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Oseltamivir-resistant influenza A(H1N1)pdm09 virus in Dutch travellers returning from Spain, August 2012

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Two Dutch travellers were infected with oseltamivir-resistant influenza A(H1N1)pdm09 viruses with an H275Y neuraminidase substitution in early August 2012. Both cases were probably infected during separate holidays at the Catalonian coast (Spain). No epidemiological connection between the two cases was found, and neither of them was treated with oseltamivir before specimen collection. Genetic analysis of the neuraminidase gene revealed the presence of previously described permissive mutations that may increase the likelihood of such strains emerging and spreading widely.

Screening for antiviral resistance in influenza viruses has become important in recent years. Before the 2007/08 season, resistance of influenza viruses to the neuraminidase (NA) inhibitors (NAIs) oseltamivir and zanamivir was only detected rarely, as the NA amino acid substitutions that conferred reduced susceptibility or resistance to these drugs had deleterious effects on the function of the neuraminidase and hence on the viruses ability to replicate and transmit [1-3]. However, increased use of antiviral drugs in certain regions of the world and stockpiling of antiviral drugs for use during a pandemic, increased the need for systematic surveillance of antiviral resistance [4].

In the Netherlands antiviral susceptibility of influenza viruses has been monitored since 2005 as part of the national influenza surveillance [5]. Likewise in Europe, many countries began conducting antiviral susceptibility monitoring in 2005/06 with the support from the two collaborating European Union (EU)-funded projects European Surveillance Network for Vigilance against Viral Resistance (VIRGIL) and European Influenza Surveillance Scheme (EISS) [4]. With this monitoring system in place, the EISS network in Europe was able to rapidly detect and monitor the emergence and spread of oseltamivir-resistant former seasonal influenza A(H1N1) viruses in the community [6-8].

The resistant viruses, which contained an H275Y substitution in the NA, were first detected in Norway and subsequently elsewhere in Europe in early 2008, and then spread globally within nine months [6-11]. In 2009 the oseltamivir-sensitive pandemic A(H1N1) virus A(H1N1)pdm09 replaced the oseltamivir-resistant seasonal A(H1N1) strain, returning the situation to that seen before 2007/08, where the vast majority of circulating viruses were sensitive to both oseltamivir and zanamivir.

In the Netherlands, only 20 NA H275Y cases infected with oseltamivir-resistant influenza A(H1N1)pdm09 virus were detected during the 2009/10 pandemic, matching the low proportions of such viruses detected world-wide [12,13]. Most of these cases involved immunocompromised patients undergoing prolonged oseltamivir treatment, with no further transmission, reflecting the poor fitness of the resistant viruses. Continued year-round influenza surveillance in the Netherlands since 2010 had not identified any more oseltamivir-resistant A(H1N1)pdm09 viruses until now.

However, elsewhere in the world there have been reports that oseltamivir-resistant A(H1N1)pdm09 viruses with an H275Y NA substitution are being detected at a higher rate in community patients who are not being treated with oseltamivir, suggesting that the resistant virus may have become more transmissible [14-16]. The largest cluster of cases reported to date occurred in Australia in 2011, and involved the detection of an oseltamivir-resistant strain in over thirty community patients, most of whom lived within 50 km of each other, but also included a case as far as 4,000 km away [15]. It was hypothesised that other mutations in the NA genes of these viruses that potentially

facilitate accommodation of the H275Y substitution had enabled the virus to retain fitness when the H275Y substitution was obtained [15].

Methods

In the Netherlands, year-round surveillance for influenza antiviral resistance is based on respiratory specimens collected by the Dutch Sentinel General Practice Network of the NIVEL Netherlands Institute for Health Services Research from patients presenting with influenza-like illness (ILI) or another acute respiratory infection (ARI), and on influenza virus isolates or influenza virus positive clinical specimens sent to the Dutch National Influenza Centre on a voluntary basis by all diagnostic laboratories in the Netherlands [17]. Sequencing or single nucleotide polymorphism RT-PCR is conducted directly on clinical specimens or viral isolates to monitor antiviral resistance or reduced susceptibility markers [5,17,18]. Viral isolates are further characterised phenotypically by NA inhibition assay and determination of the antiviral drug concentration needed to inhibit the NA enzyme activity by 50% (IC50) [5].

Since the 2009 pandemic, the Netherlands has been using a systematic approach for antiviral resistance surveillance based on a protocol and accompanying standardised questionnaire, which are available to professionals through the website of the Dutch National Institute for Public Health and the Environment (RIVM) [12]. Municipal Health Services are engaged in this epidemiological investigation to track patient travel history, course of disease, exposure to antiviral drugs, possible source of infection and possible contacts. When contacts show respiratory symptoms, specimens are collected for influenza virus detection and characterisation.

For comparison with the Dutch influenza A(H1N1)pdm09 sequences from 663 viruses collected since the 2009 pandemic, NA and haemagglutinin (HA) sequences of 150 viruses were downloaded from the EpiFlu database of the Global Initiative on Sharing All Influenza Data (GISAID) (Table 1). Phylogenetic, nucleotide mutation, and amino acid substitution analyses were performed using BioNumerics software version 6.6.4 (Applied Maths, Sint-Martens-Latem, Belgium).

Results

Through the sentinel general practice surveillance network, a teenage patient (Case 1) was diagnosed with an influenza A(H1N1)pdm09 virus infection from a specimen collected on 14 August 2012. Around the same time a hospital laboratory submitted an influenza A virus-positive clinical specimen collected on 17 August from a patient in their early 20s (Case 2) for subtyping, that was subsequently shown to be influenza A(H1N1)pdm09 virus. Their places of residence in the Netherlands were about 100 km apart. The travel history of both cases mentioned Spain as a recent holiday destination. Sequencing of the NA gene of the viruses directly from the clinical specimens showed a mutation encoding the H275Y substitution previously associated with oseltamivir resistance. The patients were interviewed by municipal health service workers and the viruses were further characterised.

Case 1 had developed ILI (high fever, cough and malaise) on the day of return and Case 2 one day after return from holiday in Catalonia, Spain, with dates of onset only one day apart: 13 August 2012 for Case 1 and 14 August 2012 for Case 2. Both cases experienced onset of mild symptoms (sore throat and cough) during their stay in Catalonia. The cases had no underlying disease and neither of them, nor their close contacts, had been exposed to oseltamivir through treatment before specimen collection.

Case 1 had stayed in Catalonia with their family between 20 July and 13 August 2012, and was possibly infected by a sibling who developed mild symptoms (sore throat and cough) on 1 August. Later, also Case 1's parent and a friend developed mild symptoms. Apart from Case 1, specimens were not taken from these individuals. Case 2 stayed in Catalonia between 3 August and 13 August. Because of ILI with high fever this patient was hospitalised for observation between 16 and 17 August after returning to the Netherlands.

Given a median incubation time for influenza of two days (range 1–7 days) [19], and taking the date of onset of mild symptoms into account, it is highly likely that both cases were infected in Catalonia. Their places of residence in Catalonia were approximately 200 km apart, and Case 2 did not visit any other places along the Catalonian coast during their stay. Apart from the two family members and friend for Case 1, neither patient could recall having met any other ill persons during their incubation period. Case 1 travelled back to the Netherlands with their family by car, whilst Case 2 travelled back to the Netherlands by coach. Travel history did not reveal a mutual stop where the cases could have met each other.

Following discussion of our findings with Spanish colleagues, they reported no detections of influenza A(H1N1)pdm09 viruses in Spain, and specifically in Catalonia, since May 2012 (personal communication, Tomàs Pumarola Suñé, National Influenza Centre Barcelona, August 2012, and Francisco Pozo Sánchez, National Influenza Centre Madrid, August 2012).

Sequencing of viruses directly from the clinical specimens of Case 1 (A/Bilthoven/4311200706/2012; GISAID accession number: EPI393738-41) and Case 2 (A/Bilthoven/4361200003/2012) showed that they had identical nucleotide sequences for partial segments of the HA, NA, matrix (M) and PB2 genes. The viruses carried an HA from genetic clade 6, while the NA genes contained mutations that encoded the H275Y substitution known to confer oseltamivir-resistance in laboratory and clinical situations (Figure). In addition, the

Table

Submitting and originating laboratories of influenza A(H1N1)pdm09 viruses for which haemagglutinin and neuraminidase sequences were downloaded from the GISAID EpiFlu sequence database

A

Submitting Laboratory	Originating Country	Originating Laboratory	Number
Centers for Disease Control and Prevention	Argentina	Instituto Nacional de Enfermedades Infecciosas	1
	Australia	WHO Collaborating Centre for Reference and Research on Influenza	1
	Bangladesh	Institute of Epidemiology Disease Control and Research (IEDCR) & Bangladesh National Influenza Centre (NIC)	2
	Canada	National Microbiology Laboratory, Health Canada	1
	Ethiopia	Ethiopian Health and Nutrition Research Institute (EHNRI)	1
	Guatemala	Laboratorio Nacional De Salud Guatemala	1
	Mexico	Laboratorio de Virus Respiratorio	8
	Paraguay	Central Laboratory of Public Health	5
	Russian Federation	Russian Academy of Medical Sciences	1
	United States	ADPH Bureau of Clinical Laboratories	1
		California Department of Health Services	3
		City of El Paso Dept of Public Health	2
		Colorado Department of Health Lab	1
		Corpus Christi-Nueces County Public Health	1
		DC Public Health Lab	1
		Delaware Public Health Lab	2
		Florida Department of Health-Jacksonville	1
		Georgia Public Health Laboratory	1
		Kansas Department of Health and Environment	1
		Kentucky Division of Laboratory Services	1
		Maine Health and Environmental Testing Laboratory	1
		Maryland Department of Health and Mental Hygiene	2
		Michigan Department of Community Health	1
		Missouri Department. of Health & Senior Services	2
		Nebraska Public Health Lab	1
		New Hampshire Public Health Laboratories	1
		New Jersey Department of Health & Senior Services	1
		New Mexico Department of Health	1
		New York State Department of Health	1
		North Carolina State Laboratory of Public Health	3
		North Dakota Department of Health	2
		Oklahoma State Department of Health	1
		Oregon Public Health Laboratory	1
		Puerto Rico Department of Health	2
		Rhode Island Department of Health	1
		San Antonio Metropolitan Health	2
		Southern Nevada Public Health Lab	1
		Spokane Regional Health District	1
		State of Idaho Bureau of Laboratories	2
		Tarrant County Public Health	1
		Tennessee Department of Health Laboratory-Nashville	1
		Texas Childrens Hospital	1
		Texas Department of State Health Services-Laboratory Services	1
		Utah Public Health Laboratory	4
		Vermont Department of Health Laboratory	2
		Washington State Public Health Laboratory	2
		West Virginia Office of Laboratory Services	1
		Wisconsin State Laboratory of Hygiene	3
		(blank)	2

GISAID: Global Initiative on Sharing All Influenza Data; WHO: World Health Organization.

viruses also contained NA substitutions V241I, N369K and N386S, the latter causing the loss of a potential glycosylation site [20]. These three mutations potentially facilitate accommodation of the H275Y substitution as suggested for the Australian oseltamivir-resistant A(H1N1)pdm09 viruses detected in a cluster of community cases in 2011 (Figure) [15]. Virus isolation from the clinical specimens of both cases is currently in progress to allow phenotypic characterisation of antiviral susceptibility.

To put these two cases into an international context, partial HA and NA gene sequences of influenza A(H1N1) pdm09 viruses detected in the Netherlands spanning

Table

Submitting and originating laboratories of influenza A(H1N1)pdm09 viruses for which haemagglutinin and neuraminidase sequences were downloaded from the GISAID EpiFlu sequence database

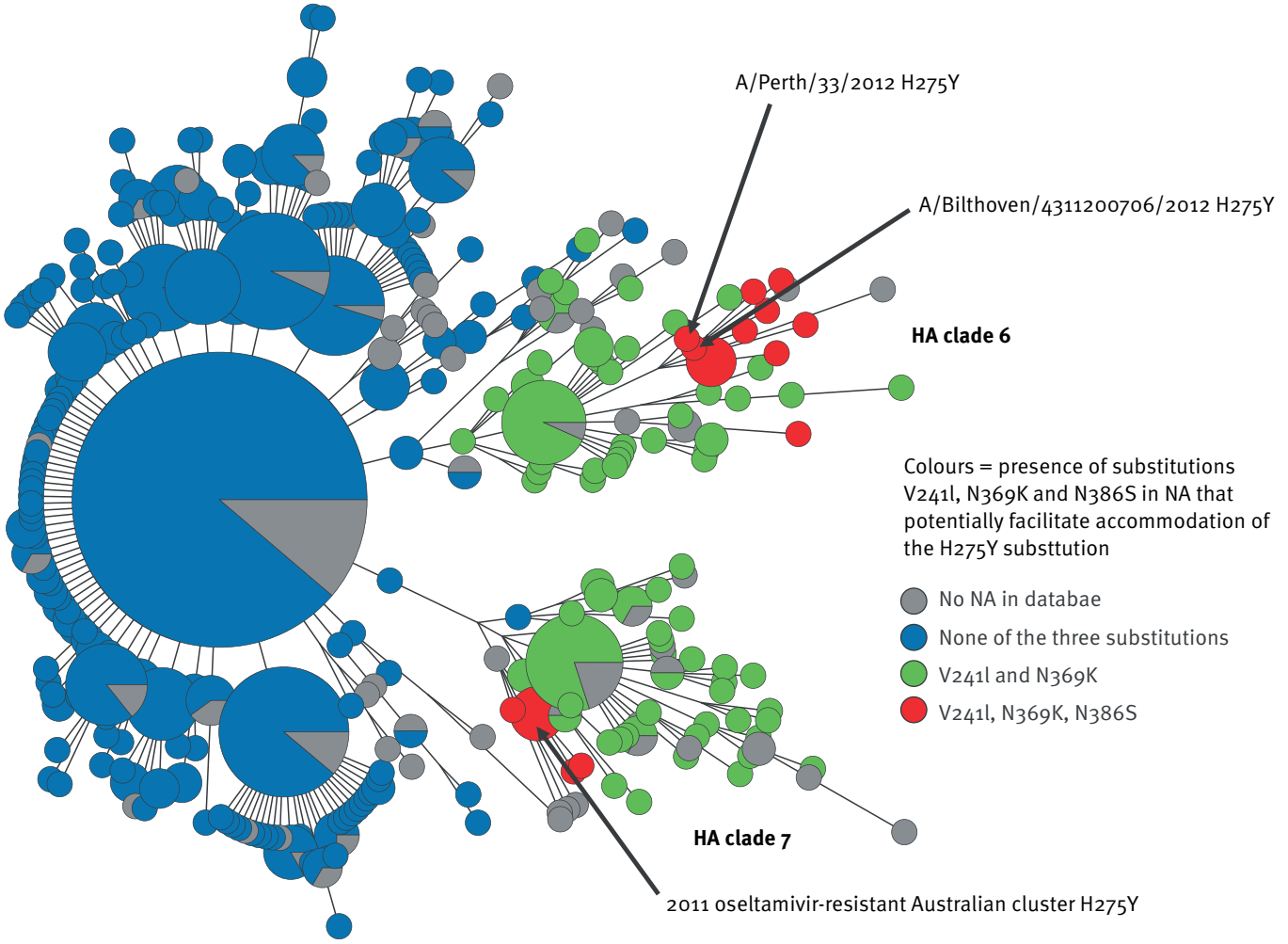
B

Submitting Laboratory	Originating Country	Originating Laboratory	Number
Institut Pasteur	France	Institut Pasteur	2
National Institute for Medical Research	Argentina	Instituto Nacional de Enfermedades Infecciosas	2
	Czech Republic	National Institute of Public Health	1
	Estonia	Health Protection Inspectorate	1
	France	CRR virus Influenza region Sud	1
	Ghana	University of Ghana	1
	Greece	Institut Pasteur Hellenique	1
	Hong Kong (SAR)	Government Virus Unit	6
	Latvia	State Agency, Infectology Center of Latvia	1
	Norway	WHO National Influenza Centre	1
	Russian Federation	Russian Academy of Medical Sciences	1
		WHO National Influenza Centre	2
	Slovenia	Laboratory for Virology, National Institute of Public Health	1
	South Africa	National Institute for Communicable Disease	2
		Sandringham, National Institute for Communicable D	1
	Sweden	Swedish Institute for Infectious Disease Control	3
National Institute of Infectious Diseases (NIID)	Japan	Sapporo City Institute of Public Health	1
Other Database Import	Denmark	(blank)	1
	Mexico	(blank)	2
	Singapore	(blank)	1
Public Health Laboratory Services Branch, Centre for Health Protection	Hong Kong (SAR)	Public Health Laboratory Services Branch, Centre for Health Protection	2
Swedish Institute for Infectious Disease Control	Sweden	Swedish Institute for Infectious Disease Control	2
		(blank)	5
WHO Chinese National Influenza Center	China	WHO Chinese National Influenza Center	1
		(blank)	1
WHO Collaborating Centre for Reference and Research on Influenza	Australia	Austin Health	1
		Canberra Hospital	1
		Childrens Hospital Westmead	1
		John Hunter Hospital, Virology Unit, Clinical Microbiology	8
		Monash Medical Centre	1
		Pathwest QE II Medical Centre	7
		Queensland Health Scientific Services	3
		Royal Hobart Hospital	1
		Westmead Hospital	2
	New Zealand	Canterbury Health Services	1
Total			**150**

GISAID: Global Initiative on Sharing All Influenza Data; WHO: World Health Organization.

Figure

Maximum parsimony network of partial haemagglutinin (HA) sequences of influenza A(H1N1)pdm09 viruses, the Netherlands, 30 April 2009–17 August 2012 (n=663) and 150 HA sequences from other countries



GISAID: Global Initiative on Sharing All Influenza Data; HA: haemagglutinin; NA: neuraminidase.

Dutch sequences spanning the entire A(H1N1)pdm09 period are combined with a selection of 150 sequences from the GISAID EpiFlu sequence database with a focus on 2012 (2009 n=3; 2010 n=12; 2011; n=40; 2012 n=90), and five from the community cluster of oseltamivir-resistant influenza A(H1N1)pdm09 viruses in 2011 in Australia [15]. Superimposed on the HA network by colour are the NA substitutions V241I, N369K and N386S that potentially facilitate accommodation of the H275Y substitution [15].

the entire A(H1N1)pdm09 period (30 April 2012 to 17 August 2012), were compared with those available in the GISAID sequence database, focusing on sequences from viruses collected in 2012 and those from the 2011 community cluster of oseltamivir-resistant A(H1N1) pdm09 in Australia. This analysis revealed a very high genetic similarity of HA and NA sequences from Cases 1 and 2 with A/Perth/33/2012, another oseltamivir-resistant virus from 2012 that contained the H275Y NA substitution. The sequences of the Dutch and Perth viruses differed by only one synonymous and one non-synonymous nucleotide change in the HA gene, and one synonymous nucleotide change in the NA gene. Influenza A/Perth/33/2012 was collected in March 2012 from a 15 month-old infant who had returned from a holiday in Bali, Indonesia. Neither the infant, nor its family, was treated with oseltamivir before specimen collection.

Discussion

Although the Dutch and Perth viruses from 2012 carry NA substitutions, V241I, N369K and N386S, that potentially facilitate accommodation of the H275Y substitution, as did the 2011 Australian oseltamivir-resistant cluster of viruses, the HA genes from these two groups form separate genetic clusters (Figure). While viruses similar to those detected in Australia in 2011 (HA clade 7, and NA carrying V241I, N369K and N386S substitutions) [15] have not been circulating recently, viruses like the Dutch and Perth strains with an HA in genetic clade 6 and NA carrying V241I, N369K and N386S substitutions represented a substantial proportion of

influenza A(H1N1)pdm09 viruses detected around the world in 2012 (Figure).

To date, the majority of oseltamivir-resistant A(H1N1) pdm09 viruses have been detected in patients undergoing oseltamvir treatment [13]. However, the cluster of resistant viruses detected in Australia in 2011 and sporadic cases reported from other continents show a recent increase in the proportion of cases of oseltamivir-resistant A(H1N1)pdm09 viruses from patients with no exposure to oseltamivir [14-16,21]. Further, the great majority of these cases were detected during periods of high influenza A(H1N1)pdm09 activity, whereas the viruses reported here were detected out of season and may suggest low-level circulation of an oseltamivir-resistant influenza A(H1N1)pdm09 strain. In the 2007/08 influenza season, former seasonal influenza A(H1N1) viruses with the same H275Y NA substitution emerged in Europe and ultimately spread around the world, leaving zanamivir as the main alternative for treatment of seasonal A(H1N1) influenza virus infections [6-11]. It is thought that the emergence of this H275Y oseltamivir-resistant seasonal A(H1N1) virus was made possible by permissive substitutions in the NA other than the three described for influenza A(H1N1)pdm09 viruses, which offset the otherwise deleterious effect of the H275Y substitution [20,22,23]. If the NA substitutions V241I, N369K and N386S do enable the A(H1N1)pdm09 virus to accommodate the H275Y substitution without a loss of fitness, then the detection of the oseltamivir-resistant strains reported here warrants close monitoring of the emergence of oseltamivir resistance among influenza A(H1N1)pdm09 viruses in light of the emergence and rapid spread of natural oseltamivir-resistant former seasonal influenza A(H1N1) viruses in 2007/08.

Rapid sharing of information on resistant viruses with regional centres for disease control and the World Health Organization is crucial to assess the threat posed by resistant viruses and ensure that treatment guidelines remain appropriate. Therefore laboratories that have the testing capacity, should conduct timely analysis of A(H1N1)pdm09 viruses for the H275Y NA mutation and refer any resistant viruses to one of the World Health Organization Collaborating Centres for Reference and Research on Influenza for further characterisation.

The surveillance results reported here illustrate the usefulness of sustained antiviral susceptibility monitoring systems that can deliver timely data to inform public health and clinical recommendations for antiviral use.

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Azole-resistant invasive aspergillosis in a patient with acute myeloid leukaemia in Germany

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We report the first culture-proven case of invasive aspergillosis (IA) caused by azole-resistant *Aspergillus fumigatus* in a patient with acute myeloid leukaemia in Germany. IA presented as breakthrough infection under posaconazole prophylaxis. Analysis of the resistance mechanism revealed the TR/L98H mutation in the cyp51A gene, which indicates an environmental origin of the strain. This case underscores the need for monitoring azole resistance in *Aspergillus* spp. and for routine susceptibility testing of moulds.

Background

Invasive aspergillosis (IA) is the most frequent invasive fungal disease (IFD) in patients with haematological malignancies and in those undergoing allogeneic stem cell transplantation, with *A. fumigatus* being its prime causative agent. Azole antifungals are recommended as first-line treatment and prophylaxis of IA in high risk patients in most international guidelines. Azole resistance is observed in patients with long-term azole therapy, but also in azole-naïve patients, suggesting an environmental origin for the latter group. Resistance to azole antifungals in clinical *A. fumigatus* isolates was reported from the Netherlands, the United Kingdom, France, the United States and recently China and India and has been associated with a mortality of up to 88% [1-4]. In Europe, resistant clinical strains were mostly isolated from patients with chronic pulmonary disease and prior long-term antifungal therapy, e.g. patients with allergic bronchopulmonary aspergillosis, chronic pulmonary aspergillosis or cystic fibrosis [5, 6]. In patients with haematological malignancies, azole resistance has rarely been reported [7].

Case report

A 35 year-old male patient was admitted to the University Hospital of Cologne with a first diagnosis of acute myeloid leukaemia (AML) in March 2012. He was started on remission-induction chemotherapy on the same day (day 0) and he received oral posaconazole prophylaxis (200 mg three times a day) from day 5. The patient was included into the ongoing study on the epidemiology of invasive aspergillosis and resistance patterns of *Aspergillus* spp. (SEPIA study) for surveillance of patients with acute leukaemia. A baseline chest computed tomography scan (CT) showed no signs of IFD. A consecutive episode of febrile neutropenia (day 13) was treated with empirical broad-spectrum antibiotics, but fever persisted.

A PCR from a throat swab revealed respiratory syncytial virus (RSV) as the possible origin of fever, cough and dyspnoea. A chest CT scan showing bilateral patchy ground glass opacities was compatible with this diagnosis. However, a broncho-alveolar lavage (BAL) performed on day 25 after admission revealed an elevated galactomannan (GM) index of ›2.5 from BAL fluid (norm: ‹0.9), which is indicative of IA. Serum levels of GM increased from 0.4 to 1.3 (norm: ‹0.5), even though posaconazole levels were adequate (2.01 mg/L; standard range not defined).

A novel PCR assay [8] performed from the same BAL fluid was positive for *A. fumigatus* and the TR/L98H mutation of cyp51A. In addition, *A. fumigatus sensu stricto* was isolated in culture, with elevated minimum inhibitory concentrations (MICs) for voriconazole (2 mg/L) and posaconazole (0.5 mg/L, Table 1). Treatment was switched to intravenous liposomal amphotericin B (LAmB) on day 35 at a dose of 3 mg/kg.

A follow-up chest CT scan on day 43, seven days after recovery from neutropenia, showed multiple larger lung nodules with air crescent sign in the right upper and left lower lobe and was strongly suggestive of pulmonary IA responding to LAmB. In addition, a new

Table

Minimum inhibitory concentrations of *Aspergillus fumigatus* isolate from a patient with acute myeloid leukaemia, and EUCAST clinical breakpoints [9], Germany 2012

Antifungal drug	Testing method	MIC [mg/L]	EUCAST breakpoint S/R
Voriconazole	EUCAST	2	-
Posaconazole	EUCAST	0.5	≤0.12/>0,25
Itraconazole	EUCAST	>16	≤1/>2
Amphotericin B	EUCAST	0.5	≤1/>2
Caspofungin	Etest	0.032	-

EUCAST: The European Committee on Antimicrobial Susceptibility Testing; MIC: minimum inhibitory concentration; R: resistant; S: susceptible.

splenic abscess was described. Splenectomy was performed on day 61. Culture of spleen tissue remained negative for bacteria and fungi, but the histological workup revealed abundant hyphae compatible with IA.

On day 140, the patient had recovered and was in complete remission from AML.

Phenotypic identification, molecular identification and resistance testing

A. fumigatus sensu stricto was identified by morphological and molecular characteristics. DNA of the *A. fumigatus* strain was isolated using the QIAamp DNA mini kit (Qiagen, Hilden, Germany). Molecular identification was done by sequencing the internal transcribed spacer (ITS) and partial beta-tubulin gene of the isolate [10]. Susceptibility testing for azole antifungals and amphotericin B was performed by microdilution testing according to EUCAST [11]. Caspofungin MIC was determined by Etest (Biomérieux, Nürtingen, Germany) according to the manufacturer's recommendation. Presence of the L98H mutation and the 34 bp tandem repeat (TR) was evidenced by sequencing the complete cyp51A gene and its promotor region [12, 13]. Direct PCR from BAL fluid for the detection of *A. fumigatus* and azole-resistance genes was carried out as previously described [8].

Discussion

A. fumigatus resistant to itraconazole and posaconazole bearing the TR/L98H mutation was identified in a patient with AML from Germany after only 14 days of azole treatment. This short duration of exposure as well as the TR/L98H mutation suggests an infection with a strain of environmental origin. The patient had not visited the neighbouring Netherlands within the last 12 months, where TR/L98H has frequently been described in azole-resistant *A. fumigatus* strains. Therefore, his respiratory tract may have been colonised with a TR/L98H mutant strain from Germany prior to development of IA.

Azole resistance is usually mediated by different point mutations in the cyp51A gene, coding for 14 alpha-demethylase, a component of the ergosterol synthesis pathway and target of azole antifungals. The frequency of different resistance mechanisms varies between study locations, patient populations and countries. In the United Kingdom, several different mutations are commonly found, such as M220 or G54 [5]. In the Netherlands, the TR/L98H mutation prevails, which was detected in more than 90% of itraconazole resistant strains [4]. TR/L98H mutant strains are reported to be of environmental origin and as a consequence of the use of 14 alpha-demethylase inhibitors as fungicides in agriculture [14]. In these strains, a leucine to histidine substitution (L98H) is associated with a 34-bp TR in the promotor region [4]. According to recently published data, this resistance mechanism is spreading across Europe [15].

Considering that prophylaxis and first-line treatment of IA is usually based on azoles, further spread of resistant isolates could jeopardise the effectiveness of prophylactic strategies and targeted treatment.

Susceptibility testing of fungi, especially of moulds, is not carried out on a routine basis in most microbiology laboratories. In comparison to susceptibility testing of bacteria, testing of moulds is more laborious, it cannot be automated today and requires sound mycological knowledge. Breakpoints for antifungals in *Aspergillus* spp. were published only recently and for a limited number of species and drugs [9]. Detection of azole resistance can be challenging, as some isolates have only slightly elevated or normal MICs for posaconazole and voriconazole. Therefore, itraconazole should be routinely tested on all isolates from patients needing antifungal therapy, because cyp51A mutant strains usually show the highest MIC to this compound.

Furthermore, reliable detection of *A. fumigatus* and cyp51A mutations can be achieved directly from clinical samples by PCR assays with subsequent sequencing [8, 16], offering faster and culture independent recognition of resistance.

More data on the susceptibility of *Aspergillus* spp., both from environmental and clinical samples is needed to reliably describe the epidemiology of azole resistance in Europe. Susceptibility testing of *Aspergillus* spp. should be routinely carried out in clinical laboratories to detect resistant strains and prevent therapeutic failure..

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Conflicts of Interest

A. Hamprecht has received a travel grant from Astellas. D. Buchheidt has received research grants from Gilead Sciences and Pfizer and served on the speakers' bureaux of Astellas, Gilead Sciences, Merck Sharp Dohme/ Merck and Pfizer. J.J. Vehreschild has received research grants from Astellas, Essex/Schering-Plough, Infectopharm, and Pfizer; and served on the speakers' bureau of Astellas, Essex/Schering-Plough, and Merck Sharp Dohme/ Merck. O.A. Cornely has received research grants from Astellas, Basilea, Bayer, Genzyme, Gilead, Merck/Schering, Merck/Serono, Optimer, and Pfizer, has been a consultant to Astellas, Basilea, F2G, Gilead, Merck/Schering, Optimer, and Pfizer. A. Steinbach has received a research grant from MSD and served on the speakers' bureau of Gilead Sciences. M.J.G.T. Vehreschild has served on the speakers' bureau of Schering-Plough/ Essex, Pfizer, MSD and Gilead Sciences. She has received a research grant from 3M.

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Clinical and virological findings in the ongoing outbreak of West Nile virus Livenza strain in northern Italy, July to September 2012

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In July–September 2012, one month earlier than in previous years, 13 confirmed human cases of West Nile virus infection were diagnosed in northern Italy, including five with neuroinvasive disease, three with West Nile fever, and five West Nile virus (WNV)-positive blood donors. In nine cases, the presence of the WNV lineage 1a Livenza strain, characterised in 2011, was ascertained. Symptomatic patients had prolonged viruria with high viral load.

In 2012, West Nile virus (WNV) infection has become a concern for many public health experts. Earlier occurrence in this year of an increased number of human cases has been reported by European countries to the European Centre for Disease Prevention and Control (ECDC) [1]. Moreover in the United States, over 1,000 WNV infection cases have been reported to the Centers for Disease Control and Prevention (CDC), the highest number for the period, since WNV was first detected in the country in 1999 [2].

We recently reported the case of a WNV ribonucleic acid (RNA)-positive blood donation identified in northern Italy in July 2012, which suggested circulation of WNV one month earlier than in previous years [3]. Partial sequencing of the WNV RNA (GenBank accession numbers: JX417422 and JX470578) demonstrated identity with the genome of a lineage 1a WNV strain, called Livenza strain, which had been identified in the same area in 2011 (WNV-Livenza/2011 strain) and which was divergent from the strain responsible for the 2008–09 outbreak in northern Italy [3]. We report here an update on the surveillance activity for WNV in northern Italy, which confirms the feared earlier occurrence of human cases and the spread of the Livenza strain. We also describe clinical and virological findings in patients with WNV infection and the rapid public health interventions that were undertaken in the affected areas.

Surveillance activities and results

In Italy, surveillance activities for human WNV neuroinvasive disease (WNND) are defined annually by the National surveillance programme [4]. In addition, in the Veneto region, special surveillance for West Nile fever has been implemented since 2010. According to the protocol, from 15 June to 30 November, patients with fever over 38.5°C and absence of concomitant diseases which could account for the febrile illness, are investigated as possible cases of WNV infection and referred to the regional reference laboratory for confirmation, as reported [5,6]. Confirmed cases of WNV disease are immediately notified from local public health departments to the regional authorities, which report cases to the Ministry of Health. The case definition of WNND and West Nile fever is as defined by the European Union [7]. Since 2012, detection of WNV RNA in urine has been included among the laboratory confirmation criteria. In the Veneto region, according to the 2012 national directives, nucleic acid amplification testing (NAAT) for screening of tissue and organ donations is carried out in the period between 15 July and 30 November in all the regional territory, while screening of blood donations is carried out during the same period in the provinces where human cases of WNND were identified in 2011, i.e. Venice, Treviso, and Belluno provinces.

By 3 September 2012, 13 confirmed cases of WNV infection, including the first previously reported case in July, have been diagnosed in the Veneto region. The 13 cases had symptom onset between 15 July and 24 August, and eight were male. Cases had an age range of 49 to 87 years. The presence of WNV lineage 1 was demonstrated in 12 cases with detectable WNV RNA by specific real-time reverse transcription polymerase chain reaction (RT-PCR) assays, as previously described [5]. For nine cases who had a relatively high viral load in blood or urine, sequencing of WNV RNA

was successful and ascertained that all these cases were infected with the WNV Livenza strain (Table). For one of these cases, the virus was isolated in cell culture and fully sequenced (GenBank accession number: JX556213). Genome sequence analysis demonstrated, that compared with the full genome sequence of the WNV-Livenza/2011 strain (GenBank accession number: JQ928174) [8], the sequence of the isolated virus harboured two non-synonymous substitutions, resulting in a Val113Ile amino acid mutation in the capsid protein and a Val45Ile mutation in the non-structural protein 5 (NS5). Eight synonymous nucleotide changes were additionally observed. The strain of the isolated virus was named WNV Livenza/2012/31.1 strain, with 31 representing the week number and one the sequential number of cases of the week.

Findings in blood donors

The first case of WNV infection in Italy in 2012, as already described [3], was identified by NAAT screening of blood donors in Venice province on 15 July. Further six positive blood donations were later detected among donors who resided in nearby areas to the first case (Figure), two of these as recently as September 2012. So far, laboratory and follow-up data are available for the first five infected blood donors detected, who are therefore included among the 13 total cases described in this communication. The most common symptom reported among the five blood donors was asthenia that occurred one to five days after donation. One donor, however, reported asthenia only at follow-up visits after retrospectively recalling having this symptom two days before donation. All but one donor were WNV IgM positive at the time of donation and developed IgG antibodies within the following week. In the urine of three donors, up to 5,000 copies/mL of WNV RNA were detectable, and this persisted for up to six days longer than in blood (Table). WNV RNA detection in urine was performed by using two different real-time RT-PCR methods, respectively targeting WNV lineage 1 [9], and both WNV lineage 1 and lineage 2 [10] on total nucleic acids purified from 1 mL urine, by using the NucliSENS EasyMAG system (bioMérieux SA, Marcy l'Etoile, France).

Findings in patients

Five cases of WNND and three of West Nile fever were confirmed (Table). A further probable case of WNND and two probable cases of West Nile fever are currently under investigation. Symptoms in patients with WNND were not severe and clinical improvement was observed in most patients. A common laboratory finding in patients with WNND or West Nile fever was detection of WNV RNA at high load (up to 100,000 copies/mL) in urine, and its persistence (over 30 days in one case) for a longer time than in blood and in the cerebrospinal fluid (CSF), even in the presence of WNV IgG antibodies. Of the eight patients, infection by WNV Livenza strain was confirmed in six. Five of these patients were resident in Venice province, in the area surrounding the Livenza river, and one in Treviso province, near the Piave river, in an area where, in 2011, a different WNV lineage 1a strain, called Piave strain, was identified in a patient and fully sequenced [8] and WNV lineage 2 was detected by veterinary surveillance [11].

Rapid public health interventions

In areas where human cases of WNV infection occurred and in neighbouring municipalities, measures for control of Culex mosquitoes, which are the prime vector for WNV transmission, were immediately implemented in accordance with the regional intervention protocol. In addition, information leaflets about WNV fever and effective protection against mosquito bites were disseminated to the population in public places and on the websites of regional and local health units.

Discussion

This report describes 13 confirmed human cases of WNV infection diagnosed in north-eastern Italy between July and September 2012. Cases occurred one month earlier than previously reported, and this could be attributable to the ongoing very hot summer season, probably responsible for the very high mosquito density, that has been observed in the affected areas. In nine cases, for whom viral RNA sequencing was successful, infection with the recently identified WNV Livenza strain was demonstrated. The new WNV strain did not appear particularly virulent and lethal, since cases had no severe symptoms and positive outcome. However, due to their small number, the virulence and lethality of the new WNV lineage 1a Livenza strain remains to be defined. To this aim, studies on animal models will be conducted with the viral isolate. The Livenza strain is classified within the Mediterranean cluster, but has several novel amino acid substitutions in non-structural proteins that are not present in other European and non-European strains, that could be relevant for virus transmissibility and neuroinvasive potential [8].

Clinical evaluation demonstrated variability of symptoms among patients, including retinitis, meningitis, gastrointestinal symptoms, respiratory failure and lower limb neuritis, besides fever, headache, asthenia, arthralgia, and myalgia. Asthenia was a common symptom in blood donors and one donor only recalled it in retrospect. Thus, asking blood donors about this symptom before donation, in areas where WNV is endemic, could help to recognise potentially infected individuals. To this end, interviews are useful and one of our patients with WN fever was identified by interview at the visit for blood donation. Detection of WNV RNA in urine was very useful for early laboratory diagnosis of WNV infection, since in some patients viral RNA was detectable only in urine and not in blood. Remarkably, patients with WNND or West Nile fever had WNV shedding at high load in urine for several days after symptom onset and seroconversion, and even after the appearance of IgG antibodies, when viral RNA was no longer detectable in blood or CSF. In addition,

Table

Clinical and virological findings in laboratory-confirmed cases of West Nile virus infection, Veneto region, Italy, 15 July–3 September 2012 (n=13)

Case	Symptoms	Time of diagnosis (in days)[a]	Laboratory data at diagnosis	Time of first follow-up (in days)[a]	Laboratory data at follow-up	Strain
Blood donor[b]	Asymptomatic	2	IgM+/IgG- in serum, WNV RNA+ in blood, WNV RNA- in urine	7	IgG appearance, WNV PRNT+	Livenza[c]
Blood donor[b]	Asthenia, fever, rash	1	IgM-/IgG- in serum, WNV RNA+ in blood and urine	7	IgM appearance at first follow-up (IgG subsequently detected at second follow-up), WNV PRNT+	Livenza[d]
Blood donor[b]	Asthenia	4	IgM+/IgG- in serum, WNV RNA- in blood and urine	10	IgG appearance, WNV RNA- in urine, WNV PRNT+	Livenza
Blood donor[b]	Asthenia	3	IgM+/IgG- in serum, WNV RNA- in blood and urine	10	IgG appearance, WNV PRNT+	ND
Blood donor[b]	Asthenia, myalgia at upper limbs and periorbital muscles	3	IgM+/IgG- in serum, WNV RNA+ in blood, WNV RNA- in urine	10	IgG appearance, WNV PRNT+	ND
WNND	Artralgia, asthenia, bilateral retinitis, fever >38.5°C, myalgia	20	IgM+/IgG+ in serum, WNV RNA+ in blood , WNV PRNT+	30	IgM+/IgG+ also in CSF, high WNV RNA load in urine	Livenza
WNND	Enchephalitis, fever, headache	6	IgM+/IgG+ in serum and CSF, WNV RNA+ in blood, CSF and urine, high WNV RNA load in urine; WNV PRNT+	15	Persistent high WNV RNA load in urine	Livenza
WNND	Arthralgia, asthenia, fever, myalgia, neuritis at lower limbs, respiratory failure	7	IgM+/IgG+ in serum and CSF, WNV RNA- in blood and CSF, WNV RNA+ in urine, high WNV RNA load in urine, WNV PRNT+	10	Persistent high WNV RNA load in urine	Livenza
WNND	Asthenia, fever, neuritis at lower limbs	7	IgM+/IgG- in serum and CSF, WNV RNA- in blood and CSF, WNV RNA+ in urine	10	IgM+/IgG- in serum, WNV RNA+ in urine, high WNV RNA load in urine	Livenza
WNND	Fever, meningitis	6	IgM+/IgG- in serum and CSF, WNV RNA- in blood and CSF, WNV RNA+ in urine, high WNV RNA load in urine	10	IgM+/IgG- in serum, persistent high WNV RNA load in urine	Livenza
WNF	Fever, gastrointestinal symptoms, headache, rash	7	IgM+/IgG+ in serum, WNV RNA- in blood, WNV RNA+ in urine, high WNV RNA load in urine, WNV PRNT+	15	WNV RNA- in urine	Livenza
WNF	Fever, headache	10	IgM+/IgG- in serum, WNV RNA- in blood, WNV PRNT+	16	IgM+/IgG- in serum, WNV PRNT+	ND
WNF	Fever, headache	6	IgM+/IgG+ in serum, WNV RNA- in blood, WNV RNA+ in urine	NA	NA	ND

NA: not available yet; ND: not determined; CSF: cerebrospinal fluid; PRNT: plaque reduction neutralisation test; RNA: ribonucleic acid; WNF: West Nile fever; WNND: West Nile neuroinvasive disease; WNV: West Nile virus.

A'+' signifies 'positive', while a '-' signifies 'negative'. High WNV RNA load refers to >10,000 copies/mL.

[a] Number of days after symptom onset except for blood donors for whom this is the number of days after blood donation.

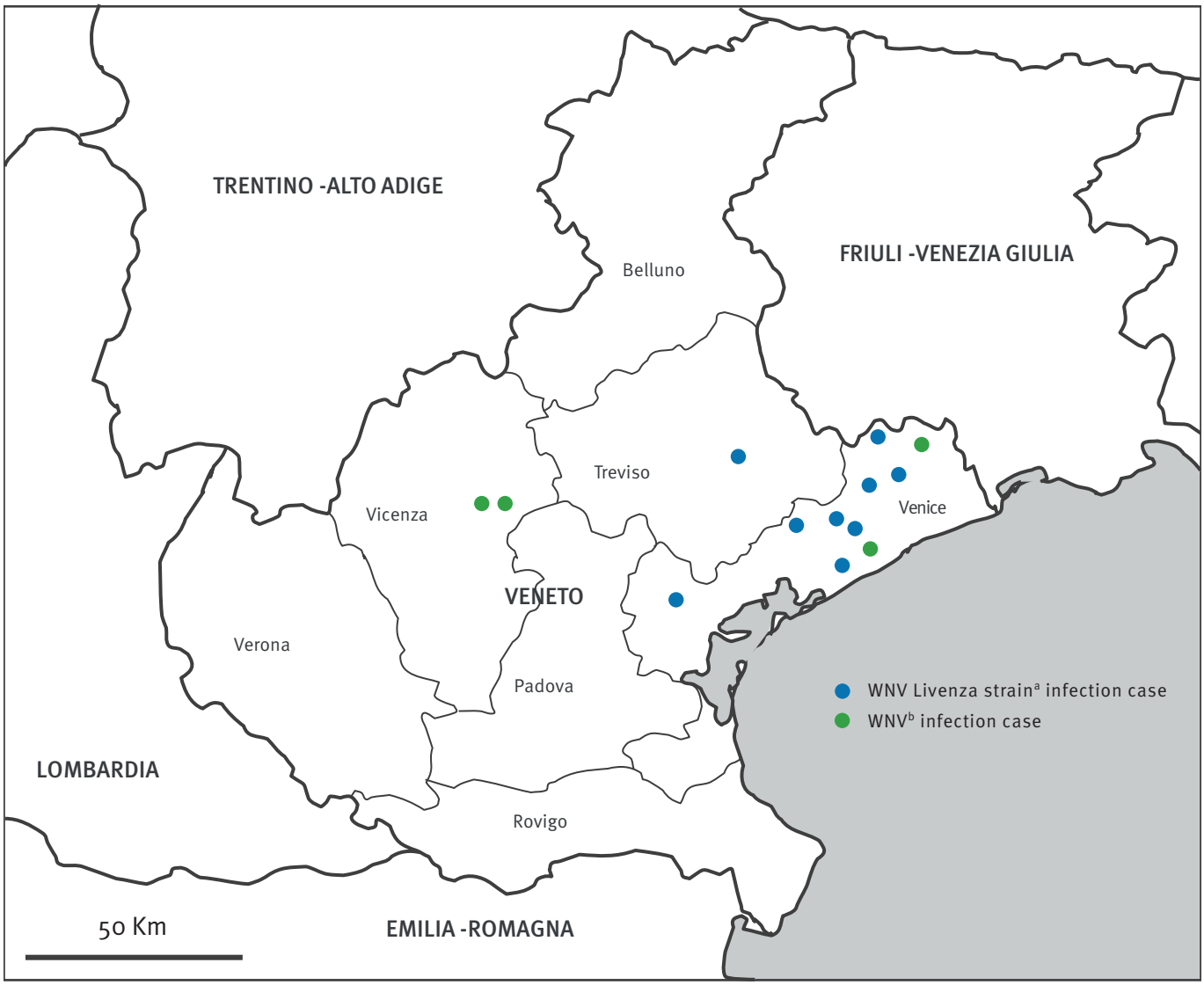
[b] WNV nucleic acid amplification testing (NAAT)-positive blood donor.

[c] Partial genome sequence, GenBank accession numbers: JX417422.1 and JX470578.1.

[d] Complete genome sequence, GenBank accession number: JX556213.

Figure

Origin of confirmed human cases of West Nile virus infection, Veneto region, Italy, 15 July–3 September 2012 (n=13)



WNV: West Nile virus

[a] WNV Livenza strain was ascertained by sequencing.

[b] WNV lineage 1 was confirmed but WNV was not sequenced.

the high viral load in urine allowed sequencing of WNV RNA in most cases.

In conclusion, this study reports an increased WNV activity in northern Italy and describes the clinical presentation of infection with the new endemic WNV Livenza strain, that appears to be responsible for the outbreak.

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Community cluster of meningococcal disease by *Neisseria meningitidis* serogroup C in Andalusia, Spain, March to May 2011

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Between March and May of 2011, a cluster of three fatal cases of meningococcal sepsis occurred in Andalusia, Spain, in a municipality with a population of around 20,000 inhabitants. The cases were in their mid-teens to early thirties and were notified to the epidemiological surveillance system of Andalusia (Sistema de Vigilancia Epidemiológica de Andalucía, SVEA) during a 68-day period from March through May 2011. All three were infected with the same strain of *Neisseria meningitidis* serogroup C genosubtype VR1:5-1;VR2:10-8. None of the cases had been previously vaccinated against *N. meningitidis* serogroup C. Antibiotic post-exposure chemoprophylaxis was administered to close contacts of every diagnosed case. Once the cluster was confirmed, the local population was informed through the media about the control measures taken by the health authorities. The vaccination history against *N. meningitidis* serogroup C of the population under 25 years-old in the municipality was checked. Vaccination was offered to unimmunised individuals younger than 25 years of age and an additional dose of vaccine was offered to those who had been vaccinated between 2000 and 2006 with a vaccination schedule of three doses before the first year of age. No further cases occurred since the beginning of these actions.

Introduction

Neisseria meningitidis is a pathogenic bacterium that colonises the nasopharynx, in most cases, in an asymptomatic way [1]. In Spain, individuals who are colonised are mainly affected by serogroups B or C, but rarely develop invasive disease. When invasive disease occurs however, it appears in acute form, frequently with clinical manifestations of meningitis or sepsis. Meningococcal disease (MD) shows high overall mortality rates [1]. The highest rates are associated with serogroup C, which affected 25% of cases in Spain in 2010 [2]. In Spain, MD is the most common cause of bacterial meningitis in children and the second most common cause in adults. Cases of MD usually appear sporadically distributed in the population during winter and spring [2]. Infrequently, there are epidemic outbreaks associated with a certain strain of N. meningitidis, which are grouped in space and time.

MD has been notifiable on clinical suspicion in Spain since 1981 [3]. In the Andalusian epidemiological surveillance system (Sistema de Vigilancia Epidemiológica de Andalucía, SVEA) included in the national centre of epidemiology (Centro Nacional de Epidemiología, CNE), a case of MD has to be urgently notified within 24 hours of diagnostic suspicion [4]. To initiate appropriate preventive actions, early confirmation of diagnosis and the identification of the serogroup are critical. These are routinely performed in the microbiology laboratory of the hospital within 48 hours of the patient's admission, or in the Neisseria reference laboratory of the national microbiology centre (Centro Nacional de Microbiología, CNM) along with the characterisation of the strain and the genosubtype identification [5].

The meningococcal C conjugate vaccine was introduced to the childhood vaccination schedule in Spain in the year 2000, administered free of charge, in three doses at two, four and six months of age. During the same year, immunisation was extended to young people of up to 18 years of age, by administering one vaccine dose. In addition to individual protection, the meningococcal C conjugate vaccine provides group immunity by decreasing the rate of nasopharyngeal carriers of N. meningitidis. This reduces the transmission of the microorganism and is important for the control of the disease [6,7]. Nevertheless, different studies have shown that the antibody response decreases in the years after vaccination [8,9] and that administration of an additional dose from the first year of age boosts antibody response [10]. In Spain, this decrease of immunity and meningococcal C conjugate

vaccine effectiveness, has been observed especially with regards to individuals born from the year 2000 onwards, who were subject to the 2000 vaccination schedule, while the effectiveness of the vaccine remained high in children between five months and 18 years of age who were born before 2000 and received one dose of vaccine [9]. This fact motivated a change of the vaccination schedule in 2006 in Spain, delaying the third dose to 15 months of age.

The meningococcal C conjugate vaccine has lead to a significant reduction of the incidence of MD by *N. meningitidis* serogroup C in Spain over the past years among vaccinated age groups and, to a lesser extent, among unvaccinated age groups. In the period from 1999 to 2009 this reduction was of 87% [2]. The incidence rate of MD by serogroup C in 2009 was 0.14 per 100,000 inhabitants in Spain and 0.21 per 100,000 in Andalusia [2]. The vaccination coverage with three doses against *N. meningitidis* serogroup C in the cohort of individuals born in 2010 in Andalusia is 90% (unpublished data).

In this paper, we present the description of a cluster of cases due to *N. meningitidis* serogroup C in a municipality of Andalusia (Spain) and the intervention to prevent further cases. The cluster occurred when different strains of *N. meningitidis* serogroup C were circulating in Andalusia, with a predominance of the strain affecting the cases in this cluster.

Methods

The case definition of an MD case in SVEA, as in the whole CNE, follows the clinical, laboratory and epidemiological criteria laid down by the European Commission [11]. The MD can occur as meningitis and/or meningococcemia that may quickly progress to purpura fulminans, shock and death. Laboratory confirmation is established by isolation or detection of *N. meningitidis* nucleic acid from a normally sterile site or a petechial aspirate, detection of *N. meningitidis* antigens from the cerebrospinal fluid (CSF) or Gram-negative diplococci in the CSF. The suspicion of the existence of a community outbreak is established with the appearance of three or more confirmed or probable cases of the same serogroup, which take place within a time interval of three months in a defined community level (e.g. municipality, neighbourhood) and in which, in addition, the incidence is over 10 cases per 100,000 inhabitants [12]. The confirmation of the outbreak is done by genosubtyping in the reference laboratory for meningococci of the CNM.

In the event reported here, the population at risk was considered to be residents or people who regularly visited and/or stayed in the municipality where the cluster of cases occurred from 10 days before hospitalisation of the first case in March to the end of May 2011 [5].

For the epidemiological investigation, close contacts were defined as people who lived with a case, people who spent the night in the same room than a case, classmates (e.g. desk mates, playmates) or colleagues (e.g. office mates) with frequent and continuing contact with a case up to 10 days before the case's hospitalisation and healthcare workers who had direct contact without protection (mask) with nasopharyngeal secretions of the case (e.g. during resuscitation and tracheal intubation procedures) [5]. To calculate vaccination coverage, the information about the vaccination status against *N. meningitidis* serogroup C in this municipality among residents born after 2000 was obtained from the computerised database of the Andalusian vaccination program (Programa de Vacunación de Andalucía, PVA). The list of residents born after 2000 in the municipality was obtained from the municipal register of inhabitants.

Results

Description of the cluster of cases

Between March and May 2011, three cases of MD serogroup C in two women in their mid-teens to early thirties and a man in this late twenties occurred in a municipality of Andalusia with a population of around 20,000 inhabitants (31% aged less than 25 years). The attack rate was 14.2 cases per 100,000 inhabitants. None of the cases had been previously vaccinated against *N. meningitidis* serogroup C and no epidemiological links were found. The three cases lived in the same municipality: two in the same neighbourhood and one in another area away from the others. None of them had relevant medical history and none had travelled recently. No underlying risk factors were identified. All three had severe sepsis with sudden onset and were treated in the intensive care units of different hospitals, where they died in the hours following admission. For the first two cases, *N. meningitidis* serogroup C was isolated in blood specimens and in CSF at the hospital laboratory and for the third case, deoxyribonucleic acid (DNA) of *N. meningitidis* serogroup C was detected in blood specimens sent to the CNM reference laboratory. In this laboratory, the same strain of *N. meningitidis* serogroup C genosubtype VR1:5-1;VR2:10-8 was subsequently identified in the three cases.

Intervention

During the first 24 hours after the notification of any case, close contacts within the 10 days before hospitalisation were sought. For all three cases, a total of 56 personal close contacts were identified in the cases' social environment (e.g. family, classmates, work mates and colleagues). All close contacts were offered post-exposure prophylaxis with oral rifampicin or ciprofloxacin, and meningococcal C conjugate vaccine, if applicable, by the local health services.

At the end of May 2011, following the notification of the third case, a review of the computerised database of PVA was started to get updated information on the immunisation status against *N. meningitidis* serogroup

C in the cohorts born since 2000 residing in the municipality. The coverage found in all of them exceeded 92% according to the schedule in force at each moment.

As an exceptional measure, the administration of one meningococcal C conjugate vaccine dose was recommended to all previously unimmunised people who were less than 25 years-old and who resided in or regularly visited the municipality, due to work, studies or family, and an additional dose was administered to children vaccinated with the schedule used between 2000 and 2006 who had not received any dose after the first year of age.

Based on the information provided by published studies on *N. meningitidis* serogroup C carriers in the population [13], vaccination was not recommended to those over 25 years of age, despite the fact that two cases were over this age. People over 25 years-old were nevertheless not excluded if they requested vaccination.

To prevent social alarm and to improve the results of the intervention, the population at risk was actively informed about the existence of the cluster, disease transmission, preventive measures, as well as the place and time for the vaccine administration. At the Regional Ministry of Health, a document with questions and answers on MD was available through a respective website, and the call centre 'Salud Responde' was also used to answer telephone enquiries on a 24/7 basis [14].

Information was also provided by the health services through the local and regional media (television, press and radio). Meetings were held with local authorities, local schools and parents of students, in order to manage the vaccination campaign. In training sessions for the healthcare professionals of the local healthcare centres, MD and MD prevention and control measures were reviewed.

From 10 days before hospitalisation of the first case in March until the end of May 2011, providing that less than 10 days had passed since their last visit, regular visitors to the municipality were offered the vaccine in their respective municipalities of residence, once they had been identified. In the affected municipality, vaccination campaign of the susceptible under 25 years of age with the meningococcal C conjugate vaccine, took place during five days at the end May 2011. Two offices for exclusive consultations and vaccination, which were attended by medical and nursing staff, were available from 9 a.m. to 8 p.m. in the healthcare centre and elsewhere in the municipality.

Vaccination was also offered to children and students younger than 25 years in an overflow room during school hours at several educational centres. All people under 25 years-old who attended these consultations were previously requested, through the information campaign, to bring their immunisation records to determine their immunisation status against *N. meningitidis* serogroup C. Those who did not bring their immunisation records and whose information was not retrievable from the PVA vaccination database were considered not immunised. Telephone numbers from local health services were made available to clarify doubts and provide additional information on vaccination.

Up to 9 June 2011, 3,818 people, not previously immunised, were vaccinated with meningococcal C conjugate vaccine, corresponding to 18.1% of the population of the municipality. The highest proportion of vaccinated (29.1%) in the municipality was reached in the 25–39 years age-group. The reason for this was that part of this age-group had an already high pre-existing vaccination coverage maintained by the PVA starting from the year 2000. Moreover, despite vaccination recommendations targeting individuals under 25 years-old, those over this age requesting vaccination could obtain it.

Discussion

N. meningitidis has a large genetic diversity, with a high rate of recombination and frequent appearance of new subtypes, associated with the virulence of the disease. In France, particularly virulent peaks associated with the isolation of phenotypes C:2a:P1.5,2 and C:2a:P1.5 were observed in 1992 and 2003. A Norwegian study [15] that compared the predominant genosubtype and virulence in the period 1985–2002 showed an increase in mortality in 1994–2002 in Norway, associated with circulating *N. meningitidis* serogroup C genosubtypes C: 15: P1.7, 16/ST -32 and C: 2a/ST-11.

In Andalusia, between July 2010 and July 2011, 42 cases of MD serogroup C were reported with an incidence rate for this period of 0.5 per 100,000 inhabitants. The mean age of these cases was 32.3 years (range: 7 months–40 years), with 67% over 19 years-old. These cases, with the exception of the cluster reported here, were sporadically distributed in the whole region. Of 27 cases with known genosubtype, 23 (85%) corresponded to the same genosubtype VR1:5-1;VR2:10-8 than that identified in the cluster. Of the 14 cases who were less than 20 years of age notified in this period, for which information on previous vaccination status was available, all but two were vaccinated. For the two cases who were considered unvaccinated, one was five weeks-old and, due to the young age, had not received the complete vaccination against *N. meningitidis* serogroup C. The other was seven months-old, from another country than Spain, without any of the first two doses established in our calendar. Six vaccine failures among the 14 cases were documented; in five of these, cases were correctly vaccinated between 2001 and 2004 and had received three doses at two, four and six months of age. The sixth case received a single dose in 2001 when he was 17 years-old. In five of these vaccine failures, the same *N. meningitidis* serogroup C genosubtype VR1:5-1;VR2:10-8 was identified.

The 42 cases of MD serogroup C in Andalusia between July 2010 and July 2011, involved 15 deaths. Of the 14 deaths for which a responsible strain was known, 12 were attributable to this dominant strain. This high mortality also coincides with an unusual distribution of cases of MD due to *N. meningitidis* serogroup C, by age group. This disease occurs mainly among children of less than 16 years of age and young adults aged between 16 and 24 year-old [12,16,17]. However, the age of the cases in the cluster reported here is strikingly higher, as well as in the total of MD cases reported in Andalusia.

An increase of cases of MD in young adults and older people has also been described in other countries [10]. Although vaccine failures due to loss of immunity is a fact already described [8-10,18], none of the cases in the cluster reported here had a history of vaccination, and vaccination failure does not seem to have occurred as no further cases were observed in the municipality where a high vaccination coverage was estimated in the population (about 92% among those born after 1998).

The relationship between carriers of *N. meningitidis* serogroup C and MD among the population is complex. A low rate of carriers is associated with a low rate of protective immunity. A recent meta-analysis [19] shows that the percentage of asymptomatic carriers is highest among young adults under 20 years of age, decreasing gradually with age. The carrier status usually lasts months and confers some immunity. In a study of carriers during an outbreak with five cases of MD due to *N. meningitidis* serogroup C, serogroup C was isolated in only 3.9% of positive samples of healthy carriers and only one of them belonged to the genosubtype implicated in the outbreak [13]. In another study of carriers of MD during an outbreak due to *N. meningitidis* serogroup C in the Netherlands, it was observed that the prevalence of carriers of the epidemic strain was lower in the affected population than in other populations with low incidence [20].

In recent years in Andalusia, which has a population of about 8.5 million, there is only one precedent of a similar cluster of MD cases due to the same *N. meningitidis* serogroup C strain. This was in 2005, when four cases in a municipality of 13,164 inhabitants were reported, three of them in the age range of 25 to 80 years. At the time that this cluster was detected, vaccination coverage for those born between 2000 and 2004 exceeded 92% and, for those born between 1987 and 1999, 78.35%. The study of the prevalence of healthy carriers of *N. meningitidis* in a representative sample of 150 individuals in the age group 20 to 25 years (unpublished data) was 13.3% and only one of the carriers (5%) had *N. meningitidis* serogroup C corresponding to the epidemic strain.

The intervention carried out following the cluster described in this paper was a challenge for health authorities in Andalusia, mainly due to the three deaths and the social alarm caused by such clusters in small populations. Community outbreaks are difficult to control, and the decision of whether and who to vaccinate, is not simple or well argued in the literature [1,12,21-24].

In general, the use of chemoprophylaxis in personal contacts of a case of MD is considered an effective measure because of its double effect: the elimination of *N. meningitidis* in carriers and the reduction in the attack rate in contacts. Regarding the use of the vaccine in outbreaks, there is less agreement about the criteria to be followed to decide who should be vaccinated in the population. As the most frequently affected are those aged between two and 25 years, this is commonly the recommended vaccination group a priori.

For outbreaks of MD, there are significant challenges for the development and implementation of intervention protocols [1,25]. First of all, the lack of evidence supporting the use of chemotherapy or vaccination, especially when there is no history of contact with a case, mainly because of inherent difficulties of the study design due to ethical issues. The second reason is the pressure that the media may come to exert in these situations, because the social alarm created can have effects on the measures taken; creating a demand by the population that does not always reflect the scientific criteria for intervention.

The decision for selecting the population to be vaccinated in the event reported here was based, as described, on the fact that rates of healthy carriers are higher in young people, as well as on the measures adopted in previous similar outbreaks, and criteria that took into account the cost–benefit of the intervention.

After the implementation of preventive measures, no new cases of MD serogroup C occurred in the area. Our results support the need to maintain a quality and comprehensive system of epidemiological and microbiological surveillance that detects, besides cases, the presence of strains that may have higher virulence in order to implement fast-acting measures.

Due to the effect that the introduction of the meningococcal C conjugate vaccine in 2000 in the vaccination schedule in children and young adults can have on the epidemiology of MD, further efforts and research are needed to improve and update knowledge on how to respond in situations similar to the cluster reported here.

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A report on the large measles outbreak in Lyon, France, 2010 to 2011

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In 2010 and 2011, the city of Lyon, located in the Rhône-Alpes region (France), has experienced one of the highest incidences of measles in Europe. We describe a measles outbreak in the Lyon area, where cases were diagnosed at Lyon University hospitals (LUH) between 2010 and mid-2011. Data were collected from the mandatory notification system of the regional public health agency, and from the virology department of the LUH. All patients and healthcare workers who had contracted measles were included. Overall, 407 cases were diagnosed, with children of less than one year of age accounting for the highest proportion (n=129, 32%), followed by individuals between 17 and 29 years-old (n=126, 31%). Of the total cases, 72 (18%) had complications. The proportions of patients and healthcare workers who were not immune to measles were higher among those aged up to 30 years. Consequently, women of childbearing age constituted a specific population at high risk to contract measles and during this outbreak, 13 cases of measles, seven under 30 years-old, were identified among pregnant women. This study highlights the importance of being vaccinated with two doses of measles vaccine, the only measure which could prevent and allow elimination of the disease.

Introduction

Measles is a one of the most highly contagious diseases. It is spread through respiratory droplets so that people in contact with the virus, who are not vaccinated or not immune due to prior infection, are at high risk of catching the disease. In France, vaccination against measles was first introduced in the vaccination programme in 1983, with a single dose for infants aged 12–15 months. The vaccination schedule was subsequently modified in 1996, whereby two doses of vaccine against measles were recommended, with the first dose still at 12–15 months. The second dose was first administered at 11–13 years of age in 1996, then at 3–6 years of age from 1997 [1]. Since 2005, the first dose is recommended at the age of 12 months and the second dose at the age of 13–24 months [1]. A catch up vaccination is recommended for individuals who did not receive two vaccine doses. Earlier vaccination is recommended for infants who are attending a daycare centre, with in this case, the first dose administered at nine months of age and second dose at 12–15 months of age.

Measles cases were sporadic in France until 2008. Since the beginning of the year 2008, France has experienced successive measles wave epidemics [2-4]. The third wave was the largest and took place from October 2010 to April 2011 in the whole country, with the highest incidence in the south-east regions of France. Since 2008, measles are also spreading out of control in many other parts of Europe, such as for example Romania, Italy, Belgium or Switzerland [5-8]. Nevertheless, France appears to be the most affected, with more than half of the European cases [9]. Measles have been mandatorily notifiable in France since 2005. Healthcare practitioners, clinicians and biologists in laboratories have to report each suspected or confirmed case using a standardised form. Between January 2008 and April 2011, more than 18,000 measles cases were reported to the French Institute for Public Health Surveillance (Institut de Veille Sanitaire). These included 10 deaths, among which nine cases aged less than 30 years-old. Neurological complications affected 26 of the total measles' cases while 808 had severe pulmonary infections. About 4,000 patients with measles were hospitalised [2,10]. The high burden of the disease in teenagers and young adults under the age of 30 years [2,3] besides children (up to 16 years-old) is a new xmain feature of this reemerging disease. Young women are also a specifically exposed population, in so far as they are at high risk to contract measles but cannot be vaccinated during pregnancy.

The Rhône-Alpes region has been the most affected area for measles in France [2,11], with an incidence of 97.9 measles cases per 100,000 population between October 2010 and September 2011. Lyon is the biggest city in this region, with about 475,000 inhabitants registered in 2008. Lyon University Hospitals (LUH) are the main hospitals in Lyon and form the second largest hospital group in France. The objective of this study was to describe this large measles outbreak through notified cases diagnosed in LUH. The survey also presents a focus on pregnant women who when exposed while not immune are at higher risk of complications.

Methods

Setting

A prospective surveillance of measles cases was instituted in LUH from 1 January 2010. For the present study, data were collected between 1 January 2010 and 8 July 2011 according to the date of disease onset. LUH form the largest group of public hospitals in the town of Lyon. Our data concerned the four main hospitals of the group: hôpital Edouard Herriot, Centre hospitalier Lyon Sud, Groupement hospitalier Nord and hôpital Femme-Mère-Enfant. The last one received children and pregnant women. For the present report, we analysed two different data sources: (i) surveillance of measles cases through all the mandatory notifications conducted by one of the LUH and (ii) virological surveillance through tested samples derived from patients and healthcare workers (HCW).

Surveillance of measles cases notified by the Lyon university hospitals

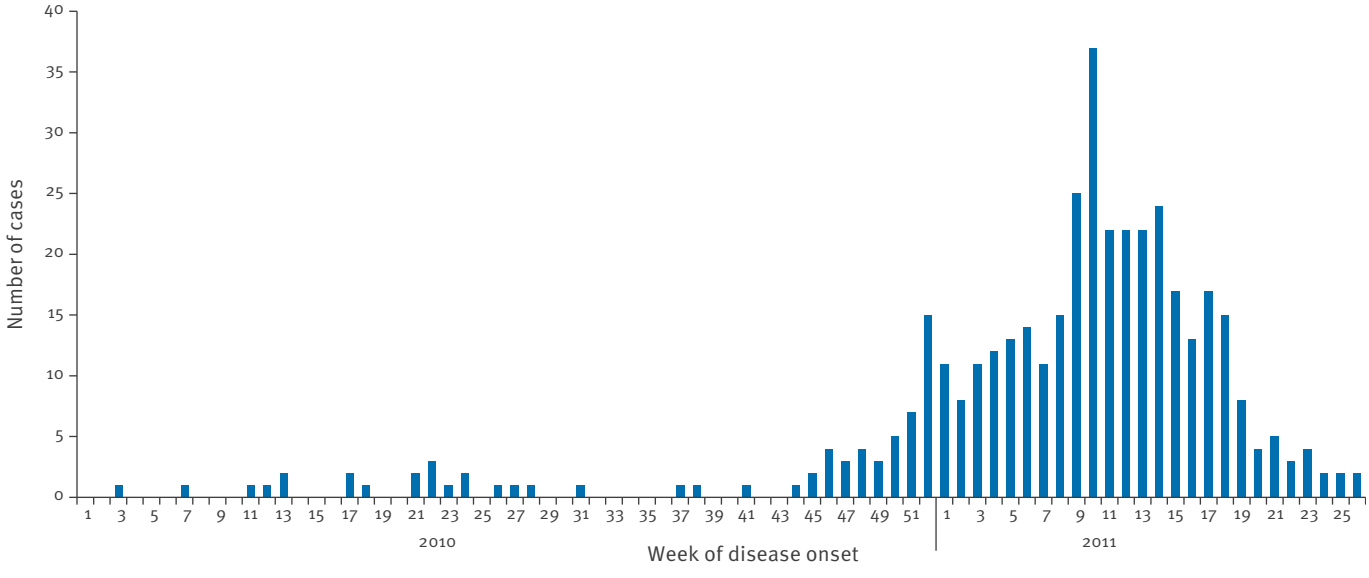
The surveillance of patients diagnosed with measles during the outbreak in LUH was performed using data from mandatory notification which was available from the regional public health authorities database (Agence Régionale de Santé de Rhône-Alpes). Clinical suspected cases were defined as having fever ≥38.5°C, maculopapular rash, and at least one of the following signs: conjunctivitis, coryza, cough, Koplik's sign. Laboratory-confirmed cases were clinical suspected cases with biological confirmation: specific IgM detected in saliva or serum and/or seroconversion with at least a four-fold increase of IgG titres and/or positive polymerase chain reaction (PCR) for measles virus. Epidemiologically-confirmed cases were defined as clinical cases who had had contact with laboratory-confirmed cases 7–18 days before onset of symptoms.

Virological surveillance of patients and healthcare workers

Data about the viro-immunological status were extracted from the hospital virological-based surveillance database. This surveillance concerned patients or HCW tested for measles IgM and/or IgG and/or measles virus by PCR in LUH. The patients were tested either because: (i) they were clinically diagnosed with measles, or (ii) they were exposed to a measles case, or (iii) they were potentially susceptible. The group of HWC represented a selected group of all the employees of LUH. These HCW were tested either because: (i) they

Figure 1

Measles cases diagnosed in Lyon University Hospitals, measles outbreak in Lyon, France, 1 January 2010–8 July 2011 (n=407)



Cases comprise clinical suspected cases, laboratory-confirmed cases and epidemiologically-confirmed cases.

Table

Characteristics of cases diagnosed with measles in Lyon University Hospitals, France, 1 January 2010–8 July 2011 (n=407)

Characteristics	Adults >16 years-old (n=181)	Children ≤16 years-old (n=226)	Overall (n=407)
	n/N (%)[a]	n/N (%)[a]	n/N (%)[a]
Male	96/181 (53)	119/226 (53)	215/407 (53)
Female	85/181 (47)	107/226 (47)	192/407 (47)
Median age in years (interquartile range)	26 (22–33)	1 (0–9)	15 (1–26)
Complications	43/181 (24)	29/226 (13)	72/407 (18)
Pneumonia	28/43 (65)	23/29 (79)	51/72 (71)
Encephalitis	1/43 (2)	1/29 (3)	2/72 (3)
Other	14/43 (33)	5/29 (17)	19/72 (26)
Laboratory confirmation requested[b]	143/181 (79)	169/226 (75)	312 (77)
IgM-positive, saliva	9/143 (6)	11/169 (7)	20/312 (6)
IgM-positive, serum	111/143 (78)	19/169 (11)	130/312 (42)
PCR-positive	60/143 (42)	153/169 (91)	213/312 (68)
Seroconversion	26/143 (18)	7/169 (4)	33/312 (11)
Vaccination status unknown	77/181 (43)	43/226 (19)	120/407 (29)
Vaccination status known	104/181 (57)	183/226 (81)	287/407 (71)
Not vaccinated[c]	74/104 (71)	150/183 (82)	224/287 (78)
Vaccinated with one dose[c]	26/104 (25)	26/183 (14)	52/287 (18)
Vaccinated with two doses[c]	4/104 (4)	7/183 (4)	11/287 (4)

[a] Unless otherwise specified.

[b] More than one laboratory method could be used to confirm a single case.

[c] Among cases whose vaccination status was known.

were clinically diagnosed with measles, or (ii) they were exposed to a measles case (patient or HCW), or (iii) they were susceptible to be frequently exposed to measles because of their workplace (i.e. emergency room), or (iv) they were potentially susceptible. Immunity status against measles was assessed by enzyme-liked immune-sorbent assay (ELISA) (Enzygnost® IgG Dade Behring, Siemens, France). Patients and HCW were considered immune if IgG titres were higher than 325 mIU/ml.

Statistical analysis

The qualitative variables are reported as number and percentage and the quantitative variables as median and interquartile range*. Characteristics of patients with measles were analysed for adults (>16 years-old) and children (≤16 years-old) separately. Qualitative variables were compared by using the chi-squared test. The significance level was $p<0.05$. Data from the notification sheet were recorded using EpiData. Statistical analyses were performed with Stata software (Stata Corp.) version 10.0.

Results

Characteristics of the patients described from the mandatory notification

Overall, 407 measles cases were diagnosed in LUH between 1 January 2010 and 8 July 2011 (Figure 1). Of these 407 cases, 149 (37%) were clinical suspected cases, 193 (47%) were laboratory confirmed and 65 (16%) were epidemiologically confirmed. Table 1 describes the characteristics of all cases diagnosed in LUH. Overall, the median age of cases was 15 years (interquartile range 1–26 years). The median age for adult cases (>16 years-old) was 26 years (interquartile range 22–33 years) while the median age for cases who were children (≤16 years-old) was one year (interquartile range 0–9 years). Among the cases, 192 (47%) were female; 181 (44%) were adults (>16 years-old) and 226 (56%) were children (≤16 years-old). The age groups with the highest incidence involved children under one year of age (n=129, 32%) and 17–29 year-olds (n=126, 31%). Among the 129 children under one year of age, 75 (58%) were male, 17 (13%) infants presented complications (14 had pneumonia), and 25 (19%) were hospitalised.

In total, 72 (18%) presented complications: 51 (13%) had pneumonia, two (<1%) had encephalitis and 19 (5%) had other complications. Other complications reported mainly involved the digestive system, like hepatitis or diarrhea, and infection of the ear-nose-throat area. No patient died because of measles. Vaccination status was available for 287 cases (71%). Among them, 63 (22%) were vaccinated, 52 (18%) with a single dose of measles vaccine and 11 (4%) with two doses.

Virology and immunology data from the hospital virology-based surveillance

Overall, 2,763 individuals were tested by serology and/or PCR in LUH: 1,398 (51%) patients and 1,365 (49%) HCW. Among 819 patients tested for IgM, 233 (28%) were IgM-positive. Among 512 tested by PCR, 317 (62%) were PCR-positive. Of 891 patients tested for IgG, 641 (72%) were IgG positive (IgG titres>325 mIU/ml). Figure 2 depicts the repartition by age groups, of the proportion of IgG positive among adults patients tested for IgG.

Among 1,227 HCW tested for IgM, 16 (1%) were IgM-positive, none was PCR-positive, while for 1,365 HCW tested for IgG, 1,304 (96%) were IgG-positive (IgG titres>325 mIU/ml). Figure 3 describes the proportion of IgG-positive against measles among HCW by age. The proportion of patients aged up to 30 years who were not considered immune (IgG titres≤325 mIU/ml) was 38% vs 16% in patients older than 30 years (p<0.001). The proportion of HCW up to 30 years of age who were not immune (IgG titres≤325 mIU/ml) was 11% vs 3% in HCW older than 30 years (p<0.001).

Measles cases among pregnant women

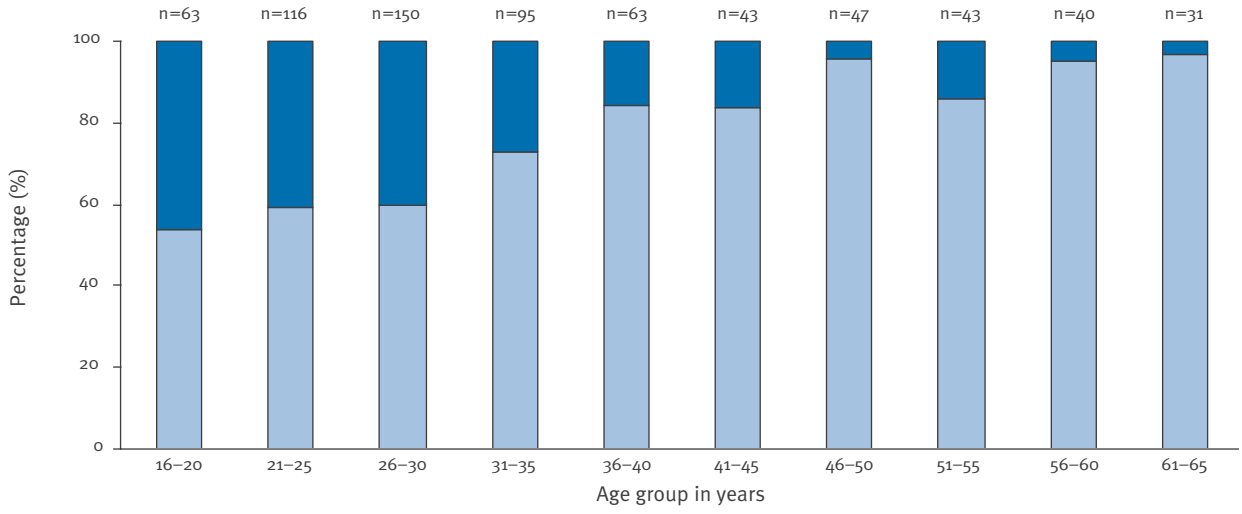
During the study period, 13 measles cases occurred among pregnant women. These cases occurred between January and July 2011. Among these women aged 25–45 years, seven were younger than 30 years-old. Among the 13 cases, one developed measles in the first trimester of gestation, six developed measles in the second trimester, five in the third trimester and one developed measles in immediate post-partum. Six (46%) of the pregnant measles cases had to be hospitalised. Four patients (31%) developed pneumonia as a consequence of the infection by measles, and one had a premature child.

Control measures

Various control measures were implemented to control the risk of hospital-acquired measles and to protect HCW. In LUH, when a case was reported to the infection control unit, an investigation was conducted to find details on this case's possible contacts with other patients or HCW. If necessary, specific prevention measures were implemented and adapted to the immune status of individuals. Patients and HCW, who had been in contact with measles cases during their infectious period, were informed about the risk of infection. People who were not immunised received a dose of measles vaccine or intravenous immune globulins, depending on their immune status, age or pregnancy status. Information about hygiene precautions was relayed by posters in all hospital wards, with special attention paid to emergency units. A questionnaire was delivered to HCW to ascertain their immune status. A blood sample was taken if immune status was unknown. If they had not received two vaccine doses, information about the high risk of transmission of the virus was delivered, and complementary vaccination was suggested. Moreover, a monthly measles surveillance report was performed by the infection control unit. This report summarised epidemiological data about the outbreak and was diffused via e-mail to all the HCW and to the hospital administration.

Figure 2

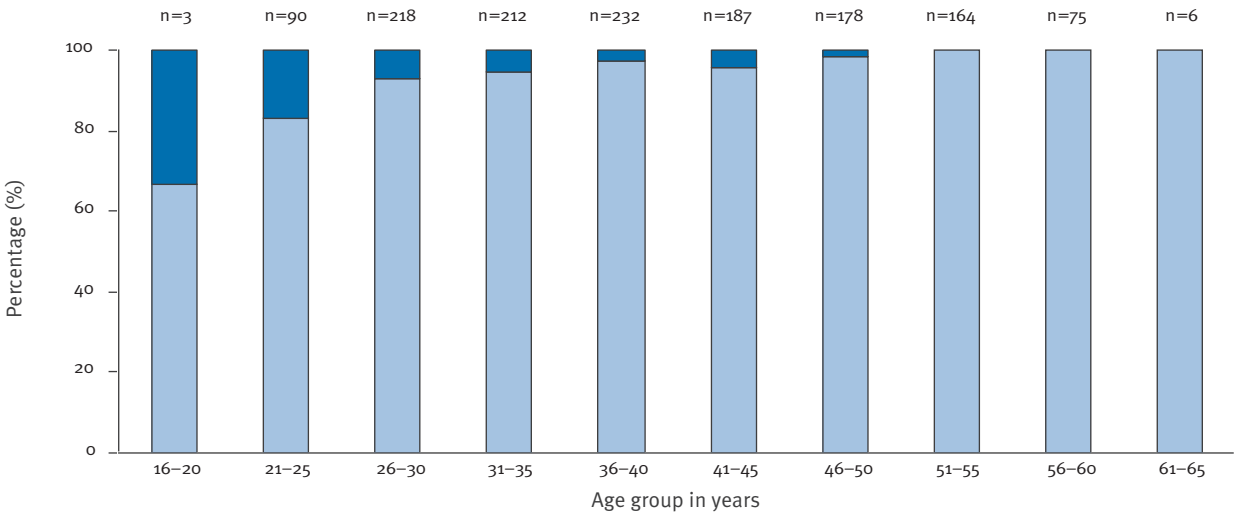
Proportion of IgG-positive against measles by age groups, among adult patients tested in Lyon University Hospitals, France, 1 January 2010–8 July 2011 (n=691)



	16–20	21–25	26–30	31–35	36–40	41–45	46–50	51–55	56–60	61–65
% IgG negative	46	41	40	27	16	16	4	14	5	3
% IgG positive	54	59	60	73	84	84	96	86	95	97

Figure 3

Proportion of IgG-positive against measles by age groups among healthcare workers tested in Lyon University Hospitals, France, 1 January 2010–8 July 2011 (n=1,365)



	16–20	21–25	26–30	31–35	36–40	41–45	46–50	51–55	56–60	61–65
% IgG negative	33	17	7	6	3	4	2	0	0	0
% IgG positive	67	83	93	94	97	96	98	100	100	100

Discussion and conclusion

It was estimated in 2009 that eight percent of people aged between six and 29 years were not immunised against measles in France [12]. The coverage is under the threshold of 95% needed for measles elimination [13]. The objective of this study was to describe the measles outbreak which occurred in Lyon, located in the Rhône-Alpes area, from 2010 to mid 2011. Our analysis focused on patients diagnosed with measles in LUH, pregnant women and HCW, and on virology and immunology data from the hospital virology-based surveillance. Overall, 407 cases of measles were diagnosed in LUH. According to 2009 estimations 92 percent of individuals between six and 29 years-old were immunised in France [12]. Moreover, in 2010–2011, the vaccination coverage for measles at 24 months-old (1 dose) was 88.8% in the Rhône-Alpes region [11]. Consequently, IgG seropositivity rates among children and young adults under 30 years-old in the Rhone-Alpes region but also nationwide are likely to reflect more vaccination coverage than contact with the virus. Although the tested population was a biased sample of the Lyon population, seroprevalence of IgG against measles was low, especially in patients and HCW under 30 years. Vaccination against measles is recommended but not mandatory for HCW in France. Their risk to contract measles appears to be much higher than the general population and they can potentially transmit the disease to their patients, especially the immunocompromised ones [14]. It appears urgent to reach a higher vaccination coverage with two doses in the French population. Eliminating measles is one of the World Health Organization's goal, which is expected for 2015 [15]. According to the results of our study, overall 78% of the measles cases were not vaccinated. A report based on French mandatory notifications between January 2008 and April 2011 [2] found similar rates concerning lack of vaccination: 86% of the cases did not receive the measles, mumps, and rubella (MMR) vaccine against measles, with differential compliance and immunisation coverage in the various districts of France. It pointed out that communications towards the general population about the need to be vaccinated in order to be protected, have to be strengthened.

Attention must be paid to newborns under one year of age because they are too young to be vaccinated and may no longer be protected by maternal antibodies. At the age of six months, 90% of the infants are not protected, irrespective of the mother's immunisation status [16]. Measles acquired during pregnancy can have deleterious effect on the mother and child outcome [17]. The most serious and frequent complication reported for pregnant women is pneumonia [17-20]. The hospitalisation and case fatality rates among pregnant women may be higher than among non-pregnant adults [20]. Concordant with these data, four cases of pneumonia among 13 pregnant cases were found in our study. Six of the pregnant women were hospitalised. An increased risk of foetal and neonatal loss is also reported [17-20]. In one case observed in this study, a premature birth occurred, however it could have been attributable to other causes. Some authors also reported an increased risk of subacute sclerosing

panencephalitis following neonate [21] or congenital [22] measles infection. Women in childbearing age should be informed of the risk of contracting measles and its possible complications. Vaccination that can only assure protection should be proposed as soon as possible in pre- or post-partum. Measles among pregnant women should be no longer considered uncommon in the regions that report outbreaks and should be systematically considered in the context of pregnant women presenting to a health practitioner with pneumonia.

In comparison with other European countries, France has been the most affected with 13,957 cases reported between January and August 2011 [9]. Italy, who reported 4,300 measles cases during the same period was the second most affected European country [6]. Four measles cases among pregnant women were reported [6] and 36% of cases were hospitalised. Overall 14% presented complications [6], which was in concordance with the complication rate of 18% in the Lyon area. Romania also experienced a large measles outbreak in 2011, with 2,072 reported cases [8]. The complication rate in Romania was much higher than in the Lyon area (respectively, 39% and 18%). Finally, the Geneva canton in Switzerland, which neighbours the Rhône-Alpes region, only reported 41 measles cases between January and May 2011, so it was far less affected than Rhône-Alpes area [5]. There, serious control measures, with quarantining and a vaccination campaign were systematically implemented. The larger number of cases that we experimented during the outbreak in Lyon area did not prevent carrying out a vaccination campaign, however, quarantining each measles case was more difficult to implement.

The main limitation of our study was a possible underestimation of the true measles incidence, as, in France, about 50% of measles cases were not reported on mandatory notification [10]. However this should not bias time-trends. Moreover, we were unable to calculate its incidence per 1,000 inhabitants because the exact origin of individuals was not known.

In conclusion, catch-up vaccination campaigns should focus on individuals aged under 30 years-old who have not received two doses of measles vaccine and on HCW. The outbreak is likely to re-occur, especially in the regions of France with low vaccine coverage. Clusters of susceptible individuals accrued over the years [10,11]. Indeed, the French Institute for Public Health Surveillance (InVS) reported that among children of 24 months old in 2008, only 90% had received one dose of the measles vaccine while according to the French vaccination programme, they should have already got two doses [23]. A fourth epidemic wave has to be expected in France and Europe. Hospital-based surveillance of measles is relevant to estimate the spread of the disease in the community and to help with early detection of healthcare-acquired cases.

* Authors' correction:

At the request of the authors, the sentence 'Quantitative variables were described as number and percentage, and qualitative variables as median and interquartile range.' was changed to 'The qualitative variables are reported as number and percentage and the quantitative variables as median and interquartile range.'. This change was made on 10 September 2012.

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ECDC guidelines for the surveillance of invasive mosquitoes in Europe

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On 28 August, the European Centre for Disease Prevention and Control (ECDC) released a technical report providing guidelines on how to implement the surveillance of invasive mosquito species (IMS), possible vectors of diseases in Europe [1]. The report was elaborated with input from a team of experts from Belgium, Italy, Serbia and the Netherlands.

The report consists of three parts. The first part addresses strategic issues and steps to be taken by the stakeholders for the decision-making process, depending on the aim and scope of surveillance, its organisation and management. Three likely scenarios have been identified: no established IMS (but with risk of introduction and establishment), locally established IMS, widely established IMS. The second part details all operational issues and steps to be implemented, i.e. key and optional procedures for mosquito collection, identification of IMS, collection of population and environmental parameters, pathogen screening, data management and analysis, and strategies for data dissemination and mapping. The third part provides models to estimate the cost of surveillance activities and suggestions to evaluate the surveillance process.

The recent notifications of autochthonous transmission of dengue and chikungunya fever in Europe [2–-5] show the vulnerability of areas where Aedes albopictus is present. Strengthening surveillance of IMS in areas at risk of importation or spread of IMS is therefore required. Consequently the targeted audience includes decision- and policy-makers, stakeholders in public health, professionals involved in surveillance or control of mosquitoes, as well as non-experts in the field. Targeted IMS are *Ae. albopictus, Ae. aegypti, Ae. atropalpus, Ae. japonicus, Ae. koreicus and Ae. triseriatus*.

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