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Spotlight on measles 2010: Measles outbreak in Ireland 2009-2010

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Measles cases are increasing in Ireland, with 320 cases notified since August 2009. Nearly two-thirds of these cases (n=206) were unvaccinated. In the early stages of the outbreak a substantial number of cases were linked to the Traveller community with some cases also reported among the Roma community, other citizens from eastern Europe and children whose parents objected to vaccination. By February 2010, there had been considerable spread to the general population.

Background

Measles is a highly infectious disease that can result in serious complications. The only way to prevent infection is through measles vaccination. Measles vaccine was introduced in Ireland in 1985; this was followed by the introduction of the combined measles-mumps-rubella (MMR) vaccine in 1988 for children aged 15 months. In 1992, a second dose of MMR was recommended for all children aged 10 to 14 years. In 1995, there was a measles and rubella vaccination campaign for children of primary school-age (5-12 years old). In 1999, the age of the second dose of MMR was changed to 4-5 years. In 2002, the age of the first dose of MMR was changed to 12-15 months, and since 2008 it is recommended at 12 months of age.

In 1985, the year when measles vaccine was introduced, 9,903 measles cases were reported, declining to 201 cases in 1987. However, despite the routine immunisation programme, further major outbreaks have occurred in 1989 (1,248 cases), 1993 (4,328 cases) and 2000 (1,603 cases).

Since the national collation of quarterly MMR₁ (first dose) immunisation uptake statistics commenced in 1999, the MMR₁ uptake rate in those aged 24 months has ranged between 69% (Quarter 4, 2001) and 91% (Quarter 3, 2009) [1]. While the immunisation uptake rate is below the target rate of 95%, measles outbreaks like the one seen in 2000 [2] will continue to occur. In addition, there are subpopulations in Ireland who are highly susceptible to measles, e.g. those who refuse the MMR vaccine and communities with low uptake of MMR due to social exclusion and disadvantage.

Methods

Measles figures presented in this report were based on data extracted from the Computerised Infectious Disease Reporting (CIDR) system on 26 February 2010 and are provisional. Incidence rates were calculated based on population data taken from the 2006 census. Crude area rates and numbers of notified cases are shown according to the eight Health Service Executive (HSE) Area Departments of Public Health.

Case classifications are assigned to notifications in Ireland as per the *Case Definitions for Notifiable Diseases* [3].

The measles case definition is as follows:

Clinical description: Clinical picture compatible with measles i.e. a generalised erythematous rash lasting for more than three days and a temperature over 38°C and one or more of the following: cough, coryza (rhinitis), Koplik's spots or conjunctivitis.

Laboratory criteria for diagnosis are one of the following:

- Detection of measles IgM antibody in the absence of recent vaccination,
- Fourfold or higher rise in measles IgG antibody level in the absence of recent vaccination
- Detection of measles virus (not vaccine strains) in a clinical specimen.

Case classification:

- Possible: clinically compatible cases,
- Confirmed: a case that is laboratory-confirmed or a clinically compatible case which is epidemiologically linked to a confirmed case. A laboratory-confirmed case does not need to meet the clinical case definition.

A measles case is epidemiologically linked if there was exposure to a laboratory-confirmed case during the infectious period (four days before to four days after rash onset) and this exposure occurred within the expected incubation period of the case under investigation, 7 to 18 days (mean 14 days) before rash onset.

while one had no case classification specified. Measles notifications and crude measles incidence rates by HSE Area are shown in Figure 1. The majority (89%) of cases were under 20 years of age with the largest number of cases (21%) in the age group of 1-2-year-olds (Figure 3).

The highest incidence rate was seen in those younger than one year (Figure 4). Of the 320 cases notified, 174 (54%) were male and 144 (45%) were female, while sex was not recorded for two cases (1%).

Of the 320 notified measles cases, 206 (64%) were unvaccinated; 45 (14%) were reported to have had one

dose of MMR; six (2%) were reported to have had two doses of MMR and for 63 (20%) the number of doses of MMR was unknown/not reported. Vaccination dates were reported for one of the six cases with two MMR doses and for 36 cases with one MMR dose (nine of these were vaccinated less than nine days before onset of illness and were probably incubating measles at the time of vaccination).

Of the 320 cases, 115 (36%) were hospitalised and 162 (51%) were not hospitalised, while hospitalisation status was unknown/not reported for 43 (13%). Length of

FIGURE 3

Measles notifications by age group and case classification, Ireland, week 31, 2009 to week 7, 2010

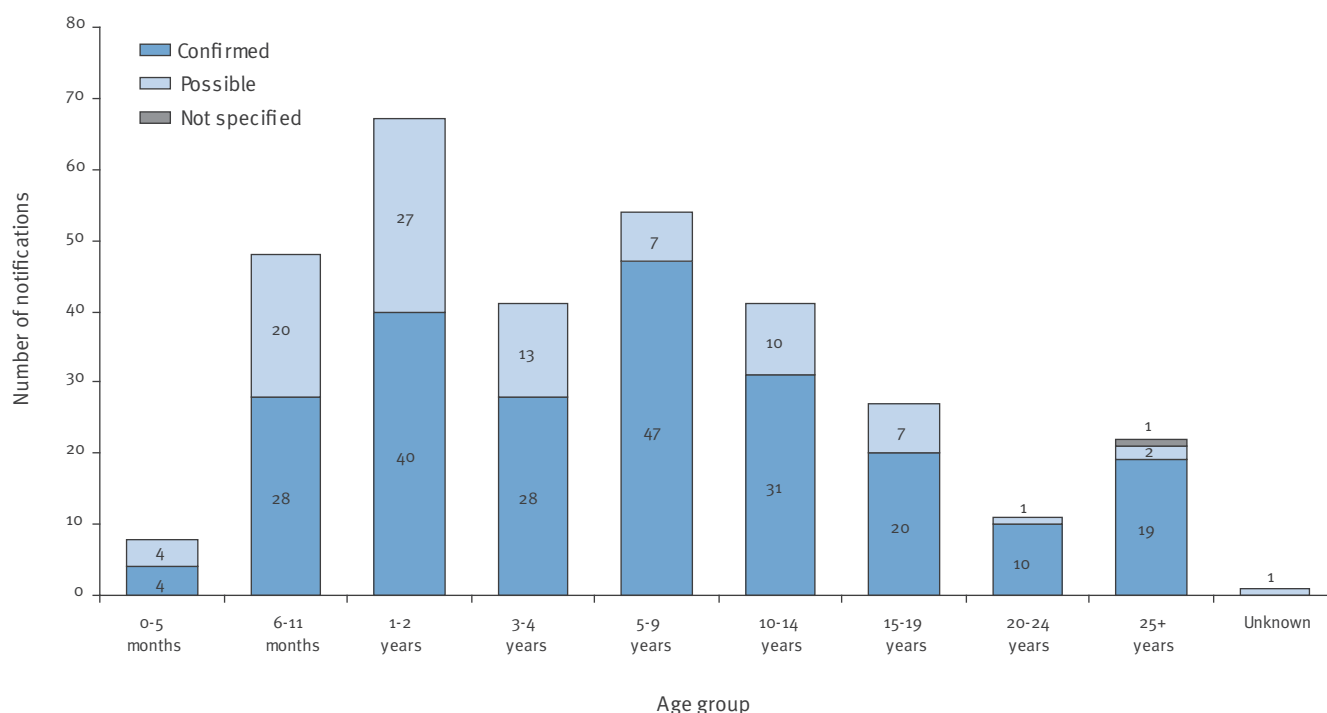
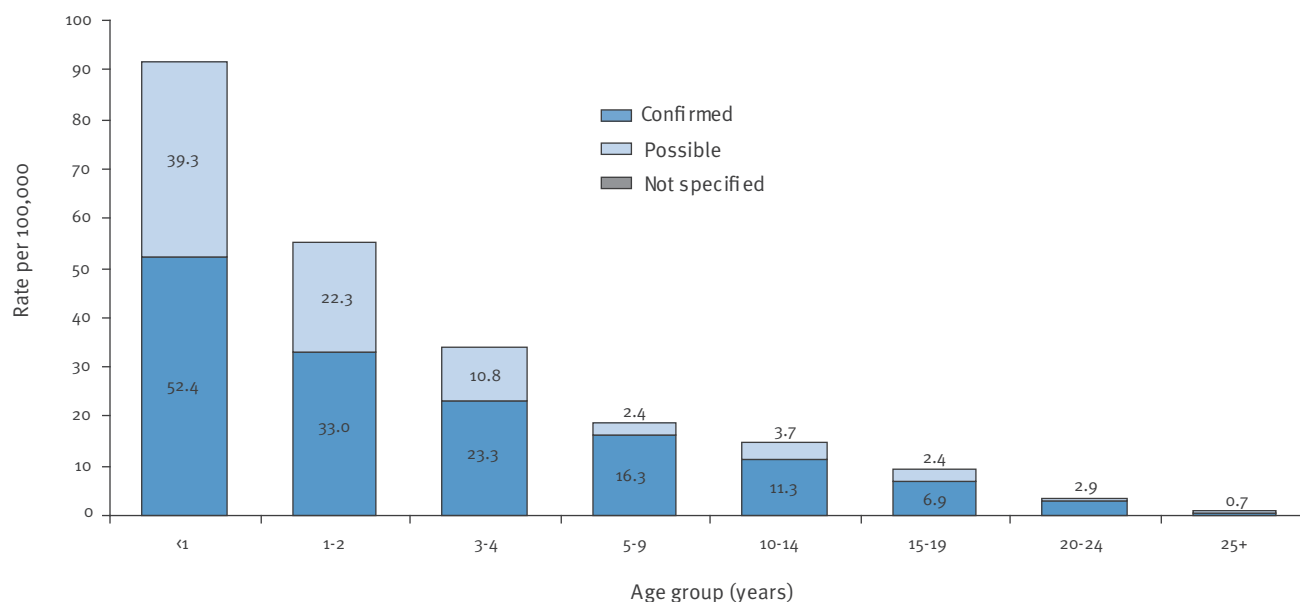


FIGURE 4

Age specific incidence rates of measles notifications, Ireland, week 31, 2009 to week 7, 2010



hospitalisation was reported for 73 cases; the median duration of stay was three days (range one to 11 days).

Complications reported included pneumonia (n=16), ear infection/otitis media (n=4), dehydration (n=2), chest infection (n=1), dehydration, nausea and vomiting (n=1), pharyngitis (n=1), pneumothorax (n=1), seizures (n=1) and tonsillitis (n=1).

National outbreak control team

At the start of the outbreak, a national outbreak control team was convened, which includes health professionals from the departments of public health in the HSE Areas, HSE-Health Protection Surveillance Centre, HSE-National Immunisation Office, HSE Population Health, HSE Social Inclusion, the Institute of Obstetricians and Gynaecologists, the National Virus Reference Laboratory and the field of Paediatric Infectious Disease. This group agreed public health strategies (vaccination and management of cases and close contacts, awareness-raising among clinicians and community) to control the outbreak at national and local level. Some of the guidance and strategies recommended by the outbreak control team are outlined here.

General guidance

All children should be vaccinated at 12 months and 4-5 years, as per the routine childhood immunisation schedule. All children who have not had two MMR vaccines by the age of five years should be offered vaccination opportunistically. Control measures for measles outbreaks were distributed to various settings and healthcare staff and are available on the HPSC website [5].

Traveller community

All Traveller children who have not had two documented doses of MMR are recommended MMR. All Traveller children aged 6-11 months during the current outbreak are recommended MMR (these children are also recommended MMR again at 12 months and at 4-5 years, as per the normal childhood immunisation schedule). Traveller children who have received MMR₁ are recommended MMR₂; MMR₂ may be given one month after the first dose (if children under 18 months of age are given MMR₂ less than three months after MMR₁, these children need a third dose at 4-5 years of age). MMR vaccine clinics and GP sites were organised to provide MMR to the Traveller community. A subgroup of the outbreak control team was established to liaise with social inclusion groups and non-governmental organisations to find ways to increase vaccination among ethnic minority groups.

Contacts of cases

MMR given within 72 hours of exposure may prevent infection. Children in outbreak situations who have received MMR₁ are recommended MMR₂; MMR₂ may be given one month after the first dose.

Healthcare staff

All healthcare staff born since 1978 should either be immune to measles or have had two documented doses

of MMR. Healthcare staff born before 1978 should be offered MMR if they are considered at high risk of exposure. Guidance on preventing measles transmission in healthcare settings (such as rapid triage and case isolation in addition to vaccination) was distributed to healthcare staff and is available on the HPSC website [5].

MMR catch-up campaign

An MMR catch-up campaign is planned for school children aged approximately four to 15 years (older school children aged were previously targeted in an MMR campaign in 2009).

Conclusion

As 29% of cases in this outbreak are currently classified as possible cases, and although the laboratory results of some of these cases are pending at the time of writing, there is a continued need to strengthen measles surveillance in Ireland and ensure rigorous case investigation and laboratory confirmation of all suspected measles cases. This outbreak highlights once again the need for an MMR vaccine uptake of at least 95% to prevent measles outbreaks and the importance of increasing coverage in all groups, in particular those groups who are hard to access. The simultaneous occurrence of the 2009 influenza A(H1N1) pandemic and the ensuing pandemic vaccination programme has put enormous pressure on vaccination teams trying to address MMR defaulters at the same time. There is a concern that this current outbreak may develop into a large outbreak similar to the one that occurred in 2000 [2].

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Observed association between the HA1 mutation D222G in the 2009 pandemic influenza A(H1N1) virus and severe clinical outcome, Norway 2009-2010

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Infection with the recently emerged pandemic influenza A(H1N1) virus causes mild disease in the vast majority of cases, but sporadically also very severe disease. A specific mutation in the viral haemagglutinin (D222G) was found with considerable frequency in fatal and severe cases in Norway, but was virtually absent among clinically mild cases. This difference was statistically significant and our data are consistent with a possible causal relationship between this mutation and the clinical outcome.

The 2009 influenza A(H1N1) pandemic has been characterised by mild and self-limiting disease in the overwhelming majority of cases. However, severe and fatal cases, many of them with primary viral pneumonia, have been occurring in age groups where such clinical outcomes are very rarely seen in seasonal influenza [1,2]. It is important to better understand what viral and host-related factors determine this dichotomy.

Genetic characterisation of clinical specimens

As part of the intensified surveillance carried out during the current influenza pandemic, the national reference laboratory for human influenza at the Norwegian Institute of Public Health collected a large number of respiratory specimens from verified and possible cases of pandemic influenza. In the present study we analysed 61 respiratory specimens from severe and fatal cases that occurred between July and December 2009, as well as from 205 cases with mild clinical outcomes collected between May 2009 and January 2010. Genetic characterisation was performed using conventional sequencing, or with a pyrosequencing assay subsequently developed to detect the particular mutations described below and which facilitated investigation of a large number of specimens.

Here we report the occurrence of an amino acid substitution, aspartic acid to glycine in position 222 (D222G) in the HA1 subunit of the viral haemagglutinin, in clinical specimens from 11 out of 61 cases analysed in Norway with severe outcome. Such mutants were not observed

in any of 205 mild cases investigated (Table), thus the frequency of this mutation was significantly higher in severe (including fatal) cases ($p < 0.001$, Fisher's exact test, two-sided) than in mild cases. D222G mutants were detected throughout the sampling period, from the first recorded severe cases in July until early December. The frequency of another substitution in the same position, D222E, did not differ significantly between mild and severe cases ($p = 0.772$). Yet another substitution, D222N, was observed in a very few cases ($n = 4$), and at a higher rate than expected among severe cases (three of four cases, $p = 0.039$). The wild type 222D was, not surprisingly, significantly less frequent in severe than in mild cases ($p < 0.001$).

In several of the patients where D222G mutant viruses were found, they coexisted with wildtype 222D viruses. Further analysis of this phenomenon is ongoing.

The cases infected with the D222G-mutated virus were not epidemiologically related to each other, and the mutated viruses do not cluster together in phylogenetic analysis (data not shown).

Validity and limitations of the analysis

Cases with severe clinical outcomes were much more likely to be included in our study for several reasons: they are more likely to seek healthcare, they are more likely to be prioritised for virological testing, and their specimens are more likely to be forwarded to the national reference laboratory where they have a higher chance of being selected for detailed analysis than viruses from mild cases. Because of this, we chose to record the frequency of a given genotype in each severity group and compare it with the corresponding frequency in other severity groups. This approach is not expected to have a selection bias.

Cases were classified as mild, severe non-fatal and fatal based on the patient information that was available to us. Some seemingly mild cases may later have exacerbated to severe outcomes without our knowledge, or the presented patient information may have

been incomplete, but we think these cases must be few. On the other hand, all severe and fatal cases were confirmed as non-mild. Thus, the fact remains that only cases confirmed as severe outcomes exhibited the D222G mutation in our investigation.

The sampling period for the cases analysed spans from the initial detections of the pandemic H1N1 virus in early May 2009 until early January 2010. The first severe and fatal cases occurred in July. By the end of December, the epidemic in Norway had largely passed, and a large proportion of cases in our data set is from the peak period in October and November. At all times an effort was made to include a reasonable number of non-severe cases in our analyses, and such cases were well represented throughout the pandemic. The fractions of severe/fatal cases among all analysed cases during the two-month periods July/August (n=21), September/October (n=84), and November/December (n=149), were within the range of 23% to 26%. Severe outcomes were not recorded among the few cases in May and June (n=11) and in January (n=1). We thus do not see a trend over time in the composition of severe versus mild cases in our dataset that could lead to an artificial difference in the frequency of the D222G substitution. Furthermore, the D222G substitution was represented also among the earliest fatal and severe cases in July and August.

Specimens from both the lower and upper respiratory tract were analysed. Lower respiratory tract specimens were available from severe/fatal cases only, and in some cases they were the only materials available. However, in all cases where we had paired upper and lower airway specimens (five cases with 222D and four cases with 222G), the wildtype-versus-D222G pattern was matching between the locations. We have therefore no reason to believe that this difference in proportion of lower airway specimens distorted the analysis.

Discussion

Amino acid position 222 resides in the receptor binding site of the HA protein and may possibly influence the binding specificity and thus the cellular tropism of the virus. The corresponding difference between

two viruses from the 1918 Spanish influenza pandemic correlates to a shift in receptor preference [3], which conceivably could make the virus prone to infect a wider range of cells in the lower respiratory tract [4,5]. However, the effect of a mutation depends on the molecular context and it is unclear whether the binding properties are affected likewise in the present pandemic virus as they were in the 1918 influenza virus.

Our observations are consistent with an epidemiological pattern where the D222G substitution is absent or infrequent in circulating viruses, with the mutation arising sporadically in single cases where it may have contributed to severity of infection. This may aid in filling some knowledge gaps identified in a recent preliminary review of this and other mutations in the pandemic virus [6]. The correlation between presence of the D222G substitution and a severe clinical outcome may reflect an increase in pathogenicity caused by the mutation, possibly related to a change in cellular tropism rendering the virus more pneumotropic. Conversely, it is possible that the likelihood of such mutations arising is higher in patients who fail to fight off the virus rapidly and have virus already colonising the lower respiratory tract. These two possibilities are not mutually exclusive. A large proportion of the fatal and severe cases had underlying risk conditions. However, some of the D222G cases manifested themselves as a rapid unexpected deterioration after a period of mild symptoms in previously healthy subjects, and we consider it likely that there is a causal relationship between the occurrence of the D222G mutation in this virus and severe disease.

It should be borne in mind, however, that the majority of severe and fatal cases investigated did not carry the D222G substitution and, clearly, this mutation is not required for a severe outcome.

Conclusions

To our knowledge, this is the first identification of a change in the pandemic virus that correlates with a severe clinical outcome. However, whereas our data lend statistically significant support to an association between the D222G mutation and severity, the number

TABLE

Pandemic influenza A(H1N1) viruses characterised for amino acid position 222 of the haemagglutinin HA1 domain, by clinical outcome, Norway, May 2009–January 2010 (n=266)

HA1 position 222 genotype ^b	Clinical outcome ^a				
	Mild (n=205)	Severe (n=34)	Fatal (n=27)	Severe plus fatal (n=61)	All cases (n=266)
222D (wt)	92% (189)	82% (28)	59% (16)	72% (44)	88% (233)
222G	0% (0)	8.8% (3)	30% (8)	18% (11)	4.1% (11)
222E	7.3% (15)	2.9% (1)	7.4% (2)	4.9% (3)	6.8% (18)
222N	0.5% (1)	5.9% (2)	3.7% (1)	4.9% (3)	1.5% (4)
Total	100%	100%	100%	100%	100%

^a Clinical outcome based on patient information, assigned into categories by a medical specialist according to WHO guidance criteria [1].

^b Percentage of genotype within each clinical category is given, with number of cases per category in parentheses.

of mild cases would need to be larger to determine whether mutant viruses are indeed circulating at a very low frequency also in non-severe cases. Provided that D222G mutant viruses are not circulating, i.e. that they are less transmissible, the immediate public health impact of this finding is limited. However, it may have implications for the management of severe cases where the virus, if transmitted through massive exposure, may be more virulent than the commonly circulating variant. Furthermore, it may serve as a reminder that the generally very low virulence of the current pandemic virus is not a fixed characteristic, and that there is no reason for complacency in carrying out measures that limit infection with this virus at individual and population level.

Further virological, clinical and epidemiological investigations are needed to ascertain the role of this and other mutations that may alter the virulence and transmissibility of the pandemic influenza A(H1N1) virus.

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Contribution of human metapneumovirus to influenza-like infections in North Greece, 2005-2008

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Following its detection in 2001, human metapneumovirus (hMPV) has repeatedly been reported as a respiratory pathogen, especially in children. This study was aimed at determining the proportion of hMPV infections in patients with influenza-like illness (ILI) during the three influenza seasons 2005-6, 2006-7, 2007-8 in northern Greece. We collected 380 nasopharyngeal swabs or aspirates from ILI patients during the winter seasons 2005-2008 and examined them for influenza viruses and hMPV by one-step real time RT-PCR and nested RT-PCR. Influenza viruses were detected in 151 of the 380 specimens (39.7%) and hMPV in 23 of them (6.05%). Co-infections with hMPV and influenza viruses were observed in seven cases. The majority of the ILI patients (60.5%) were 0-18 years old. However, the incidence of influenza was slightly higher in the age group of 19-60-year-olds, while the incidence of hMPV infections was higher in the age group of 0-18-year-olds. We conclude that hMPV plays an important role as a contributor in influenza-like infections, especially in children. It circulates in the community during influenza seasons and its clinical appearance can be confused with influenza symptoms. However, further research is needed to elucidate the quantitative and qualitative importance of hMPV infections.

Introduction

In 2001, van den Hoogen *et al.* isolated in cultures of tertiary monkey kidney cells a novel paramyxovirus and identified it by random arbitrary polymerase chain reaction (PCR) [1]. The virus was classified tentatively as a new member of the genus *Metapneumovirus* and assigned the provisional name of human metapneumovirus (hMPV). Detection of viral gene sequences by reverse transcription-PCR (RT-PCR) directly from respiratory secretions made it possible to demonstrate rapidly that the virus was occurring worldwide and in all age groups [2].

Influenza viruses, which also circulate in the winter season, are the leading aetiological agents of respiratory tract illness in young adults [3]. In Greece, the influenza season starts in late December and lasts until early April, peaking in January and February [4-6],

and the seasonal surveillance programme on cases of influenza-like illness (ILI) starts earlier in November. The National Influenza Centre for North Greece, one of two National Influenza Centres in Greece, examines clinical samples from ILI patients, including infants and children, every year. The sentinel surveillance system has been responsible for ILI surveillance in Greece since December 1999. The collected data are reported by e-mail to the Hellenic Centre for Diseases Control and Prevention.

The aim of this study was to determine the contribution of hMPV infections to ILI during the three influenza seasons 2005-6, 2006-7 and 2007-8 in North Greece.

Materials and methods

During the three influenza seasons 2005-2008, the National Influenza Centre for North Greece collected 380 nasopharyngeal swabs or aspirates from ILI patients, 214 male and 166 female patients between one month and 85 years of age (mean age 19.94 years). Of these specimens, 158 were collected during the 2005-6 local influenza season, 129 in the season 2006-7) and 93 in the season 2007-8 (Table 1). The ILI patients were further divided into five age groups (0-5 years, 6-10 years, 11-18 years, 19-60 years and >60 years) (Table 2).

The specimens were collected within three days of the onset of symptoms by general practitioners and paediatricians participating in the Greek sentinel influenza surveillance programme, and they were transported to the laboratory in sucrose phosphate medium within 24 hours at a temperature of 4°C. All specimens were accompanied by a sentinel standard form with information on age, sex, date of onset, date of specimen collection, place of residence and clinical features of each patient. All cases reported in this study met the Greek case definition for ILI, which comprises the following symptoms: acute onset, high fever (>38°C), cough, muscle ache and/or headache and malaise. These data were extracted from the summaries prepared by the reporting physicians. As the purpose of this study was

to test only the ILI cases, only patients who met these criteria were included.

Virus detection and typing

Following RNA extraction from clinical specimens, one-step real time RT-PCR for the detection of influenza A and B viruses was performed, using primers specific for matrix protein and nucleoprotein genes of influenza A and B [7]. To subtype the influenza A viruses, a nested RT-PCR was performed targeting the haemagglutinin gene using specific primers provided by the Influchek kit (Euroclone, Italy).

For the detection of hMPV in clinical specimens, a one-step real-time RT-PCR method was used following the protocol by Bonroy *et al.* [8].

Statistical analysis

Statistical analysis of the results was performed with SPSS (version 11.0). For the different age groups, the median age and the infection rates were estimated by means of descriptive statistics. The chi-square test was used to compare the infections rates for both influenza and hMPV infection among different age groups.

Results

The median age of the 380 ILI patients was 19.94 years. They had mostly upper respiratory infections and none of them was hospitalised. Influenza viruses were detected in 151 specimens (39.7%) and hMPV in 23 (6.05%) (Table 1). Co-infections with hMPV and influenza virus were observed in seven cases.

In the 2005-6 season (158 ILI cases), 54 (34.1%) of the specimens were positive for influenza viruses (median age of the patients 16.6 years). The predominant type

during that season was influenza B (44 of 54, 81.4%). hMPV was the causative agent of ILI in eight cases (5.06% with median age of the patients 8.8 years). Co-infections with influenza virus and hMPV were identified in three of the eight cases [two with influenza B and one with influenza A(H3)].

During the 2006-7 season (129 ILI cases), 51 (39.5%) of the specimens were positive for influenza or hMPV infection. Influenza A(H3) was the predominant subtype (88.2%), while influenza B contributed to 11.7% of the infections. hMPV was detected in eight cases (6.2%) and two of them were co-infections, both with influenza A(H3) virus. The median age of the infected patients during this season was 24.1 years for influenza and 10.5 years for hMPV infection. Influenza A(H1) was not isolated in either of the winters 2005-6 or 2006-7.

In the 2007-8 influenza season, 46 of 93 specimens (49.4%) were positive for influenza viruses. Influenza A(H1) was the predominant subtype (67.3%), while influenza B virus was detected mostly at the end of the season at a rate of 32.6%. Seven specimens were identified as positive for hMPV (7.53%) (Table 1). Viral co-infections between hMPV and influenza A(H1) virus were observed in two of the seven positive cases. The median age of influenza- and hMPV-infected patients during this season was 22.3 and 21.5 years,

According to the age distribution (Table 2), the majority (n=230, 60.5%) of the ILI patients were 0-18 years old. Only 21 of the 380 patients examined were older than 60 years (5.5%). The incidence of influenza infection was slightly higher in the age group of 19-60-year-olds, as 58 of the 129 influenza-infected

TABLE 1

Detection of influenza virus and human metapneumovirus in ILI cases, North Greece, influenza seasons 2005-2008 (n=380)

Influenza season	ILI cases (n)	Influenza-positive (n)	Influenza A(H3) (n)	Influenza A(H1) (n)	Influenza B (n)	hMPV (n)
2005-6	158	54	10	0	44	8
2006-7	129	51	45	0	6	8
2007-8	93	46	0	31	15	7
Total for 2005-2008	380 (100%)	151 (39.7%)	55	31	65	23 (6.05%)

hMPV: human metapneumovirus; ILI: influenza-like illness.

TABLE 2

Age distribution of ILI cases and confirmed influenza virus and hMPV infections, North Greece, influenza seasons 2005-2008 (n=380)

Age group (years)	ILI cases (n)	Influenza-positive (n)	hMPV-positive (n)
0-5	94	23	12
7-10	82	26	4
11-18	55	37	1
19-60	128	58	5
>60	21	7	1
Total	380	151	23

hMPV: human metapneumovirus; ILI: influenza-like illness.

patients (44.9%) belonged to this age group. The median age of influenza- and hMPV-infected patients was 20.9 years and 13.6 years, respectively. The incidence of hMPV infection was highest in the age group of 0-18-year-olds, with 17 of the 23 hMPV-infected patients (73.9%) in this age group. Finally, 16 of the 23 hMPV-infected patients (69.5%) were under 10 years old.

Statistical analysis of the results demonstrated that there was a statistically significant difference between the age groups with respect to influenza and hMPV infection in the 2005-6 season. Younger patients up to the age of 18 years were more likely to be infected by these two viruses ($p=0.03$ for influenza and $p=0.005$ for hMPV). On the contrary, there was no statistically significant difference in the following influenza season 2006-7 in the ages of the patients with respect to hMPV infection ($p=0.472$), while there was a statistically significant difference between the age groups with respect to influenza infection, with older patients (19-60 years old) more likely to get influenza in that season ($p=0.026$). No statistically significant difference between the age groups was found for influenza or hMPV infections in the third period 2007-8 ($p=0.161$ for influenza and $p=0.247$ for hMPV). We did not observe any statistically significant correlation of between sex and the probability of being infected by either hMPV or influenza virus ($p=0.500$ for influenza and $p=0.061$ for hMPV).

We further analysed the clinical severity of the 23 cases infected and co-infected with hMPV. The most common clinical findings in patients infected only with hMPV were fever lasting on average three days ($n=21$), cough ($n=18$), rhinorrhoea ($n=17$) and wheezing ($n=15$). The duration of the fever was ascertained during follow-up by the physicians, done for all 23 hMPV patients to monitor symptoms that developed later. Chest radiographs were obtained for 10 patients. Abnormal findings such as peribronchial cuffing, prominent hilum and focal infiltrates were noted. It should be noted that the chest x-rays were taken in adults with severe symptoms, as radiological examination is unusual for children with common respiratory infections as ILI in most European countries. Patients co-infected with hMPV and influenza viruses had almost the same symptoms as the ones who were infected only with hMPV.

Despite the fact that none of the patients was hospitalised, four children with hMPV and six with influenza virus infection required supplemental oxygen as a preventive measure, according to the physicians attending to them at the outpatient clinic. Seven hMPV-infected children were treated with antibiotics for a median of five days (range: 1-14 days). The most frequently reported symptoms of the eight patients aged over 60 years (seven with influenza and one with hMPV infection) were mostly high fever over 38.5°C ($n=8$), muscle ache ($n=8$), dyspnoea ($n=6$) and sore throat ($n=8$). No

hMPV-infected patient required mechanical ventilation or administration to the intensive care unit.

In the present study we screened only influenza virus and hMPV infections. Co-infections with respiratory syncytial virus (RSV) and other respiratory pathogens require additional studies.

Discussion

Human metapneumovirus is an emerging pathogen which has been associated with symptoms ranging from mild upper respiratory tract infections to severe pneumonia, exacerbation of asthma and chronic obstructive pulmonary disease [9-14]. Serological studies show that the virus has been circulating undetected in humans for at least 50 years [1]. It is thus an important pathogen and it is essential to obtain a better understanding of its contribution to acute respiratory infections.

As the virus can cause clinical signs and symptoms that resemble influenza [15], the aim of the present study was to determine the contribution of hMPV to ILI during the three influenza seasons 2005-8 in North Greece. According to our results, hMPV contributed to ILI at a rate of 6.05%, while influenza viruses were the main cause of the disease (39.7%). Although the overall prevalence of hMPV was low, it played an important role as a contributor to ILI, especially in children, as the majority of the hMPV-infected patients (69.5%) were under 10 years old.

In the influenza season 2005-6, 5.06% of ILI patients were hMPV-positive, in 2006-7, it was 6.2% and 6.05% in 2007-8. In a similar study conducted in Japan from 2002 to 2004, hMPV positivity rates in patients with ILI were 5.7% in 2002-3 and 5.2% in 2003-4 [16], while in another study in Finland from 2000 to 2002, hMPV was responsible for 7% of all respiratory infections in children, even though influenza was circulating in the community at the same time [17].

Co-infections of hMPV with RSV, influenza and various other viruses have been reported in many studies, at a rate of 4-70% [17]. In the present study, hMPV was detected in 23 ILI cases, seven of which were co-infections with influenza viruses. Co-infections with hMPV occurred with all the subtypes of the influenza viruses detected (influenza A(H3), A(H1) and B). As in previous studies, this report confirms that co-infections are possible, but the clinical implications of hMPV in these cases is still unknown, as so little is known about its contribution as a co-pathogen [13,14,19]. In agreement with the majority of studies, there was no evidence that patients co-infected with hMPV and influenza viruses had more severe disease, although Semple *et al.* have recently suggested that dual infection by hMPV and RSV is associated with increased severity as judged by mechanical ventilation and intensive care unit admission [20].

Our findings demonstrate that the effect of hMPV is greatest in children, as the median age of hMPV-infected patients was 13.6 years during the three investigated influenza seasons. Sixteen of the 23 hMPV-infected patients (69.5%) were under 10 years old and about 50% of them belonged to the age group 0-5 years. According to previous studies, hMPV accounts for more than 4% of all respiratory infections in children during the winter season and for 1-2% of all respiratory infections annually [16]. This estimate agrees with the results of a recent 20-year study in which the prevalence of hMPV ranged from 1% to 5% of all upper respiratory infections in a given year in children under the age of five years [21].

In the present study, younger patients (0-18 years old) were more likely to be infected by influenza virus or hMPV in the 2005-6 season, in contrast to 2006-7, when older patients (19-60 years old) had a higher probability of getting influenza. According to information from the former European Influenza Surveillance Scheme (EISS), the highest consultation rates for ILI during the 2006-7 winter season were generally observed among children aged 0-4 years and 5-14 years. However, in some countries the population under surveillance was skewed to the younger ages (partly due to a high proportion of paediatricians participating in EISS) and/or older ages [22].

hMPV was detected from the beginning to the end of each influenza season examined, and was circulating during the whole period. These findings are consistent with previous studies that demonstrate the seasonal distribution of hMPV infections, which resembles that of influenza with recurrent epidemics during the winter [12,13]. In conclusion, our results show that hMPV is an emerging cause of acute respiratory infection in ILI patients and may have a significant clinical impact, especially in children. However, further research is needed to elucidate the quantitative and qualitative importance of hMPV infection, its seasonal distribution, the groups at risk of severe complications, and strategies for its diagnosis, treatment and prevention.

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Estimating the impact of the 2009 influenza A(H1N1) pandemic on mortality in the elderly in Navarre, Spain

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To the editor: We read with particular attention the article “Estimating the impact of the 2009 influenza A(H1N1) pandemic on mortality in the elderly in Navarre, Spain” by Castilla and colleagues [1].

We were surprised by the results and conclusion of the authors. They refer to a significant excess of deaths (Table 1: +4.9%, $p=0.0268$) among adults aged 65 years or older during the pandemic period (weeks 24 to 52, 2009). When considering the average annual mortality rate for 2006–2008 compared to the same rate in 2009, the excess of deaths is non-significant (Table 1: +2%, $p=0.47$). Because of the marked growth of the elderly population (the authors mention an increase of 10% in people aged 85 and more from 2006 to 2009), it would be preferable to use mortality rates rather than the numbers of deaths to compare the observed with the estimated deaths.

In Table 2, the authors estimate a significant excess of mortality of 9.9% in the population aged 65 years or older during the 12-week first pandemic wave (weeks 24 to 35) and a new non-significant excess of mortality during the second 10-week pandemic wave (weeks 40 to 49). If we compare those two periods on a weekly basis we observe 56 deaths per week (weeks 24 to 35) and 60 deaths per week (weeks 40 to 49). The authors report 208 cases of pandemic influenza A(H1N1) per week and 1,757 cases of pandemic influenza A(H1N1) per week, respectively, for these periods (Table 2). It would appear strange to have 1.07 times more deaths per week while at the same time 8.5 times more cases of influenza were observed per week. Furthermore, the numbers for laboratory-confirmed cases of influenza presented in Table 2 are higher in week 40 to 49 than for all other periods, which is not consistent with the conclusions. In the same table, the authors show a decrease of 4% in the number of deaths between week 36 and 39 during which 52 deaths per week and 346 cases of influenza per week were observed (see Table 2) but do not elaborate on those results.

The authors do not provide information about the percentage of the population aged 65 years or older in the number of cases during the two pandemic waves, while it is known that the elderly seem to have suffered less from the 2009 influenza pandemic than the younger adults [2-3].

In the Figure, the weekly observed number of deaths is higher than the expected number of deaths from week 24 to week 35 (summer period). The authors mention that the heat-alert threshold in their region was not reached during the summer, while it is known that a slight increase in mortality is possible even if temperatures remain below the heat-alert threshold. In the summer period, temperatures and mortality fluctuations are closely related and temperatures just below the heat-alert threshold can have already a marked effect on mortality among the elderly [4]. We wonder whether the observed temperatures during the summer 2009 were more elevated than those recorded during the three previous summers, which would partly explain the inconsistency of the observed mortality results during the two pandemic waves?

The Poisson fluctuation interval around the observed weekly numbers of deaths would have facilitated the identification of the weeks in which there was a statistically significant excess of deaths.

As the study was done on the basis of all-cause mortality data, it is difficult to deduce a causal relationship between pandemic influenza and mortality in our opinion. A temporal relationship should have been discussed more in-depth. The monitoring of mortality is a part of measuring the burden of disease in a population and needs to be done cautiously.

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Authors' reply: Estimating the impact of the 2009 influenza A(H1N1) pandemic on mortality in the elderly in Navarre, Spain

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To the editor: We appreciate the comments by L Jossieran and A Fouillet [1] and agree with many of them. A number of the aspects they comment on were mentioned in our work, but we appreciate having another opportunity to discuss them.

Demonstrating the impact of influenza on general mortality entails considerable difficulty, and has been the object of interesting methodological discussions [2,3]. The difficulty lies largely in finding an adequate baseline reference for the comparison, ruling out the effect of other causes. An ecological study like ours, conducted in a small region, has limited capacity to provide definitive evidence for this association. Aware of this limitation, we restrict ourselves to describing the excess mortality observed in older people coinciding with the weeks of highest circulation of the pandemic influenza – and for which we have found no other arguments that could completely explain it. Similar excess mortality has also been observed to coincide with circulation of the seasonal influenza virus, but not in periods with little or no influenza activity.

In table 1 we showed the comparison between observed and expected values, both for the number of cases and for the crude and adjusted rates. Coinciding with the downward secular trend in mortality, the crude rate in the period before circulation of pandemic influenza was 1.3% lower than expected, despite ageing of the population, and the standardised mortality ratio indicated a reduction of 4%. In contrast, the crude rate observed in the pandemic period was 2% higher than expected, and the standardised mortality ratio of 1 indicated a stabilisation. We suggested that this different trend in the pandemic period could be related with the circulation of 2009 influenza A(H1N1).

The deaths occurring during the summer were compared with those of other summers when influenza activity was not detected, which facilitates detection of the impact of influenza on mortality, in the event that it occurred. In contrast, the deaths that occurred from week 47 on were compared with those of weeks with

some influenza activity in previous years. Thus, only if the impact of influenza on mortality is greater in the study year than in the reference years, can we say that there was an excess of deaths. In any case, mortality during the second pandemic wave was higher than in the first, which is consistent with the incidence of medically-attended influenza-like illness (MA-ILI).

In weeks 36 to 39 we continued to detect a non-negligible incidence of MA-ILI, but only 3% of the cases analysed were positive for influenza virus, indicating low virus circulation.

In 2009 the weekly or daily mortality thresholds were exceeded in June, July and August. In June and July the mean maximum and minimum temperatures were not significantly different from the means for the same months in the three previous years. In August, however, the mean maximum temperature was 4.0 °C higher (30.8 versus 26.8) and the mean minimum was 1.5 °C higher (15.8 versus 14.3) with respect to the three previous years, although at no time did it exceed the thresholds established for a heat alert. Daily mortality exceeded the threshold of deaths on six occasions during the summer of 2009, but on only two of them had the temperatures in the three previous days exceeded a maximum of 33 °C or a minimum of 18 °C. Although we do not totally rule out the effect of heat on mortality during the summer of 2009, it does not appear to completely explain the excess mortality detected.

The incidence of MA-ILI in persons aged 65 and older was 0.73 per 1,000 during the first pandemic wave and 3.67 per 1,000 in the second wave. Nonetheless, the repercussion of influenza on mortality in older people may be partly due to deaths in persons not previously diagnosed with ILI.

Finally, there are some arguments that would explain a certain impact of the influenza A(H1N1) pandemic on mortality in older people that may have gone unnoticed. The incidence of seasonal influenza is usually low in the elderly, but when its impact on mortality is

evaluated, relatively high values with respect to incidence are generally estimated. Mortality from pandemic A(H1N1) influenza has been evaluated in several studies, but most of them have focused on deaths occurring in laboratory-confirmed cases [4-5]. Although the number of influenza tests has increased greatly, it is still small in relation to the number of MA-ILI cases. Previous studies have suggested that influenza can trigger or exacerbate non-infectious pathologies such as cardiovascular diseases [6], and influenza may be hidden by the underlying pathology. It is also possible that some of the influenza-related deaths occurred outside the hospital.

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