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Outbreak of West Nile virus infection in humans, Romania, July to October 2010

A Sirbu (anca.sirbu@insp.gov.ro)[1], C S Ceianu[2], R I Panculescu-Gatej[2], A Vázquez[3], A Tenorio[3], R Rebreanu[4], M Niedrig[5], G Nicolescu[2], A Pistol[1]

1. National Institute of Public Health - National Centre for Surveillance and Control of Communicable Diseases, Bucharest, Romania
2. Cantacuzino National Institute for Research and Development in Microbiology and Immunology, Bucharest, Romania
3. Institute of Public Health 'Carlos III', Madrid, Spain
4. National Institute of Public Health - Regional Centre of Public Health, Cluj, Romania
5. Robert Koch Institute, Berlin, Germany

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A total of 57 cases of West Nile virus infection (54 with neuroinvasive infection and three with fever) were identified in Romania between July and October 2010. The median age of the cases was 53.4 years, with the highest incidence in the age group 60–69 years. The case fatality rate was 8.8%. Cases were distributed in 19 districts in the southern, western, central and eastern parts of the country. Molecular investigation revealed lineage 2 West Nile virus, related to the Volgograd 2007 strain.

Introduction

On 28 August 2010, the National Reference Laboratory for Vector-borne Diseases in the Cantacuzino Institute in Romania reported to the National Centre for Surveillance and Control of Communicable Diseases (NCSCCD) 10 positive results for West Nile virus (WNV) in samples of patients distributed in nine different Romanian districts. Six of these 10 cases were male and four were female, with a median age of 56 years (range: 32–79 years).

Romania recorded the first large outbreak of West Nile neuroinvasive disease (WNND) in Europe in 1996, with 393 confirmed cases. This was also the first such outbreak in urban settings. Cases were confined to Bucharest, its rural surroundings and 14 districts in the Danube Plain [1,2]. In response to this outbreak, in 1997, the Ministry of Health set up a regional, hospital-based surveillance system and sporadic cases were recorded every year in the districts neighbouring the Danube River [3]. In 2009, the surveillance system was extended at national level, following the confirmation of two cases of West Nile fever (WNF) in humans in the central part of Romania and the detection of WNV-specific IgG antibodies among horses in many other areas of the country.

Within the routine WNV surveillance activities in Romania, the following case definition is used for a suspected case with WNV infection: a person over 15 years of age who presents with fever and meningitis, encephalitis or meningoencephalitis between May and October and who reports a history of mosquito bites.

Two sets of samples are collected for each suspected case: for patients with acute symptoms both cerebrospinal fluid (CSF) and serum are taken. For patients in convalescence phase, a second serum sample is taken 14–21 days later. A probable or confirmed case of WNV infection is defined as a person who met the relevant clinical and the laboratory criteria for probable or confirmed cases described in the European Union case definition [4]. A suspected case is considered not to be a case if WNV-specific IgM was not detected in CSF and serum.

Data were obtained from infectious disease hospitals and reported using a standardised form containing information on symptoms, onset date and possible risk factors.

Following an outbreak of WNV infection in Greece in July to August 2010 [5], surveillance for WNND was enhanced in all districts in Romania from 12 August 2010. All districts were asked to increase their vigilance. In addition, the case definition for suspected cases used for routine surveillance was modified: a history of travel in the Danube Delta and/or in Greece was added.

Outbreak description

On 30 August 2010, after the 10 WNND cases had been reported on 28 August, the NCSCCD further reinforced the WNV surveillance activities in humans at national level and the case definition was modified once more: all persons aged over 15 years presenting with fever and meningitis or encephalitis or meningoencephalitis and clear CSF were considered suspected cases and were tested for WNV-specific antibodies.

After a cluster of five cases was recorded on 28 August 2010 in a newly affected area in Central Transylvania (Alba district – Blaj city, Mures and Sibiu districts), active perifocal surveillance was set up locally for WNF cases as part of the enhanced surveillance: epidemiologists were involved in retroactively identifying persons who presented to general practitioners with fever and rash during August 2010. Samples from these patients were tested for the presence of WNV.

The WNV surveillance season starts every year from early May and ends on 30 October. In 2010, the surveillance season was exceptionally extended by two weeks in two places that were most affected by the outbreak (Bucharest city and Constanta district). From 10 May to 15 November 2010, a total of 170 suspected cases with WNV infection were reported in Romania. Of these, 52 were confirmed cases (49 with WNND and three with WNF), five were probable cases and 113 were negative for WNV.

The first confirmed WNND case had symptom onset on 4 July 2010 and the last on 11 October 2010. The distribution of the probable and confirmed cases of WNV infection by date of symptom onset is presented in Figure 1.

The first case was diagnosed retroactively, during the investigation of a cluster of unexpected deaths in Constanta district, thought to be caused by hyperthermia due to high temperatures (39 °C) in early July 2010. For the rest of the cases, most (n=28) had symptom onset during the second half of August, 19 in September and only three cases had symptom onset in October (Figure 1).

Among the 57 cases, the sex ratio (male:female) was 1.7:1 (36 male:21 female). The median age was 53.4 years (age range: 12–81 years). The highest number of cases (n=15) belonged to the age group 60–69 years (Figure 2).

The incidence per age group ranged from 0.1 to 0.8 per 100,000 population, with the highest values being for the age groups 60–69 years (0.8 per 100,000 population) and 70 years and above (0.5 per 100,000 population). The lowest incidence was in the age groups under 20 years and 20–29 years (0.1 per 100,000 population).

All confirmed and probable cases were hospitalised with WNV infection (31 with meningitis, 19 with meningoencephalitis and four with encephalitis). Three had non-neuroinvasive symptoms: two had fever and maculopapular exanthema and one had prolonged febrile syndrome. Among the severe cases, eight entered into a coma. Clinical symptoms included: fever (n=53), headache (n=50), stiff neck (n=42), shivering (n=26), confusion (n=21) vomiting (n=22), myalgia (n=25), disorientation (n=17), photophobia (n=12), Kernig sign (n=14), Brudzinski sign (n=8), memory loss (n=3), maculopapular exanthema (n=2).

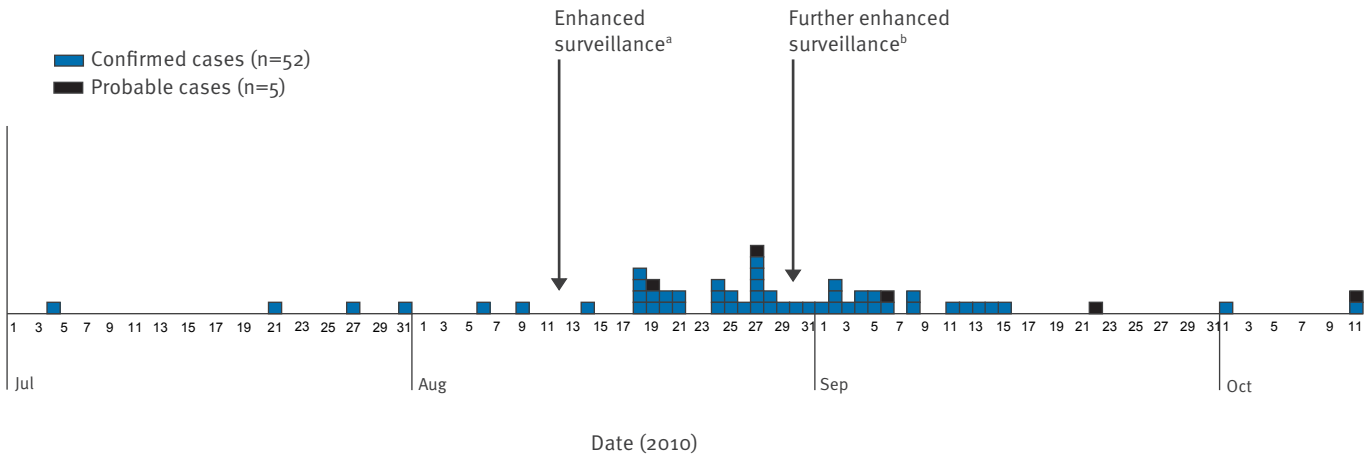
Five deaths were recorded among the 57 identified cases, giving a case fatality rate of 8.8%. All deceased patients were aged over 65 years, and had underlying conditions (hypertension, diabetes).

Of the 57 cases, 30 lived in urban settings and 27 in rural areas, giving an urban: rural ratio of 1.1:1.

Most cases (n=35) were recorded in the southern part of the country, an area known to be endemic for WNV from previous years. However, WNV infections were reported in humans in previously unaffected areas, such as districts in central Transylvania, and in the Moldavian Plateau (Figure 3).

Figure 1

Distribution of cases of West Nile virus infection (probable and confirmed) by date of symptom onset, Romania, July – October 2010 (n=57)



[a] Increased vigilance and amendment of case definition.

[b] Reinforced surveillance activities and second amendment of the case definition.

Laboratory investigation

Serum and CSF samples were tested for the presence of IgM and IgG antibodies specific for WNV, using a commercial enzyme-linked immunosorbent assay (ELISA) kits (Focus Technologies, USA). A total of 45 WNV neuroinvasive cases were confirmed by IgM-capture ELISA, based on the presence of WNV-specific IgM antibodies in CSF.

In nine cases with neurological clinical picture, CSF samples were either not available or were negative or borderline positive for WNV-specific IgM by ELISA. Serum samples from these cases were tested also by seroneutralisation assay using a lineage 1 WNV strain from Israel: four additional neuroinvasive cases were confirmed by the presence of WNV neutralising antibodies in serum, while in the other five cases, the seroneutralisation assay was negative. In the three cases with non-neuroinvasive WNV infection, the infection was confirmed by the presence of WNV neutralising antibodies in serum.

Cases from Transylvania were also tested for the presence of tick-borne encephalitis virus-specific antibodies because this virus had previously been found to be circulating in this area.

Serum and CSF samples were collected within five days from symptom onset from 16 of the 49 confirmed neuroinvasive cases; tissue samples were collected from one fatal case at the autopsy. Reverse transcription (RT) and real-time polymerase chain reaction (PCR) was used to detect the WNV genome in these samples.

Figure 2

Incidence rate of cases of West Nile Virus infection (probable and confirmed) by age group, Romania, July – October 2010 (n=57)

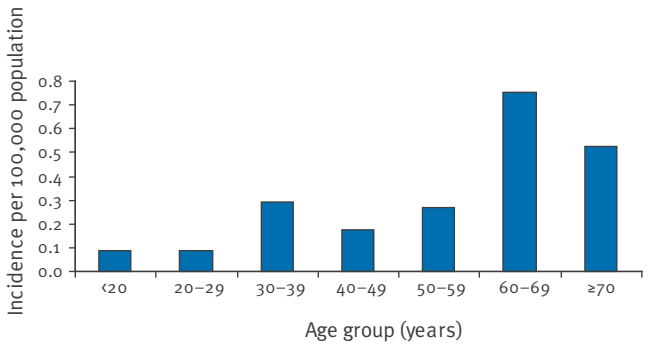
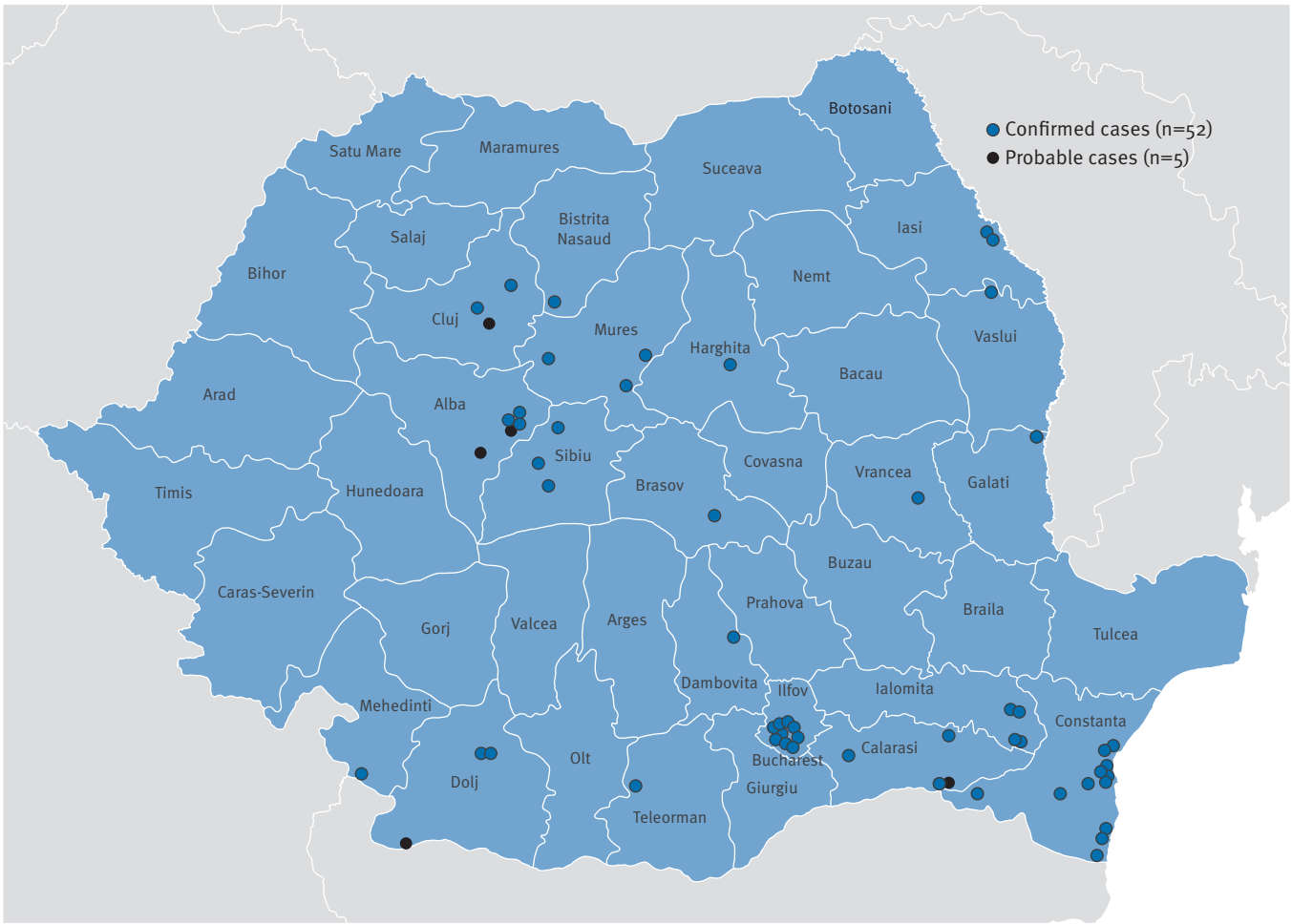


Figure 3

Distribution of cases of West Nile virus infection (probable and confirmed) by place of exposure, Romania, July – October 2010 (n=57)



One dot represents one case.

The target sequence was a conserved region of the 3´ non-coding region of WNV (Vázquez *et al.*, unpublished data). In addition, partial sequence of the flavivirus NS5 gene was obtained following a generic RT-nested-PCR to detect flaviviruses [6]. New degenerate internal primers were designed for sequencing. Virus culture was performed for the same cases using Vero and C6-36 cells, and gave negative results after three blind passages.

Molecular investigation detected the WNV genome in the brain tissue of the fatal case, and in the serum and/or CSF of four of the 16 cases tested. Partial sequencing of the NS5 gene was performed for only one positive (approximately 1,200 genome equivalents/ml)) serum sample: analysis of 780 nucleotides of the NS5 gene demonstrated that the virus was a WNV lineage 2 strain, with 99.3% sequence identity to the virus circulating in Volgograd in 2007 (GenBank: FJ425721.1).

Public health measures

Surveillance has been gradually increased following reports of the outbreak of WNV infection in Greece and the detection of the first cases in Romania. The Ministry of Health and the regional public health authorities informed the local authorities about recommended measures for mosquito control and communicated data on the evolution of the outbreak to the general population on a weekly basis. The population was also informed about measures to reduce exposure to mosquitoes and to prevent mosquito bites.

The Ministry of Health informed the National Institute of Haematology on a daily basis about the situation of the confirmed human cases of WNV infection and about the places where they have been identified. The National Institute of Haematology deferred donations from blood donors in rural areas until 1 December 2010. Initially, donations from affected urban areas were also deferred, but at a later stage, in order to maintain a sufficient blood supply, only donors from these areas presenting with a history of fever were excluded. In addition, those who donated blood were required to report to the Blood Donation Centre any symptoms of fever in the 15 days after giving blood. Donated blood was stored and not used before the five-day period had elapsed. Donors who had spent at least one night in areas with human cases of WNV infection were excluded from donation for a period of 28 days after having left the affected area.

Veterinary doctors were informed about the occurrence of WNV infection in humans and were requested to provide information on WNV infection in animals. According to the information received from the national veterinary authority, no dead birds infected with WNV and no cases of encephalomyelitis or recent WNV infection in horses have been recorded during the outbreak in humans. Seroprevalence studies found WNV-specific antibodies in poultry from two districts in the eastern and western parts of the country. WNV-specific IgG antibodies were detected in horses from 22 districts across the country, including nine districts in which human cases of WNV infection occurred in 2010.

Discussion

With 52 confirmed cases of WNV infection widely distributed in the country, the 2010 transmission season was associated with the most important WNV infection outbreak since 1997, when the WNV surveillance system was implemented in Romania.

Weather conditions (rainfalls, high temperatures) in 2010 were favourable to the increase of mosquito populations. Culex pipiens had already been identified as the vector of WNV in the 1996 outbreak [7]. In late summer, at least in urban areas, Cx. pipiens is the main mosquito biting humans, and we may assume that in this type of environment, this species was the WNV vector in 2010 also.

A specific feature of this outbreak was its extended area in the country: cases were distributed in 19 districts, with some concentration of cases in the south-eastern district of Constanta and in urban areas such as Blaj (western part) and Bucharest. Although most cases occurred in the already known endemic area in the south (in the Danube lowland and Delta neighbouring counties), in the 2010 transmission season, cases were also recorded in previously unaffected areas, from the valleys of other major rivers, known to be bird migration pathways.

Partial sequencing of the NS5 gene from a WNV-positive serum of a Bucharest resident revealed a virus strain belonging to the genetic lineage 2, highly similar (99.3%) to the Volgograd strain involved in the 2007 WNV outbreak in the Volga Delta area [8]. It is unsure whether the same WNV strain was involved in the outbreak beyond the Carpathian Mountains in Transylvania in 2010. Lineage 2 WNV strains were previously thought to be of low virulence. Nevertheless recent studies in South Africa suggest that lineage 2 WNV strains are a cause of neurological disease in horses and humans [9]. The WNV strain circulating in Romania from the 1996 epidemic belonged to the genetic lineage 1 [7] and was associated with a case fatality rate of 4.3% (an 8.8% rate was recorded in 2010) [1]. In conclusion, a change in the epidemiological profile of WNV infection was recorded in 2010 in Romania, with emergence of cases in previously unaffected areas in western and eastern parts of the country, and the emergence of a neuroinvasive lineage 2 WNV strain.

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Measles among healthcare workers: a potential for nosocomial outbreaks

E Botelho-Nevers[1,2], N Cassir[1,2], P Minodier[3], R Laporte[3], P Gautret[1], S Badiaga[4], DJ Thiberville[1], L Ninove[5], R Charrel[5], P Brouqui (philippe.brouqui@ap-hm.fr)[1]

1. Pôle de Maladies Infectieuses, IFR 48, Assistance Publique Hôpitaux de Marseille, France
2. These two authors contributed equally to this work
3. Pôle mère-enfants, Assistance Publique Hôpitaux de Marseille, France
4. Pôle AUR, Assistance Publique Hôpitaux de Marseille, France
5. Laboratoire de virologie, IFR 48, Assistance Publique Hôpitaux de Marseille, France

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We report here 14 cases of measles among healthcare workers (HCWs) in Public Hospitals of Marseilles, France that occurred between April and November 2010. All cases but one were under 30 years of age. Following the identification of these cases, we checked the immune status among 154 HCWs who volunteered to take part in the study and showed that 93% and 88% were immune against measles and mumps respectively. HCWs non-immunised against measles were all under 30 years of age.

Introduction

France has been experiencing a measles outbreak since 2008. A recrudescence of cases among children and young adults has been related to insufficient vaccine coverage [1]. The risk of acquiring measles in non-immune healthcare workers (HCWs) is estimated to be 13 times higher than in general population [2]. Consequently, young non-immune HCWs are highly at risk occupational measles and when infected constitute a risk of transmission to non-immune or immunocompromised patients [3-5]. Indeed measles is a highly contagious disease [6]; therefore, strict adherence to alcohol-based hand rub and rapid implementation of appropriate respiratory isolation measures are essential but insufficient to prevent measles outbreaks in hospital settings [7]. Vaccination is consequently the only reliable protection against nosocomial spread of measles [3]. In France, five vaccines are mandatory for HCWs: vaccines against diphtheria, tetanus, poliomyelitis, hepatitis B virus, and tuberculosis. Other vaccines such as the vaccine against measles are only recommended [8]. According to the recently released national guidelines regarding measles vaccination for HCWs, getting vaccinated against measles or completing measles vaccination is recommended but not mandatory [9]. Few reports described seroprevalence against measles among HCWs in general: a high level of immunity has been reported in Italy and the United Kingdom [10,11]. However, it has been described to be lower among nurse and medical students in Switzerland [12]. The last European measles serosurvey including French data on different age groups was published in 2001 [13].

In 2010, at total of 122 cases of measles were managed in the three of Public Hospitals of Marseilles (PHM) and since April 2010, cases of measles appeared among HCWs of the PHM. Following notification of the first case, we evaluated the immune status among the HCWs who volunteered to participate. We describe here the measles cases that occurred among HCWs from April 2010 to November 2010 and the immunity against measles of 154 volunteer HCWs working in three wards at high risk for transmission of contagious diseases (such as infectious disease, emergency room, paediatric, maternity and oncology wards). In the same period, a cluster of three mumps cases occurred among medical students at the associated School of Medicine and therefore we also checked the immune status for mumps of the 154 HCWs.

Identification of measles cases

Measles cases among the HCWs of the PHM were identified in three different ways: through (i) the infectious diseases specialist of PHM (6 cases), (ii) the occupational medicine or infection control unit (5 cases), (iii) the laboratory database of the hospital (3 cases) that contained information for all HCWs of PHM for the period from January to November 2010.

PHM has approximately 15,000 staff members including all statutory personnel and medical and nurse students.

Seroprevalence of IgG against measles and mumps in HCWs

Between April and November 2010, all HCWs (n=363) of the infectious disease ward, the paediatric and the adult emergency rooms of North Marseille Hospital one of the three locations of PHM were invited to participate in a study aimed to clarify their immune status against measles and mumps. A short questionnaire recording occupation, age and history of measles and mumps

immunisation or past infection was distributed to the participants. Answers to questions were collected in Excel, frequencies, means and univariate analysis were performed with EpiInfo version 3.5.1 August 2008 (Centers for Disease Prevention and Control, Atlanta, USA). HCWs who accepted to participate in the study were invited to have a blood test for measles and mumps and to sign a written consent to take part in the study. HCWs were screened for measles and mumps IgG on serum sample by enzyme-linked immuno-sorbent assay (ELISA) (Siemens, France).Test was considered positive for measles and mumps if antibody titers were above 500 mIU/mL. HCWs with negative measles or mumps IgG were informed about the result and were offered immunisation with either a measles vaccine or measles-mumps-rubella (MMR) vaccine according to availability of vaccine and sex (MMR vaccines were used for women).

Results

Measles cases among healthcare workers

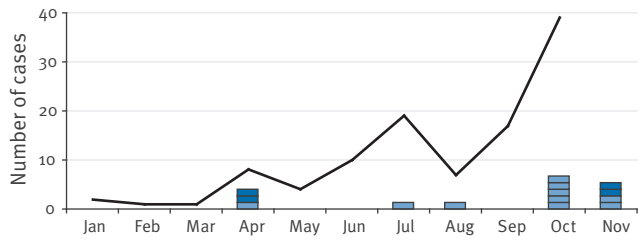
Fourteen laboratory-confirmed measles cases occurred among HCWs at PHM. The mean age was 27.54 +/- 4.70 years (range 22-39) and nine were women. The approximate attack rate of measles was of 93 cases per 100,000 HCWs. Ten cases occurred in medical staff: five were residents, three were medical students and two were medical doctors. The four remaining cases occurred in two nurses and two nurse assistants. Distribution of cases among HCWs and patients of PHM during the year 2010 is presented in Figure 1.

During the same period, 108 cases of measles were diagnosed at our institution among patients.

To the best of our knowledge, no transmission from HCWs to patients occurred. Measles vaccination status was available for 10 cases: six HCWs were unvaccinated and four had received only one dose of measles-containing vaccine in childhood. Place of infection was considered the hospital for 12 cases (certain in eight cases (i.e. HCWs working in a ward where cases of measles in patients have been managed in the previous 15 days), probable in four cases (i.e, HCWs with no direct contact with patients infected with measles but contact with HCWs managing these patients) and the community for two cases. Two cases acquired despite the post-exposure measles vaccination performed in 48 hours.

Figure 1

Measles cases among healthcare workers and patients of Public Hospitals of Marseilles, France, January – November 2010



The black curve represents measles cases among patients.
Each box represents one measles case among healthcare workers.
Dark blue boxes represent related measles cases among healthcare workers.

Table 1

Immune status for measles and mumps by occupation and age among healthcare workers participating in the study, Public Hospitals of Marseilles, France, April – November 2010 (n=154)

Occupation	Number (%)	Number of HCWs immune to measles (%)	Number of HCWs immune to mumps (%)
Medical Doctor	19 (12)	19 (100)	18 (95)
Resident	19 (12)	18 (95)	16 (84)
Medical student	55 (36)	50 (91)	47 (85)
Nurse	32 (21)	30 (94)	28 (87)
Nurse assistant	27 (17)	25 (93)	25 (93)
Other	2 (1)	2 (100)	2 (100)
Age (years)			
19-24	52 (34)	45 (86)	43 (83)
25-29	34 (22)	31 (91)	30 (88)
30-34	15 (10)	15 (100)	14 (93)
35-39	12 (8)	12 (100)	11 (92)
40-44	12 (8)	12 (100)	11 (92)
45-49	13 (8)	13 (100)	12 (92)
50-54	9 (6)	9 (100)	8 (89)
55-59	6 (4)	6 (100)	6 (100)
60-65	1 (1)	1 (100)	1 (100)
Total	**154**	**144 (93)**	**136 (88)**

HCW: healthcare worker.

Serosurvey in healthcare workers at the Public Hospitals of Marseilles

A total of 154 HCWs took part in the study, representing a participation rate of 42.4% (154/363); 74 in the infectious diseases department, 57 and 23 in the paediatric and the adult emergency rooms respectively. All 154 HCWs answered to the questionnaire and had blood tests for measles and mumps. The breakdown of participants by occupation and age are shown in Table 1.

The mean age of the participating HCWs was 32.4 years +/- 11.1 (range 19-65 years), 118 were women (76%). Of the 154 HCWs, 144 (93%) and 136 (88%) had a positive IgG serology for measles and mumps respectively. The HCWs in the age groups of 19-24 and 25-29 years had a seroprevalence of 86.5% and 91.2% respectively (Figure 2).

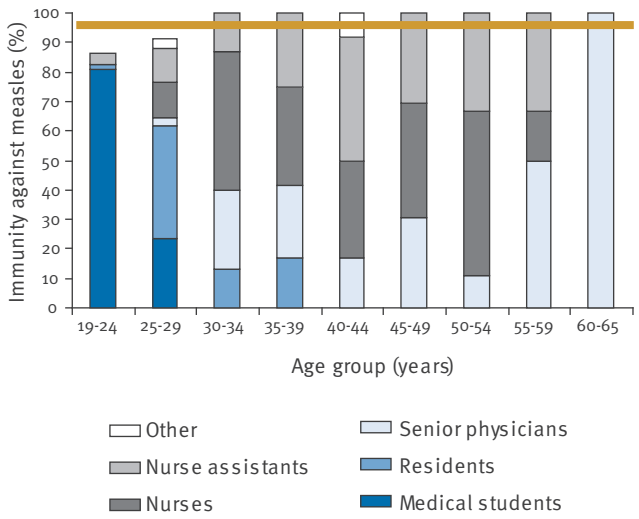
The absence of immunity against measles (naturally acquired or through vaccination) was significantly associated with younger age groups (mean age 23.9 +/-2.4 years for non-immune HCWs vs. 32.9 +/-11.3 years for immune HCWs, p=0.011).
Around a quarter and a third of the HCWs did not know their immune status for measles and mumps, respectively (Table 2). The number of vaccine doses was often unknown among vaccinated HCWs.

Discussion

Our study reports a series of measles cases among healthcare workers during 2010. Measles spreads in Europe and in France with large outbreaks among general population since 2008 but an increase in the number of cases was noticed in 2010 in France [1].

Figure 2

Healthcare workers with immunity against measles by age group and occupation, Public Hospitals of Marseilles, France, April – November 2010



The age groups of 19-24 and 25-29 years had a seroprevalence of 86.5% and 91.2% respectively.

The orange line represents the target country-wide vaccination coverage (95%) suggested by the World Health Organization for the elimination of the disease [14].

Table 2

History of vaccination or naturally-acquired immunity for measles and mumps among immune healthcare workers, by age group, Public Hospitals of Marseilles, France, 2010 (n=154)

Age group (years)	Number (%)	History of measles				History of mumps			
		Vaccinated N (%)	Naturally-acquired N (%)	Not known N (%)	No immunisation	Vaccinated N (%)	Naturally acquired N (%)	Not known N (%)	No immunisation
19-24	52 (34)	31 (60)	4 (8)	11 (21)	6 (12)	28 (54)	3 (6)	16 (31)	5 (10)
25-29	34 (22)	20 (59)	7 (21)	7 (21)	0	16 (47)	7 (21)	10 (29)	1 (3)
30-34	15 (10)	7 (47)	6 (40)	2 (13)	0	4 (27)	3 (20)	6 (40)	2 (13)
35-39	12 (8)	4 (33)	6 (50)	2 (17)	0	3 (25)	3 (25)	2 (17)	4 (33)
40-44	12 (8)	2 (17)	6 (50)	4 (33)	0	3 (25)	3 (25)	5 (42)	1 (8)
45-49	13 (8)	0	8 (62)	5 (39)	0	0	5 (39)	5 (39)	3 (23)
50-54	9 (6)	0	3 (33)	5 (56)	1 (11)	0	1 (11)	6 (67)	2 (22)
55-59	6 (4)	0	4 (67)	2 (33)	0	0	5 (83)	1 (17)	0
60-65	1 (1)	0	1 (100)	0	0	0	1 (100)	0	0
Total	**154**	**64 (42)**	**45 (29)**	**38 (25)**	**7 (5)**	**54 (35)**	**31 (20)**	**51 (33)**	**18 (12)**

N: number

Marseilles is one of the areas in France that experienced a high incidence of measles in 2010, with an incidence of 14.8 per 100,000 population [15]. The incidence of measles among HCWs in PHM seems much higher than in the general population. One can assume that the identification of measles cases among HCWs is more exhaustive than in the general population where the incidence is largely underestimated [15]. However it cannot be excluded that measles cases among HCWs may have been missed. The high attack rate of measles among HCWs presented here indicates the high risk for transmission of measles in healthcare settings among non-immune persons [1-3,16,17]. In our study as in the general population, cases of measles affected mainly young adults aged between 20 and 30 years [1,15] who had not been vaccinated against measles or who had received only one dose of measles-containing vaccine. Our report shows that at least eight measles cases among HCWs would have been prevented if national guidelines had been applied [9]. Although the selection of HCWs on a voluntary basis may have introduced a bias in the participation rate, the sample selected here remains representative for PHM staff. However, the data may be extrapolated only to teaching hospitals in France where young students and HWCs are usually employed. Occupational medicine and infection control unit checked the immune status of staff and patients exposed and suggested post-exposure prophylaxis when necessary as recommended in the national guidelines [9]. In this outbreak, post-exposure vaccination performed in the 72 hours after exposure as recommended [8,9] failed to prevent measles in two cases. Therefore, all susceptible exposed HCW had to stay at home even if prophylaxis measures were undertaken. While post-exposure prophylaxis (immune globulines) had been given for immunocompromised patients [9], transmission to patients could not been excluded notably due to prolonged incubation period of measles.

Our seroprevalence study revealed that 6.5% of HCWs participating in the study were susceptible to measles. All susceptible HCWs were younger than 30 years with a significant association between susceptibility to measles and younger age but no link could be established with the HCW occupation. This observation confirms the need to focus the attention on high-risk age groups among HCWs. Susceptibility for mumps is 11.7 % among HCWs and is also higher in younger HCWs but with no significant difference among age groups. This susceptibility to measles and mumps among younger population is due to a suboptimal vaccination coverage with often a single vaccine dose [1] compared to adults older than 30 years that had nearly all acquired natural immunisation [13]. Our results on susceptibility to measles and mumps among HCWs are similar to those found in the literature from other European countries [10,11]. Moreover, as described elsewhere [18], we showed that at least a fourth of the HCWs do not know their immune status for measles and mumps. Therefore, in our opinion, each HCW, irrespective of their occupation, younger than 30 years should be tested for measles antibodies. All susceptible HCWs should be promptly vaccinated. Vaccination of all susceptible HCWs should be implemented in hospitals by the occupational medical staff during the medical check-up before recruitment but also by preventive medical staff before enrolling in the school of medicine or in schools for nurses. As immunisation remains the only reliable protection against the spread of measles [3] we suggest HCWs refusing vaccination should be deferred from caring for immunocompromised patients.

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Effectiveness of pandemic and seasonal influenza vaccine in preventing pandemic influenza A(H1N1)2009 infection in England and Scotland 2009-2010

P Hardelid (pia.hardelid@hpa.org.uk)[1], D M Fleming[2], J McMenamin[3], N Andrews[1], C Robertson[3,4], P SebastianPillai[1], J Ellis[1], W Carman[5], T Wreghitt[6], J M Watson[1], R G Pebody[1]

1. Health Protection Agency Centre for Infections, London, United Kingdom
2. Royal College of General Practitioners Research and Surveillance Centre, Birmingham, United Kingdom
3. Health Protection Scotland, Glasgow, United Kingdom
4. University of Strathclyde, Glasgow, United Kingdom
5. West of Scotland Specialist Virology Centre, Glasgow, United Kingdom
6. Health Protection Agency Regional Microbiology Network, Cambridge, United Kingdom

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Following the global spread of pandemic influenza A(H1N1)2009, several pandemic vaccines have been rapidly developed. The United Kingdom and many other countries in the northern hemisphere implemented seasonal and pandemic influenza vaccine programmes in October 2009. We present the results of a case–control study to estimate effectiveness of such vaccines in preventing confirmed pandemic influenza infection. Some 5,982 individuals with influenza-like illness seen in general practices between November 2009 and January 2010 were enrolled. Those testing positive on PCR for pandemic influenza were assigned as cases and those testing negative as controls. Vaccine effectiveness was estimated as the relative reduction in odds of confirmed infection between vaccinated and unvaccinated individuals. Fourteen or more days after immunisation with the pandemic vaccine, adjusted vaccine effectiveness (VE) was 72% (95% confidence interval (CI): 21% to 90%). If protection was assumed to start after seven or more days, the adjusted VE was 71% (95% CI: 37% to 87%). Pandemic influenza vaccine was highly effective in preventing confirmed infection with pandemic influenza A(H1N1)2009 from one week after vaccination. No evidence of effectiveness against pandemic influenza A(H1N1)2009 was found for the 2009/10 trivalent seasonal influenza vaccine (adjusted VE of -30% (95% CI: -89% to 11%)).

Introduction

Following the emergence and rapid global spread of pandemic influenza A(H1N1)2009 virus in April 2009 [1], several vaccines against this virus were quickly developed [2-6]. Clinical trials, including products with a new squalene adjuvant (MF59 or AS03) demonstrated that these novel pandemic vaccines were immunogenic in various target populations [2-6]. Published work on the possible effect of prior trivalent seasonal influenza vaccination on the subsequent risk of pandemic influenza infection has been conflicting: some have suggested a protective effect [7], others have found no association [8-10], and recent work from Canada has reported an increased risk of subsequent pandemic infection [11].

The United Kingdom (UK), as many other countries in the northern hemisphere, implemented its seasonal and pandemic influenza vaccine programmes in autumn 2009. Two pandemic vaccines were introduced in the UK: Pandemrix (GlaxoSmithKline), an inactivated low-dose influenza vaccine with one dose containing 3.75g haemagglutinin (HA) equivalent of the influenza A/California/7/2009 isolate combined with the AS03 adjuvant) and Celvapan (Baxter), a whole-virion, Vero cell-derived influenza vaccine with a dose of 7.5 µg of influenza A(H1N1) HA antigen of the A/California/07/2009 isolate. The pandemic vaccine programme was initially targeted at clinical risk groups older than six months, pregnant women and healthcare workers [12] and later extended to all healthy children six months to five years of age. Pandemrix was the main vaccine administered through the UK pandemic vaccine programme: by late February 2010, provisional uptake for the first dose of Pandemrix in England was 37.1% for clinical at-risk groups, 20.4% for healthy children six months to five years of age and 39.9% for healthcare workers [13].

The UK has an established surveillance system to monitor the effectiveness of the annual seasonal influenza vaccine programme. The system uses routine epidemiological data generated through swabbing of cases of influenza-like illness (ILI) presenting in primary care in England and Scotland [14]. Using this approach, this study sets out to provide estimates of the effectiveness of the pandemic and seasonal influenza vaccine

programmes in preventing infection with pandemic influenza A(H1N1)2009.

Methods

Study population and period

This study uses data from three influenza sentinel surveillance schemes in England and Scotland: the Royal College of General Practitioners' surveillance scheme (RCGP) covers 96 practices and ca. 900,000 patients throughout England (65 practices contribute to the swabbing programme), the Health Protection Agency (HPA) Regional Microbiology Network (RMN) surveillance scheme includes 45 contributing general practices and covers around 400,000 patients, and the Health Protection Scotland (HPS) scheme covers 101 general practices and 640,931 patients in Scotland (90 practices contribute to swabbing).

In all three schemes, clinicians are instructed to provide nose and throat swabs from a convenience sample of patients presenting with acute onset of respiratory illness, i.e.rapid development of appropriate symptoms usually with fever. No particular age group is specifically targeted and swabbing is undertaken regardless of prior influenza vaccination status of the patient.

This study covers samples collected in the period from 1 November 2009 (the pandemic influenza vaccination programme was rolled out across the UK on the 21 October) to 29 January 2010.

Cases were defined as individuals presenting with ILI in one of the participating practices in the defined study period who were swabbed and tested positive for pandemic influenza A(H1N1)2009 by RT-PCR. Controls were individuals presenting with ILI in the same period who were swabbed and tested negative. If they tested positive for other non-influenza respiratory viruses they were still included in the control group. Individuals who tested positive for other subtypes of influenza A or for influenza B were excluded from the vaccine effectiveness (VE) estimates.

A standard specimen request form provided demographic and clinical information on cases and controls including date of birth, gender, date of onset, date of specimen collection, influenza vaccination status and vaccination date. Information on type of vaccine and dose was also collected.

Laboratory methods

Samples were sent to the HPA Centre for Infections (RCGP scheme), local HPA Regional Microbiology Network laboratories (RMN scheme) or the West of Scotland Specialist Virology Centre (HPS scheme) for molecular testing. Laboratory confirmation was undertaken using RT-PCR assays for circulating influenza A viruses, influenza B viruses and other respiratory viruses including respiratory syncytial virus and adenovirus [15-17].

Statistical methods

The two exposures of interest were vaccination with 2009/10 seasonal trivalent influenza vaccine and vaccination with either Pandemrix or Celvapan. Respiratory samples with a delay greater than 29 days between illness onset and sample collection were excluded as viral load is likely to be substantially reduced so long after disease onset. Although any such reduction in sensitivity (provided specificity remains high) is unlikely to affect VE estimates [18], a sensitivity analysis was undertaken restricting the VE estimation to a maximum of seven days between illness onset and sample collection. Only two individuals (both controls) had received a second dose of pandemic vaccine at the time of this study; these were not categorised differently to those who had received one dose.

Individuals were considered vaccinated if their date of seasonal or pandemic vaccination was 14 days or more before the date of onset [2]. As there is some evidence that the immune response induced by pandemic vaccines is more rapid than for seasonal vaccines (E. Miller, HPA, personal communication), sensitivity analyses were carried out including individuals with a date of pandemic vaccination seven or more days before onset of symptoms.

For individuals whose date of onset was missing, the date of sample minus the median delay between illness onset and sample collection (three days) was assumed. As this assumption may affect the estimate of VE (if the exposure of interest is misclassified), we also investigated the effect of using the actual date of sample, or date of sample minus seven days for individuals with a missing date of onset. For the small number of samples (0.5%) for which the date of sample collection was missing, the date of receipt in the laboratory was used instead.

VE was estimated using logistic regression models with pandemic influenza A(H1N1)2009 PCR result as outcome and seasonal or pandemic vaccination status as the linear predictor. VE can then be estimated as 1-[odds ratio] [18]. Age (coded into five standard age groups, <5 years, 5-14 years, 15-44 years, 45-64 years and 65 years and above), sex, seasonal influenza vaccination status, country (England or Scotland), surveillance scheme (HPS, RCGP or RMN), date of sample collection (month) and the number of days delay between onset of symptoms and sample collection (coded into five categories: 0-1 day, 2-4 days, 5-7 days, 8-14 days and 15-29 days) were investigated as potential confounding variables.

Model selection for seasonal or pandemic VE estimation was performed by initially including age, date and vaccination status as covariates in the regression model. Other variables were added if they were significant and changed the vaccination odds ratios by 20% or more. Subgroup analyses by age group (<15 years and ≥ 15 years), for individuals who had received only

one dose of vaccine, and for samples collected within seven days of onset were carried out.

As there were a large number of individuals with missing pandemic vaccination status, including only complete case data could potentially have lead to bias if the missing information was not completely at random. Instead, these observations were coded as 'vaccination status unknown' and included in the logistic regression models. The effect of excluding these individuals or classifying them as unvaccinated was also investigated. Individuals coded as vaccinated with pandemic vaccine, but with an unknown date of vaccination, were initially excluded from the logistic regression models. A sensitivity analysis was then carried out by refitting the final model assuming that those with missing vaccination dates for seasonal vaccine had all been vaccinated before 17 October (implying they would all have had an immune response by 1 November), and that those with missing pandemic vaccination dates had all been vaccinated on 21 October. We also investigated the effect of using week rather than month of sample collection as an indicator of time period. All statistical analyses were carried out in R version 2.10.1[19].

Vaccination status information collected on the swab request forms was validated by linking swab records from the HPS and RCGP swabbing schemes to electronic records from a subset of the practice team information database from HPS and electronic database records from RCGP network practices, respectively [20,21]. Linkage was achieved using age, sex, date of swab collection and practice post code for RCGP and the community health index (CHI) number for the HPS scheme. This also allowed an investigation of the vaccination status of persons with missing vaccination information on the swab request form. Validation was not possible for swabs collected through the RMN scheme.

Ethics approval

In England, ethics approval was not required and informed consent was not sought. The work was carried out under National Health Service (NHS) Act 2006 (section 251) for England, which provides statutory support for disclosure of such data by the NHS, and their processing by the HPA, for purposes of communicable disease control. In Scotland, ethics approval was not required and informed consent was not sought. HPS remains a constituent part of the NHS and coordinates the investigation and management of all national outbreaks.

Results

This report comprises information on 5,985 individuals whose samples were collected through the three surveillance systems in the study period, and who had a known PCR result. Two persons were positive for influenza B and one other person was positive for influenza A(H3): these three individuals were not included at any stage of the analysis. Of the remaining 5982, 1,746 (29.2%) were positive for influenza A(H1N1), 630 individuals (10.5%) were positive for other respiratory viruses, and 3,606 individuals (60.3%) were negative for all viruses tested. Table 1 shows the distribution and completeness of the baseline characteristics of the study participants according to whether they were cases or controls.

For the 663 individuals (11.1%) for whom the date of onset was missing, the date of sample minus the median delay (three days) was used. The proportion with missing date of onset was not significantly higher among those positive for pandemic influenza A(H1N1)2009 than among those who were negative: 174 of 1,746 (10.0%) compared with 487 of 4,236 (11.5%), chi-square test p=0.09. The proportion of individuals with unknown pandemic vaccination status (Table 1) was significantly higher among cases than controls (chi-square test p<0.001). The proportion of individuals with unknown pandemic vaccination status decreased between November (1,982 of 3,572 with unknown vaccination status, 55.5%) and January (207 of 640, 32.3%).

Of the 186 individuals who had received pandemic vaccine, only two (1.1%) had received two doses of vaccine: the remainder had received one dose of pandemic vaccine. Of the 97 vaccinated individuals for whom vaccine brand was known, only one had received Celvapan (one dose) and the rest Pandemrix.

One hundred and thirty individuals had received both seasonal and pandemic vaccines. This amounted to 69.9% of the 186 pandemic vaccinees and 21.6% of the 601 individuals who had received seasonal vaccination

Pandemic vaccine effectiveness

Among individuals who had received the pandemic vaccine, four of 85 (4.7%) were positive for pandemic influenza A(H1N1)2009 14 days after vaccination, compared with 870 (28.4%) of 3,067 unvaccinated individuals who were positive. This difference was statistically significant (chi-square test p<0.0001), giving a crude pandemic VE estimate in preventing confirmed pandemic influenza A(H1N1)2009 infection of 88% (95% confidence interval (CI): 66% to 95%).

The four vaccine failures occurred in people aged between 15 and 64 years. Three of them had received Pandemrix, and for one vaccine brand was unknown. All had received one dose.

The VE of the pandemic vaccine, adjusted for age group and sampling date (month) was 72% (95% CI: 21% to 90%) (Table 2). These were the only two variables which altered the crude VE estimate by more than 20%. As the vaccine failures all occurred in adults, the unadjusted pandemic VE point estimate in children aged less than 15 years was 100% (binomial exact 95% CI: 74% to 100%), and in adults aged 15 years and over, the pandemic VE estimate was 67% (95% CI: 6% to 88%).

Adjusted seasonal influenza VE was -30% (95% CI: -89% to 11%). This estimate was adjusted for age group, sampling date (month) and pandemic vaccination status; these were the only variables which were significantly associated with a positive test result for pandemic influenza A(H1N1)2009 and altered the crude odds ratio for seasonal influenza vaccination status by more than 20%. If all individuals with an unknown date of seasonal influenza vaccination were assumed to be vaccinated on 17 October (and should therefore have developed protection by 1 November), the adjusted VE of the seasonal influenza vaccine was -22% (95% CI: -60% to 8%).

As a number of individuals included with a missing date of onset (n=616) were included in the final model, we examined the effect of setting the date of onset as equal to the date of sampling or date of sampling minus seven days if the date of onset was missing. The point estimates of the VE for either seasonal or pandemic vaccination remained the same. Several other sensitivity analyses were also carried out, with varying assumptions about the vaccination status of individuals with missing vaccination status (Table 2).

The adjusted VE estimate remained robust to varying assumptions about the true vaccination status and date of vaccination of individuals for whom this information was missing, and restriction to various subgroups. If vaccine protection was assumed to be induced after seven or more days rather than 14 days, 120 individuals could be classified as vaccinated with pandemic vaccine, among whom seven (5.8%) were positive for pandemic influenza A(H1N1)2009. This gave an adjusted pandemic VE estimate of 71% (95% CI: 37% to 87%). There was only a minimal effect on VE when using week of sample collection rather than month (as a factor variable) in controlling for time period.

In order to validate data on pandemic vaccination status, RCGP and HPS swab data were linked to general practitioner (GP) records. Linkage was successful for a total of 1,468 individuals (of whom 910 were in the HPS scheme and 558 in the RCGP scheme). Of the 41 individuals recorded as vaccinated in the dataset from the swabbing programme, four (9.8%) did not have a record of vaccination in GP databases; however vaccination could have occurred in a hospital setting. Among the 606 individuals who were unvaccinated according to the swabbing dataset, only two (0.3%) were vaccinated according to the GP records and 604 were unvaccinated. Among the 821 individuals for whom there was no information on pandemic vaccination status in the swabbing dataset, only seven (0.9%) were vaccinated according to their GP records, the rest (99.1%) were unvaccinated. The proportion of vaccinated individuals in this group was significantly (chi-square test p<0.001) lower than among individuals with a known vaccination status, among whom 3.1% (95% CI: 2.7%, to 3.6%) were vaccinated (Table 1).

Discussion

This study has demonstrated high effectiveness of the newly developed monovalent pandemic influenza vaccine against confirmed pandemic influenza A(H1N1)2009 infection one week after vaccination – although the proportion of the study population that had received vaccination was low. No significant association, protective or otherwise, between trivalent seasonal influenza vaccination and confirmed pandemic influenza A(H1N1)2009 infection has been identified.

The case–control design employed in this study is an established method to estimate effectiveness of seasonal influenza vaccine in several countries [14, 22-26] and its robustness has been validated [21]. There are, however, potential limitations: Firstly, a convenience sample was used because random sampling of patients for a routine surveillance system based on GP-provided care is not feasible. It is unlikely, however, that the sampling would have caused substantial bias: although it is conceivable that a GP might selectively sample patients based on their vaccination status, their case or control status would not have been known at the time of sampling. Thus any selection bias would be randomly distributed. Selection bias could occur if severity of symptoms was related to influenza A(H1N1)2009-positive status, and GPs selectively sampled from persons with more severe symptoms whom they also know were vaccinated (although instructions are to sample the first few cases seen every week, regardless of vaccination status). This scenario would lead to an underestimation of VE. Secondly, as the vast majority of vaccinated individuals in this study for whom the vaccine brand was known had received Pandemrix, our results will not be applicable to Celvapan. Indeed, the study reflects the distribution of doses by vaccine brand delivered in the UK. Consequently, the estimated VE presented here is mainly applicable to Pandemrix. Thirdly, there were no data available on whether an individual had a chronic condition and therefore was in a target group for pandemic influenza vaccination. As the presence of a chronic condition may increase the severity of illness associated with influenza (compared to other respiratory infections) and thus the likelihood of seeking treatment in primary care, this may have lead to an underestimation of VE. A larger, more detailed study based on individual data from general practices would provide the possibility to adjust for such potential confounders. Fourthly, the impact of the influenza A(H1N1)2009 pandemic was greatest in children and young people, very few of whom had received the seasonal vaccine. For this reason, the effect of seasonal vaccination cannot be measured with precision. Finally, a number of samples lacked information on vaccination status. Several sensitivity analyses were carried out to examine the effect of various assumptions regarding vaccination status for those with missing vaccination status information. The pandemic VE estimates, however, appeared robust in these scenarios. Furthermore, validation of a sample of the RCGP and HPS swab data showed agreement of 99.1% between the information provided on the swab request form and

Table 1

Personal and clinical characteristics of pandemic influenza A(H1N1) cases and controls, United Kingdom, 1 November 2009 – 29 January 2010 (N=5,982)

Variable	Number of cases (% of cases *N*=1,746)	Number of controls (% of controls *N*=4,236)
Received pandemic vaccine		
Vaccinated ≥14 days before onset	4 (0.2)	81 (1.9)
Vaccinated 7-13 days before onset	3 (0.2)	32 (0.8)
Vaccinated <7 days before onset	10 (0.6)	45 (1.1)
Vaccinated – date unknown	0 (0)	11 (0.3)
Unvaccinated[a]	877 (50.2)	2,225 (52.5)
Vaccination status unknown	852 (48.8)	1,842 (43.5)
Received seasonal vaccine		
Vaccinated ≥14 days before onset	52 (3.0)	234 (5.5)
Vaccinated<14days before onset	15 (0.9)	85 (2.0)
Vaccinated – date unknown	45 (2.6)	170 (4.0)
Unvaccinated[a]	1,476 (84.5)	3,313 (78.2)
Vaccination status unknown	158 (9.0)	434 (10.2)
Sex		
Female	934 (53.5)	2,486 (58.7)
Male	797 (45.6)	1,708 (40.3)
Unknown	15 (0.9)	42 (1.0)
Age group (years)		
<5	211 (12.1)	824 (19.5)
5-14	597 (34.2)	550 (13.0)
15-44	723 (41.4)	1,790 (42.3)
45-64	192 (11.0)	790 (18.6)
65+	21 (1.2)	265 (6.3)
Unknown	2 (0.1)	17 (0.4)
Date of sample		
November 2009	1,308 (74.9)	1,399 (33.0)
December 2009	371 (21.2)	2,264 (53.4)
1-29 January 2010	67 (3.8)	573 (13.5)
Interval (days between onset and sample collection)		
0-1	384 (22.0)	616 (14.5)
2-4	844 (48.3)	1,773 (41.9)
5-7	247 (14.1)	823 (19.4)
8-14	72 (4.1)	378 (8.9)
15-29	17 (1.0)	110 (2.6)
≥30	8 (0.5)	47 (1.1)
Unknown	174 (10.0)	489 (11.5)
Surveillance scheme		
RCGP	608 (34.8)	1,581 (37.3)
RMN	186 (10.7)	548 (12.9)
HPS	952 (54.5)	2,107 (49.7)

HPS: Health Protection Scotland RCGP: Royal College of General Practitioners' surveillance scheme; RMN: Health Protection Agency (HPA) Regional Microbiology Network.

[a] By date of onset.

the GP electronic record. The proportion of persons recorded as vaccinated by their GP was significantly lower among those with missing pandemic vaccination information on the swab request form compared to those where this information was available.

This study demonstrates that the pandemic influenza vaccine was highly effective in reducing confirmed pandemic influenza infection in persons consulting in primary care. In addition, it provides evidence of protection from as early as seven days after vaccination. This discovery corroborates findings of the high immunogenicity of pandemic vaccines in clinical trials: a UK study has reported that 79% of participants had seroconverted by 14 days after receiving a single dose of MF-59-adjuvanted vaccine [2]. More recent published work done after introduction of the pandemic vaccine into the German national programme has demonstrated it to be highly effective using the screening method [27]. However, although the investigators adjusted for the confounding effect of age, the screening method should be treated cautiously due to potential unrecognised confounding [28]. Our VE findings have been adjusted for various confounders. The results are similar to the estimated effectiveness of the traditional trivalent non-adjuvanted seasonal influenza vaccine during periods in which the vaccine is well matched with the circulating influenza strain [26,29], and the pandemic VE estimated here is considerably higher than in seasons of vaccine mismatch [23].

The peak of pandemic influenza activity during the second wave was in October 2009, at which stage the pandemic vaccine programme had only just started. Thus only a small proportion of the eligible population had been vaccinated at a time when pandemic virus was circulating widely. Consequently, although the observed pandemic VE was high in this study, because uptake was relatively low at this stage, any impact of the programme on disease at the population level would be more limited. This highlights the challenge of rapidly developing a new vaccine and implementing a new vaccine programme.

This study found no evidence that vaccination with 2009/10 trivalent seasonal influenza vaccine was associated with increased or decreased risk of subsequent pandemic influenza A(H1N1)2009 infection in the UK. This contrasts with conflicting published reports that seasonal influenza vaccine might either increase subsequent risk of pandemic influenza [11] or alternatively provide protection against pandemic influenza, particularly severe disease [7]. This study replicates findings from case–cohort studies in Australia and the United States, in which no protective effect was reported from the 2008/09 seasonal vaccine [8,9]. This observation suggests that cross protection from earlier seasonal vaccination cannot be assumed.

In conclusion, this study provides evidence that the pandemic influenza A(H1N1)2009 vaccine provided good protection against infection with pandemic influenza A(H1N1)2009 seven days or more after vaccination during the pandemic period. Further work is required to ascertain the effectiveness of the pandemic vaccine in children, in specific clinical risk groups and by individual vaccine brand.

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Table 2

Adjusted pandemic vaccine effectiveness under various assumptions and exclusion criteria, United Kingdom, 1 November 2009 – 29 January 2010

Assumption or exclusion criterion	Adjusted[a] pandemic vaccine effectiveness (95% confidence interval)	*n* in model
Individuals with missing vaccination dates excluded, individuals with missing vaccination status included as separate category	72% (21%–90%)	5,808
All individuals with missing vaccination status are assumed unvaccinated	71% (20%–90%)	5,808
All individuals with missing vaccination dates are assumed vaccinated on 21 October	74% (28%–91%)	5,819
Including only those individuals who received one dose of vaccine	71% (20%–90%)	5,806
Excluding individuals with missing pandemic vaccination status	73% (26%–90%)	3,147
Excluding individuals with an interval between onset and sampling of more than seven days	70% (15%–89%)	4,601
Pandemic vaccination protection begins after seven days	71% (37%–87%)	5,843
Using week rather than month as indicator of time period	73% (24%–90%)	5,808

[a] Adjusted for age group and sampling date (month).

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Conflicts of interest
All authors have completed the Unified Competing Interest form at http://www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare that DMF has received funding to attend influenza related meetings and has received consultancy fees from influenza vaccine manufacturers who might have an interest in the submitted work in the previous there years. In addition, The Virus Reference Department of the Health Protection Agency receives funding from a variety of vaccine manufacturers who might have an interest in the submitted work. All other authors declare they have no conflicts of interest.

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Effectiveness of the 2009 seasonal influenza vaccine against pandemic influenza A(H1N1)2009 in healthcare workers in New Zealand, June-August 2009

S Jefferies[1,2], D Earl[2], N Berry[2], T Blackmore[2], S Rooker[2], N Raymond[2], A Pritchard[1], M Weatherall[2,3], R Beasley (Richard.Beasley@mrinz.ac.nz`)[1,2], K Perrin[1,2]

1. Medical Research Institute of New Zealand, Wellington, New Zealand
2. Capital and Coast District Health Board, Wellington, New Zealand
3. University of Otago Wellington, Wellington, New Zealand

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There is uncertainty whether the 2009 seasonal influenza vaccination influences the risk of infection with the 2009 pandemic influenza A(H1N1) virus. This issue was investigated in 548 healthcare workers from Capital and Coast District Health Board, Wellington, New Zealand, presenting with influenza-like illness during the influenza pandemic between June and August 2009. All workers completed an assessment sheet and had a nasopharyngeal swab tested by real-time RT-PCR. The risk of pandemic influenza A(H1N1) infection associated with the 2009 seasonal inactivated trivalent influenza vaccine was determined by logistic regression, with adjustment for potential confounding variables. In 96 workers pandemic influenza A(H1N1) RNA was detected and 452 tested negative. The multivariate analysis did not show any effect of vaccination on PCR-confirmed influenza A(H1N1)2009 infection (odds ratio 1.2, 95% confidence interval 0.7–1.9, p=0.48). We conclude that 2009 seasonal influenza vaccination had no protective effect against influenza A(H1N1)2009 infection amongst healthcare workers. To protect against further waves of the current pandemic influenza or future pandemics in which the influenza virus is antigenically distinct from contemporary seasonal influenza viruses, it would be necessary to vaccinate with a specific pandemic influenza vaccine, or a seasonal influenza vaccine that includes the pandemic influenza serotype.

Introduction

One of the important public health issues emanating from the global response to control the influenza pandemic was whether the seasonal trivalent inactivated influenza vaccination provided any protection. The novel reassortment of the influenza A(H1N1)2009 virus, combining swine, avian and human influenza genetic sequences, suggested that seasonal vaccination would confer little or no protection against this new virus [1-3]. This view was supported by a report from the United States that vaccination with seasonal influenza vaccines, regardless of whether they contained adjuvant, induced little or no cross-reactive antibody response to pandemic influenza A(H1N1) in any age group [4,5]. Consistent with these data, a case-cohort study from the United States [6], a case-control study from Australia [7], and a case series from Canada [8] have reported that the 2008/09 seasonal trivalent influenza vaccine provided no protective effect against pandemic influenza A(H1N1) infection.

In contrast, epidemiological studies from Mexico suggested that the seasonal trivalent inactivated influenza vaccine, administered as part of a national vaccination programme in 2009, provided partial protection against the 2009 pandemic influenza A(H1N1) [9,10]. In the case-control study [9], evidence was also provided that seasonal vaccination might protect against the most severe forms of the disease. It was proposed that these findings were consistent with an older report that showed that the 1967 seasonal influenza vaccine contributed towards preventing disease in the 1968/69 influenza pandemic in those who had not received the pandemic vaccine [11]. Furthermore, studies have reported variable levels of protection among infants, children and adults at times when seasonal influenza vaccine strains were not antigenically well matched to circulating endemic strains [12-17]. However, a case-control study based on Canada's sentinel vaccine effectiveness monitoring system reported that receipt of the 2008/09 seasonal influenza vaccine decreased the risk of seasonal influenza infection as expected, but was associated with an increased risk of pandemic influenza A(H1N1) infection [18]. In the same publication, two further Canadian case-control studies and one prospective cohort study were described in which seasonal influenza vaccination was associated with a 1.4 to 2.5-fold increased risk of medically attended illness due to pandemic influenza A(H1N1) [18]. Thus, epidemiological evidence exists to suggest that the 2009 seasonal influenza vaccination may increase, decrease

or have no effect on the risk of pandemic influenza A(H1N1) infection [19].

The provision of a comprehensive occupational health programme and the availability of occupational, virology and clinical databases of healthcare workers at Capital and Coast District Health Board (CCDHB) provided a unique opportunity to investigate this issue. In this prospective study, we report the potential effect of the 2009 seasonal influenza vaccine on the likelihood of acquisition of influenza A(H1N1)2009 in healthcare workers in New Zealand.

Methods

CCDHB has a comprehensive occupational health service which established an acute on-call programme for the investigation and treatment of workers who developed symptoms suggestive of influenza-like illness (ILI) during the 2009 influenza pandemic. The programme was activated in the second week of June 2009 within six weeks of the first confirmed case of pandemic influenza A(H1N1) infection in New Zealand [20]. In accordance with CCDHB policy, all staff who developed influenza-like symptoms, at work or elsewhere, were required to consult the occupational health service. The influenza-like symptoms included, but were not limited to, fever, runny nose, sore throat and cough. They completed a standardised influenza assessment sheet, provided a nasopharyngeal swab and were prescribed oseltamivir. The influenza assessment sheet collected information on variables such as age, sex, area of work, co-morbidity, pregnancy, the time between the onset of symptoms and nasopharyngeal swab, and whether the staff member self-reported having received the 2009 seasonal trivalent influenza vaccine. Travel from New Zealand in the four weeks prior to ILI was also recorded, although the virus had become largely endemic in the community by the time the data recording started.

The swabs were combined into one tube of viral and PCR transport medium and viral RNA was extracted using the High Pure Viral Nucleic Acid kit (Roche Diagnostics). Viral RNA specimens were analysed by realtime

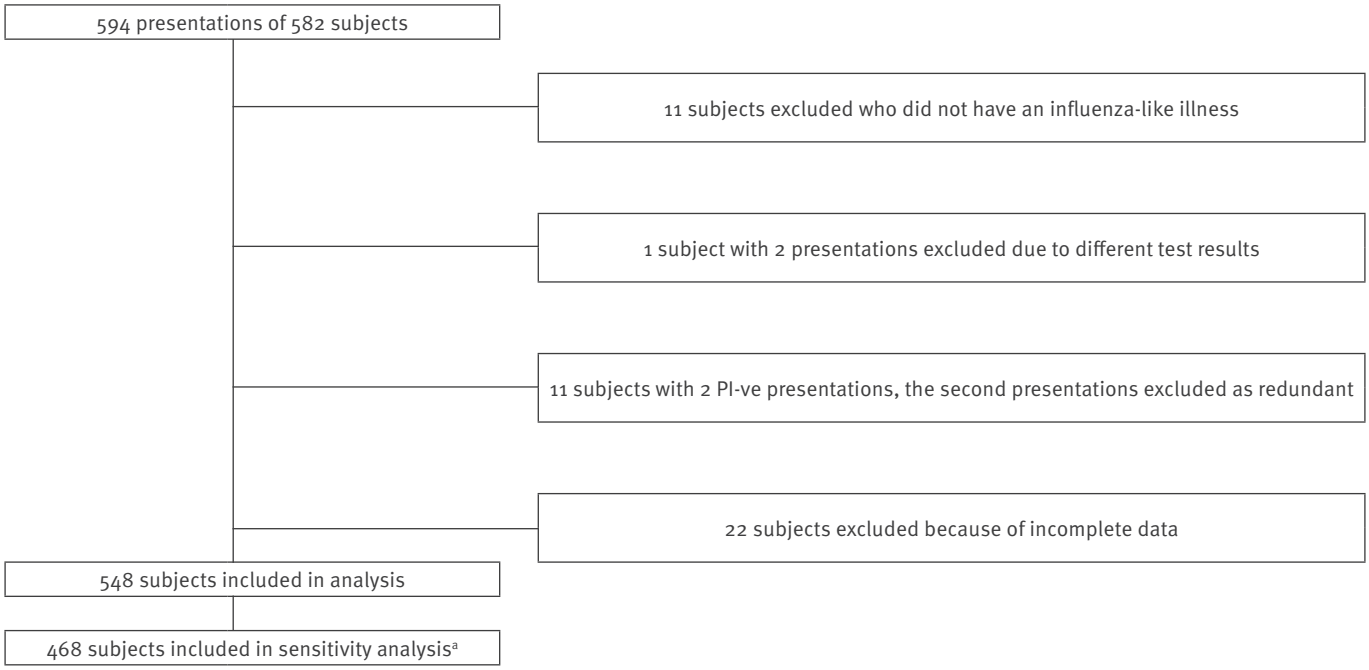
Table 1

Definition of comorbidities of study participants, New Zealand, 15 June–31 August 2009

Disorders included as comorbidity		
Respiratory	Cardiovascular	Other systemic
Asthma	Arrhythmias	Addison's disease
Bronchitis	Angina	Breast cancer on chemotherapy
Chronic obstructive pulmonary disease	Cardiomyopathy	Chronic renal failure
	Stroke	Diabetes mellitus
	Hypertension	Hepatitis B/C
	Pulmonary stenosis	Hypo/hyperthyroidism
		Inflammatory bowel disease
		Renal transplant
		Rheumatoid arthritis
		Scleroderma
		Systemic lupus erythematosus
		Thalassaemia
Disorders not included as comorbidity		
Chronic backpain/spinal fusion		
Cyclic vomiting syndrome		
Depression		
Eczema		
Epilepsy		
Fibromyalgia		
Gout		
Hypercholesterolaemia		
Irritable Bowel Syndrome		
Marfan's Syndrome		
Obstructive Sleep Apnoea		
Osteoarthritis		
Psoriasis		
Reflux gastritis		

Figure

Inclusion criteria for study participants, New Zealand, 15 June–31 August 2009 (n=582)



PI+ve: pandemic influenza A(H1N1) RNA detected by rRT-PCR; PI-ve: pandemic influenza A(H1N1) RNA not detected by rRT-PCR.

[a] 80 subjects had no documentation of OHS administered seasonal influenza vaccine

Table 2

Characteristics of healthcare workers presenting with influenza-like illness, New Zealand, 15 June–31 August 2009 (n=548)

	Mean (standard deviation)		
Variable	PI+ve N=96	PI-ve N=452	All N=548
Age (years)	37.3 (10.8)	39.5 (11.3)	39.1 (11.3)
Deprivation decile	5.4 (2.9)	5.1 (2.9)	5.1 (2.9)
Days between symptom onset and swab	1.3 (1.1) N=92	1.5 (1.6) N=418	1.5 (1.5) N=510
	n/N (%)		
	PI+ve	PI-ve	All
Male sex	30/96 (31.3)	99/452 (21.9)	129/548 (23.5)
Ethnicity			
• Not stated	8/96 (8.3)	19/452 (4.2)	27/548 (4.9)
• Māori	8/96 (8.3)	31/452 (6.9)	39/548 (7.1)
• Pacific island	9/96 (9.4)	28/452 (6.2)	37/548 (6.8)
• Other	71/96 (74.0)	374/452 (82.7)	445/548 (81.2)
Patient contact	83/96 (86.5)	353/452 (78.1)	436/548 (79.6)
Travel[a]	2/96 (2.1)	15/452 (3.3)	17/548 (3.1)
Pregnancy (women only)	1/66 (1.5)	5/353 (1.4)	6/419 (1.4)
Comorbidities	31/96 (32.3)	114/452 (25.2)	145/548 (26.5)
Hospital admission	0/96 (0)	2/452 (0.4)	2/548 (0.4)
Emergency department attendance	6/96 (6.3)	9/452 (2.0)	15/548 (2.7)
Self-reported vaccination[b]	53/96 (55.2)	233/451 (51.7)	286/547 (52.3)
OHS-documented vaccination[c]	44/83 (53.0)	186/385 (48.3)	232/468 (49.6)

OHS: occupational health service; PI+ve: pandemic influenza A(H1N1) RNA detected by rRT-PCR; PI-ve: pandemic influenza A(H1N1) RNA not detected by rRT-PCR; realtime reverse transcription PCR.

[a] International travel within four weeks before influenza-like illness symptoms.

[b] One participant missing data.

[c] Documentation of 2009 seasonal influenza vaccination in occupational health service personal files. For 80 subjects a file was not available.

reverse transcription PCR (rRT-PCR) using the Capillary Lightcycler instrument version 1.2 (Roche Diagnostics) following protocols provided by the World Health Organization Collaborating Centre for the Surveillance, Epidemiology and Control of Influenza at the United States Centers for Disease Control and Prevention [21]. Swab specimens were tested using primers targeting the influenza A matrix gene, designed for universal detection of type A influenza viruses, and the influenza A haemagglutinin (H) gene (SwH1), specifically designed to detect pandemic influenza A(H1N1)2009. A sample was defined as positive for pandemic influenza A(H1N1) when both genes were detected. Specimens testing positive for the matrix gene but with no detectable levels of SwH1 were tested for seasonal human influenza A(H1) and A(H3) virus by rRT-PCR using primers and probes from version 2007 of the CDC protocol [21]. For the purposes of the analyses in this study, participants in whom pandemic influenza A(H1N1) RNA was detected (PI+ve) were compared with participants in whom no pandemic influenza A(H1N1)2009 or seasonal strains were detected (PI-ve).

The seasonal influenza vaccine used in New Zealand in 2009 was the inactivated trivalent vaccine Fluarix (GlaxoSmithKline), containing 15µg haemagglutinin each of the three strains A/Brisbane/59/2007, IVR-148 (H1N1), A/Uruguay/716/2007, NYMCX-175C (H3N2) and B/Brisbane/60/2008.

The CCDHB and Hutt Valley District Health Board (HVDHB) patient information systems of the participants were accessed to obtain information on ethnicity and deprivation decile. In New Zealand, deprivation decile is derived from nine variables descriptive of socio-economic status relative to the location of the home, such as income, home ownership and access to transport. It ranges from 1 (least deprived) to 10 (most deprived) [22]. We also used these databases to identify whether any of the participants were admitted to or attended the emergency department of Wellington,

Table 3

Univariate associations between study participants' characteristics and confirmed pandemic influenza A(H1N1) infection, New Zealand, 15 June–31 August 2009 (n=548)

Variable	Odds ratio for association (95% confidence interval)	p value
Age (per decade older)	0.8 (0.7 to 1.0)	0.08
Deprivation decile (per level)	1.0 (0.96 to 1.1)	0.45
Male sex	1.6 (1.0 to 2.6)	0.05
Ethnicity		0.18 [a]
• Not stated	2.2 (0.9 to 5.3)	0.26 [a]
• Māori	1.4 (0.6 to 3.1)	0.76 [a]
• Pacific island	1.7 (0.8 to 3.7)	0.71 [a]
• Other	Reference level	
Patient contact	1.8 (1.0 to 3.4)	0.07
Travel[b]	0.6 (0.1 to 2.8)	0.53
Pregnancy (women only)	1.1 (0.1 to 9.3)	0.95
Comorbidities	1.4 (0.9 to 2.3)	0.15
Hospital admission	Not applicable	0.51
Emergency room attendance	3.3 (1.1 to 9.4)	0.02
Self-reported vaccination	1.2 (0.7 to 1.8)	0.53
OHS-documented vaccination[c]	1.2 (0.7 to 1.9)	0.49

OHS: occupational health service.

[a] Compared to 'Other'.

[b] International travel within four weeks before influenza-like illness symptoms.

[c] Documentation of 2009 seasonal influenza vaccination in occupational health service personal files. For 80 subjects a file was not available.

Table 4

Multivariate association between study participants' vaccination status and confirmed pandemic influenza A(H1N1) infection[a], New Zealand, 15 June–31 August 2009 (n=548)

Variable	Odds ratio for association (95% confidence interval)	p value
Self-reported vaccination	1.2 (0.7 to 1.9)	0.48
OHS-documented vaccination[b]	1.2 (0.7 to 1.9)	0.49

OHS: occupational health service.

[a] Adjusted for age, sex, ethnicity, deprivation decile, patient contact, relevant travel, pregnancy (all men coded as not-pregnant), comorbidities.

[b] Documentation of 2009 seasonal influenza vaccination in Occupational Health Service personal files. In 80 subjects no file was available.

Kenepuru and Hutt hospitals for an ILI in the two days before and the two weeks after the swab was taken. These three government-funded hospitals represent the only hospitals in the greater Wellington region which provide acute medical services. Workers admitted to hospital with an ILI were considered to have experienced a severe influenza illness.

The CCDHB occupational health service keeps the records of the assessment and treatment of healthcare workers presenting with suspected pandemic influenza A(H1N1) (including the influenza assessment sheet, PCR results and prescribed treatment). The personal files of all healthcare workers employed at CCDHB were checked for documentation of the 2009 seasonal influenza vaccination. The sensitivity analysis of the effect of the 2009 seasonal influenza vaccination was based on these records. The demographic, clinical, occupational, vaccination and virological data was entered in a database where every subject was given a unique identifier. The dataset was coded and anonymised prior to analysis.

Statistical power

With 100 cases and 450 controls and assuming a 50% immunisation rate in the controls, the study had 80% power to detect an odds ratio of 0.52.

Statistical analysis

Logistic regression was used to determine the strength of association between PCR-confirmed pandemic influenza A(H1N1) infection and self-reported seasonal influenza vaccination, unadjusted and adjusted for potential confounding variables. The variables included age, sex, ethnicity (Maori, Pacific, other, not stated), deprivation decile, relevant overseas travel, comorbidity (yes/no) (Table 1), and pregnancy (yes/no, all men coded as not pregnant). SAS version 9.1 was used for the statistical calculations.

This analysis was restricted to subjects who presented with an ILI and had documentation of the influenza assessment sheet and PCR results. Subjects who presented on more than one occasion and had different PCR results from the different presentations were excluded. In subjects who presented on more than one occasion and pandemic influenza A(H1N1) was not detected on any presentation, the data from the first presentation was included.

Results

There were 582 healthcare workers who presented on 594 occasions to the CCDHB occupational health service between 15 June and 31 August 2009 (Figure). After application of the exclusion criteria, 548 workers who had presented with an ILI were included in the analysis.

The characteristics of these participants are shown in Table 2. The mean age of the participants was 39 years (range: 20 to 69 years) and 24% were male. People of Maori and Pacific origin made up 14% of the study group. The majority of participants (80%) had clinical patient contact as part of their work. Overall, 52% of the participants self-reported having received the 2009 seasonal influenza vaccination. In 27% of participants comorbidities were reported, of which the most common were asthma and hypertension. Among the 145 healthcare workers with documented comorbidities, 82 self-reported having received the 2009 seasonal vaccine, 62 self-reported not having received it, and for one the information was missing. The mean time from the onset of symptoms to nasopharyngeal swab was 1.5 days.

Influenza A was detected by PCR in 103 of the 548 included participants. In 96 of those pandemic influenza A(H1N1) was detected, in five seasonal human influenza A(H1), in one seasonal human influenza A(H3) and in one an untypable strain of influenza A. We therefore determined 96 (17.5%) participants with confirmed pandemic influenza A(H1N1) infection (PI+ve) and 452 (82.5%) in whom pandemic influenza A(H1N1) was not detected (PI-ve).

There was no difference in the proportion of workers with and without proven pandemic influenza A(H1N1) infection who reported having received the 2009 seasonal influenza vaccination, with 53 of 96 (55.2%) infected and 233 of 451 (51.7%) not infected at an odds ratio of 1.2 (95% confidence interval (CI): 0.7–1.8, p=0.53) (Table 2 and 3). The multivariate analysis, adjusted for age, sex, ethnicity, deprivation decile, patient contact, overseas travel, comorbidity and pregnancy, did not indicate any significant risk of pandemic influenza A(H1N1) being associated with the 2009 seasonal influenza vaccine (odds ratio: 1.2, 95% CI: 0.7–1.9, p=0.48) (Table 4).

Personal files of 468 of the participants were held by the occupational health service. In a sensitivity analysis based on the documentation from these files, we saw no significant effect of 2009 seasonal influenza vaccination on the risk of pandemic influenza A(H1N1) neither in the univariate analysis (odds ratio: 1.2, 95% CI: 0.7–1.9, p=0.49) (Table 3) nor multivariate analysis (odds ratio: 1.2, 95% CI: 0.7–1.9, p=0.49) (Table 4).

PI+ve participants were similar to PI-ve participants with regard to age, deprivation decile, pregnancy, comorbidities, relevant travel, and time between symptom onset and swab (Tables 2 and 3). There was no statistically significant difference in ethnicity between the swab-negative and swab-positive group, however this analysis was limited by the small numbers of people of Maori and Pacific origin, and the point estimates were consistent with an increased risk. Likewise, the point estimate for patient contact was consistent with an increased risk, but the difference was not statistically significant (odds ratio: 1.8, 95% CI: 1.0–3.4, p=0.07).

Fifteen people with an ILI visited an emergency department in the two days before and two weeks

after presentation to the occupational health service. Participants who attended an emergency department were more likely to be PI+ve (odds ratio: 3.3, 95% CI: 1.1–9.4, p=0.02). Two people were admitted to hospital with an ILI, both of whom were PI-ve.

Discussion

In our prospective study the 2009 seasonal influenza vaccination had no protective effect against pandemic influenza A(H1N1) infection amongst healthcare workers in New Zealand. This suggests that to obtain protection against influenza A(H1N1)2009 in the current season 2010, it would be necessary to vaccinate with a specific pandemic influenza A(H1N1) vaccine, or to include the influenza A(H1N1)2009 antigenic group in the 2010 seasonal influenza vaccine.

A number of methodological issues are relevant to the interpretation of the study findings. Firstly, by recruiting healthcare workers, we were able to study a population with a high prevalence of seasonal influenza vaccination; about half of the workers included in the study had received the 2009 seasonal influenza vaccine. Secondly, by studying workers, all of whom were under 70 years-old, we were able to investigate a group that did not have prior widespread immunity to pandemic influenza, assuming that the age-specific rates of pre-existing protective antibodies in New Zealand are similar to those in the United Kingdom [23]. All subjects presenting to the occupational health service with an ILI provided nasopharyngeal swabs which were assessed by rRT-PCR. The mean time between onset of symptoms and nasopharyngeal swab was 1.5 days, with no significant difference between groups, suggesting that delay in viral sampling was unlikely to be a confounding factor [24].

Another issue is the accuracy of the seasonal vaccination records. For the primary analysis, information on vaccination status was provided by the workers when completing the influenza assessment sheet at the time of presentation to the occupational health service. As this information was provided without knowledge of the PCR results, and the seasonal influenza vaccinations had taken place in the three months before the study, we consider the findings unlikely to be influenced by recall bias. For the sensitivity analysis, seasonal influenza vaccination status was also determined from documentation in the participants' personal files held by the occupational health service. While this approach was limited by the fact that not all workers had personal files and some workers may have been vaccinated through community services, the comparable results provided internal validity to the study findings.

Pandemic influenza infection results in disease with a wide spectrum of severity, from asymptomatic to life-threatening illness [24-26]. All participants included in our analysis presented with a symptomatic ILI, which means that asymptomatic workers with influenza infection were not included in the study. Due to the low frequency of severe illness requiring hospital admission (none among the confirmed pandemic influenza A(H1N1) cases in our study) we were unable to determine whether seasonal influenza vaccination may protect against the most severe forms of the disease.

Thanks to the prospective collection of comprehensive data at the time of presentation and the availability of clinical databases, we were able to undertake multivariate analyses in which we adjusted for variables that could have influenced the association between 2009 seasonal influenza vaccination and infection with pandemic influenza A(H1N1)2009. These factors included age, sex, ethnicity, work-related patient contact, overseas travel, pregnancy and comorbidities. This approach lent strength to our statistical analysis.

Our findings add to recent data from studies that have identified no risk [6-8], a decreased risk [9,10], or an increased risk [18] of pandemic influenza A(H1N1) infection associated with seasonal influenza vaccination. An Australian study found no evidence in any age group of seasonal influenza vaccination providing significant protection against pandemic influenza A(H1N1) virus infection [7]. In that study the population had been vaccinated with an inactivated trivalent vaccine which contained the A/Brisbane/59/2007 antigenic group as the H1N1 component, the same subtype variant included in the trivalent vaccine in our study. The strength of their study was the validity of vaccination records, virological confirmation of influenza infection in subjects presenting with ILI and the age-stratified and age-adjusted analyses.

A case-control study from Mexico demonstrated that seasonal influenza vaccination had 73% effectiveness against pandemic influenza A(H1N1) [9]. This study was limited by the choice of controls, who had a higher rate of co-morbidity and for that reason may have been more likely to receive seasonal influenza vaccination, and by the fact that the vaccination status was retrospectively collected and there was no microbiological verification of the absence of influenza infection [27,28]. Similar limitations apply to a cohort study from the United States, which did not find any protective effect of seasonal influenza vaccination on pandemic influenza infection [6].

However, these potential limitations do not apply to a subsequent large surveillance study of pandemic influenza A(H1N1) virus infection in Mexico, which showed that the risk of infection was reduced by about one third in those who had been vaccinated for seasonal influenza [10]. Although it has been suggested that these study results could have been confounded by selection bias, if elderly people who are more likely to be vaccinated were less likely to be infected with pandemic influenza due to pre-existing immunity [29], this was not supported by subsequent stratified analysis [30]. Based on data from the first and second waves

of the pandemic in Mexico up to 30 November 2009, the negative association between seasonal vaccination and risk of testing positive for pandemic influenza A(H1N1) was present across all age groups, including those younger than 60 years [30].

In contrast, three case-control studies and a prospective cohort study demonstrated a statistically significant 1.4 to 2.5-fold increased risk of medically attended illness due to pandemic influenza A(H1N1) [18]. The first of these studies, based on Canada's well established sentinel vaccine effectiveness monitoring system identified that seasonal influenza vaccination increased the risk of pandemic influenza infection to a similar extent as it reduced the risk of seasonal influenza infection (+68% versus -56%) [18]. A study of an outbreak of pandemic influenza A(H1N1) infection amongst United States military personnel also identified an increased risk of infection, although this association was limited to personnel on active duty and not their family members or retired staff [33].

The reasons for these contrasting results are uncertain. It is possible that they may be due to methodological differences between the studies, or to differences in the effect of the specific vaccines, in the immunisation programmes or in population immunity [18,34]. Regardless of the underlying reasons, these epidemiological studies suggest that seasonal influenza vaccination cannot be considered or recommended as an effective strategy for the prevention of pandemic influenza infection.

In conclusion, this study has shown that the 2009 seasonal influenza vaccination provided no protection against pandemic influenza A(H1N1) infection in healthcare workers in New Zealand. To obtain protection against subsequent waves of the pandemic influenza A(H1N1)2009 by vaccination, it would therefore be necessary to either vaccinate with a specific pandemic influenza vaccine or a seasonal influenza vaccine which includes the influenza A(H1N1)2009 subtype. The findings also suggest that in future influenza pandemics in which the virus is antigenically and genetically distinct from contemporary human seasonal influenza viruses, development of a specific pandemic influenza vaccine is a high priority, as partial protection by the contemporary seasonal influenza vaccines cannot be assumed.

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