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Clinical presentation and laboratory findings for the first autochthonous cases of dengue fever in Madeira island, Portugal, October 2012

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An outbreak of dengue fever in Madeira island was reported in 2012. Clinical and laboratory findings of the first two laboratory-confirmed autochthonous cases are reported. Both cases had fever (≥38 °C) and petechial rash. Symptoms also included myalgia, asthenia, nausea, vomiting, anorexia, diffuse abdominal pain, and diarrhoea. The two cases were confirmed by serology and one tested positive for a dengue viral sequence. Dengue virus serotype DEN-1 was identified with probable Central or South American origin.

Dengue virus (family *Flaviviridae*, genus *Flavivirus*) is the aetiological agent of dengue fever, a mosquito-borne infection endemic in the tropics and subtropics. The National Institute of Health Dr. Ricardo Jorge (INSA) performs reference laboratory diagnosis of dengue in Portugal. Human cases identified annually in this laboratory are imported from endemic areas. In terms of number of positive samples, these areas include by order of importance, mainly Brazil, but also Timor-Leste, India, Cape Verde, Mexico, Thailand, Angola, Pakistan and Vietnam. In the beginning of October 2012, for the first time, two autochthonous cases of dengue fever from Madeira archipelago, Portugal were diagnosed. These signaled the beginning of an outbreak and the latest published data includes 2,144 reported autochthonous cases of dengue fever in Madeira island [1]. Clinical and laboratory findings of these first two cases are reported.

Case reports

Case 1

On 20 September, 2012 a 16 year-old woman developed febrile illness with temperature up to 38.5°C, nausea, vomiting, anorexia, diffuse abdominal pain and diarrhoea. Five days later she presented at the local hospital, with prostration, and, in the second day after admission developed petechial rash at the upper and lower limbs that spread to the lower abdomen within 24 hours.

The patient, who lives in Caniço in the neighbourhood of the town of Funchal and studies in Funchal, mentioned traveling to Algarve, south of mainland Portugal, six weeks before and had not been vaccinated against flaviviruses such as yellow fever, tick-borne encephalitis and Japanese encephalitis viruses.

The first laboratory findings in the hospital showed thrombocytopenia with minimal platelet count ($65x10^9$/L, norm: 150–$400x10^9$/L), leucopenia ($2.4x10^9$/L, norm: 4.5–$13.5x10^9$/L) and elevated transaminases (alanine/glutamic pyruvic transaminase (ALT/GPT): 117U/L, norm: 17–63 U/L; aspartate/glutamic-oxaloacetic (AST/GOT): 95 U/L, norm: 10–50 U/L). In the Funchal hospital the screening test for dengue was performed by immunochromatography (NADAL dengue fever IgG/IgM) followed by dengue IgM and IgG capture enzyme-linked immunosorbent assay (ELISA) (Panbio). The serum sample taken on day 6 after the onset of febrile illness was positive by immunochromatography, ELISA IgM positive (5.91) and IgG negative (0.07) (cut-off >1.1).

In the National Institute of Health, sera samples were tested by immunofluorescent assay in-house (IgG and IgM) and Euroimmun commercial test (IgM). The serum sample (day 11 post onset) was positive for dengue virus specific IgM with a titre of 256 (cut-off =16) and IgG with a titre of 1,024 (cut-off =32). The serum sample was also tested for immunoglobulins specific to other flaviviruses, such as yellow fever, tick-borne encephalitis, West Nile and Japanese encephalitis viruses by immunofluorescent assays and all assay results were negative. The patient recovered without complications and was discharged from the hospital on the eighth day of hospitalisation.

Case 2

On 27 September 2012, a 44 year-old man developed febrile illness with temperature up to 38.0°C, myalgia

and asthenia. Two days later he visited the local hospital and presented also with petechial rash at the upper and lower limbs.

The patient who lives and works in Santa Luzia, a local administrative unit of the city of Funchal, did not travel abroad and reported no vaccination against yellow fever, tick-borne encephalitis and Japanese encephalitis viruses.

The first laboratory findings in the hospital showed thrombocytopenia with minimal platelet count ($74x10^9$/L, norm: $150–400x10^9$/L), leucopenia ($2.7x10^9$/L, norm: $4.5–13.5x10^9$/L), very high creatine kinase (CK) (1,146 ng/ml, norm: 20–200 ng/ml), and slightly elevated AST/GOT (58 U/L, norm: 10–50 U/L), high lactate dehydrogenase (LDH) (395 U/L, norm: <246 U/L). A sample collected on day 2 post onset was positive by immunochromatography, ELISA IgM positive (5.32), unusually at day 2, and IgG negative (0.18). The IgM antibodies in cases of dengue infection are usually detected after the fifth day following the onset of symptoms suggesting that maybe, in this case, the date of the onset was not properly determined.

In the National Institute of Health the serum sample (day 4 post symptom onset) tested by immunofluorescent assay was positive for dengue virus specific IgM with a titre of 1,024 (cut-off =16) and IgG with a titre of 512 (cut-off =32). The serum sample was also subjected to immunofluorescent assays for immunoglobulins specific to other flaviviruses, such as yellow fever, tick-borne encephalitis, West Nile and Japanese encephalitis viruses and all assay results were negative.

The patient recovered without complications and was discharged from the hospital on the fourth day of hospitalisation. Twelve days after the onset of symptoms he returned for further evaluation, and the blood count had returned to normal (leukocytes ($7.2x10^9$/L, norm: $4.5–13.5x10^9$/L) and platelets ($326x10^9$/L, norm: $150–400x10^9$/L)).

Molecular laboratory analysis

In the National Institute of Health laboratory, nucleic acids were extracted from ethylenediaminetetraacetic acid (EDTA) blood samples using NucliSens easyMAG platform (bioMérieux). The presence of dengue virus RNA was checked by one-step conventional reverse transcription-polymerase chain reaction (RT-PCR) using generic flavivirus primers targeting the non-structural protein 5 (NS5) gene [2,3] followed by dengue specific assays including multiplex RT-PCR targeting the core-pre-membrane (CprM) region [4,5] and real time RT-PCR [6].

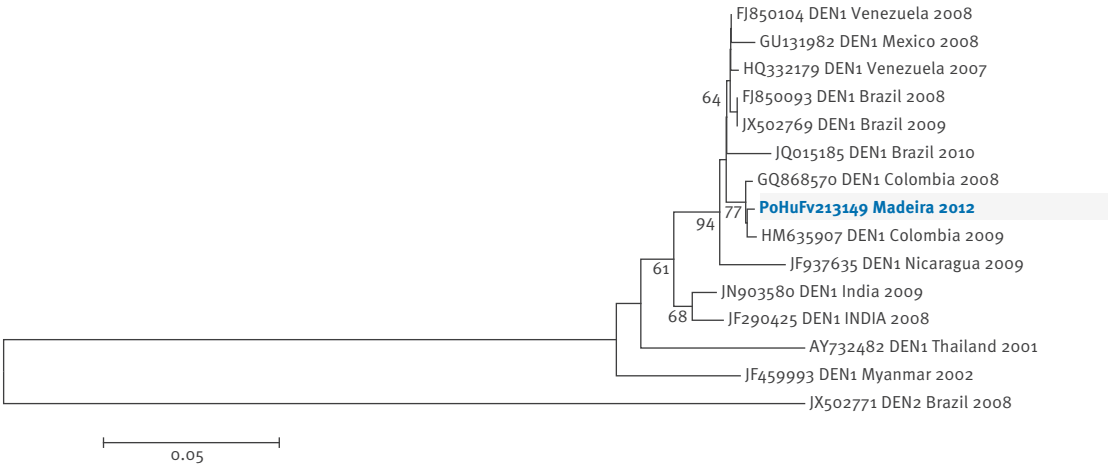
Fragments obtained by nested-PCR (NS5, 163 bp and CprM region, 454 bp) were sequenced bi-directionaly. The 454 bp CprM partial polyprotein gene sequence was deposited on the GenBank database under accession number KC248375. Similarity searches were made within the GenBank data set using the basic local alignment search tool (BLAST) BLASTN algorithm [7].

For case 1, RT-PCR for flaviviruses was negative as well as the results from both dengue specific assays (conventional multiplex and real time RT-PCR) on day 11 post onset of disease. For case 2, RT-PCR for flaviviruses was positive as well as dengue specific real time RT-PCR and conventional multiplex RT-PCR on day 5 post onset.

Four distinct dengue virus serotypes (DEN-1, DEN-2, DEN-3 and DEN-4) are recognised [8]. Sequence

Figure

Phylogenetic analysis of a viral sequence derived from an autochthonous case of dengue fever in Madeira, Portugal, October 2012



The phylogeny was inferred based on the core-pre-membrane (CprM) nucleotide sequences (454 bp) by neighbour-joining method using molecular evolutionary genetics analysis (MEGA) version 5 software. Distance matrices were calculated using Kimura two-parameter model. Bootstrap values obtained from 1,000 replicate trees are shown for key nodes. The sequence derived from the autochthonous case of dengue fever in Madeira is highlighted in blue.

analysis of NS5 and CprM partial sequences derived from case 2 by BLASTN and a preliminary phylogenetic analysis based on CprM nucleotide sequences ascertain DEN serotype 1 identified as related to viruses circulating in latin America namely Colombia, Venezuela, and the Roraima region in northern Brazil [7] (Figure). The virus isolated in Madeira is also associated with DEN-1 strains recognised as belonging to genotype V (e.g. in the Figure, GenBank accession numbers: JX502769, JQ015185), a genotype that represents most of the strains collected in the Americas, West Africa and a limited number of strains collected from Asia [9].

For the case 2, the viral isolation in Vero E6 cells was achieved and the full genome sequence is in progress.

Discussion and conclusions

Infection with dengue virus causes a wide spectrum of human disease, from asymptomatic infections, to classic dengue fever and to haemorrhagic disease. More severe disease is usually associated with secondary infections with heterologous serotypes [10,11]. Between early October 2012 and the beginning of 2013, 2,144 autochthonous dengue virus infections were reported in the island of Madeira. There were no reports of severe disease and a decrease of new cases in the last weeks of 2012 was observed [1].

Dengue virus is transmitted by *Aedes* mosquitoes, namely *Aedes (Ae.) aegypti* and *Ae. albopictus*. *Ae. aegypti* was introduced in Madeira probably from a Caribbean country (personal communication, M Melim, 2009) and its presence was detected for the first time in 2005, in the city of Funchal [12]. Although vector control measures to eradicate or to reduce the spread of this invasive mosquito species were taken, it became the most abundant mosquito species namely in the sites selected for the national vector surveillance programme in 2010 and 2011 [13].

The first cases of the dengue fever outbreak in Madeira, as the majority of all cases, happened in Funchal, the urban environment where the mosquito Ae. aegypti was first found [12]. In 2012, the combination of the high vector density with multiple breeding sites in the city [13] and lack of immunity of the population are likely to explain the dimension of the outbreak.

After the initial cases presented here and as of the end of November 2012, the National Institute of Health laboratory tested an additional 43 samples by RT-PCR, 28 of which were found positive. In all of these cases DEN-1 serotype was identified.

The circulation of the four serotypes of dengue in the Caribbean region and South America is described [8]. Hence as it has not yet been identified how the mosquito and the virus were introduced in Madeira, we can assume that there is a risk of new introductions, and spread of new virus serotypes in the next years. As it is well known, the absence of long-term cross-immunity among the different dengue serotypes allows for multiple sequential infections with heterologous serotypes and the occurrence of cases with more severe disease.

Vector control and surveillance in the territory to avoid the mosquitos' introduction to other regions of Madeira archipelago and to the mainland is necessary and has already been implemented by the local health authorities.

After 2012, the clinical cases should also be evaluated and serotyped due to the risk of new virus introductions.

In cases of dengue outbreaks, the laboratory has an essential role for early detection and reporting of the first cases. This contributes to the decision to implement vector control measures. Laboratory tests are important for precise identification of the aetiological agent and to retrace its origin. The reference laboratory also provides a quality control for other laboratories and helps with the continuous monitoring of suspected cases

Conflict of interest

None

Authors' contributions

All authors collaborated in the work and in the manuscript presented here. MJA: laboratory coordination at INSA and manuscript preparation; PLF, GA: clinical data and laboratory techniques in Madeira; MJA, FA, HO,LZZ: molecular diagnosis at INSA; MJA, TL, PP: serological diagnosis at INSA; LZZ, HZ: data analysis and suggestions to the manuscript.

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Early and unexpectedly severe start of influenza epidemic in the Czech Republic during influenza season 2012–13

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A sudden increase in severe influenza has been registered in the Czech Republic since the end of 2012, with 264 cases requiring intensive care, including 51 deaths. Most patients had at least one risk factor. Severe influenza in patients with obesity, smoking and/or haematological disorders including haematological cancers was more frequent than in the pre-pandemic period. The seasonal influenza vaccination status of the cases indicates indirect efficiency of the current vaccine in preventing severe influenza.

Influenza virus activity in Europe is detected each season, yet the precise timing and magnitude of this activity remain highly unpredictable. There was increased media attention regarding the re-occurrence of severe influenza A(H1N1)pdm09 cases at the beginning of 2013 in the Czech Republic. This led to a higher demand of antiviral drugs. In order to clarify the current influenza situation, we provide here representative data from the case-based reports of severe influenza cases and combine them with the data received from routine surveillance.

Influenza surveillance in the Czech Republic

Epidemiological and virological surveillance of influenza and other viral acute respiratory infections (ARI) is well established in the Czech Republic (population: 10.5 million) [1]. The surveillance system is active throughout the year and uses the European Union case definition for influenza [2]. Data are collected weekly and analysed at national level. The information is provided to the European Centre for Disease Prevention and Control (ECDC) and the World Health Organization (WHO), where it is analysed together with the data from other countries of the European Union and the WHO European Region, respectively [3,4]. There is no routine reporting of severe acute respiratory infection (SARI) from hospitals. Nevertheless, due to the occurrence of increased numbers of severe influenza cases during the pandemic in 2009, the Regional Public Health Authorities started, on request of the Ministry of Health of the Czech Republic to provide case-based information about hospitalised patients with influenza illness who require treatment at intensive or resuscitation care units.

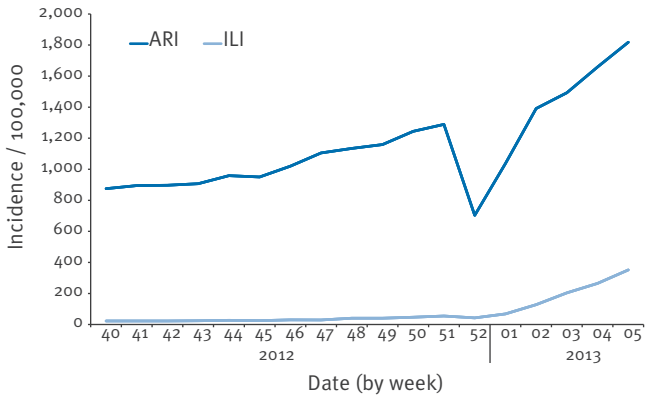
To gain insight into the genetic characteristics of the influenza A viruses circulating in the Czech Republic, the National Influenza Reference Laboratory in Prague sequences the full coding region of the genome from selected isolates or positive clinical specimens and subsequently performs phylogenetic analysis of representative A(H1N1)pdm09 influenza virus strains.

Influenza season 2012/13

Since mid-November 2012, the incidence of ARI and influenza-like illness (ILI) has been increasing in all monitored age groups (0–5, 6–14, 15–24, 25–59, ≥60 years and total population). There was an artificial decrease in reporting during the Christmas and New Year holidays that is seen every year. Since the beginning of January 2013, the reported ARI and ILI rates have not been unusual, and the influenza epidemic threshold of 1,600 ARI cases per 100,000 population

Figure 1

Weekly morbidity from acute respiratory infection and influenza-like illness, Czech Republic, influenza season 2012/13



Rates per 100,000 population; data up to 1 February 2013.

per week was reached as late as during week 4 of 2013 (week ending 25 January 2013) (Figure 1).

Nevertheless, two other indicators signalised significant influenza activity in the Czech Republic. Firstly, the absolute numbers as well as the percentage of influenza-positive samples among the tested specimens (sentinel sampling consists of two swabs, one child and one adult, collected per week from each of the 14 regions in the Czech Republic) has been considerably higher after week 51, 2012 (Table 1).

Secondly, hospital surveillance has noted a clear increase of very severe influenza illness since the beginning of the year 2013, compared to non-epidemic period. By 1 February 2013, a total of 264 hospitalised patients (159 male and 105 female) with severe influenza illness that required treatment at intensive or resuscitation care units (ICU) have been reported to public health authorities (Table 2). Among those were 51 deaths (34 men and 17 women).

Of the 264 ICU patients, 174 (66%) were positive for influenza A(H1N1)pdm09 virus, 76 (29%) were positive for influenza A(unknown sub-type), six were positive for influenza A(H3N2) virus, one for influenza B virus, and seven cases remained unconfirmed by virology (determined by clinical diagnosis only). The virological results for influenza ICU patients were similar to the data from combined sentinel and non-sentinel surveillance.

The mean age of the influenza ICU patients was 56 years (min: one month; max: 92 years; median: 59 years). The mean age of the fatal influenza ICU cases was 64 years (min: 27 years; max: 92 years; median: 67 years). The main risk factors included: obesity with body mass index above 30 (79/264), smoking (65/264), chronic cardiovascular (77/264), respiratory (46/264) or haematological illness (21/264), often in combination. Only seven patients were vaccinated against seasonal influenza during autumn 2012.

Phylogenetic analysis of the haemagglutinin (HA) gene of the first three strains in the 2012/13 season, representing the influenza A(H1N1)pdm09 viruses currently circulating the Czech Republic, indicate that they cluster within the H1N1pdm09 group 6. This group, along with H1N1pdm09 group 7, represents the currently predominating phylogenetic lineages of this influenza virus strain in Europe (Figure 2; Table 3). Comparing our HA sequences with the A/California/7/2009(H1N1) strain revealed up to 12/549 amino acid changes (A/Czech Republic/140/2012(H1N1); 97.8% identity), three of which were localised within the known antigenic sites (in H3 numbering): Ca2 (H141R), Sb (S188T) and Ca1 (S206T).

Discussion and conclusions

Influenza A(H1N1)pdm09 and unsubtyped influenza A viruses were found in almost all cases of influenza ICU patients. Since the A(H1N1)pdm09 virus was the predominant strain we suppose that the majority of unsubtyped influenza A viruses also belonged to this subtype. The majority of deaths were linked primarily to rapidly developing respiratory failure as already described [5]. In particular, progressive pneumonitis caused by A(H1N1)pdm09 virus is difficult to explain since the virus is neither shown to have markedly increased virulence over other seasonal influenza viruses, nor it is a particularly strong cytokine inducer in vitro [6]. The potential for increased pathogenicity could be a result of the combination of other genetic markers [7].

The current influenza situation in the Czech Republic is not exceptional as such. It is relatively similar to the season 2010/11 when the influenza A(H1N1)pdm09 virus dominated the epidemic. However, other European

Table 2

Number of severe influenza patients at intensive care units and deaths by week, Czech Republic, 22 December 2012–1 February 2013 (n=264)

	Number of new cases	Of which deaths
week 52, 2012	12	3
week 1, 2013	28	3
week 2, 2013	40	5
week 3, 2013	60	13
week 4, 2013	56	16
week 5, 2013	68	11
Total	**264**	**51**

Table 1

Sentinel and non-sentinel influenza virus detections, Czech Republic, influenza season 2012/13 (n=994)

Week	40	41	42	43	44	45	46	47	48	49	50	51	52	1	2	3	4
Influenza A		1				3	1	1	2		5	16	35	59	61	117	129
Influenza A(H1N1)pdm09							2			1	5	10	10	67	133	95	164
Influenza A(H3N2)		1	1									4	3		12	10	16
Influenza B		1		2			1				1	2	1	3	6	5	8
Total influenza-positive	**0**	**3**	**1**	**2**	**0**	**3**	**4**	**1**	**2**	**1**	**11**	**32**	**49**	**129**	**212**	**227**	**317**
Samples tested	**15**	**32**	**37**	**40**	**40**	**37**	**35**	**33**	**61**	**58**	**81**	**82**	**75**	**260**	**529**	**681**	**697**
Positivity in %	0	9	3	5	0	8	11	3	3	2	14	39	65	50	40	33	45

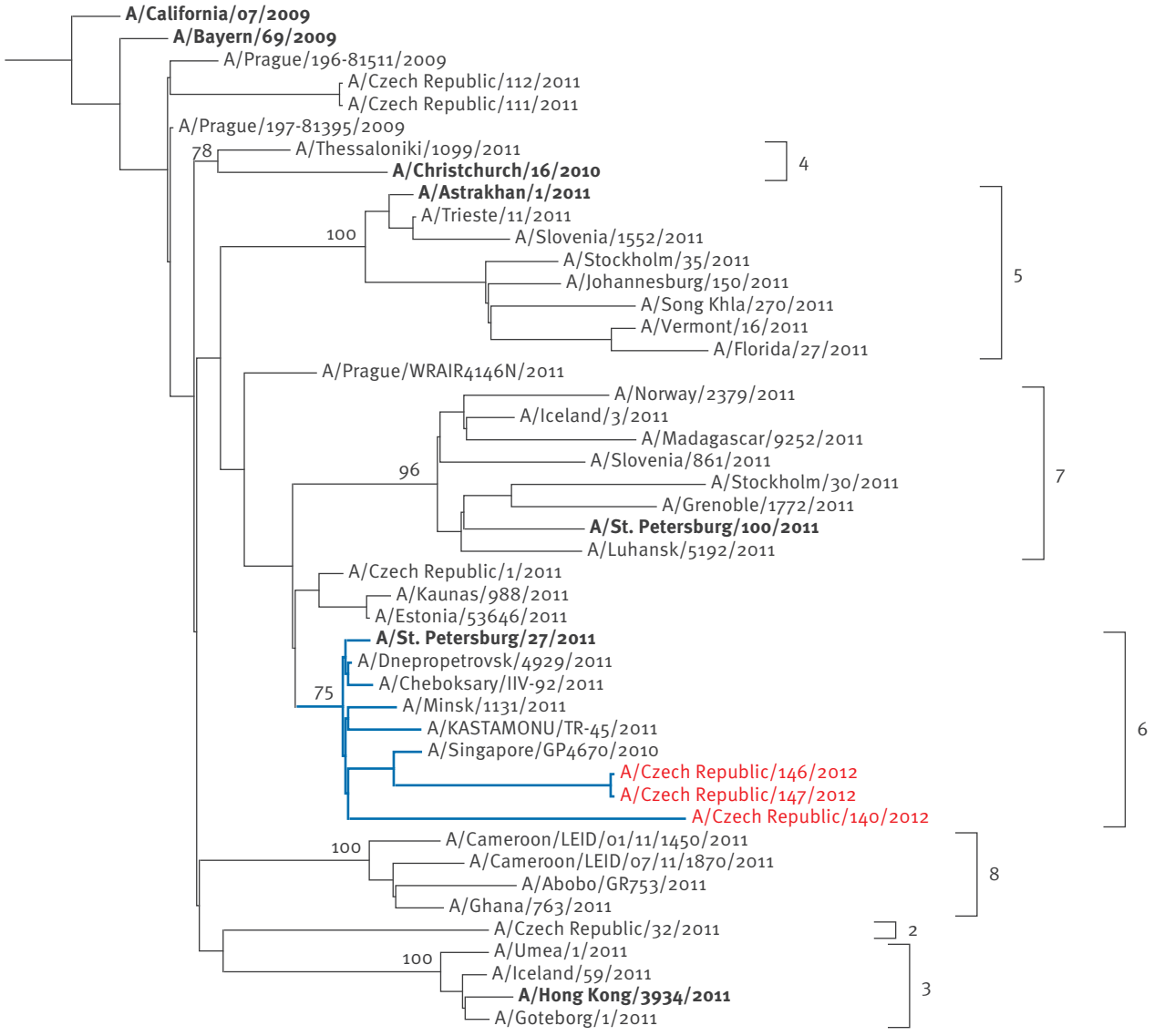
countries do not report such high numbers of severe influenza this year [3,4]. So far during this season, only 14 fatalities have been reported from EU countries in which hospital surveillance of severe influenza disease is established, 12 of these occurred in France [3]. With regards to circulation of influenza viruses in Europe, the situation is varied. For example, influenza B virus is dominant mainly in the United Kingdom (UK), influenza A(H3N2) in Denmark and A(H1N1)pdm09 in Austria, Lithuania and Norway [3]. This report may serve as an alert that a sudden increase in severe influenza cases may soon be seen also in other, especially Eastern European, countries this season.

The observations presented here prompted us to build up the following hypothesis: the proportion of people vaccinated against influenza in the Czech Republic is small, only approx. 5% of the whole population. As this year's seasonal vaccine is matching the circulating viruses well, which resulted in a vaccine effectiveness of 62% in the United States [8], the proportion of people protected against influenza in other countries should be higher than in the Czech Republic, especially in Western Europe where the influenza epidemic started earlier.

In addition, we have to consider that the effectiveness of the trivalent vaccine may well be different for the different components. If the virus mix circulating in one country was considerably different from what is

Figure 2

Phylogenetic tree of influenza A(H1N1) haemagglutinin gene



The H1 tree was constructed from representative sequences collected from 2009 to 2012 and obtained from the Global Initiative on Sharing All Influenza Data (GISAID) and the Influenza Virus Resource databases. The tree was generated with the maximum-likelihood algorithm in the PHYLIP programme (Felsenstein, 2004) [9] on the basis of 1,650 nt sequences and rooted to the A/California/7/2009(H1N1) influenza strain. Bootstrap values (100 re-samplings) in percentages are indicated at key nodes. The viruses sequenced in this report are highlighted in red and the reference strains in bold. The cluster representing the influenza A(H1N1)pdm09 group 6 is coloured blue.

Table 3

Origin of the haemagglutinin sequences of pandemic influenza A(H1N1) isolates included in the phylogenetic analysis

Isolate name	Collection date	Isolate ID		Originating laboratory
		IVR	GISAID	
A/Prague/197-81395/2009(H1N1)	2009-06-27	GU290047		National Institute of Public Health,Prague, Czech Republic
A/Prague/196-81511/2009(H1N1)	2009-07-15	GU290055		
A/Czech Republic/32/2011(H1N1)	2011-01-18		EPI_ISL_90718	
A/Czech Republic/1/2011(H1N1)	2011-01-25	JF682629		
A/Czech Republic/111/2011(H1N1)	2011-10-25	JQ693484		
A/Czech Republic/112/2011(H1N1)	2011-10-25	JQ693492		
A/Czech Republic/140/2012(H1N1)	2012-11-16		EPI_ISL_133958	
A/Czech Republic/146/2012(H1N1)	2012-12-27		EPI_ISL_133959	
A/Czech Republic/147/2012(H1N1)	2012-12-24		EPI_ISL_133960	
A/Prague/WRAIR4146N/2011(H1N1)	2011-01-18	CY098004		Walter Reed Army Institute of Research, Maryland, United States
A/California/07/2009(H1N1)	2009 (month and day unknown)	CY121680		New York Medical College/NCBI, NIH, Bethesda, United States
A/Thessaloniki/1099/2011(H1N1)	2011-03-08		EPI_ISL_90764	Institut Pasteur Hellenique, Athens, Greece
A/Bayern/69/2009(H1N1)	2009 (month and day unknown)		EPI_ISL_73686	Robert Koch-Institute, Berlin, Germany
A/Cheboksary/IIV-92/2011(H1N1)	2011-02-22	JN704791		The D.I.Ivanovsky Institute of Virology, Moscow, Russian Federation
A/St. Petersburg/27/2011(H1N1)	2011-02-14		EPI_ISL_90760	WHO National Influenza Centre, St. Petersburg, Russian Federation
A/Astrakhan/1/2011(H1N1)	2011-02-28		EPI_ISL_90787	
A/St. Petersburg/100/2011(H1N1)	2011-03-14		EPI_ISL_90954	Russian Academy of Medical Sciences, St. Petersburg, Russian Federation
A/Dnepropetrovsk/4929/2011(H1N1)	2011-03-10		EPI_ISL_99894	Institute of Epidemiology and Infectious Diseases AMS of Ukraine, Kiev, Ukraine
A/Luhansk/5192/2011(H1N1)	2011-03-07		EPI_ISL_99895	
A/Minsk/1131/2011(H1N1)	2011-02-15		EPI_ISL_94707	Laboratory of Influenza and ILI, Minsk, Belarus
A/Estonia/53646/2011(H1N1)	2011-02-01		EPI_ISL_94695	Health Protection Inspectorate, Tallin, Estonia
A/Kaunas/988/2011(H1N1)	2011-02-15		EPI_ISL_99909	Lithuanian AIDS Center Laboratory, Vilnius, Lithuania
A/Stockholm/35/2011(H1N1)	2011-11-22		EPI_ISL_100460	Swedish Institute for Infectious Disease Control, Solna, Sweden
A/Stockholm/30/2011(H1N1)	2011-11-09		EPI_ISL_99784	
A/Goteborg/1/2011(H1N1)	2011-04-12		EPI_ISL_93766	
A/Umea/1/2011(H1N1)	2011-01-28		EPI_ISL_90424	
A/Norway/2379/2011(H1N1)	2011-12-08		EPI_ISL_100455	St. Olavs Hospital HF, Trondheim, Norway
A/Slovenia/861/2011(H1N1)	2011-02-05		EPI_ISL_95550	National Institute of Public Health, Ljubljana, Slovenia
A/Slovenia/1552/2011(H1N1)	2011-04-30		EPI_ISL_95564	
A/Kastamonu/TR-45/2011(H1N1)	2011-02-12		EPI_ISL_95555	Refik Saydam National Public Health Agency, Ankara, Turkey
A/Iceland/3/2011(H1N1)	2011-01-10		EPI_ISL_99914	Landspitali - University Hospital, Reykjavik, Iceland
A/Iceland/59/2011(H1N1)	2011-03-24		EPI_ISL_99924	
A/Trieste/11/2011(H1N1)	2011-01-11		EPI_ISL_90758	Istituto Superiore di Sanità, Rome, Italy
A/Grenoble/1772/2011(H1N1)	2011-09-21		EPI_ISL_103048	CRR virus Influenza region Sud, Cedex, France
A/Vermont/16/2011(H1N1)	2011-12-22		EPI_ISL_103212	Vermont Department of Health Laboratory, Burlington, United States
A/Florida/27/2011(H1N1)	2011-10-30		EPI_ISL_99811	Florida Department of Health-Tampa Bureau of Laboratories, Tampa, United States
A/Christchurch/16/2010(H1N1)	2010-07-12		EPI_ISL_79722	WHO Collaborating Centre for Reference and Research on Influenza, Victoria, Australia
A/Singapore/GP4670/2010(H1N1)	2010-12-30	CY091676		National Public Health Laboratory, Singapore,
A/Song Khla/270/2011(H1N1)	2011-09-12		EPI_ISL_99814	WHO National Influenza Centre, National Institute of Medical Research (NIMR), Nonthaburi, Thailand
A/Hong Kong/3934/2011(H1N1)	2011-03-29		EPI_ISL_93746	Government Virus Unit, Kowloon, Hong Kong
A/Johannesburg/150/2011(H1N1)	2011-07-10		EPI_ISL_99903	National Institute for Communicable Disease, Sandringham-Johannesburg, South Africa
A/Ghana/763/2011(H1N1)	2011-05-13		EPI_ISL_94709	University of Ghana, Accra, Ghana
A/Cameroon/LEID/07/11/1870/2011(H1N1)	2011-07-07		EPI_ISL_99899	Centre Pasteur du Cameroun, Yaoundé, Cameroon
A/Cameroon/LEID/01/11/1450/2011(H1N1)	2011-01-19		EPI_ISL_99900	Centre Pasteur du Cameroun, Yaoundé, Cameroon
A/Abobo/GR753/2011(H1N1)	2011-09-12		EPI_ISL_103049	Pasteur Institut of Côte d'Ivoire, Abidjan, Cote d'Ivoire
A/Madagascar/9252/2011(H1N1)	2011-08-10		EPI_ISL_99934	Institut Pasteur de Madagascar, Antananarivo, Madagascar

circulating in another country, that could affect severity of disease in these countries. Regardless of the reasons behind the current situation, early admission, prompt diagnosis and early antiviral treatment improve the outcome of patients infected with influenza A(H1N1) pdm09.

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Conflict of interest

None declared.

Authors' contributions

JK wrote the manuscript and is responsible for analysis of data from epidemiological surveillance. MH and HJ are responsible for analysis of data from virological surveillance, both contributed to the manuscript. AN did the genome sequencing and phylogenetic analysis and contributed to the manuscript. IP run the hospital ICU database and contributed to the manuscript.

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Low vaccine effectiveness against influenza A(H3N2) virus among elderly people in denmark in 2012/13 – a rapid epidemiological and virological assessment

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In Denmark, the 2012/13 influenza season has been dominated by influenza A(H3N2). We estimated the vaccine effectiveness (VE) of the trivalent influenza vaccine by linking national registers in a test-negative case–control study of patients tested for influenza aged ≥65 years. The adjusted VE against laboratory-confirmed influenza A and B was -11% (95% CI: -41 to 14) and 69% (95% CI: 26 to 87), respectively. Genetic characterisation of the influenza A(H3N2) viruses indicated genetic drift, with seven substitutions at key antigenic sites.

Background

In Denmark, consultation rates for influenza-like illness and the number of patients testing positive for influenza increased in week 51 (starting 17 December) and peaked in week 52 (starting 24 December) 2012. Compared with the previous three influenza seasons, the activity was particularly high among people aged ≥65 years. From week 51, excess mortality was observed in this age group [1]. Influenza A(H3N2) virus has to date been the dominant subtype.

Trivalent influenza vaccine (TIV) was offered free of charge to Danish citizens aged ›65 years between weeks 40 and 52. We took advantage of unique national registries to obtain a rapid within-season estimate of influenza vaccine effectiveness (VE) in people of this age group. Furthermore, we describe the vaccination coverage among influenza A patients in intensive care units (ICUs), and characterised circulating influenza A(H3N2) viruses genetically.

Estimating vaccine effectiveness against laboratory-confirmed influenza

Information on patients aged ≥65 years tested for influenza A and B virus by PCR was obtained from the Danish Microbiology Database; information on administered TIV from weeks 39 to 48 was obtained from the Danish vaccination register [2,3]. The study period ran from week 40 (1 October 2012) to week 4 (27 January 2013).

The Danish Microbiology Database comprises real-time data on microbiological diagnostic test results for the entire Danish population. The Danish healthcare sector is public and all influenza testing is done according to national guidelines.

The VE against influenza A and B was estimated in a test-negative case–control design, using the formula (1 – odds ratio) x 100%.

Cases were defined as patients who tested positive for influenza A or B virus. Controls were patients who tested negative for both influenza A and B viruses. As our study is based on results of diagnostic samples, we do not have information on symptoms or indications for testing.

Influenza cases were included when they first tested positive. Patients were considered vaccinated if they received the TIV at least two weeks before the sample was taken; otherwise they were unvaccinated. In sensitivity analyses, the cut-off was changed to three weeks.

Data were analysed in SAS using PROC LOGISTIC. Adjustment was made for age group (65–69, 70–74, 75–80, ≥80 years) and place of sampling (general practitioner vs hospital).

Estimating vaccination coverage

Vaccination coverage among influenza A patients aged ≥65 years in all Danish ICUs was estimated by linking data from the national influenza ICU surveillance system [4] with that in the vaccination register.

Virus characterisation

Nucleic acid was extracted from 200 µl of clinical samples (sentinel, surveillance and diagnostic samples), full-length haemagglutinin (HA) genes were sequenced and analysed as described previously [5]. All samples

were initially screened for influenza A in a real-time reverse transcription (RT)-PCR reaction targeting the matrix gene [6]. If up to five samples were positive for influenza A(H3) within one week, all samples were set up for sequencing. If more than 5 samples were positive within one week, one sample from each of the five regions of Denmark with an influenza A real-time RT-PCR cycle threshold <35 was picked blindly for sequencing, if available.

Vaccine effectiveness results

A total of 1,443 patients aged ≥65 years were tested during the study period for influenza: 364 and 35 tested positive for influenza A and B, respectively (Table 1). Those who tested negative were considered as controls. Some 95% (1,374/1,443) of the patients were sampled at hospitals. Vaccination coverage increased with age among influenza A cases and controls, and the proportion of patients who tested positive for influenza A also generally increased with age (Table 1).

Vaccination coverage among the cases was 45%, which was similar to the coverage among the controls (41%), as well as the estimated national coverage among people aged ≥65 years (44%, data not shown). Most of the vaccinated study participants received TIV between weeks 40 to 44; cases of influenza A mainly occurred from week 52 onwards (Figure 1).

In the study population, VE against laboratory-confirmed influenza A was −11% (95% CI: −41 to 14), whereas VE against laboratory-confirmed influenza B was 69% (95% CI: 26 to 87) (Table 2).

In the main analysis, patients were considered vaccinated if they received the TIV at least two weeks before

Figure 1

Trivalent influenza vaccines received in week 39 (24 September 2012)–week 47 (25 November 2012) and laboratory-confirmed influenza A cases in week 40 (1 October 2012)–week 4 (27 January 2013) among 1,443 patients aged ≥65 years tested for influenza, Denmark

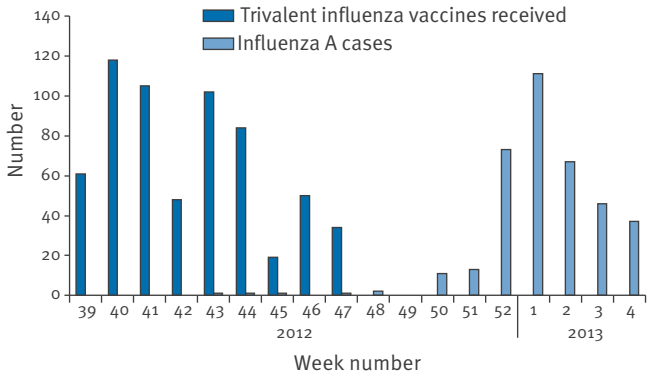


Table 1

Laboratory-confirmed influenza A and B cases (n=399) and influenza A and B test-negative controls (n=1,044) aged ≥65 years by trivalent influenza vaccination statusa, age group and sex, and vaccination coverage among influenza cases and controls by age group and sex, Denmark, week 40 (1 October 2012)–week 4 (27 January 2013)

Characteristic	Influenza A			Influenza B			Controls		
	Vaccinated (n)	Not vaccinated (n)	Vaccination coverage (%)	Vaccinated (n)	Not vaccinated (n)	Vaccination coverage (%)	Vaccinated (n)	Not vaccinated (n)	Vaccination coverage (%)
Age group									
65–69	24	51	32.0	2	11	15.4	117	211	35.7
70–74	26	44	37.1	2	4	33.3	92	153	37.6
75–79	43	41	51.2	1	4	20.0	90	111	44.8
≥80	72	63	53.3	2	9	18.2	129	141	47.8
Sex									
Male	81	108	42.9	3	16	15.8	231	326	41.5
Female	84	91	48.0	4	12	25.0	197	290	40.5
Total	**165**	**199**	**45.3**	**7**	**28**	**20.0**	**428**	**616**	**41.0**

[a] Information on trivalent influenza vaccination was obtained for week 39 (24 September 2012) to week 47 (25 November 2012). Patients were considered vaccinated if they received the vaccine at least two weeks before the sample was taken; otherwise they were considered unvaccinated.

Table 2

Crude and adjusted odds ratios and adjusted vaccine effectiveness estimates against laboratory-confirmed influenza A and B among 1,443 patients aged ≥65 years and tested for influenza, Denmark, week 40 (1 October 2012)–week 4 (27 January 2013)

Influenza type	Crude odds ratio (95% CI)	Adjusted odds ratio[a] (95% CI)	Adjusted vaccine effectiveness[a] (95% CI)
A	1.19 (0.94 to 1.52)	1.11 (0.86 to 1.41)	−11% (−41 to 14)
B	0.36 (0.16 to 0.83)	0.31 (0.13 to 0.74)	69% (26 to 87)

[a] Adjusted for age and place of sampling (general practitioner vs hospital).

the sample was taken. In sensitivity analyses, the cut-off was changed to three weeks, which did not change the estimates.

Vaccination coverage among intensive care unit patients

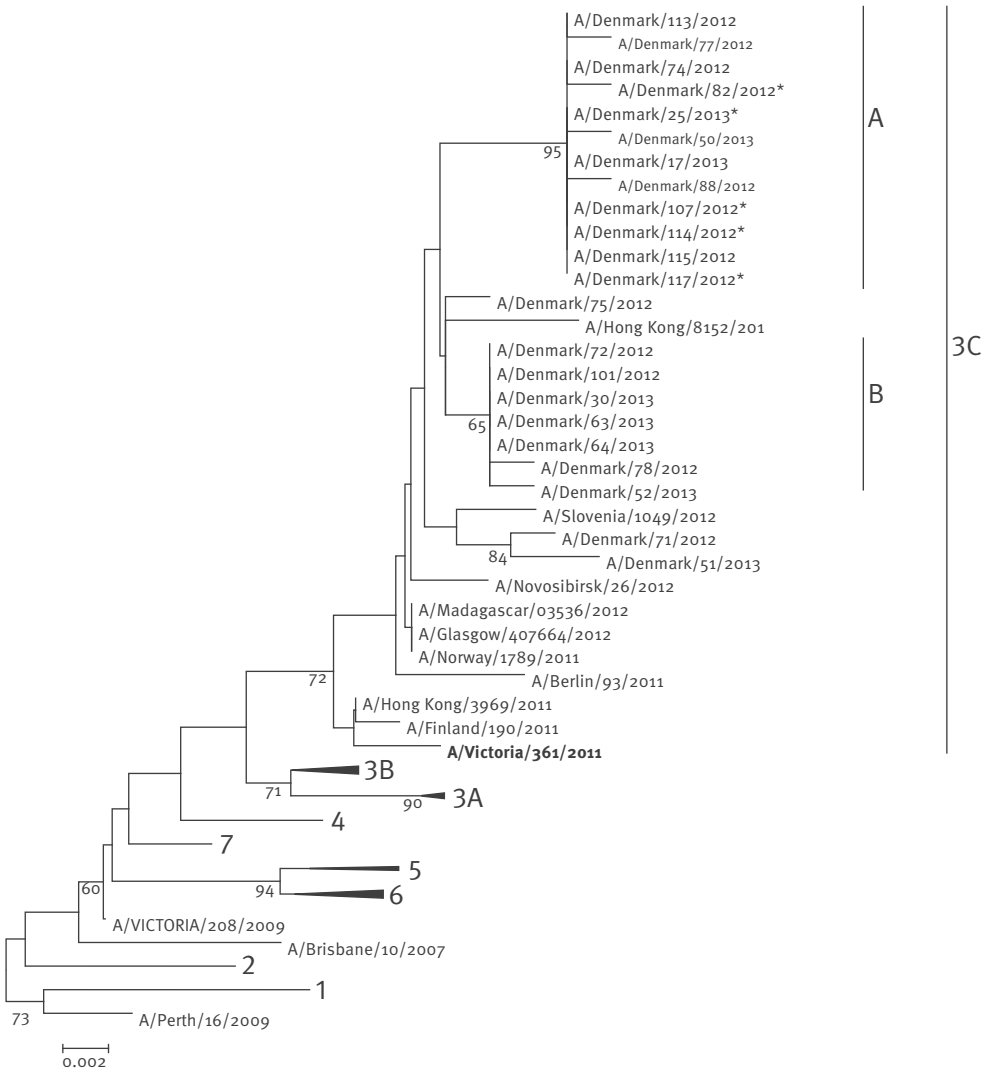
A total of 53 influenza A patients in ICUs were reported during the study period, of whom at least 22 were vaccinated with TIV at least two weeks before admission. Of the 53 ICU patients, 33 were aged ≥65 years. Samples from 16 of these elderly patients were subtyped: all contained influenza A(H3N2) virus. Of 32 elderly patients with known vaccination status, 15 were vaccinated at least two weeks before admission (47%). Nine were aged 65–69 years, seven were 70–74 years, six were 75–79 years and 10 were ≥80 years. Information on comorbidity was available for 22 of the elderly patients: the two most common comorbidities were cardiovascular and chronic pulmonary disease, reported for 17 of the 22 patients.

Virus characterisation results

The National Influenza Centre in Denmark, Statens Serum Institut, subtyped 487 clinical specimens (those positive for influenza A or B) from week 40 (1 October 2012) to week 4 (27 January 2013). Of these, 395 (81%) were A(H3N2) viruses, 59 (12%) B-Yamagata viruses, 30 (6%) A(H1N1)pdm09 and 3 (<1%) B-Victoria. Based on HA amino acid sequence analysis, the Danish A(H3N2) viruses formed two main clades, A and B (Figure 2). Clade B is within the defined 3C A(H3) phylogenetic clade, where the 2012/13 A(H3N2) vaccine component

Figure 2

Phylogenetic tree of 22 influenza A(H3N2) virus sequences coding for 523 amino acids of the viral haemagglutinin, Denmark, week 46 (14 November 2012)–week 2 (13 January 2013)



A distance-based neighbor-joining phylogenetic tree was generated using Molecular Evolutionary Genetics Analysis (MEGA) software v.5 [14] with 1,000 bootstrap replicates (values ≥60 shown on branch) and rooted to A/Perth/16/2009. The Danish isolates were collected countrywide. Reference A(H3) sequences (Table 3) were included for comparison.

Collapsed branches are given for all clades except for clade 3C, for simplicity. Clade designations are given at collapsed branch nodes or on the side of the clade for clade 3C. The influenza A(H3N2) vaccine component for the northern hemisphere influenza season 2012/13 is shown in bold. Sequences from samples from patients vaccinated before week 47 2012 are marked with an asterisk.

Table 3

Haemaglutinin sequences of influenza A(H3) virus isolates included in the phylogenetic analysis[a]

Segment ID	Country	Collection date	Isolate name	Originating laboratory	Submitting laboratory	Authors
EPI165489	Australia	2007-Jan-01	A/Brisbane/10/2007		Other Database Import	
EPI272062	Australia	2009-Jun-02	A/VICTORIA/208/2009	Victorian Infectious Diseases Reference Laboratory	WHO Collaborating Centre for Reference and Research on Influenza	Deng Y-M, Iannello P, Caldwell N, Leang S-K, Komadina N
EPI379585	Australia	2012-May-09	A/VICTORIA/802/2012	Austin Health	WHO Collaborating Centre for Reference and Research on Influenza	Deng Y-M, Iannello P, Caldwell N, Jelley L, Komadina N
EPI349106	Australia	2011-Oct-24	A/Victoria/361/2011	Melbourne Pathology	WHO Collaborating Centre for Reference and Research on Influenza	Deng Y-M, Caldwell N, Iannello,P, Komadina N
EPI377369	Russian Federation	2012-Apr-09	A/Novosibirsk/26/2012	WHO National Influenza Centre	National Institute for Medical Research	
EPI377291	Russian Federation	2012-Apr-19	A/Ekaterinburg/4/2012	WHO National Influenza Centre	National Institute for Medical Research	
EPI326139	Sweden	2011-Mar-28	A/Stockholm/18/2011	Swedish Institute for Infectious Disease Control	National Institute for Medical Research	
EPI302231	Norway	2010-Dec-03	A/Norway/1330/2010	WHO National Influenza Centre	National Institute for Medical Research	
EPI346595	Finland	2011-Nov-25	A/Finland/190/2011	National Institute for Health and Welfare	National Institute for Medical Research	
EPI392250	Ghana	2012-Jun-20	A/Ghana/ARI 1494/2012	University of Ghana	National Institute for Medical Research	
EPI392307	France	2012-Mar-08	A/Paris/972/2012	CRR virus Influenza region Sud	National Institute for Medical Research	
EPI392294	Madagascar	2012-Jun-12	A/Madagascar/03619/2012	Institut Pasteur de Madagascar	National Institute for Medical Research	
EPI392292	Madagascar	2012-Jun-04	A/Madagascar/03536/2012	Institut Pasteur de Madagascar	National Institute for Medical Research	
EPI319276	Madagascar	2011-Feb-21	A/Madagascar/0648/2011	Institut Pasteur de Madagascar	National Institute for Medical Research	
EPI377311	Estonia	2012-Mar-09	A/Estonia/66234/2012	Health Protection Inspectorate	National Institute for Medical Research	
EPI392272	Hong Kong (SAR)	2012-Jun-26	A/Hong Kong/8152/2012	Government Virus Unit	National Institute for Medical Research	
EPI331093	Hong Kong (SAR)	2011-May-19	A/Hong Kong/3969/2011	Government Virus Unit	National Institute for Medical Research	
EPI377386	Slovenia	2012-Apr-02	A/Slovenia/1049/2012	Laboratory for Virology, National Institute of Public Health	National Institute for Medical Research	
EPI379788	Iceland	2012-Apr-14	A/Iceland/24/2012	Landspitali - University Hospital	National Institute for Medical Research	
EPI358885	Greece	2012-Feb-01	A/Athens GR/112/2012	Institut Pasteur Hellenique	National Institute for Medical Research	
EPI379845	Ukraine	2012-Apr-01	A/Ukraine/5398/2012	Ministry of Health of Ukraine	National Institute for Medical Research	
EPI335714	Norway	2011-Aug-02	A/Norway/1789/2011	Norwegian Institute of Public Health	National Institute for Medical Research	
EPI326137	Norway	2011-Mar-16	A/Norway/1186/2011	Norwegian Institute of Public Health	National Institute for Medical Research	
EPI377330	United Kingdom	2012-Apr-03	A/Glasgow/407664/2012	Gart Naval General Hospital	National Institute for Medical Research	
EPI379766	Denmark	2012-Apr-21	A/Denmark/42/2012	Statens Serum Institut	National Institute for Medical Research	
EPI360950	Germany	2011-Jul-03	A/Berlin/93/2011	National Institute for Medical Research	Centers for Disease Control and Prevention	

Segment ID	Country	Collection date	Isolate name	Originating laboratory	Submitting laboratory	Authors
EPI211334	Australia	2009-Jan-01	A/Perth/16/2009	WHO Collaborating Centre for Reference and Research on Influenza	Centers for Disease Control and Prevention	
EPI278808	United States	2010-Jul-13	A/Alabama/05/2010	U.S. Air Force School of Aerospace Medicine	Centers for Disease Control and Prevention	
EPI335923	United States	2010-Dec-30	A/Iowa/19/2010	Iowa State Hygienic Laboratory	Centers for Disease Control and Prevention	
EPI376512	United States	2012-May-03	A/Minnesota/10/2012	Minnesota Department of Health	Centers for Disease Control and Prevention	
EPI416258	Denmark	2012-Dec-11	A/Denmark/82/2012	Statens Serum Institut	Statens Serum Institut	Bragstad K
EPI416257	Denmark	2012-Dec-01	A/Denmark/77/2012	Statens Serum Institut	Statens Serum Institut	Bragstad K
EPI416256	Denmark	2012-Nov-23	A/Denmark/74/2012	Statens Serum Institut	Statens Serum Institut	Bragstad K
EPI416255	Denmark	2013-Jan-03	A/Denmark/30/2013	Statens Serum Institut	Statens Serum Institut	Bragstad K
EPI416254	Denmark	2012-Dec-15	A/Denmark/115/2012	Statens Serum Institut	Statens Serum Institut	Bragstad K
EPI416253	Denmark	2012-Dec-28	A/Denmark/113/2012	Statens Serum Institut	Statens Serum Institut	Bragstad K
EPI416251	Denmark	2012-Dec-31	A/Denmark/88/2012	Statens Serum Institut	Statens Serum Institut	Bragstad K
EPI416250	Denmark	2012-Dec-05	A/Denmark/78/2012	Statens Serum Institut	Statens Serum Institut	Bragstad K
EPI416249	Denmark	2012-Nov-22	A/Denmark/75/2012	Statens Serum Institut	Statens Serum Institut	Bragstad K
EPI416248	Denmark	2013-Jan-02	A/Denmark/52/2013	Statens Serum Institut	Statens Serum Institut	Bragstad K
EPI416247	Denmark	2013-Jan-08	A/Denmark/51/2013	Statens Serum Institut	Statens Serum Institut	Bragstad K
EPI416246	Denmark	2013-Jan-13	A/Denmark/50/2013	Statens Serum Institut	Statens Serum Institut	Bragstad K
EPI416245	Denmark	2013-Jan-02	A/Denmark/25/2013	Statens Serum Institut	Statens Serum Institut	Bragstad K
EPI416244	Denmark	2013-Jan-01	A/Denmark/17/2013	Statens Serum Institut	Statens Serum Institut	Bragstad K
EPI416243	Denmark	2012-Dec-27	A/Denmark/117/2012	Statens Serum Institut	Statens Serum Institut	Bragstad K
EPI416242	Denmark	2012-Dec-19	A/Denmark/114/2012	Statens Serum Institut	Statens Serum Institut	Bragstad K
EPI416241	Denmark	2012-Dec-26	A/Denmark/107/2012	Statens Serum Institut	Statens Serum Institut	Bragstad K
EPI416240	Denmark	2012-Dec-30	A/Denmark/101/2012	Statens Serum Institut	Statens Serum Institut	Bragstad K
EPI406237	Denmark	2012-Nov-08	A/Denmark/71/2012	Statens Serum Institut	Statens Serum Institut	Bragstad K
EPI406235	Denmark	2012-Nov-14	A/Denmark/72/2012	Statens Serum Institut	Statens Serum Institut	Bragstad K

[a] From the EpiFlu database of the Global Initiative on Sharing Avian Influenza Data (GISAID).

(A/Victoria/361/2011) is assigned, but the Danish clade A should probably be assigned a new A(H3) clade. Five of the 22 Danish HA sequences included in the phylogenetic analysis, collected from week 46 (11 December 2012) to week 1 (2 January 2013), were from patients who had been vaccinated before week 47: all five were located in clade A (Figure 2). Altogether, seven amino acid substitutions in the HA defined the Danish clade A, compared with egg-grown/tissue grown A/Victoria/361/2011 – at antigenic site B: T128A, R/Q156H and V186G; site A: R142G and N145S; site C: N278K; and site D: S/Y219S. Additional substitutions were Q33R, E/D190D and V347M. The clade B viruses did not possess the substitutions at positions 128, 142 and 347. The clade A viruses have lost the 126 N-linked glycosylation seen in A/Victoria/361/2011 and clade B (predicted by NetNGlyc 1.0 server [7]. For comparison, we included reference HA sequences from influenza A(H3) viruses, obtained from the EpiFlu database of the Global Initiative on Sharing Avian Influenza Data (GISAID) (Table 3).

Discussion

We took advantage of unique newly established national registries to obtain a rapid within-season VE estimate. In the current season, which to date has been dominated in Denmark by influenza A(H3N2) virus, we calculated an adjusted VE point estimate of –11% (95% CI: –41 to 14) against laboratory-confirmed influenza A in patients aged ≥65 years tested for influenza. Given the confidence interval, we cannot exclude a VE of up to 14%, which is still considerably lower than expected. By contrast, vaccination did protect against influenza B (adjusted VE point estimate of 69%). As the National Influenza Centre has subtyped 93% of the influenza A viruses in Denmark as influenza A (H3N2), we expect the poor VE against influenza A to be associated with this subtype. The poor VE against influenza A was supported by a comparable vaccination coverage among

ICU patients with influenza A and among Danish people in general in the same age group (≥65 years). We also identified genetic drift of circulating influenza A(H3N2) viruses, which may explain the low VE in elderly people (aged ≥65 years).

The study was observational, with the biases this study type may entail. We were unable to adjust for comorbidity, but we adjusted VE for age group – which was associated with both vaccination uptake and testing positive for influenza A – and place of sampling, which are both likely to be correlated with comorbidity. Although other test-negative VE studies have shown that adjustment for comorbidity only resulted in minor changes in the VE estimates [8], we cannot exclude the possibility that some residual confounding with comorbidity does exist; however, it is unlikely that the effect of confounding is of a magnitude that would dramatically change the estimates. Furthermore, the VE against influenza B, estimated on only a few cases, was comparable to early estimates from the United States Centers for Disease Control and Prevention [9]. It is possible that some cases and controls classified as unvaccinated had in fact been vaccinated after week 47. However, by way of comparison, in 2011, 95% of the vaccinations were given before week 48.

Our study design has a number of advantages. It was cost-effective because data were obtained from existing sources, and information on vaccination was registered prospectively and independently of outcome. The specificity of the outcome was high and independent of patient recall. The patients tested for influenza may differ from the general population of elderly people but the test-negative design reduces the risk of selection bias, since cases and controls presumably share the same health-seeking behaviours. As 95% of the samples were taken in a hospital setting, the study may indicate a poor protection against severe outcomes of influenza (requiring hospitalisation).

Elderly people who are tested for influenza may represent a more vulnerable group, who have a weaker response to vaccination compared with the elderly population in general; however, if this would explain the findings, we would also expect a low VE against influenza B.

Genetic characterisation revealed that the Danish clade A viruses may be the cause of the low VE observed in elderly people. Four or more substitutions in two or more antibody binding sites are predicted to give an antigenically different virus [10] – thus the seven differences described for the Danish isolates are a cause for concern. Key substitutions causing the genetic drift are at positions 128 and 142, which are rare substitutions. The A128 substitution causes loss of an N-linked glycosylation site and amino acid changes in the 140–146 region of HA antigenic site A is characteristic for antigenically distinct viruses of epidemic significance [11]. These two substitutions should therefore be considered carefully when antigenic drift is investigated further.

In conclusion, our epidemiological data suggest a low VE against influenza A(H3N2) among elderly people in Denmark; this is in contrast with early VE estimates in the range of 45–55% reported from other studies including all age groups [8,9,12].

Our molecular investigations indicate genetic drift; however, antigenic data on the clade A viruses are needed to document antigenic drift. Therefore, virus neutralisation and haemagglutination inhibition assays are in progress, along with an investigation of the role of the neuraminidase, as the neuraminidase also plays an important role in the genetic and antigenic drift of influenza viruses [13].

Matching of the influenza A(H3N2) vaccine virus with circulating influenza strains needs further investigation but should be taken into consideration before the coming selection of influenza vaccine strains for the next season, 2013/14. Influenza vaccination is still considered useful among Danish citizens this season due to the high protection against influenza B.

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Conflict of interest

None declared.

Authors' contributions

K Bragstad: Conceived the idea for the study and made the genetic and antigenic characterisations, she drafted the first version of the paper, made revisions, and approved the final version of the paper. H-D Emborg: Cleaned the data from the microbiology database and the vaccination database and made the statistical analysis of VE. She contributed to the interpretation of the data and reviewed the first draft of the paper and approved the final version of the paper. M Voldstedlund: Has established the microbiology database and retrieved the influenza data from the database. She assisted in the data cleaning and interpretation of the results,

she reviewed the first draft of the paper and approved the final version of the paper. T K Fischer: Contributed with the interpretation of the virological and epidemiological data and revised the first draft of the paper critically and approved the final version of the paper. S Gubbels: Is in charge of the surveillance of influenza at ICUs in Denmark and contributed with the data on vaccination coverage among ICU patients. She contributed to the interpretation of the results and reviewed and approved the final draft of the paper. B Andersen: Contributed considerably in the laboratory, she approved the final version of the paper. K Mølbak: Has established the microbiology database, he conceived the idea and the design of the VE study and contributed to the interpretation of the results. He revised the first draft of the paper critically and approved the final version of the paper. T G Krause: Conceived the idea and the design of the VE study, she was involved in the data analysis and the interpretation of the results. She wrote the first draft of the paper together with K Bragstad, revised the paper and made the final draft of the paper.

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Large measles outbreak in Geneva, Switzerland, January to August 2011: descriptive epidemiology and demonstration of quarantine effectiveness

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Between January and August 2011, the canton of Geneva, Switzerland, experienced a large measles outbreak with 219 cases (47 cases per 100,000 inhabitants) in the context of an extensive epidemic in a neighbouring region of France. Most cases were young adults (median age: 18 years), often unaware of their vaccination status. The vast majority of cases were either not (81%) or incompletely vaccinated (8%). Thirty clusters with a total of 119 cases and a median cluster size of three (range: 2–15 cases) were identified. Overall, 44 cases were imported or linked to imported cases. Of 73 contacts of cases who were quarantined, 50 developed measles and caused six secondary cases. This compares to 81 secondary cases among 173 non-quarantined cases (relative risk: 0.26; 95% confidence interval: 0.06–0.65), demonstrating the effectiveness of well targeted quarantine measures in reducing transmission.

Introduction

At the end of 2010, the objective of the World Health Organization (WHO) to interrupt the endemic transmission of measles appeared to be within reach in the canton of Geneva, Switzerland. Although several outbreaks had occurred between 2003 and 2008, the number of cases decreased from the summer 2008 until 2010. Fifty measles cases had been notified in 2003 (12 cases per 100,000 inhabitants), mostly isolated or in small clusters related to imported cases, and in 2010, only nine cases were notified (two cases per 100,000 inhabitants) [1-3]. During the same period, measles immunisation coverage with two doses of measles-mumps-rubella vaccine (MMR) was close to the 95% elimination threshold, with 91.7% for children aged 28 months and 92.3% for children aged between five and six years [1,4-7].

Against this context, however, a large outbreak started in January 2011 [8]. It lasted seven months, and finally 219 cases were notified to local health authorities (47 cases per 100,000 inhabitants). In the same period, a large outbreak was occurring in the neighbouring region of Rhône-Alpes, France, where 6,037 cases were reported from October 2010 to September 2011 [9]. Geneva canton (population 467,000) shares 96% (103 of 107.5 km) of its border with France, and approximately 80,000 persons cross the border every day.

The aim of this report is to describe the measles outbreak that occurred in Geneva between January and August 2011, measures taken to reduce its extension and the impact of quarantine on disease transmissions.

Methods

Measles case notification

In Switzerland, measles notification has been mandatory since 1999. Physicians report to local health authorities within 24 hours any patient presenting with maculopapular rash associated with fever and any of the following symptoms: cough, coryza or conjunctivitis. Initial notification is followed by a more detailed description of the case including self-reported vaccination status. Notification of confirmed cases by laboratories is also mandatory within 24 hours. In general, there are therefore two nominative notifications per case. Patients who do not seek medical attention are not officially reported, but during an outbreak, they are identified through contact investigation by the cantonal health authority and counted as cases if they fit the case definition (active case finding).

Case definition and classification

The following case definitions, slightly more sensitive than those recommended by the European Centre for Disease Prevention and Control (ECDC), were used in this investigation [10]. A confirmed case was a person i) with a positive laboratory test and at least one of the clinical criteria of measles listed above (laboratory-confirmed case) or ii) who met the clinical case definition and was epidemiologically linked to a laboratory-confirmed case (epidemiologically linked case). A probable

case was a person who met the clinical case definition with no epidemiological link to a laboratory-confirmed case. A possible case was a person without a positive laboratory result who did not meet all clinical case definition criteria.

Laboratory tests

Laboratory confirmation tests included serum IgM and IgG measurements in combination to distinguish between acute infection and immunity, or PCR for measles virus RNA in throat swab or oral fluid. Genetic characterisation was carried out either at the Central Virology Laboratory of Geneva University Hospital or at the Robert-Koch Institute in Berlin, Germany, to determine genotype by sequence analysis of the variable part of the neuraminidase (N) gene and the haemagglutinin (H) gene.

Suspected cases with two negative IgM tests or one negative IgM test and with a negative PCR result, and those with a single positive IgM test without any clinical symptoms of measles, were excluded. Cases included in this report were residents of the canton Geneva presenting with clinical symptoms of measles between 1 January and 31 August 2011.

Outbreak control evaluation

An evaluation of the ways this outbreak was managed was performed in September and October of 2011 using a standard questionnaire. Its aim was to assess how partner institutions perceived the various aspects of outbreak response including communication, surveillance, control and contact tracing activities. All 38 institutions involved in controlling the outbreak or their representatives received this questionnaire. All questionnaire items were rated on a scale of 1 (not satisfactory) to 5 (very satisfactory).

The statistical comparison of incidence following exposure to quarantined and non-quarantined cases was conducted using StatXact version 4.0.1 software [11].

Outbreak description

From 1 January until 31 August 2011, 219 cases were reported, 182 (83%) through the notification system and 37 (17%) by active case finding. There were 195 (89%) confirmed, 16 (7%) probable and eight (4%) possible cases. Among the confirmed cases, 138 were laboratory-confirmed and 57 were epidemiologically linked cases.

An additional 62 cases diagnosed in Geneva and reported to the health authorities are not included in this report because they were either French residents (n=44), lived in the canton of Vaud (n=14) or in another canton or country (n=4). These patients, however, all worked, attended school or consulted a physician in Geneva. Another 37 reported cases were ruled out as non-measles cases.

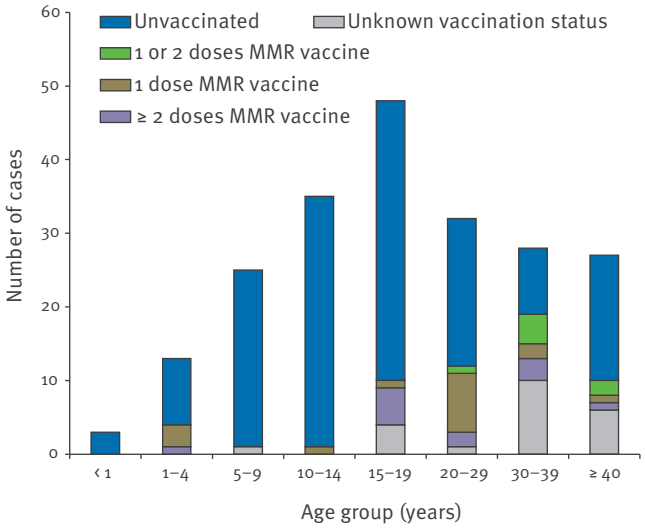
Sequencing of 31 measles viruses isolated during the outbreak was performed. Genotype D4 was isolated in all 23 samples obtained from residents of the canton Geneva as well as in seven samples from French residents. One D9 serotype was identified in a tourist from the Philippines visiting Geneva.

The epidemic curve is presented in Figure 1. Of the 219 cases, 44 (20%) could be documented as imported cases (n=21) or epidemiologically linked, directly or indirectly, to an imported case (n=23). These cases came from or were epidemiologically linked to cases from the French département of Haute-Savoie (n=25), the French département of Ain (n=4), other départements of France (n=7), the canton of Vaud (n=6), Argentina (n=1) and Poland (n=1). During the first eight weeks of the epidemic, a substantial proportion of the cases (19 of 49) were imported or linked to imported cases.

Among the 211 confirmed and probable cases, 98 (46%) were male. The median age was 18 years (range: 11 months–59 years). For 189 (90%) of those self-reported information on immunisation status was available. Some 154 (81%) had not been vaccinated with MMR vaccine, 16 (8%) had received one dose, 12 (6%) at least two doses and seven were vaccinated with an unknown number of doses. Among the 154 unvaccinated cases all but five who were older than 47 years, (i.e. born in or before 1963, the age at which measles vaccination is no longer recommended in Switzerland) were eligible for vaccination (97%). Cases are presented by age group and vaccination status in Figure 2. No catch-up campaigns were done during this epidemic. However, catch-up is recommended in the Swiss vaccination plan for all persons born after 1963.

Figure 2

Confirmed and probable measles cases by age and self-reported vaccination status, Geneva, Switzerland, 1 January–31 August 2011 (n=211)



MMR: measles-mumps-rubella.

Figure 1

Measles cases by month of rash onset, Geneva, Switzerland, 2003-11 (n=219)

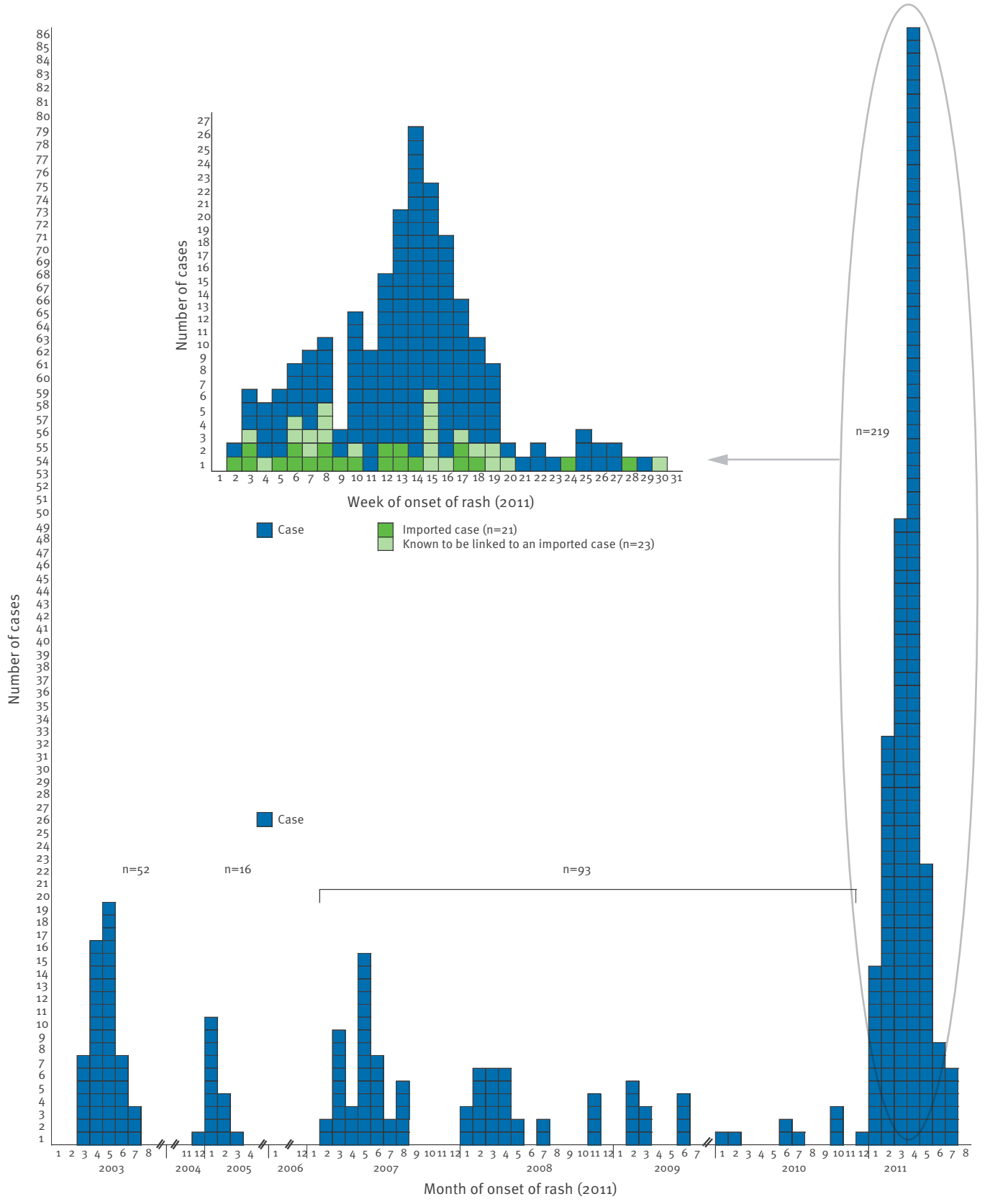
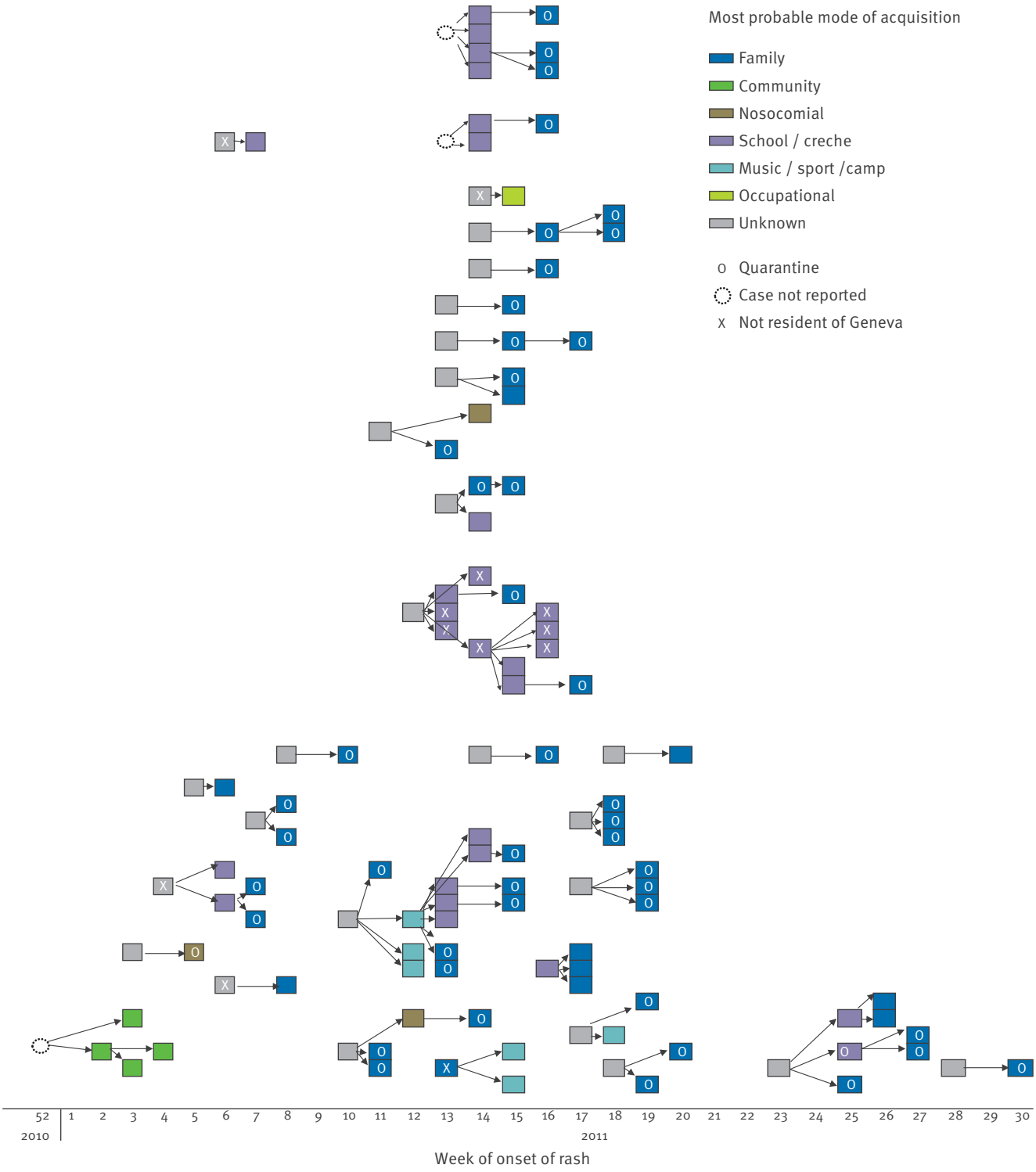


Figure 3

Mode of acquisition of measles, Geneva, Switzerland, 1 January–31 August 2011 (n=123)



Cases not resident in Geneva are included for completeness of cluster description.

Of 211 cases, 18 (9%) presented with at least one complication such as pneumonia (n=11), encephalitis (n=1), bronchitis (n=1), other lower respiratory disease (n=2), otitis (n=2), keratitis (n=1) and exacerbation of cystic fibrosis (n=1). Seventeen (8%) patients were hospitalised, one of them for 10 days in intensive care with respiratory failure. The other causes of hospitalisations were encephalitis (n=1), pneumonia (n=4), hypoxaemia (n=4), general alteration of heath status (n=4), and two cases stayed overnight for clinical observation. The precise cause of hospitalisation remains unknown for two patients. There were no deaths and all patients recovered. The median duration of hospitalisation was four days (range: 1–14 days). The median age of hospitalised patients was 33 years (range: 7–52 years). Among the 14 (82%) hospitalised patients for whom the vaccination status was known, 11 had not been vaccinated and one had been completely vaccinated (two doses).

We identified 31 transmission clusters (Figure 3) with 123 cases including 12 who were not residents of Geneva. There were two clusters of three generations of cases including 13 and 15 cases. The other clusters included two to eight cases in one or two generations (median cluster size: 3). Transmission among residents of Geneva occurred within families (n=54 cases), in schools (n=20) and daycare (n=1), among friends (n=4), in health service (n=3), during sporting or musical events (n=5), in the workplace (n=1) and during a one-week camp (n=1). There were an additional 22 single cases (index case of each cluster) with unknown mode of acquisition.

Control measures

Control measures in the Geneva canton have been previously described [8,12]. They were implemented as early as possible by local health authorities and school health services without waiting for laboratory confirmation (Figure 4). Extensive and rapid contact tracing was conducted as an emergency measure so that contacts and relatives of cases could be informed and their vaccination or immunisation status assessed. When a case had unvaccinated or non-immune close contacts, either siblings or classmates, these were quarantined at home for 18 days after last contact or after onset of the case's rash. Although immediate post-exposure vaccination may prevent measles, it does not later during quarantine. In addition, because vaccine-related symptoms may mimic measles, it may discredit vaccination itself in this often reluctant population. For these reasons, unless immediate post exposure (<72 h) vaccination was done, vaccination was recommended at the end of the quarantine period if measles had not occurred. Early in the course of the epidemic, all parents of children attending school or nursery were sent a letter informing them about the outbreak and the importance of vaccinating their children. They were also informed about quarantine of unvaccinated contacts.

Of 73 exposed unvaccinated or non-immune persons who were quarantined, 50 developed measles. Only six instances of subsequent transmission occurred, all among household members and none in the community. The 173 cases which occurred among non-quarantined cases were associated with 81 secondary cases, of which 48 occurred among household members and 33 in the community. As indicated in the Table, quarantine reduced the overall risk of transmission by 74% (12% versus 47%; relative risk (RR): 0.26; 95% confidence interval (CI): 0.06–0.56). The reduction of the risk of transmission was obviously lower among household members (12% versus 28%; RR: 0.43; 95% CI: 0.09-1.00) and was major, 95%, in the community (0% versus 19%; RR: 0.05; 95% CI: 0.00-0.69). Case finding and contact tracing was identical regardless of quarantine status.

Health authorities regularly informed emergency medical services, the media and the public. In addition, local physicians were sent by email epidemiological updates and practical information in connection with their role in outbreak control. Early in the epidemic, they were advised to reduce the age of first measles vaccination from 12 to nine months. Press releases, individual emails to all students at Geneva University, information letters to highschool students, directors of schools and daycare centres were also sent out. Young adults were the main target of mass communication and they were advised to verify their immunisation status and be vaccinated if necessary.

Of the 38 partner institutions involved in outbreak control who received the evaluation questionnaire, 19 responded. Their overall level of satisfaction was fairly

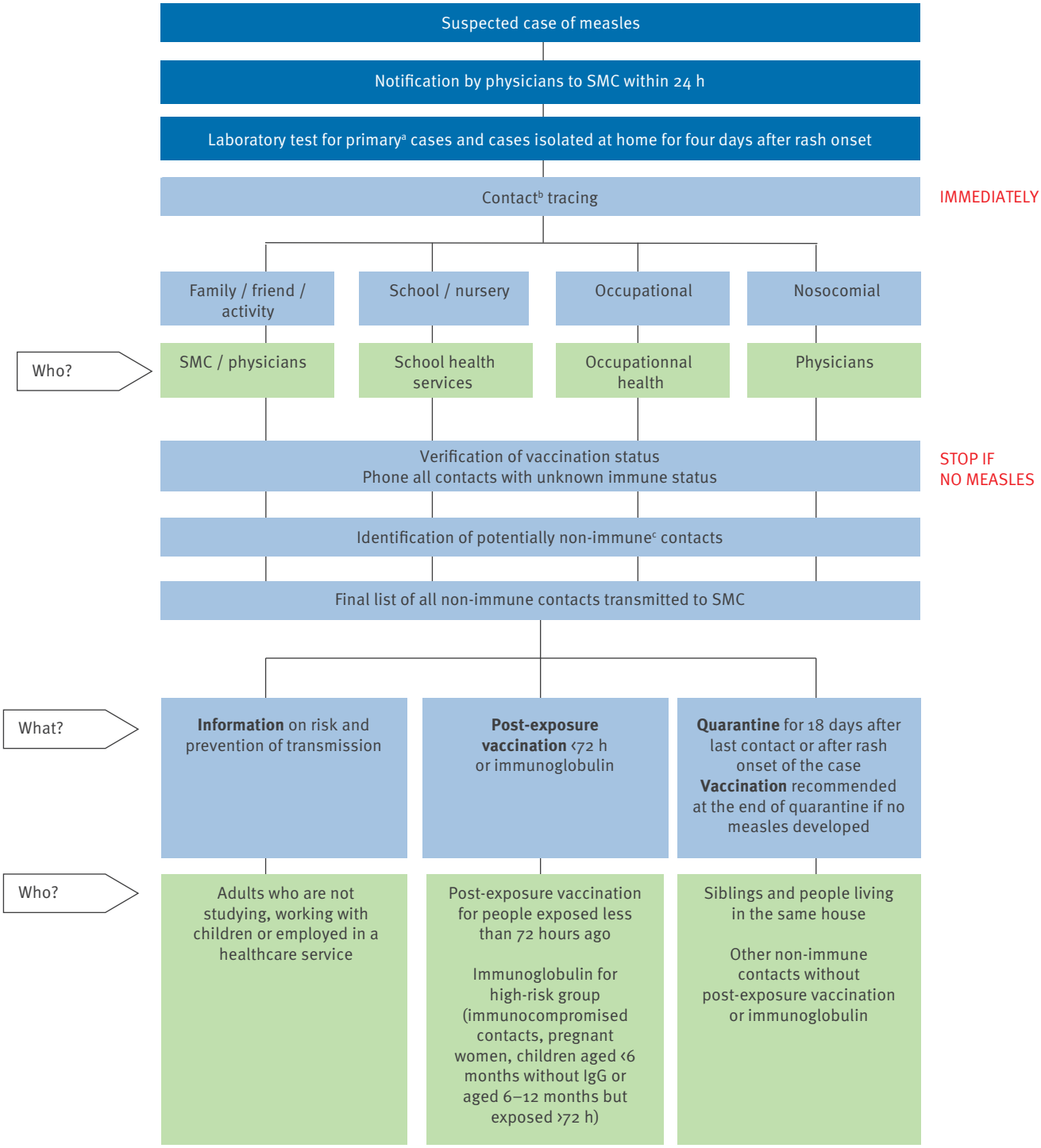
Table

Rate of transmission of measles by measures of quarantine, risk ratios and p value, Geneva, Switzerland, 1 January–31 August 2011

Type of transmission	Quarantine (n=50)		No quarantine (n=173)		Risk ratio (95% confidence interval)	p value
	Transmission	Rate	Transmission	Rate		
Total	**6**	**12%**	**81**	**47%**	**0.26 [0.06–0.56]**	**0.002**
Within household	6	12%	48	28%	0.43 [0.09–1.00]	0.051
Outside household	0	0%	33	19%	0.05 [0.00–0.69]	0.01

Figure 4

Control measures during the measles outbreak, Geneva, Switzerland, 1 January–31 August 2011



SMC: Cantonal Health Service.

[a] A primary case was defined as a measles case not known to be related to other cases.

[b] Contacts were defined as people who were exposed to the case during the contagious period (four days before to four days after rash onset).

[c] Non-immune contacts were defined as people born after 1963 and without vaccination or IgG or proven histoy of disease.

high (average: 4.2/5) with a large variability in the responses. Epidemiological reports and cantonal communication were particularly well noted (4.5 and 4.6) as was the collaboration with the partners. The most critical points related to media information and the lack of well-defined roles and responsibilities for contact tracing and control measures (3.4 and 3.6).

Discussion

This outbreak was the largest ever documented in the canton of Geneva. It occurred in the context of an extensive measles epidemic in the neighbouring Rhône-Alpes region in France. About one fifth of the cases were imported or related to imported cases, mostly from the bordering département of Haute-Savoie. This does not come as a surprise as the epidemiology of infectious diseases in the canton of Geneva is often closely related to its neighbouring regions for obvious demographic, economic and geographic reasons.

Although national MMR vaccination coverage remains below the threshold required for measles elimination in Switzerland [13-15], it is currently higher in Geneva: 91.7% for children aged 28 months for two doses, and 92.3% for children aged between five and six years [1,5-7]. Data collected locally indicated that the main reason for not vaccinating children were concern for side effects and the belief that natural infection contributed more to better health than vaccination [16]. Progressive accumulation of non-immune persons, however, is inevitable. Combined with multiple introductions of infectious patients into the Geneva community, outbreaks or at least small clusters of cases seem currently unavoidable.

Most cases in this outbreak were adults, many of whom were not aware of their vaccination status. There was a delay in the diagnosis of several cases during the seasonal influenza period between weeks 1 and 7 of 2011 in Geneva, as early presentation of measles can be quite similar to influenza. In some instances, there were multiple consultations before measles was diagnosed. Early consultation of adults presenting with non-specific symptoms prior to the rash may also have contributed to delay in diagnosis. However, only three healthcare-related cases were observed.

Control measures were implemented early for all cases including those whose measles diagnosis had not yet been confirmed. Nevertheless, post-exposure vaccination was often ineffective as it was done too late, especially in siblings. At least 17 close contacts received post-exposure vaccination. Six of them developed measles, of whom five were vaccinated more than 72 hours after exposure.

Although, as expected, the secondary attack rate among unvaccinated household members was high, quarantine of non-immunised relatives, close contacts and classmates, a measure previously implemented in Geneva [1,2], was very effective. The large majority (68%) of exposed non-vaccinated or non-immune persons who were quarantined developed measles, but no transmission outside their own families occurred. Data collected during this outbreak documented a 95% reduction in the risk of community transmission. Even when household transmission was included, quarantine decreased the risk of transmission by 74%. Compliance to quarantine was good and this measure was well accepted. This may, at least in part, have been due to the support from school health services and because parents had been previously informed of this possible consequence of their refusal to have their child vaccinated. Exclusion of children with measles was strictly enforced by school authorities.

The results from the evaluation questionnaire helped us to further define the operational roles of the various partner institutions in case management, contact tracing and contact management. It also confirmed the value of rapid and regular analysis and dissemination of local epidemiological information.

Conclusion and recommendations

Fairly high MMR vaccination coverage in children as well as early and effective control measures including quarantine probably contributed to reducing the magnitude of this outbreak, especially among school-age children. Although Switzerland adheres to the WHOs objective of eliminating measles in the European region by 2015, this will require, in addition to a national strategy [17], a common effort of all European countries and regions. Sustained high vaccination coverage, effective surveillance and early control measures including quarantine of non-vaccinated exposed persons should be implemented by all European countries and regions if outbreaks such as this one are to be prevented and virus circulation interrupted.

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