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

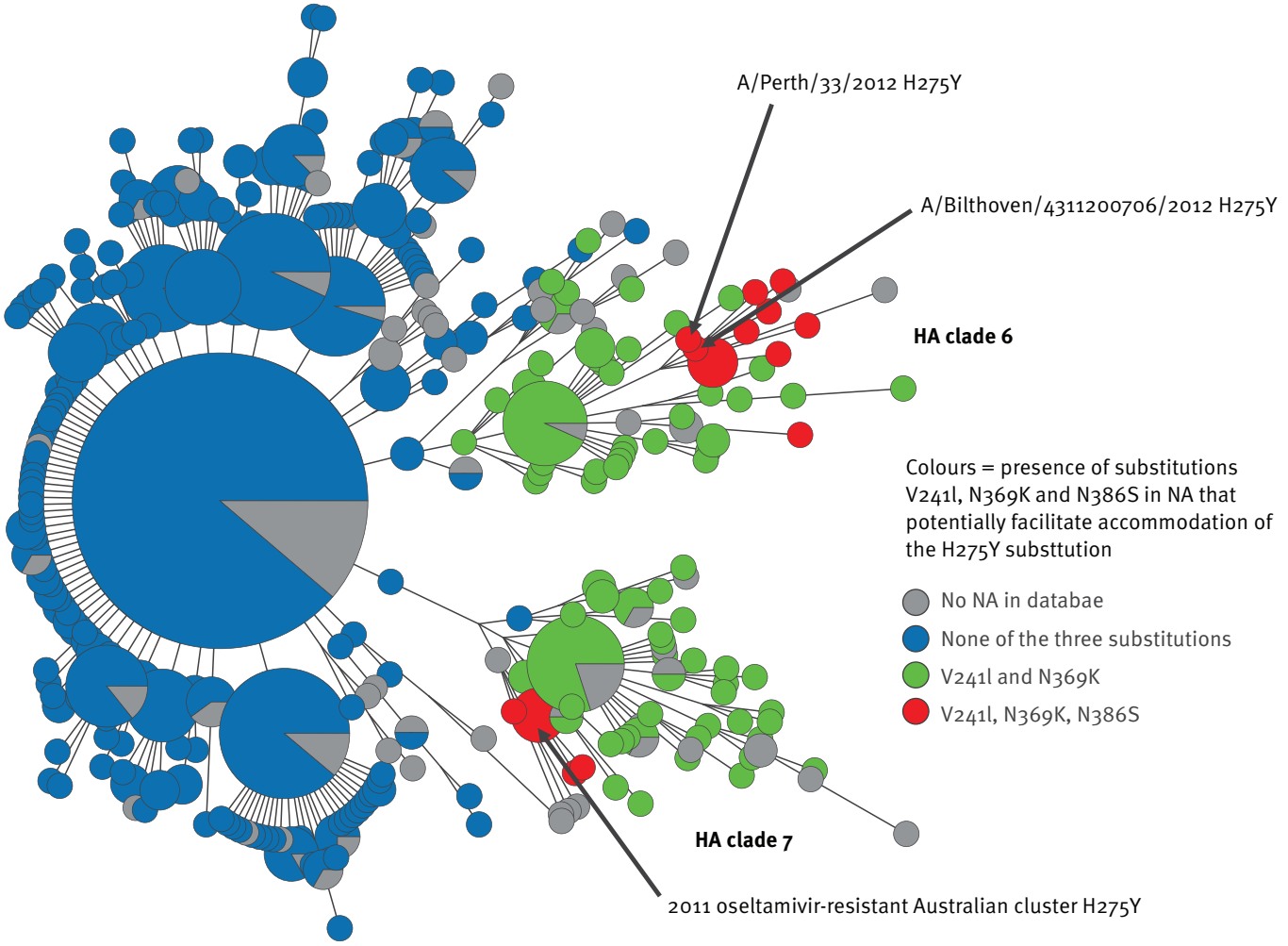
B

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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