccination scheme, it predicted a relative reduction of about 6% in the total number of people infected with influenza A(H1N1)v virus by the end of the year 2009 (95% CI: 1%,17%). The predictions of the modified model are shown in Figure 2.

## Discussion

Based on a model with simple harmonic seasonal forcing, the peak of the H1N1 influenza pandemic was predicted to occur between weeks 39 to 43 with 95% confidence. However, it should be noted that the actual periodic function underlying seasonal forcing of influenza has not been well studied, and the uncertainties in the model predictions arising from seasonal forcing assumptions are difficult to quantify.

The 95% confidence interval for $t_0$ predicted by this analysis was [8 Feb 2009, 7 Mar 2009], which is in good agreement with the genetic analysis presented in Fraser *et al.* that found $t_0$ between 3 November 2008 and 2 March 2009 with 95% confidence [9]. Further, the value of $R_0$ predicted by the model between mid-March and the end of April 2009 was between 1.3 and 1.7. This is in agreement with the results presented in Fraser *et al.* , who estimate $R_0$ to be in the range 1.4 to 1.6, based on an analysis of Mexican H1N1 influenza data collected during that time period [9].

We predict that almost two thirds of the US population will be infected with pandemic H1N1 influenza by the end of 2009. However, the serological analysis presented in King *et al.* showed that up to 60% of seasonal influenza infections are asymptomatic [10]. If the same is true of the current pandemic influenza, about a quarter of the population will fall ill.

The most optimistic assumptions about the CDC vaccination campaign yielded a relative reduction of only 6% in the total number of infected individuals. If we assume a 40% symptomatic infection rate, and a mortality rate of between 0.05% and 0.5%, this corresponds to an estimated prevention of between 2,500 and 25,000 deaths. The actual reduction would certainly be lower because 10-30% of adults vaccinated will not achieve immunity [7,8]. Also a large fraction of the population targeted by influenza A(H1N1) vaccinations are children. Vaccination immunity in

children develops at least four weeks after vaccination and would occur too late in the pandemic to make a significant difference to the number of infected in that age group.

The cost benefit analysis involved in devising a pandemic influenza vaccination campaign is extremely complicated, especially due to the ever evolving nature of the pandemic. What we learn from the successes and mistakes of vaccination programmes developed during the current H1N1 influenza pandemic will greatly aid us in decision making during future influenza pandemics.


## Acknowledgements

The authors wish to gratefully acknowledge the partial support of this research by the National Science Foundation via grant DMS-0719697.*


*Authors' correction: On request of the authors, the following corrections were made on 14 January 2010: The population of the US used in the studies was 305,000,000, not 350,000,000 as originally written. The assumed initial number of infective individuals was I0=1, not I0=1/N. $\beta_0$ and $\beta_1$ are expressed in units of gamma. An acknowledgement was added.

## References


1. Questions and Answers: Seasonal Influenza. Atlanta: Centers for Disease Control and Prevention. [accessed 2009 Oct 2]. Available from: http://www.cdc.gov/flu/about/qa/disease.htm
2. FluView: 2008-2009 Influenza Season Week 38 ending September 26, 2009. Atlanta: Centers for Disease Control and Prevention. [accessed 2009 Oct 2]. Available from: http://www.cdc.gov/flu/weekly/fluactivity.htm
3. Influenza-like illness in the United States and Mexico. Geneva: World Health Organization; 22 April 2009. Avaliable from: http://www.who.int/csr/don/2009_04_24/en/index.html
4. Dushoff J, Plotkin JB, Levin SA, Earn DJ. Dynamical resonance can account for seasonal influenza epidemics. Proc Natl Acad Sci USA. 2004;101(48):16915-6.
5. Colizza V, Barrat A, Barthelemy M, Valleron A-J, Vespignani A. Modeling the worldwide spread of pandemic influenza: baseline case and containment interventions. PloS Med. 2007;4(1):e13.
6. Orders for Live Attenuated Influenza Vaccine. Washington: US Department of Health and Human Services [accessed 2009 Oct 2]. Available from: http://www.flu.gov/individualfamily/vaccination/orders.html
7. Hannoun C, Megas F, Piercy J. Immunogenicity and Protective Efficacy of Influenza Vaccination. Virus Res. 2004 Jul;103(1-2):133-8.
8. Clark TW, Pareek M, Hoschler K, Dillon H, Nicholson KG, Groth N, et al. Trial of influenza A (H1N1) 2009 monovalent MF59-adjuvanted vaccine -preliminary report. N Engl J Med. 2009;361. DOI: 10.1056/NEJMoa0907650.
9. Fraser C, Donnelly CA, Cauchemez S, Hanage WP, Van Kerkhove MD, Hollingsworth TD, et al; WHO Rapid pandemic Assessment Collaboration. Pandemic potential of a strain of influenza A (H1N1): early findings. Science. 2009;324(5934):1557-61.
10. King JC Jr, Haugh CJ, Dupont WD, Thompson JM, Wright PF, Edwards KM. Laboratory and Epidemiological Assessment of a Recent Influenza B Outbreak. J Med Virol. 1988;25(3):361-8.

# Resistance of turkeys to experimental infection with an early 2009 Italian human influenza A(H1N1)v virus isolate

**C Terregino[1], R De Nardi[1], R Nisi[1], F Cilloni[1], A Salviato[1], M Fasolato[1], I Capua (icapua@izsvenezie.it)[1]**
1. OIE/FAO and National Reference Laboratory for Avian Influenza and Newcastle disease, OIE Collaborating centre for infectious diseases at the human-animal interface, Istituto Zooprofilattico Sperimentale delle Venezie (IZSVe), Legnaro, Italy

This article was published on 15 October 2009.
Citation style for this article: Terregino C, De Nardi R, Nisi R, Cilloni F, Salviato A, Fasolato M, Capua I. Resistance of turkeys to experimental infection with an early 2009 Italian human influenza A(H1N1)v virus isolate. Euro Surveill. 2009;14(41):pii=19360. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19360

We performed an experimental infection of 21- and 70-day-old meat turkeys with an early human isolate of the 2009 pandemic H1N1 influenza virus exhibiting an α-2,3 receptor binding profile. Virus was not recovered by molecular or conventional methods from blood, tracheal and cloacal swabs, lungs, intestine or muscle tissue. Seroconversion was detected in a limited number of birds with the homologous antigen only. Our findings suggest that in its present form, the pandemic H1N1 influenza virus is not likely to be transmitted to meat turkeys and does therefore not represent an animal health or food safety issue for this species.

## Introduction

Following the emergence of the human pandemic influenza A(H1N1)v virus in spring 2009, questions about the circulation of this virus in an animal reservoir were raised by international organisations. In particular, three aspects appeared to be of relevance, namely implications on animal health, aspects of food safety, and epidemiological aspects related to animals being infected with a human virus and perpetuating a parallel channel of infection in the animal reservoir.

Turkeys (*Meleagris gallopavo*) are highly susceptible to type A influenza virus infection and have been infected in the past with viruses of swine origin [1-4]. In August 2009, infection of two turkey flocks in Chile with the human influenza A(H1N1)v virus was reported [5]. The genetic profile of the virus appeared to be closely related (similarity ranging between 99.7% and 100%) to the strain that was circulating in the human population in Chile at the time [6]. The aim of this experiment was to establish the susceptibility of turkeys of different ages to infection with the human virus and to assess whether it would be detectable in the blood or in tissues of meat birds following administration of a high viral dose.

## Materials and methods

### Animals

Commercially available turkeys were used in this study. The birds originated from a flock that was serologically negative for all avian influenza subtypes, including influenza A(H1N1)v, by agar gel immunodiffusion test (AGID) and enzyme-linked immunosorbent assay (ELISA) and negative for influenza A virus by real-time reverse transcription-PCR (RRT-PCR) on cloacal and tracheal swabs [7]. All animals were identified by means of wing tags and received feed and water *ad libitum*. Birds were housed in negative pressure, high efficiency particulate air (HEPA) filtered isolation cabinets for the duration of the experimental trial.

### Challenge virus and protocol

Challenge of turkeys was carried out with the influenza A virus isolate A/Italy/2810/2009(H1N1). The virus was isolated from a human case detected in Verona, Italy, in specific pathogen-free (SPF) embryonated hens' eggs via the amniotic cavity and was characterised according to chapter on swine influenza in the World Organisation for Animal Health (OIE) Manual of Diagnostics Tests and Vaccines for Terrestrial Animals [8]. The number of virus passages in SPF embryonated hens' eggs was limited to the minimum (two) in order to limit laboratory manipulation and adaptation.

The haemagglutinin (HA) and neuraminidase (N) genes of the virus obtained from nasal swabs of the patient and the HA of the virus obtained from the allantoic fluid after the second passage in eggs, were genetically analysed and sequences were deposited in the EpiFlu database of the Global Initiative on Sharing Avian Influenza Data (GISAID), accession numbers EPI181386, EPI181387 and EPI211620. The A/Italy/2810/2009(H1N1) virus isolate has 99.6% homology with influenza A/California/4/09. The HA gene of the strain grown in eggs, which was used for the infection, contains arginine (Arg) instead of glutamine (Gln) at position 226. This substitution is associated with a receptor binding affinity to α-2,3 sialic acid receptors which are typical of avian viruses and thus bind preferably to avian cells [9].

For viral titration, 100 µl of 10-fold diluted A/Italy/2810/2009 virus was inoculated into five SPF embryonated hens' eggs and the median embryo infectious dose ($EID_{50}$) was calculated according to the Reed and Muench formula [10].

### Molecular tests

*Extraction of RNA*

Viral RNA was extracted from 200 µl of blood using the commercial kit 'NucleoPsin RNA II' (Macherey-Nagel) and from 200 µl of phosphate-buffered saline (PBS) suspension of cloacal and tracheal swabs and homogenised organs using the 'High Pure RNA Isolation Kit' (Roche®) commercial kit.

*Real time RT-PCR (RRT-PCR)*

Published primers and probes [7] targeting the Matrix (M) gene of type A influenza virus were used. The reverse primer M-124 was modified in order to have a perfect match with the M gene sequence of the influenza A(H1N1)v virus isolates. The forward, M-25, and reverse primers were used at the optimised concentration of 300 nM each, the specific fluorescent-labelled probe, M+64, was used at the final concentration of 100 nM. RNA was amplified in a final volume of 25 µl using a QuantiTect Multiplex® RT-PCR kit (Qiagen, Hilden, Germany). The PCR reaction was performed using the RotorGene 6000 (Corbett, Australia) apparatus with the following protocol: 20 minutes at 50 °C and 15 minutes at 95 °C followed by 40 cycles at 94 °C for 45 sec and 60 °C for 45 sec. All samples were also analysed using the RRT-PCR protocols for the M and HA genes recommended by WHO [11].

*Serology*

Type- and subtype-specific antibodies were detected by means of a commercial ELISA (ID-VET®) and AGID tests and by haemagglutination inhibition (HI) test according to the European Union (EU) diagnostic manual [12] using 1% chicken red blood cells. For the HI test, the detection of antibodies to the H1 subtype of avian influenza A virus was performed using four haemagglutinating units of the homologous antigens of the human H1N1v strain (A/Italy/2810/2009), an H1N1 strain of swine origin (A/swine/Italy/711/06) or an avian H1N1 strain (A/duck/Italy/1447/05).

Naïve animals were considered positive with a serologic titre of ≥ 4 log2, as indicated by the EU guidelines.

**Experimental design**

*Experiment 1: Evaluation of the presence of virus in blood, meat and viscera*

A group of 10 70-day-old turkeys were oro-nasally infected with 100µl of the challenge virus containing 107 $EID_{50}$. On days 1, 2, 3, 4 and 5 post infection (p.i.), blood was collected from each bird from the wing vein, mixed with anticoagulant (Alsever's solution 1:1), and the establishment of viraemia was evaluated by RRT-PCR. If blood samples yielded positive results, up to two birds presenting viraemia were killed humanely on the day of testing. When blood samples yielded negative results and no animals showed clinical signs, two turkeys were killed humanely on a random basis. In case of any death, lungs, intestine, superficial and deep pectoral muscles and thigh muscles were collected on the day of death.

*Experiment 2: Evaluation of clinical signs, tracheal and cloacal shedding and seroconversion following experimental infection*

A group of 12 21-day-old turkeys were used in this experiment. All animals were experimentally infected oro-nasally with 100µl of challenge virus containing 107 $EID_{50}$. Twice a day clinical signs were recorded. On days 2, 4, 6, 10 15 p.i. tracheal and cloacal swabs were collected from each bird. On day 14 and 21 p.i. blood samples were collected to evaluate seroconversion.

## Results

Mild, non-specific clinical signs were observed in the 21-day-old birds a few days following administration of the challenge virus. These signs were considered to be non-specific because the birds did not exhibit the conjunctivitis, sinusitis or nasal discharge typical of low pathogenicity avian influenza infection. In both experimental groups, the virological and molecular results from all collected samples were negative. Seroconversion was detected in 41.6%, 8.3% and 33.3% of birds belonging to the younger age group by ELISA, AGID and HI tests (only with the homologous antigen), respectively. The results are presented in detail in the Table.

## Discussion

The data reported here indicate that both 21- and 70-day-old turkeys are resistant to infection with early strains of the human influenza A(H1N1)v virus. Notwithstanding the high infectious dose and the mutation Arg to Gln in 226 of the HA gene, it was not possible to achieve infection or to detect virus in blood, respiratory and enteric organs or in muscles of experimentally infected birds. What is surprising is the evidence of seroconversion in a proportion of the infected poults. Since active infection was not achieved, it is likely that the seroconversion is related to the high viral dose administered. In any case, antibodies were detectable only with the homologous virus, thus indicating that intra-subtypic cross-reactivity was below HI detection limits.

Our findings indicate that unless the human influenza A(H1N1) v virus undergoes substantial changes, the risk that meat turkeys become infected with the virus is negligible. Therefore, there is no reason to be concerned about the animal health or food safety implications of this infection in this species.

**Table**

**Results of serological tests after infection (n=12)**

| | 14 days post infection | | | 21 days post infection | | |
|---|---|---|---|---|---|---|
| Turkey (ID number) | HI* | ELISA | AGID | HI* | ELISA | AGID |
| 71 | n | p | d | n | p | d |
| 72 | 1:64 | p | d | 1:32 | p | d |
| 73 | n | n | n | n | n | n |
| 74 | 1:16 | p | d | 1:16 | d | d |
| 75 | n | n | n | n | n | n |
| 76 | 1:4 | p | d | 1:8 | p | d |
| 79 | n | n | n | n | n | n |
| 80 | n | n | n | n | n | n |
| 81 | 1:32 | p | p | 1:64 | p | p |
| 82 | n | n | n | n | n | n |
| 83 | n | n | n | n | n | n |
| 84 | 1:16 | p | n | 1:16 | p | n |

AGID: agar gel immunodiffusion test; ELISA: enzyme-linked immunosorbent assay.
*Haemagglutination inhibition (HI) test performed with homologous antigen; n= negative; p= positive; d= doubtful.

## References


1. Mohan R, Saif YM, Erickson GA, Gustafson GA, Easterday BC. Serologic and epidemiologic evidence of infection in turkeys with an agent related to the swine influenza virus. Avian Dis. 1981;25(1):11-6.
2. Andral B, Toquin D, Madec F, Aymard M, Gourreau JM, Kaiser C, et al. Disease in turkeys associated with H1N1 influenza virus following an outbreak of the disease in pigs. Vet Rec. 1985;116(23):617-8.
3. Ludwig S, Haustein A, Kaleta EF, Scholtissek C. Recent influenza A (H1N1) infections of pigs and turkeys in northern Europe. Virology. 1994;202(1):281-6.
4. Choi YK, Lee JH, Erickson G, Goyal SM, Joo HS, Webster RG, et al. H3N2 influenza virus transmission from swine to turkeys, United States. Emerg Infect Dis. 2004;10(12):2156-60.

5. ProMED-Mail. Chile finds swine flu in turkeys. Available from: http://www.promedmail.org/pls/otn/f?p=2400:1001:19224::::F2400_P1001_BACK_PAGE,F2400_P1001_ARCHIVE_NUMBER,F2400_P1001_USE_ARCHIVE:1001,20090821.2961,Y.

6. ProMED-Mail. Transmission of A (H1N1) 2009 virus from human to birds. Available from: http://www.promedmail.org/pls/otn/f?p=2400:1001:19224::NO::F2400_P1001_BACK_PAGE,F2400_P1001_PUB_MAIL_ID:1004,78988.

7. Spackman E, Senne DA, Myers TJ, Bulaga LL, Garber LP, Perdue ML, et al. Development of a real-time reverse transcriptase PCR Assay for type A influenza virus and the Avian H5 and H7 hemagglutinin subtypes. J Clin Microbiol. 2002;40(9):3256-60.

8. World Organisation for Animal Health (OIE). Manual of Diagnostic Tests and Vaccines for Terrestrial Animals, pp.1128-1138. Paris; 2009. Available from: http://www.oie.int/eng/normes/mmanual/A_summry.htm

9. Mochalova L, Gambaryan A, Romanova J, Tuzikov A, Chinarev A, Katinger D, et al. Receptor-binding properties of modern human influenza viruses primarily isolated in Vero and MDCK cells and chicken embryonated eggs. Virology. 2003;313(2):473-80.

10. Reed LJ, Muench H. A simple method of estimating fifty percent endpoints. American Journal of Hygiene. 1938;27:493-7.

11. World Health Organization. WHO information for laboratory diagnosis of pandemic (H1N1) 2009 virus in humans-update. Geneva: WHO; 2009. Available from: http://www.who.int/csr/resources/publications/swineflu/WHO_Diagnostic_RecommendationsH1N1_20090521.pdf.

12. Commission Decision 2006/437/EC of 4 August 2006 approving a Diagnostic Manual for avian influenza as provided for in Council Directive 2005/94/EC. Official Journal of European Union. 31 August 2006. Available from: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2006:237:0001:0027:EN:PDF

# Pandemic influenza A(H1N1) 2009 vaccines in the European Union

**K Johansen (Kari.Johansen@ecdc.europa.eu)[1], A Nicoll[1], B C Ciancio[1], P Kramarz[1]**
1. European Centre for Disease Prevention and Control, Stockholm, Sweden

This article was published on 15 October 2009.
Citation style for this article: Johansen K, Nicoll A, Ciancio BC, Kramarz P. Pandemic influenza A(H1N1) 2009 vaccines in the European Union. Euro Surveill. 2009;14(41):pii=19361. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19361

Pandemic vaccines from four manufacturers are now available for use within the European Union (EU). Use of these vaccines will protect individuals and reduce the impact on health services to more manageable levels. The majority of the severely ill will be from known risk groups and the best strategy will be to start vaccinating in line with the recommendation from the European Union Health Security Committee prioritising adults and children with chronic conditions, pregnant women and healthcare workers. The composition of authorised vaccines is reviewed in this article. The vaccine strain in all authorised pandemic vaccines worldwide is based on the same initial isolate of influenza A/California/7/2009 (H1N1)v but the vaccines differ in conditions for virus propagation, antigen preparation, antigen content and whether they are adjuvanted or not. The vaccines are likely to be effective since no significant genetic or antigenic drift has occurred and there are already mechanisms for estimating clinical effectiveness. Influenza vaccines have good safety records and no safety concerns have so far been encountered with any of the vaccines developed. However, special mechanisms have been devised for the early detection and rigorous investigation of possible significant side effects in Europe through post-marketing surveillance and analysis. Delivery of the vaccines to the risk groups will pose difficulties where those with chronic illnesses are not readily identifiable to the healthcare services. There is considerable scope for European added value through Member States with excess vaccines making them available to other states.

## Introduction

Vaccines from four manufacturers are now becoming available for protection against pandemic influenza A(H1N1) 2009 infection. Three vaccines have been authorised through the central European Medicines Agency (EMEA) mechanism for use in any European Union (EU) Member State (MS) and a fourth vaccine was recently authorised by the Hungarian National Regulatory Agency for use in Hungary (Table 1). The central mechanism was streamlined by rehearsal through use of mock-up protocols and experience of the development of human avian influenza vaccines including human clinical trial data. Within Europe, vaccination is known to have started in the Nordic countries and Hungary and will shortly begin in other EU countries. Pandemic vaccines have during the last few weeks been authorised for use in China, Australia and the United States (US), where vaccination campaigns have also begun.

The new vaccines are important countermeasures to mitigate the effects of pandemic waves in Europe however they are arriving too late and in too low quantities to stop population transmission. Instead, the vaccination strategy will have to be the usual one of influenza vaccination in Europe, namely that of protecting the vulnerable [1,2].

Adherence to pandemic vaccine recommendations issued in the vaccine campaigns will be dependent on the current view of the pandemic in the general public, and more specifically among target groups recommended by the European Union Health Security Committee (HSC) / Early Warning and Response System (EWRS) for the initial rounds of vaccinations: healthcare workers, risk groups with underlying conditions and pregnant women [2]. Availability of sound data on safety and effectiveness will also be of importance.

## Vaccine composition

The composition of the authorised European pandemic vaccines differ significantly in conditions for virus propagation, antigen preparation, antigen content and whether they are adjuvanted or not (Table 1).

The vaccine strain in pandemic vaccines worldwide is based on the initial isolate of influenza A/California/7/2009 (H1N1)v or a reassortment based on the same isolated strain and a more fast-growing influenza A(H1N1) strain (PR8) which is called influenza A/California/7/2009 (H1N1)v-like. No significant genetic or antigenic drift has occurred since the virus first was isolated in April 2009, which is why these vaccines are expected to be effective against the pandemic waves expected in Europe this winter season. However, the ability of a pandemic influenza vaccine to evoke an immune response against drifted influenza viruses that are different from those included in the formulation would obviously be of major clinical value [3,4] - if such a drift should occur.

Due to limitations in vaccine supply worldwide in the case of a pandemic and the propensity of influenza viruses to antigenic drift, the World Health Organization encouraged development of vaccines with adjuvants when avian flu vaccines were developed. The term is derived from the Latin 'adjuvans' meaning 'to help'. Adjuvants have been used for many years in many vaccines with good effect. In influenza vaccines they can reduce the dose of antigen needed to produce the same immunological (protective) response and improve their ability to provide longer-lasting protection broad enough to cover many antigenic drifted variants. They work naturally by prolonging the exposure time of antigen to the immune system, enhancing the delivery of antigen to antigen-presenting cells, and

providing immunostimulatory signals that potentiate the immune response [5]. In the three current adjuvanted pandemic vaccines the oil-in-water adjuvants (squalene-based) and the aluminium phosphate adjuvant have allowed reduction of the haemagglutinin content per dose by a factor of between two and eight (7.5 µg to 1.875 µg /dose) compared to seasonal influenza vaccines (15 µg/dose) (see Table 1). Squalene is both a natural intermediate product of endogenous human cholesterol metabolism and a component of human cell membranes. It is constantly detected in human blood. It is also found in fish liver oil and vegetable oil (~0.7% in olive oil). When ingested, about 60-80% of squalenes are absorbed from the intestinal tract. The product for vaccine production is isolated from shark liver. There is already a large body of experience from their use in vaccines for humans. No safety concerns of clinical significance have arisen in more than 70 clinical trials with squalene-containing adjuvants. A seasonal influenza vaccine containing the MF59 adjuvant, Fluad, has been used since 1997 with over 40 million doses distributed. The MF59 safety database includes to this date information on more than 20,000 individuals [6]. The AS03 adjuvant contains two oils, squalene and DL-α-tocopherol (vitamin E), both with immunostimulating capacity. DL-α-tocopherol is a nutrient and the daily requirement for humans is 20-30 mg. The safety database for AS03 includes more than 10,000 individuals [personal communication GSK Biologicals].

Both squalene-based adjuvants, MF 59 and AS03, have been shown to induce more local or systemic reactions within three days of vaccination than non-adjuvanted vaccines but there are no major reactions reported [6,7].

The aluminium phosphate adjuvant has been used extensively in vaccines for the past 5-6 decades, and particularly in Hungary in the seasonal influenza vaccine, and has enabled the manufacturer to reduce the dose almost three-fold (see Table 1) [8].

One of the European pandemic vaccines is non-adjuvanted. This is an inactivated wild-type whole-virion vaccine. To reduce early experiences with seasonal influenza vaccines with increased reactogenicity seen with vaccines based on the whole-virion concept compared to split and subunit vaccines, current manufacturer have made a dose-reduction of the haemagglutinin from 15 µg to 7.5 µg per dose (see Table 1) and shown that they still provide a robust immune response [9-10].

Three pandemic vaccines contain thiomersal thiosalicylate (ethylmercury, containing 49.6% mercury per weight), a long-used mercury-containing preservative needed to maintain sterility in many vaccines during production and in their final injectable form. The pandemic vaccines contain thiomersal in varying concentration from 5 to 50 µg per dose (see Table 2). Mercury is commonly found as an environmental contaminant in foods, notably in fish and seafood, principally in the form of methylmercury. While exposure to methylmercury varies by country, intake estimates for European consumers are close to internationally established safe intake limits. The Joint FAO/WHO Expert Committee on Food Additives (JECFA) has established a Provisional Tolerable Weekly Intake (PTWI) of 1.6 µg/kg body weight [11]. Acknowledging that there are different chemical forms of mercury: elemental, inorganic and organic, the conclusion is that in view of the recommendations for food products the total dose of thiomersal provided in one or two doses of pandemic vaccine is regarded to be of little significance and harmless to those vaccinated, which is also the experience from many years of its use in other vaccines [12-16].

### Induced immunogenicity

The current European recommendation of two doses for the three centrally authorised vaccines (see Table 1) separated by at least three weeks are based on clinical trials with the avian flu vaccines when two doses were generally needed to achieve a good immunological response [17-19]. Initial reports on immunogenicity

**Table 1**

**Overview of vaccines against pandemic influenza A(H1N1) available in the European Union in October 2009**

| Name, producer | Product description | Culture medium | Haemagglutinin-content | Adjuvant emulsion | Number of doses |
|---|---|---|---|---|---|
| Celvapan, Baxter | Inactivated, whole wild-type virus A/California/7/2009 (H1N1)v | Cell-culture | 7.5 µg | None | All > 6 months 2 x 0.5 mL |
| Pandemrix, GSK | Inactivated, split-influenza, reassortant, A/California/7/2009 (H1N1)v-like strain | Egg-culture | 3.75 µg (per adult dose) | AS03 | >10 years 2 x 0.5 mL |
| | | | 1.875 µg (per pediatric dose) | | 6 months – 9 years 2 x 0.25 mL |
| Focetria, Novartis | Inactivated, surface-influenza antigens (haemagglutinin and neuraminidase), reassortant, A/California/7/2009 (H1N1)v-like strain | Egg-culture | 7.5 µg | MF59 | All > 6 months 2 x 0.5 mL |
| Fluval P, Omninvest | Inactivated, whole reassortant virus A/California/7/2009 (H1N1)v-like strain | Egg-culture | 6 µg (per adult dose) 3 µg (per pediatric dose) | aluminium phosphate | Adults and adolescents > 12 years 1 x 0.5 mL Children 3-12 years 1 x 0.25 mL Children 6 months - 3 years* 1 x 0.25 mL (*decision pending) |

using non-adjuvanted and adjuvanted pandemic vaccines from several companies have concluded that a single dose of pandemic vaccine provides an unexpectedly good immune response [20,21]. It is good news that the vaccine strain is so immunogenic and most probably provides rapid protective immunity in the majority of vaccinated individuals. Immunogenicity data from clinical trials using the current pandemic vaccines authorised in Europe will soon become available and if possible the Committee for Medicinal Product for Human Use (CHMP) at the EMEA will then consider whether to adjust the recommendations for all or specific age groups. However, it will be important to determine how long-lasting this immune response will be and EMEA has therefore so far taken a safe course of relying on the evidence from the clinical trials with avian flu vaccines that two doses are needed for a robust long-term immune response.

The long-term immune response will be followed closely in vaccinated individuals and if subsequently one dose is deemed enough to provide a sustained protective immunity at least in healthy adults, more vaccine doses will become available for populations currently not targeted for the initial vaccine doses. However, it is quite possible based on previous experience that young children, individuals with congenital or acquired immunodeficiences and susceptible elderly will need two doses for obtaining a good long-term immune response that will protect them through the whole 2009-10 season.

One European manufacturer of pandemic vaccine (Omninvest, Hungary) recommends one dose to all age groups based on trials with the avian and H1N1 influenza vaccine (Table 1) [8,22].

### Vaccine effectiveness

Immunogenicity does not directly reflect high effectiveness but with the use of specific pandemic vaccines against viruses that are not drifted, vaccine effectiveness is expected to be good. In a pandemic context vaccine effectiveness data should be provided by age group, by number of doses received, and by vaccine brand. This requires very large sample sizes in order to produce reliable effectiveness data in time to contribute to the success of vaccination campaigns. Vaccine effectiveness will be studied on a European level through a project funded by the European Centre for Disease Prevention and Control (ECDC) involving study centres in ten countries (I-MOVE project, coordinated by a research group EpiConcept) [23]. These studies will be based on networks of physicians reporting influenza-like illness (ILI) cases undergoing laboratory testing for influenza. Manufacturers may also undertake separate studies of pandemic vaccine effectiveness as recommended by EMEA. They may use study protocols developed as part of the I-MOVE project and posted on ECDC web portal to improve comparability between studies [24,25].

### Vaccine safety

The safety of the vaccines is of prime concern to the authorities and the public. The safety profiles already observed with seasonal and the human avian flu vaccines containing similar compounds including adjuvants will be applicable to the corresponding vaccines containing the influenza A(H1N1) 2009 pandemic strain and they have been well tolerated. The pandemic H1N1 vaccines from all European manufacturers used in the ongoing clinical trials in healthy children, adults and elderly have so far been well tolerated with only minor side effects. The authorised pandemic H1N1 vaccines undergo the same rigorous manufacturing oversight, product quality testing and lot release procedures that apply to seasonal influenza vaccines. EMEA has in its reviewing process evaluated all available published and unpublished safety data [26] for the three centrally authorised pandemic vaccines and so far has found no safety signals that might indicate an increased risk following the use of these vaccines.

At this stage longer-term safety data cannot be available and associations with very rare conditions can only be ruled out by careful post-marketing surveillance. This is always the case with new vaccines and medicines in general at the moment of their introduction. Those monitoring vaccine safety, will keep a special watch for increased incidence of Guillain-Barre syndrome (GBS). GBS is a rare condition and may be associated with several infections; campylobacter, influenza and Epstein-Barr virus [27]. GBS was observed with one crude A(H1N1) vaccine derived from an influenza of swine origin and used in the US in the 1976-7 influenza season. The observed attributable risk for all age groups in the six weeks after vaccination was around nine cases per million vaccines [28]. As the exact causal mechanism of this phenomenon has never been elucidated health officials worldwide will be on alert for reports of GBS this year. However, the overwhelming evidence, including the best study to date in Europe, points to no association of GBS with seasonal influenza vaccines, but instead a documented significant association of GBS with influenza infection itself [29].

Post-marketing surveillance is therefore crucial and will take a number of forms. The routine spontaneous pharmacovigilance system within EU Member States will continue and reports will be sent as usual to the EMEA Eudravigilance database. In addition manufacturers are required to send simplified periodic safety update reports (PSURs) to EMEA. These are usually required on a six-month basis but that has been reduced to monthly reporting. In addition, ECDC in collaboration with a consortium of researchers

**Table 2**

**Overview of thiomersal and immunostimulating compounds* included in vaccines against pandemic influenza A(H1N1) available in the European Union in October 2009**

| | Thiomersal | Adjuvant emulsion |
|---|---|---|
| Celvapan, Baxter | No | None |
| Pandemrix, GSK | 5 µg (per adult dose)<br>2.5 µg (per pediatric dose) | **AS03**<br>squalene* 10.69 mg<br>α-tocopherol* 11.86 mg<br>polysorbate 80 4.86 mg<br>per adult dose;<br>half the above amounts per pediatric dose |
| Focetria, Novartis | 50 µg | **MF59**<br>squalene* 9.75 mg<br>polysorbate 80 1.175 mg<br>sorbitan trioleate 1.175 mg |
| Fluval P, Omninvest | 50 µg (per adult dose)<br>25 µg (per pediatric dose) | aluminum phosphate<br>0.33 mg $Al^{3+}$<br>(per adult dose)<br>0.165 mg $Al^{3+}$<br>(per pediatric dose) |

(VAESCO) are developing complementary vaccine safety monitoring and hypothesis testing through linkage of large computerised clinical databases and immunisation registries (http://vaesco.net/internet/en/index.html) [30].

As with many vaccines, several of the pandemic vaccines are being produced in formulations that contain thiomersal. Multiple analyses showed no increased risk of adverse events associated

**Table 3**

**Recommendations and guidance of various bodies concerning priority groups / target groups for specific pandemic vaccines against pandemic influenza A(H1N1) 2009**

| Key contents from the three organisations | World Health Organization Strategic Advisory Group of Experts (7 July 2009) | United States Centers for Disease Control and Prevention Advisory Committee on Immunization Practices (28 August 2009) Limited supply | United States Centers for Disease Control and Prevention Advisory Committee on Immunization Practices (28 August 2009) Plentiful supply option | European Union Health Security Committee (25 August 2009) |
|---|---|---|---|---|
| **General considerations and criteria for selecting the priority and target groups** | 'SAGE suggests the following groups for consideration, noting that countries need to determine their order of priority based on country-specific conditions:' | 'ACIP recommends that vaccination efforts should focus initially on persons in five target groups (below). In the event that vaccine availability is unable to meet initial demand, priority should be given to a subset of the five target groups (below).' **No priority order between the categories below** | 'ACIP recommends that vaccination efforts should focus initially on persons in five target groups (below).' **No priority order between the categories below** | 'It should be stressed that it is within the mandate and responsibility of Member States to develop a vaccination strategy for influenza A(H1N1) 2009.' **No priority order between the categories below** |
| **Priority and target groups** | **Healthcare workers** - all countries should immunise their healthcare workers as a first priority to protect the essential health infrastructure | **Healthcare workers and emergency medical services personnel** - who have direct contact with patients or infectious material | **Healthcare and emergency medical services personnel** | **Healthcare workers** |
| | **Pregnant women** – since this group appears to be at increased risk for severe disease. | **Pregnant women** | **Pregnant women** | **Pregnant women** |
| | **Individuals aged >6 months with one of several chronic medical conditions** – in order to reduce morbidity and mortality | **Children and adolescents aged 5–18 years who have medical conditions** that put them at higher risk for influenza-related complications | **Persons aged 25-64 years who have medical conditions that put them at higher risk for influenza-related complications.** | **All persons from 6 months of age up with underlying chronic conditions** - increasing the risk for severe disease, starting with the ones who have a severe underlying condition (e.g. severe asthma, unstable coronary heart disease, uncompensated heart failure, etc.) |
| | **Healthy young adults (aged >15 years and <49 years)** to reduce morbidity and mortality | Persons who **live with or provide care for infants aged <6 months** | **Persons who live with or provide care for infants aged < 6 months** (e.g. parents, siblings and daycare providers) | |
| | **Healthy children** | **Children aged 6 months to 4 years** | **Persons aged 6 months to 24 years** | |
| | **Healthy adults aged >49 years and <65 years** to reduce morbidity and mortality | | | |
| | **Healthy adults aged >65 years** to reduce morbidity and mortality | | | |

with thiomersal-containing vaccines. Based on a recent review, Global Advisory Committee on Vaccine safety (GACVS) concluded that "there is no evidence supporting any change in WHO's recommendations for thiomersal-containing vaccines" [31].

### Risk benefit analyses and risk communication for making informed choices

Risk benefit analysis is more difficult than usual given an infection that has mild effect on most people but causes severe disease in some individuals, nevertheless it is clear that people in the target groups should be immunised including healthcare workers [32,33]. A European strategy for benefit-risk monitoring of the pandemic influenza A(H1N1) vaccine has been agreed upon by EMEA and ECDC. It is important that those being offered the vaccines are given clear guidance and information on the likelihood of them being affected by the pandemic influenza A(H1N1) 2009 virus and of experiencing severe outcomes to enable them to make informed choices. The most recent risk assessment from ECDC reports the experience from countries in the southern hemisphere temperate zone. These are countries that have experienced the first winter of transmission [33]. While it cannot be assumed that the experience in Europe will be identical they give the best broad idea of what can be expected [34]. In countries such as Australia, Chile and New Zealand clinical attack rates were not high. However, there were pressures experienced by primary care and hospital services, especially intensive care units [35,36]. The demand on secondary and higher levels of care have mostly, though not entirely, come from sick people from the risk groups (Table 3). Hence the emphasis on these groups recommended by the European Union Health Security Committee (HSC) / Early Warning and Response System (EWRS) [2,37]. Individuals with chronic underlying diseases are at greater risk of developing severe disease. Among the hospitalised and fatal cases, 60-70% suffer from some underlying condition [38]. Estimates for case fatality rates are under 0.1% but it is still expected that most pandemic influenza-associated deaths will be in younger adults (those under the age of 60 years) [36]. This estimated case fatality rate is lower than seen in any of the 20th century pandemics. It should be mentioned here that 12-22 deaths per week have been observed in EU and EEA Member States since 1 September 2009.

Among healthy individuals, pregnant women and young children are at greatest risk of severe disease [39]. In the US the estimated rate of admission to hospital has been four to five times higher in pregnant women than in the non-pregnant women general population (0.32 per 100,000 pregnant women, 95% CI 0.13 – 0.52 vs 0.076 per 100,000 population at risk, 95% CI 0.07-0.09). Whether the risk of severe disease increases with gestational age, as it does for seasonal influenza, is not known yet [40]. Providing vaccines to pregnant women will also protect their infants through maternal antibodies as these children cannot be immunised until six months of age. The description of the first fatal case series in children has been published in the US and it is expected that this information will inform parents' decisions [41]. Similarly to cases in adults, chronic underlying conditions were a risk factor and only a third of the children who died had previously been healthy.

These kinds of data are not yet available from Europe and apart from the above US study concerning pregnant women, more analyses are necessary to answer the questions EU citizens offered vaccination will reasonably ask: If I am affected what is my risk of going into hospital or dying from the infection? What is the risk for my asthmatic son? My handicapped sister? My elderly father? We also need to be sure that the risk groups are the same for Europe as they are for North America and the southern hemisphere [42].

The overall picture is complicated by the fact that although there are some healthy people who experience severe disease in this pandemic (usually they constitute up to 30% of a series of severe cases) the indications are that most of those infected will experience a mild self-resolving disease. Hence the challenge for those promoting vaccination to healthy people is considerable. They have to convey that if healthy adults and children are infected they will most likely not get very ill, however, at the same time there is a small risk of severe disease or even death. For healthcare workers it is important to ensure that vaccines are readily available and to remind them of their responsibility not to infect their much more vulnerable patients [43].

### Vaccination scares

With the implementation of the vaccination campaigns there will be vaccine scares because of coincidence alone, i.e. temporal but not necessarily causal association [44]. For example with the average background incidence of GBS of 1-2 cases per 100,000 population per year it can be expected that in a country of 20 million inhabitants 200-400 cases of GBS per year or four to eight cases per week are registered [45]. If some of these cases occur in temporal proximity to vaccination, concerns may be raised about the association with the vaccine. Special challenges for safety surveillance are related to the fact that some of the groups being immunised initially, such as pregnant women and people with chronic illnesses, are anyway more likely to experience complications including spontaneous abortion or reactivation of the chronic disease. Proper and timely investigation of suspected cases and rapid assessment will be crucial. From recent experience, for example with the HPV vaccines, it can be expected that once proper investigations are undertaken the scares will most often turn out to be the result of coincidence not causation. However that will not be assumed and plausible (and probably some non-plausible), observed associations will be investigated and tested. One attractive prospect of European added value is that observations and a hypothesised relationships from one country can be tested in several other countries enlarging the sample size to test and data may be shared.

### Vaccine availability and delivery

The newly authorised pandemic vaccines are now available to European populations. The challenging problem is that much of the manufacturing capacity is already spoken for through advance purchasing contracts held by some but not all European countries. In addition, vaccines will be produced gradually, so initially there will be a limited supply of vaccine doses in Europe and elsewhere. Prioritisation activities have therefore been viewed necessary.

Several governmental and other official organisations worldwide have provided guidance or recommendations on who should be offered vaccine first [46] (Table 3). The priority groups identified in the Table should serve as indication only and countries may wish to adapt, and some have already done so, the prioritisation in line with their epidemiology, health service provision and resources. All organisations have listed healthcare workers, pregnant women and persons with underlying medical conditions as the first three priority groups. These groups were also agreed on by EU Member States through the Health Security Committee (HSC) and Early Warning and Response System (EWRS) [2]. Vaccinating people with chronic

conditions will be difficult in countries where primary care services do not maintain ready lists of such individuals.

The World Health Organization has asked wealthy countries to help poorer ones to purchase limited amounts of these vaccines – cost should not be a barrier to access. A number of the best provisioned European countries and vaccine manufacturers have stated that they would make available vaccine doses to WHO for further distribution. What will be equally challenging is the distribution of vaccines within Europe. Risk will be distributed more evenly than supply. Seasonal influenza vaccines are used very unevenly in Europe. For example, vaccine coverage among people aged 65 years and older varies 40-fold on a per capita basis [47]. If only single doses are needed after review of immune responses to the various vaccines then there will be reasonable expectations that countries ordering late may be able to purchase vaccines from countries that ordered early in large volumes. This possibility was envisaged at the extraordinary EU Health Council under the Swedish Presidency on 12 October [48]. There are contractual and liability barriers that will need to be solved but it should be hoped that the sharing of influenza vaccines will show a good example of European added value.

## References


1. Nicoll A, Ciancio BC, Tsolova S, Blank PR, Yilmaz C. The scientific basis for offering seasonal influenza immunisation to risk groups in Europe. Euro Surveill. 2008;13(43):pii=19018. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19018
2. European Union Health Security Committee (HSC) / Early Warning and Response System (EWRS). HSC/EWRS statement on influenza A(H1N1) 2009: target and priority groups for vaccination. 25 August 2009 [cited 15 October 2009]. Available from: http://ec.europa.eu/health/ph_threats/com/Influenza/docs/HSC_EWRS_statement_en.pdf
3. Leroux-Roels I, Borkowski A, Vanwolleghem T, Dramé M, Clement F, Hons E, et al. Antigen sparing and cross-reactive immunity with an adjuvanted rH5N1 prototype pandemic influenza vaccine: a randomised controlled trial. Lancet. 2007;370(9587):580-9.
4. Chu DW, Hwang SJ, Lim FS, Oh HM, Thongcharoen P, Yang PC, et al. Immunogenicity and tolerability of an AS03(A)-adjuvanted prepandemic influenza vaccine: A phase III study in a large population of Asian adults. Vaccine. 12 August 2009 [Epub ahead of print]. doi:10.1016/j.vaccine.2009.07.102.
5. Atmar RL, Keitel WA. Adjuvants for pandemic influenza vaccines. Curr Top Microbiol Immunol. 2009;333:323-44.
6. Pellegrini M, Nicolay U, Lindert K, Groth N, Della Cioppa G. MF59-adjuvanted versus non-adjuvanted influenza vaccines: Integrated analysis from a large safety database. Vaccine. 12 September 2009 [Epub ahead of print]. doi:10.1016/j.vaccine.2009.08.101.
7. Rümke HC, Bayas JM, de Juanes JR, Caso C, Richardus JH, Campins M, et al. Safety and reactogenicity profile of an adjuvanted H5N1 pandemic candidate vaccine in adults within a phase III safety trial. Vaccine. 2008;26(19):2378-88.
8. Fazekas G, Martosne-Mendi R, Jankovics I, Szilvasy I, Vajo Z. Cross-reactive immunity to clade 2 strains of influenza virus A subtype H5N1 induced in adults and elderly patients by Fluval, a prototype pandemic influenza virus vaccine derived by reverse genetics, formulated with a phosphate adjuvant, and directed to clade 1 strains. Clin Vaccine Immunol. 2009;16(4):437-43.
9. Ehrlich HJ, Müller M, Fritsch S, Zeitlinger M, Berezuk G, Löw-Baselli A, et al. A cell culture (Vero)-derived H5N1 whole-virus vaccine induces cross-reactive memory responses. J Infect Dis. 2009;200(7):1113-8.
10. Ehrlich HJ, Müller M, Oh HM, Tambyah PA, Joukhadar C, Montomoli E, et al. A clinical trial of a whole-virus H5N1 vaccine derived from cell culture. N Engl J Med. 2008;358(24):2573-84.
11. European Food Safety Authority. Opinion of the Scientific Panel on contaminants in the food chain [CONTAM] related to mercury and methylmercury in food. 24 February 2004 [cited 15 September 2009]. Available from: http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1178620763245.htm
12. Thompson WW, Price C, Goodson B, Shay DK, Benson P, Hinrichsen VL, et al. Early thimerosal exposure and neuropsychological outcomes at 7 to 10 years. N Engl J Med. 2007;357(13):1281-92.
13. Tozzi AE, Bisiacchi P, Tarantino V, De Mei B, D'Elia L, Chiarotti F, et al. Neuropsychological performance 10 years after immunization in infancy with thimerosal-containing vaccines. Pediatrics. 2009;123(2):475-82.
14. Aschner M, Ceccatelli S. Are neuropathological conditions relevant to ethylmercury exposure? Neurotox Res. 16 September 2009 [Epub ahead of print]. doi:10.1007/s12640-009-9113-2.
15. Burbacher TM, Shen DD, Liberato N, Grant KS, Cernichiari E, Clarkson T. Comparison of blood and brain mercury levels in infant monkeys exposed to methylmercury or vaccines containing thimerosal. Environ Health Perspect. 2005;113(8):1015-21.
16. Institute of Medicine of the National Academies. Immunization Safety Review: Vaccines and Autism. Washington, DC: The National Academies Press; 2004. Available from: http://books.nap.edu/openbook.php?record_id=10997
17. European Medicines Agency. Pandemrix. Summary of product characteristics. [cited 15 October 2009]. Available from: http://www.emea.europa.eu/humandocs/PDFs/EPAR/pandemrix/Pandemrix-PU-17-en.pdf
18. European Medicines Agency. Celvapan. Summary of product characteristics. [cited 15 October 2009]. Available from: http://www.emea.europa.eu/humandocs/PDFs/EPAR/celvapan/spc/emea-spc-h982pu17en.pdf
19. European Medicines Agency. Focetria. Summary of product characteristics. [cited 15 October 2009]. Available from: http://www.emea.europa.eu/humandocs/PDFs/EPAR/focetria/spc/emea-spc-h385en.pdf
20. Greenberg ME, Lai MH, Hartel GF, Wichems CH, Gittleson C, Bennet J, et al. Response after one dose of a monovalent influenza A (H1N1) 2009 vaccine - preliminary report. N Engl J Med. 10 September 2009 [Epub ahead of print]. doi:10.10656/NEJMoa0907413.
21. Clark TW, Pareek M, Hoschler K, Dillon H, Nicholson KG, Groth N, et al. Trial of influenza A (H1N1) 2009 monovalent MF59-adjuvanted vaccine - preliminary report. N Engl J Med. 10 September 2009 [Epub ahead of print]. doi:10.1056/NEJMoa0907650.
22. Országos Gyógyszerészeti Intézet (National Institute of Pharmacy). Fluval P szuszpenziós injekció. Betegtajékoztató. (Fluval P suspension for injection. Information for patients) [cited 15 October 2009]. Hungarian. Available from: http://www.ogyi.hu/fluval_p_ogyi_t_20970/
23. Valenciano M, Ciancio BC, Moren A, the influenza vaccine effectiveness working group. First steps in the design of a system to monitor vaccine effectiveness during seasonal and pandemic influenza in EU/EEA Member States. Euro Surveill. 2008;13(43):pii=19015. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19015
24. European Centre for Disease Prevention and Control. Protocol for cohort database studies to measure influenza vaccine effectiveness in the European Union and European Economic Area Member States. Technical document. Stockholm: ECDC; 2009. Available from: http://ecdc.europa.eu/en/publications/Publications/ 0907_TER_Influenza_AH1N1_Measuring_Influenza_Vaccine_Effectiveness_Protocol_Cohort_Database_Studies.pdf
25. European Centre for Disease Prevention and Control. Protocol for case-control studies to measure influenza vaccine effectiveness in the European Union and European Economic Area Member States. Technical document. Stockholm: ECDC; 2009. Available from: http://ecdc.europa.eu/en/publications/Publications/0907_TED_Influenza_AH1N1_Measuring_Influenza_Vaccine_Effectiveness_Protocol_Case_Control_Studies.pdf
26. European Medicines Agency. Pandemic influenza A(H1N1)v vaccines authorised via the core dossier procedure. Explanatory note on scientific considerations regarding the licensing of pandemic A(H1N1)v vaccines. 24 September 2009 [cited 15 October 2009]. Available from: http://www.emea.europa.eu/pdfs/human/pandemicinfluenza/60825909en.pdf
27. Schonberger LB, Bregman DJ, Sullivan-Bolyai JZ, Keenlyside RA, Ziegler DW, Retailliau HF, et al. Guillain-Barre syndrome following vaccination in the National Influenza Immunization Program, United States, 1976-1977. Am J Epidemiol. 1979;110(2):105-23.
28. Tam CC, O'Brien SJ, Petersen I, Islam A, Hayward A, Rodrigues LC. Guillain-Barré syndrome and preceding infection with campylobacter, influenza and Epstein-Barr virus in the general practice research database. PLoS One. 2007;2(4):e344.
29. Stowe J, Andrews N, Wise L, Miller E. Investigation of the temporal association of Guillain-Barre syndrome with influenza vaccine and influenza-like illness using the United Kingdom General Practice Research Database. Am J Epidemiol. 2009;169(3):382-8.
30. Eurosurveillance editorial team. ECDC in collaboration with the VAESCO consortium to develop a complementary tool for vaccine safety monitoring in Europe. Euro Surveill. 2009;14(39):pii=19345. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19345
31. World Health Organization. Meeting of Global Advisory Committee on Vaccine Safety, 18-19 June 2008. Wkly Epidemiol Rec. 2008;83(32):287-92.
32. Jordan R, Hayward A. Should healthcare workers have the swine flu vaccine? BMJ. 2009 Aug 25;339:b3398. doi: 10.1136/bmj.b3398.

33. European Centre for Disease Prevention and Control. Pandemic H1N1 2009. ECDC interim risk assessment. 25 September 2009 [cited 15 October 2009]. Available from: http://ecdc.europa.eu/en/healthtopics/Documents/0908_Influenza_AH1N1_Risk_Assessment.pdf

34. European Centre for Disease Prevention and Control. Revised pandemic 2009 planning assumptions for Europe. 18 September 2009 [cited 15 October 2009]. Available from: http://ecdc.europa.eu/en/activities/sciadvice/Lists/ECDC%20Reviews/ECDC_DispForm.aspx?List=512ff74f%2D77d4%2D4ad8%2Db6d6%2DbfOf23083f30&ID=650

35. Australian Government Department of Health and Ageing. Australia's response and data. [cited 15 October 2009]. Available from: http://www.healthemergency.gov.au/internet/healthemergency/publishing.nsf/Content/resources

36. Baker MG, Wilson N, Huang QS, Paine S, Lopez L, Bandaranayake D, et al. Pandemic influenza A(H1N1)v in New Zealand: the experience from April to August 2009. Euro Surveill. 2009;14(34):pii=19319. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19319

37. European Commission. Influenza A (H1N1): EU Health Security Committee agrees statement on target and priority groups for vaccination. Press release. 25 August 2009 [cited 15 October 2009]. Available from: http://europa.eu/rapid/pressReleasesAction.do?reference=IP/09/1252

38. Jain S, Kamimoto L, Bramley AM, Schmitz AM, Benoit SR, Louie J, et al. Hospitalized patients with 2009 A1N1 influenza in the United States, April-June 2009. N Engl J Med. 8 October 2009 [Epub ahead of print]. doi:10.1056/NEJMoa0906695.

39. Jamieson DJ, Honein MA, Rasmussen SA, Williams JL, Swerdlow DL, Biggerstaff MS, et al. H1N1 2009 influenza virus infection during pregnancy in the USA. Lancet. 2009;374(9688):451-8.

40. Skowronski DM, De Serres G. Is routine influenza immunization warranted in early pregnancy? Vaccine. 2009;27(35):4754-70.

41. Centers for Disease Control and Prevention (CDC). Surveillance for pediatric deaths associated with 2009 pandemic influenza A (H1N1) virus infection - United States, April-August 2009. MMWR Morb Mortal Wkly Rep. 2009;58(34):941-7.

42. The ANZIC Influenza Investigators. Critical Care Services and 2009 H1N1 Influenza in Australia and New Zealand. N Engl J Med. 8 October 2009 [Epub ahead of print]. doi:10.1056/NEJMoa0908481.

43. European Centre for Disease Prevention and Control. Why healthcare workers are a priority group for pandemic influenza A(H1N1) vaccination? 6 October 2009 [cited 15 October 2009]. Available from: http://ecdc.europa.eu/en/activities/sciadvice/Lists/ECDC%20Reviews/ECDC_DispForm.aspx?List=512ff74f%2D77d4%2D4ad8%2Db6d6%2DbfOf23083f30&ID=664

44. Griffin MR, Braun MM, Bart KJ. What should an ideal vaccine postlicensure safety system be? Am J Public Health. 2009 Oct;99 Suppl 2:S345-50.

45. van Doorn PA. What's new in Guillain-Barré syndrome in 2007-2008? J Peripher Nerv Syst. 2009;14(2):72-4.

46. European Centre for Disease Prevention and Control. ECDC Interim Guidance. Use of specific pandemic influenza vaccines during the H1N1 2009 pandemic. Stockholm: ECDC; August 2009 [cited 15 October 2009]. Available from: http://www.ecdc.europa.eu/en/publications/Publications/ 0908_GUI_Pandemic_Influenza_Vaccines_during_the_H1N1_2009_Pandemic.pdf

47. Mereckiene J, Cotter S, Nicoll A, Lévy-Bruhl D, Ferro A, Tridente G, et al. National Seasonal Influenza Vaccination Survey in Europe, 2008. Euro Surveill. 2008;13(43):pii=19017. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19017

48. Swedish Presidency of the European Union. EU health ministers on the new influenza. Press release. 12 October 2009 [cited 15 October 2009]. Available from: http://www.se2009.eu/en/meetings_news/2009/10/12/eu_health_ministers_on_the_new_influenza

# A foodborne outbreak of norovirus gastroenteritis associated with a Christmas dinner in Porto, Portugal, December 2008

**J R Mesquita[1,2], M SJ Nascimento (saojose@ff.up.pt)[1]**
1. Department of Microbiology, Faculty of Pharmacy, University of Porto, Portugal
2. Veterinary Section, Agrarian Superior School, Polytechnic Institute of Viseu, Portugal

This article was published on 15 October 2009.
Citation style for this article: Mesquita JR, Nascimento MS. A foodborne outbreak of norovirus gastroenteritis associated with a Christmas dinner in Porto, Portugal, December 2008. Euro Surveill. 2009;14(41):pii=19355. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19355

An outbreak of acute norovirus gastroenteritis was detected and epidemiologically linked to a Christmas dinner reunion of 22 recent graduate students in a restaurant in Porto, Portugal, in December 2008. A retrospective cohort study was carried out using online standardised questionnaires. Sixteen primary and three secondary cases were identified and the risk ratios with 95% confidence intervals for each food item were calculated. The response rate to the online questionnaires was 96%. The outbreak met all four Kaplan's criteria and the attack rate was 73%. Norovirus GII.4 2006b was detected in stools and emesis samples of two primary cases. The ingestion of soup and lettuce salad was considered a risk factor for this norovirus outbreak, as determined by statistical analysis. Our investigation demonstrated two routes of transmission of norovirus starting with foodborne exposure followed by secondary person-to-person spread. To our knowledge this is the first study identifying norovirus as the causative agent of a foodborne outbreak in Portugal.

## Background

Noroviruses are the leading cause of foodborne outbreaks of acute gastroenteritis and the most common cause of sporadic infectious gastroenteritis among persons of all ages [1-6]. In the present study we describe the investigation by statistical and virological methods of what we think to be the first report of a foodborne norovirus outbreak in Portugal. On 27 December 2008, a group of 22 former students of the University of Porto, now living in different regions of Portugal and abroad, gathered at a Christmas dinner party. This meeting was the only person-to-person contact that this group had had in months. They sat at two different tables (with 4 and 18 individuals, respectively) and were served separately without any contact between the two tables during the meal. Symptoms of loose stools and vomiting appeared 24 hours after the dinner in a 28-year-old couple from the group. This couple had not shared any other meal since they had spent Christmas holidays away from each other. The dehydration was so severe that they required hospitalisation. They received intravenous fluid therapy and oral loperamide in order to recover fluid balance, oral metoclopramide for nausea and emesis and oral omeprazol for gastric and duodenal protection. Both developed fever (39.0ºC - 39.5ºC) and received intravenous paracetamol and antibiotic therapy with oral ciprofloxacin, which was maintained for seven days. At that time no laboratory diagnosis was made for gastroenteritis pathogens. The two patients spent the night in the hospital for observation and received further intravenous fluids now with acetylsalicylic acid for the fever. At that time and based on the symptoms the possibility of a foodborne outbreak was considered. Preliminary investigations of the couple led to the Christmas dinner served to another 20 persons as the most probable origin of infection. A retrospective study was initiated in order to find the full extent of the outbreak and its probable source.

## Methods

### Epidemiological investigation

A list of people who attended the Christmas dinner was retrieved from the index cases, the 28-year-old couple who presented with vomiting, diarrhoea, abdominal pain, nausea and fever. A structured questionnaire was developed and emailed to the 22 participants of the dinner to obtain information about sex, age, food intake,

Table 1

**Comparison of Kaplan's criteria with the primary cases of an outbreak of gastroenteritis in Porto, Portugal, December 2008 (n=16)**

| Kaplan's criteria | Outbreak in Porto |
|---|---|
| 1) Vomiting in > 50% cases | Vomiting in 94% of the cases |
| 2) Duration of illness 12-60 hours | 81% of cases had duration of illness between 12 - 60 hours* |
| 3) Incubation period of 15-36 hours | 94% of cases had incubation period of 15-36 hours |
| 4) Bacterial pathogens not present | Stool samples found negative for bacteria |

*This study questionnaire asked for the duration of illness in terms of days and not in hours. 81% of the cases presented duration of illness between 12 and 60 hours and 19% had duration of illness between 60 and 72 hours.

**Figure 1**

**Geographic distribution of the primary and secondary cases of an outbreak of gastroenteritis in Portugal, December 2008**

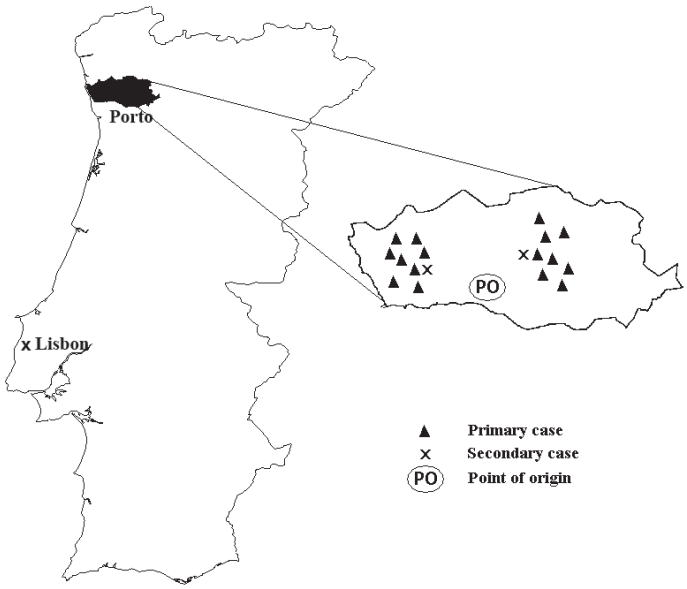



**Figure 2**

**Cases associated with an outbreak of gastroenteritis in Porto, Portugal, December 2008 - January 2009, by date of onset of symptoms (n=19)**

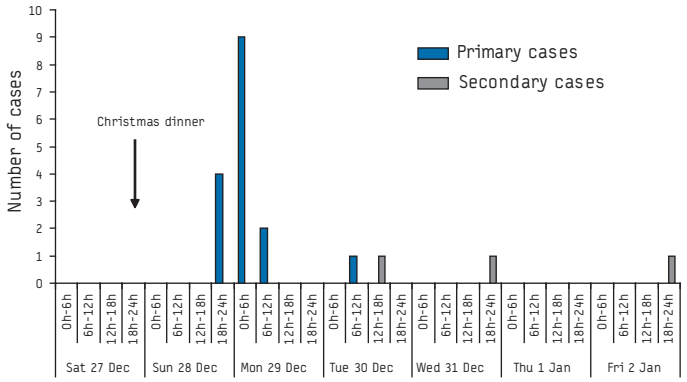



**Table 2**

**Univariate analysis of risk attributed to specific food items consumed during a dinner party associated with an outbreak of gastroenteritis in Porto, Portugal, December 2008**

| Food item | Univariate analysis | | |
|---|---|---|---|
| | RD | RR | 95% CI (RR) |
| Lettuce salad | 0.197 | 1.31 | 0.74-2.32 |
| Iced-cake | 0.197 | 0.76 | 0.43-1.35 |
| French-fries | 0.06 | 0.92 | 0.47-1.79 |
| Soup | 0.385 | 1.63 | 1.06-2.50 |
| Cheese | 0.058 | 0.92 | 0.47-1.81 |
| Bread | 0.047 | 1.07 | 0.45-2.55 |

RD: risk difference; RR: risk ratio; CI: confidence interval;

onset and nature of symptoms and duration of illness. They were also asked to report similar cases in their households and close environment during the same or the following week in order to obtain details about possible secondary cases caused by person-to-person transmission.

Primary case was defined as a person who ate at the restaurant on the night of 27 December 2008 and experienced diarrhoea (alone) or a vomiting episode plus one or more of the following symptoms: abdominal pain, nausea, and fever within 72 hours after the restaurant meal. Secondary case was defined as a close contact (household member) of a primary case who did not participate in the dinner of 27 December and experienced diarrhoea (alone) or a vomiting episode plus one or more of the following symptoms: abdominal pain, nausea, and fever within a two week period after the meal.

The primary attack rate (AR) was calculated as the number of primary cases divided by the total number of people dining at the restaurant on 27 December and therefore possibly exposed to the causative agent.

To measure the association between eating specific food items served at the Christmas dinner and developing illness, Mantel-Haenszel estimates of the risk ratio (RR) with 95% confidence intervals for each food item were calculated.

### Laboratory investigation

Two stool samples and one emesis sample were collected from the couple 36 hours after the Christmas dinner and tested for bacterial, parasitic and viral enteric pathogens. Routine bacterial culture for *Salmonella* and *Shigella* was performed according to standard procedures and microscopic methods were used to screen for protozoa and helminths. Stool specimens were examined for rotavirus and adenovirus by a commercial immunochromatographic test. All samples were examined for the presence of norovirus by reverse-transcription polymerase chain reaction (RT-PCR) using JV12y/JV13i oligonucleotide primers [7] followed by nucleotide sequencing of the RT-PCR products.

## Results

### Epidemiological and clinical characteristics of cases

Of the 22 dinner participants, 21 completed the questionnaire (response rate 96%) and 16 met the primary case definition yielding an overall attack rate of 73%. All cases (nine female and seven male) reported symptoms in compliance with Kaplan's criteria [8,9] (Table 1).

Based on the answers to the questionnaires three further persons were identified who met the definition of secondary case, two of these were parents of two primary cases living in Porto, the third was identified in Lisbon and was a close contact of an asymptomatic person who had participated in the dinner (Figure 1).

The 16 primary cases reported the following clinical symptoms: diarrhoea (n=12, 75%), vomiting (n=15, 94%), abdominal pain (n=8, 50%), nausea (n=7, 44%), fever (n=5, 31%), fainting (n=1, 6%) and asthenia (n=7, 44%). Two persons (the 28-year-old couple) had to be hospitalised because of the severity of dehydration and received intravenous fluids. Among the five dinner participants who did not fully meet the case definition criteria, two had abdominal pain, two reported nausea and three reported asthenia.

Clinical symptoms in the primary cases started abruptly 24-36 hours after the Christmas dinner, on Sunday and Monday, 28-29 December 2008. The mean incubation period was 28 hours (Figure 2). The duration of illness ranged from 12 to 76 hours (mean 45 hours). The last case associated with this outbreak was a secondary case in Lisbon who had onset of symptoms on Friday 2 January 2009, six days after the dinner. This person had contact with one of the asymptomatic guests of the dinner who traveled from Porto to Lisbon on 1 January.

**Food risk assessment**

From the data obtained through the questionnaires on food items consumed at the dinner soup was identified as the most likely source of the outbreak with a RR of 1.63 (95% CI: 1.06-2.50), followed by lettuce salad with a RR of 1.31 (95% CI: 0.74-2.32) (Table 2).

**Laboratory investigation**

Macroscopic analysis of one stool sample revealed live blood. This was confirmed by the presence of erythrocytes by optical microscopy. Both stool samples tested negative for *Salmonella* and *Shigella* and for rotavirus and adenovirus. The two stool samples and the emesis sample tested positive for norovirus. Nucleotide sequencing of the RT-PCR products demonstrated that all three isolates were identical and belonged to genotype GII.4 2006b.

## Discussion

In the present study we describe a foodborne outbreak associated with a dinner in a restaurant in Porto, Portugal. Our combined epidemiological data and virological findings suggested that the causative pathogen was norovirus which was detected from the faecal and vomit specimens obtained from the couple who required hospitalisation. This strain was identified as a GII.4 2006b which has been predominant at a global scale for the past three years [10,11]. The involvement of other enteric pathogens in this outbreak cannot be ruled out with the exception of *Salmonella, Shigella,* enteric protozoa, helminths, rotavirus and adenovirus for which the faecal samples tested negative. The treatment of the hospitalised couple with loperamide is questionable since the use of antimotility agents in severe gastroenteritis may be harmful [12]. Normally, except the rehydration therapy, no further drugs are necessary in viral gastroenteritis treatment. The clinical and epidemiological characteristics of this outbreak including an attack rate of 73%, a mean incubation period of 28 hours, and a mean duration of illness of 45 hours as well as the occurrence of secondary cases are in accordance with a norovirus outbreak. Moreover, this cluster of cases met all four epidemiological criteria for a norovirus outbreak [8,9].

No definitive conclusion on the source of this outbreak could be reached, since food samples were not available for norovirus detection. However a foodborne origin was supported by the analysis performed with the web-based tool developed by the Foodborne Viruses in Europe (FBVE) network for the investigation of norovirus food-related outbreaks [13]. Risk associated with individual food item revealed, unexpectedly, that soup, despite being a warm product, was the most likely source of the outbreak based on its highest RR (1.63, 95% CI: 1.06-2-50). Lettuce salad has been frequently associated with norovirus outbreaks [14] and in the present study was also associated with a high RR (1.31, 95% CI: 0.74-2.32). French fries, cheese and bread were not considered a risk factor given their RR (~1). Whether the food was contaminated before arriving at the restaurant or infection was due to poor food handling practices could not be determined since information on hygiene conditions, food handling practices and health status of the restaurant staff were not available.

Our data indicated that there were two routes of transmission in this outbreak. The origin was a foodborne transmission which caused infection in the primary cases who, subsequently, through person-to-person transmission, infected secondary cases among household and close contacts. The last case associated with this outbreak was detected six days after the dinner in a person resident in Lisbon who had contact with one of the participants of the Christmas dinner group. Although no laboratory confirmation was performed, the Lisbon case met in full the definition of secondary case, but the possibility that this patient was not associated with the outbreak cannot be ruled out.

To our knowledge this is the first study identifying norovirus as the causative agent of a foodborne outbreak in Portugal.


## Acknowledgements

We thank Jan Vinjé and Leslie Barclay of the National Calicivirus Laboratory of the Centers for Disease Control and Prevention, Atlanta, GA, United States, for helpful discussions and for sequencing and phylogenetic analysis.


## References


1. Green KY. Caliciviridae: the noroviruses. In: Knipe DM, Howley PM, editors. Fields Virology. Philadelphia: Lippincott Williams & Wilkins; 2007. p. 949-79.
2. Patel MM, Hall AJ, Vinje J, Parashar UD. Noroviruses: a comprehensive review. J Clin Virol. 2009;44(1):1-8.
3. Patel MM, Widdowson MA, Glass RI, Akazawa K, Vinje J, Parashar UD. Systematic literature review of role of noroviruses in sporadic gastroenteritis. Emerg Infect Dis. 2008;14(8):1224-31.
4. Lopman BA, Reacher MH, Van Duijnhoven Y, Hanon FX, Brown D, Koopmans M. Viral gastroenteritis in Europe: 1995-2000. Emerg Infect Dis. 2003;9(1):90-6.
5. Verhoef L, Boxman I, Duizer E, Rutjes SA, Vennema H, Friesema IH, et al. Multiple exposures during a norovirus outbreak on a river-cruise sailing through Europe, 2006. Euro Surveill. 2008;13(24):pii=18899. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=18899
6. Widdowson MA, Monroe SS, Glass RI. Are noroviruses emerging? Emerg Infect Dis. 2005;11(5):735-7.
7. Vennema H, Bruin E, Koopmans M. Rational optimization of generic primers used for Norwalk-like virus detection by reverse transcriptase polymerase chain reaction. J. Clin. Virol. 2002; 25(2):233-5.
8. Kaplan JE, Feldman R, Campbell DS, Lookabaugh C, Gary GW. The frequency of a Norwalk-like pattern of illness in outbreaks of acute gastroenteritis. Am. J. Public Health. 1982; 72(12):1329-32.
9. Turcios RM, Widdowson MA, Sulka AC, Mead PS, Glass RI. Reevaluation of epidemiological criteria for identifying outbreaks of acute gastroenteritis due to norovirus: United States, 1998–2000. Clin Infect Dis. 2006;42(7):964–9.
10. Verhoef L, Depoortere E, Boxman I, Duizer E, van Duynhoven Y, Harris J, et al. Emergence of new norovirus variants on spring cruise ships and prediction of winter epidemics. Emerg Infect Dis. 2008; 14(2):238-43.
11. Kanerva M, Maunula L, Lappalainen M, Mannonen L, von Bonsdorff CH, Anttila VJ. Prolonged norovirus outbreak in a Finnish tertiary care hospital caused by GII.4-2006b subvariants. J Hosp Infect. 2009;71(3):206-13.
12. Li ST, Grossman DC, Cummings P. Loperamide therapy for acute diarrhea in children: systematic review and meta-analysis. PLoS Med. 2007;4(3):e98.
13. Verhoef L, Kroneman A, van Duynhoven Y, Boshuizen H, van Pelt W, Koopmans M, et al. Selection tool for foodborne norovirus outbreaks. Emerg Infect Dis. 2009;15(1):31-8.
14. Fumian TM, Leite JP, Marin VA. Miagostovich MP. A rapid procedure for detecting noroviruses from cheese and fresh lettuce. J Virol Methods. 2009;155(1):39-43.

# Interference between outbreaks of respiratory viruses

**G Ånestad (gabriel.anestad@fhi.no)[1], S A Nordbø[2,3]**
1. Department of Virology, Division of Infectious Disease Control, Norwegian Institute of Public Health, Oslo, Norway
2. Department of Medical Microbiology, St.Olavs Hospital, Trondheim University Hospital, Trondheim, Norway
3. Institute of Laboratory Medicine, Children´s and Women´s Health, Norwegian University of Science and Technology, Trondheim, Norway

This article was published on 15 October 2009.
Citation style for this article: Ånestad G, Nordbø SA. Interference between outbreaks of respiratory viruses. Euro Surveill. 2009;14(41):pii=19359. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19359

**To the editor:** Norway, like several other European countries, has experienced a delay in the expected outbreaks with pandemic H1N1 influenza. In a recent paper from Sweden it has been postulated that this delay, at least partly, was caused by interference with other respiratory viruses. This view is supported by the fact that a relatively high rhinovirus activity was registered in late summer and early autumn in Sweden [1].

St. Olav's University Hospital in Trondheim, Norway has for several years conducted extensive laboratory surveillance of respiratory viruses including rhinoviruses. The Figure shows the rhinovirus infections diagnosed in Trondheim in the past three years. An increase in diagnosed rhinovirus infections was observed during late summer and early autumn in 2007 and during autumn 2009.

Compared with the complex and enveloped influenza virus particle, rhinoviruses may have advantages at times of the year when the climatic conditions are suboptimal for respiratory viruses. Thus, if the interference theory is correct, rhinoviruses will usually not have any competition with other respiratory viruses during late summer and early autumn, and the interference effect will be obscured. On the other hand, if a competing virus is introduced, the interference activity will be apparent in a delayed outbreak development. As an illustration of this, pandemic H1N1 influenza virus was first diagnosed at St. Olav's Hospial in May 2009, and although a little peak in influenza cases was observed near the end of July 2009, only 5-10% of specimens from patients with influenza-like illness have tested positive for pandemic H1N1 influenza virus. The great majority of these patients were infected with rhinoviruses and to a lesser extent with parechovirus.

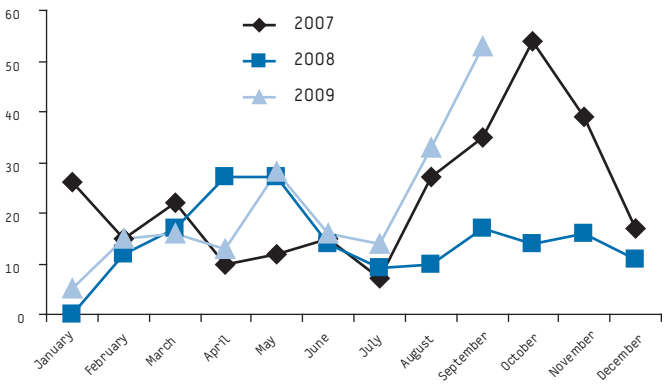
Greer *et al*. observed that co-infections with rhinoviruses and other respiratory viruses were more uncommon than expected, indicating that rhinovirus infection may render the host less likely to be infected with other viruses [2].*

Based on observations in Norway, epidemiological interference between several epidemic viruses including influenza virus has been suggested [3-5]. The present observations may lend some further support to this hypothesis.

***Author's** correction: On request of the authors, the number of rhinovirus infections in September 2009 was corrected in the figure on 21 October 2009, and one sentence was added introducing a new reference.

**Figure**

**Laboratory-confirmed rhinovirus infections, January 2007-September 2009, Trondheim, Norway (n=646)***




## References

1. Linde A, Rotzén-Östlund M, Zweygberg-Wirgart B, Rubinova S, Brytting M. Does viral inteference affect spread of influenza? Eurosurveillance Eurosurveill. 2009;14(40):. pii=19354. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19354
2. Greer RM, McErlean P, Arden KE, Faux CE, Nitsche A, Lambert SB, et al. Do rhinoviruses reduce the probability of viral co-detection during acute respiratory tract infections? J Clin Virol. 2009;45(1):10-5.
3. Ånestad G. Interference between outbreaks of respiratory syncytial virus and influenza virus infection. Lancet. 1982;1(8270):502.
4. Ånestad G. Surveillance of respiratory viral infections by rapid immunofluorescence diagnosis, with emphasis on virus interference. Epidemiol Infect. 1987;99(2):523-31.
5. Ånestad G, Vainio K, Hungnes O. Interference between outbreaks of epidemic viruses: additional Norwegian observations. Scand J Infect Dis. 2009;41(5):381-2.