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The current state of introduction of human papillomavirus vaccination into national immunisation schedules in Europe: first results of the VENICE2 2010 survey

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The Venice 2 human papillomavirus vaccination survey evaluates the state of introduction of the HPV vaccination into the national immunisation schedules in the 29 participating countries. As of July 2010, 18 countries have integrated this vaccination. The vaccination policy and achievements vary among those countries regarding target age groups, delivery infrastructures and vaccination coverage reached. Financial constraints remain the major obstacle for the 11 countries who have not yet introduced the vaccination.

Background

In early 2010, a survey on human papillomavirus (HPV) vaccination status in Europe was conducted within the European Centre for Disease Prevention and Control (ECDC) funded Vaccine European New Integrated Collaboration Effort (VENICE) 2 project [1]. One of the main objectives of the VENICE projects is to collect data on vaccination programmes, including information on status of introduction and implementation of new vaccinations, and to share that information amongst the participating countries. All the 27 European Union (EU) Member States plus Iceland and Norway participate in VENICE 2, which is the continuation of VENICE 1 (n=28 participating countries) [2].

Two surveys on HPV vaccination in Europe had been carried out in early 2007 and 2008 within VENICE 1 [3,4]. This new round-up has updated the status of introduction of HPV vaccination in the 29 countries participating in VENICE 2 and explored the target population, the main modalities of vaccination implementation and provision, the funding mechanism and, when available, the vaccination coverage reached. All 29 countries completed the VENICE 2 online questionnaire.

Human papillomavirus vaccination introduction

The process of introducing a new vaccine into a national immunisation schedule in the European countries occurs in two steps. A recommendation from a national advisory body is first made, followed by an official decision taken by the national health authorities. As of July 2010, the vaccination advisory bodies in 21 of the 29 countries had made a recommendation in favour of HPV vaccination, compared to 12 out of 27 countries in February 2008. Of those 21 countries, 18 had actually integrated the HPV vaccination in their national immunisation programme (Figure).

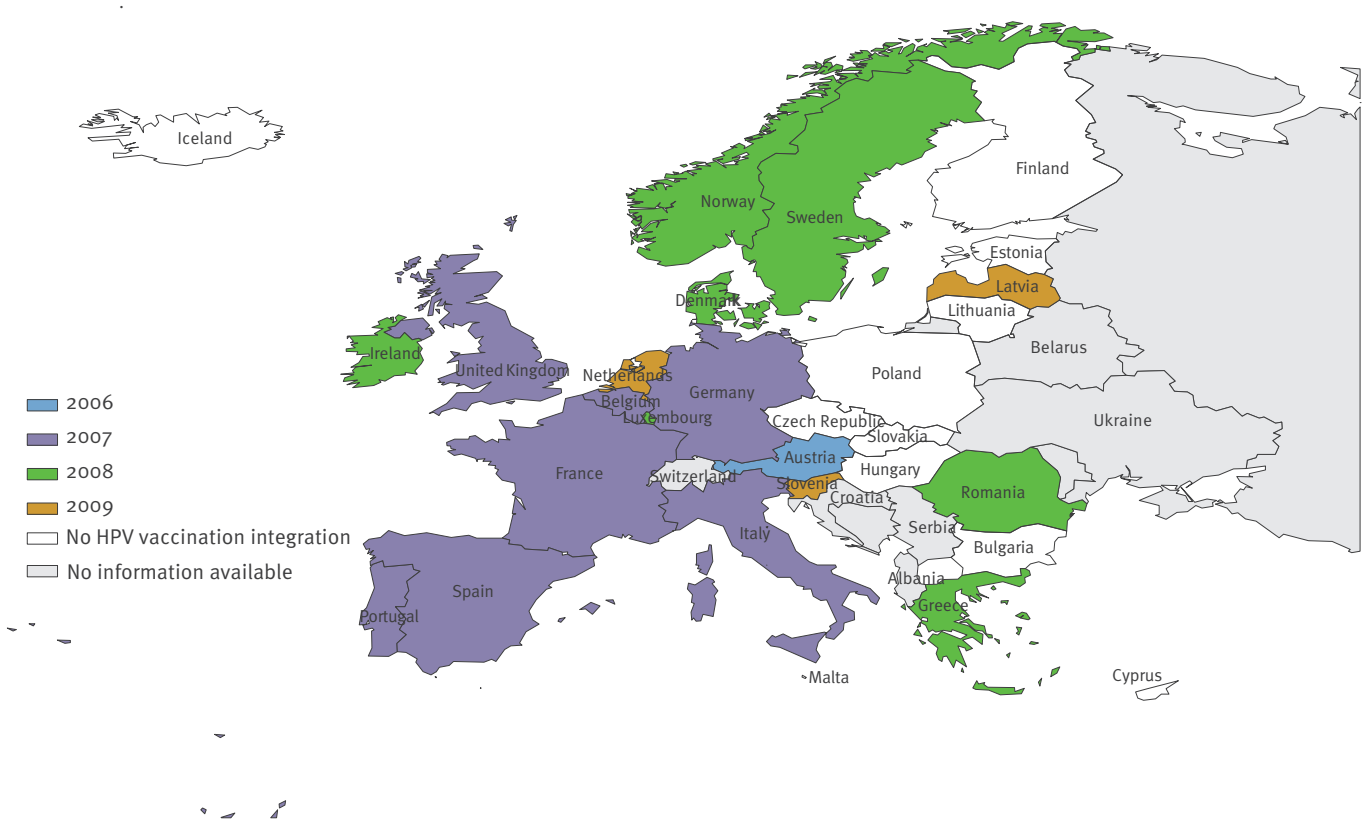
The HPV vaccination integration process has occurred in one country in 2006 (Austria), seven countries in 2007 (Belgium, France, Germany, Italy, Portugal, Spain and the United Kingdom), seven countries in 2008 (Denmark, Greece, Ireland, Luxembourg, Norway, Romania, and Sweden) and three countries in 2009 (Latvia, the Netherlands, and Slovenia). Of these 18 countries where routine immunisation had been implemented, nine countries had decided to implement a catch up programme. In two of the 11 countries where no decision of integration has been taken yet, a tentative schedule for the decision of integration or not of the HPV vaccination has been set up.

At least one *ad hoc* study to support the decision process about HPV vaccine introduction was undertaken by 12 of the surveyed countries (seven completed and five ongoing) and six countries plan to carry out such a study. Studies included disease burden evaluations, mathematical modelling projects or

economical assessments. Respectively 11 and 17 countries have either completed or are currently conducting HPV mathematical modelling studies or economical assessments to support the decision-making process

Figure

Human papillomavirus vaccination integration in the national immunisation schedules in Europe



HPV: human papillomavirus.

Table 1

Vaccination policy and target population (routine immunisation) in Europe, 2010 VENICE 2 human papillomavirus vaccination survey

Countries (N=18)[a]	Gender	Target age group	Coverage (3 doses, %)	Date of start
Austria	Female /Male	Girls/Women - Boys/Men before sexual debut	-	November 2006
Belgium	Female	12-18	-	November 2007
Denmark	Female	12	58 (2010)	January 2009
France	Female	14	24 (2008)	July 2007
Germany	Female	12-17	-	March 2007
Greece	Female	12-15	-	January 2008
Ireland	Female	12-13	-	May 2010
Italy	Female	11	56 (2009)	July 2007 – November 2008[b]
Latvia	Female	12	-	September 2010
Luxemburg	Female	12	17 (2009)	March 2008
Netherlands	Female	12	-	April 2010
Norway	Female	12	30 (2010)	August 2009
Portugal	Female	13	81 (2009)	October 2008
Romania	Female	12	-	November 2009
Slovenia	Female	11-12	-	September 2009
Spain	Female	11-14	-	January 2008
Sweden	Female	10-12	-	January 2010
United Kingdom	Female	12	80 (2009)	September 2008

[a] The 18 countries that have human papillomavirus in the national immunisation schedule.

[b] Depending on the region.

for the introduction of the HPV vaccination. A Health Technology Assessment has been fully performed by six countries, partially by one country and planned but not performed yet by two countries [5-9].

Vaccination policy targets

The adopted vaccination policy targeted only females in all the countries where HPV vaccine has been introduced except in Austria, where both females and males are targeted. A striking feature is the heterogeneity in the target populations for both routine and catch-up vaccination strategies. Adolescents aged 12 years were chosen as target population for routine vaccination in eight of the 18 countries (Austria, Denmark, Latvia, Luxemburg, the Netherlands, Norway, Romania and the United Kingdom), while girls aged 11 (Italy), 13 (Portugal), 14 (France) or an age range including several birth cohorts were chosen in the seven other states. Age ranges for catch-up vaccination were even more heterogeneous: only two countries (Belgium and Luxembourg) opted for the same age group (13-18 years) for catch-up campaigns (Tables 1 and 2).

In most of the countries, virtually all HPV vaccinations were performed in the public sector, either in public health centres (Denmark, Italy, Netherlands and Portugal), school health services (Ireland, Norway, Slovenia, Sweden) or both (Latvia, Romania, Spain and United Kingdom). Five countries provided HPV vaccinations mainly through the private sector and one country combined both public and private structures. Of the nine countries with catch-up campaigns, HPV vaccination was mainly administered through public health infrastructures in four countries, through the private sector in three countries or through both channels and/or school health services in two countries.
Routine vaccination was offered free of charge in most countries (15/18), partially at the expense of the vaccinee or private insurance in two countries (Belgium and France) and fully at the vaccinee's expenses in one country (Austria). For catch-up campaigns, vaccination was offered free of charge in seven out of nine countries and partially at the expenses of the vaccinee or private insurance in the two remaining countries.

Reasons for not introducing human papillomavirus vaccination

The main reason provided by the countries who had not introduced HPV vaccination into their national immunisation schedule was financial constraints. Indeed, nine of the 11 concerned countries quoted a lack of funding for the vaccination or a prohibitive vaccine cost. Two of those 11 countries also mentioned uncertainty on the duration of protection and insufficient anticipated epidemiological impact beyond the current screening programme as contributing reason.

Vaccination coverage data

Thirteen countries of the 18 where HPV vaccination has been introduced declared that a vaccination monitoring system had been implemented. However, only seven of these 13 countries provided vaccination coverage data. Indeed, half of the six remaining countries who had not provided any data yet have only recently introduced HPV vaccination into their national immunisation schedule (2009). Coverage for routine vaccination with three doses varied between 17% and 81% in 2010. Three countries reached a vaccination coverage between 17% and 30% (France, Luxemburg and Norway), two at 56% and 58% (Denmark and Italy) and two at 80% and 81% (Portugal and the United Kingdom). For the catch-up vaccination programmes, apart from one country with a coverage of 73% (Denmark), the five other countries with available data reached a vaccination coverage between 29% and 56% (France, Luxemburg, the Netherlands, Portugal, and the United Kingdom).

Conclusions

This survey, thanks to the participation of all the countries involved in VENICE 2, provided a comprehensive overview of the status of HPV vaccination introduction throughout Europe. Several interesting aspects have emerged: since the last survey, 13 additional countries have integrated HPV vaccination into their national immunisation programme. The vast majority

Table 2

Vaccination policy and target population (catch-up programme) in Europe, 2010 VENICE 2 human papillomavirus vaccination survey

Countries (N=9)[a]	Gender	Target age group	Coverage (3 doses, %)	Date of start
Belgium	Female	13-18	-	May 2008
Denmark	Female	15, 16, 17	73 (2010)	October 2008
France	Female	15-23	30 (2008)	July 2007
Italy	Female	14/15/16/17/24[b]	-	July 2007- January 2010[b]
Luxemburg	Female	13-18	29 (2009)	March 2008
Netherlands	Female	13-16	45 (2009)	March 2009
Portugal	Female	17	56 (2009)	January 2009
Romania	Female	12-24	-	January 2010
United Kingdom	Female	13-17	32 (2009)	September 2008

[a] The nine countries that have catch-up immunisation programme.
[b] Depending on the region.

of the countries with no HPV vaccination routine were from the eastern part of the EU for which the cost appears to be a major impediment. In the 18 countries where HPV vaccination has been introduced, 50% have implemented catch-up campaigns. Preadolescent females have been chosen as target populations but the selected age differed. The majority of the countries fully subsidise the HPV vaccine and two thirds of the countries use public health infrastructures or school health services to offer routine HPV vaccination to the target population. According to available data, only two countries have so far reached a vaccination coverage above 60% for routine vaccination and only one country a vaccination coverage above 60% for catch-up vaccination programme. Further analysis of the collected data is currently ongoing, focusing on sub-national HPV vaccination characteristics and other determinants underlying the decision to introduce HPV vaccination.

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Type 1 wild poliovirus and putative enterovirus 109 in an outbreak of acute flaccid paralysis in Congo, October-November 2010

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An outbreak of flaccid paralysis syndrome in adults is ongoing in Congo. Molecular analysis of faecal, throat and cerebrospinal samples identified wildtype 1 poliovirus and an additional enterovirus C strain related to enterovirus 109 as the cause. As of 22 November, the cumulative number of cases was 409, of which 169 (41.3%) were fatal. This is one of the largest wild type 1 poliovirus outbreaks ever described associated with an unusually high case fatality rate.

Background

Following mass vaccination campaigns organised through the Global Polio Eradication Initiative, the World Health Organization (WHO) declared that poliomyelitis had been eradicated from many regions of the world. However, although transmission of wild poliovirus type 2 (WPV2) has been interrupted, isolated human cases and outbreaks of WPV1 and WPV3 are still being reported in many countries [1]. Epidemiological investigations showed that all these clinical cases during the last decade were due to importation of WPV from one of the four countries where indigenous WPV transmission is still ongoing, namely Afghanistan, India, Nigeria and Pakistan [1]. In the past decade, several outbreaks and isolated clinical cases resulting from WPV1 and/or WPV3 importation from Nigeria or India were reported in 15 polio-free countries in Africa. Two small WPV1 outbreaks occurred recently, in Namibia in 2006 and Angola in 2010, after importation of WPV1 of Indian origin [2]. In order to prevent these episodic cases of imported and indigenous WPV transmission, polio immunisation campaigns were conducted that targeted some 72 million children in 15 countries across western and central Africa.

Outbreak description

Congo had recorded its last official case of indigenous polio in 2000 [3]. On 5 November 2010 the Ministry of Health of Congo declared an outbreak of poliovirus centred in the second largest town, Pointe-Noire. The outbreak presumably started in mid-October 2010, with an unusual accumulation of cases of acute flaccid paralysis (AFP) syndrome in patients between 15 and 72 years-old. Most cases occurred in Pointe-Noire and some in Cabinda province. Cases exported from Pointe-Noire were then reported in several towns and villages of Congo. As of 22 November the cumulative number of cases was 409, of which 169 (41.3%) were fatal. Direct contact between cases was rare, and there was no apparent spatial pattern. Likewise, there was no evidence of a common source such as food or water. In most hospitalised patients the disease started with influenza-like symptoms four to seven days before the onset of AFP of the legs. AFP then ascended rapidly (within a day), frequently leading to death from cardiac and/or respiratory failure. The large number of severe cases and the high case fatality rate contrast sharply with previous WPV outbreaks and point to a role of unknown viral/host features or to the existence of massive numbers of mild and therefore unreported additional cases.

Laboratory investigations

The Centre International de Recherches Médicales de Franceville (CIRMF), Gabon, received three plasma samples for aetiologic investigation on 29 October. Real-time and conventional reverse transcription (RT)-PCR testing was negative for neurologic, enteric and respiratory viral pathogens, namely the genera *Enterovirus, Flavivirus, Alphavirus, Phlebovirus*, human mastadenoviruses, the family *Paramyxoviridae* (mumps virus, measles virus, parainfluenza viruses 1-4, respiratory syncytial virus, human metapneumovirus) and the subfamily *Coronavirinae* (human coronavirus Nl63, HKU1, OC43 and 229E. Also negative were species-specific real-time RT-PCR tests for West Nile virus, tick-borne

encephalitis virus, cytomegalovirus, human herpesvirus type 6, herpes simplex virus type 1, varicella zoster virus, rotavirus serogroup A, norovirus genogroups 1 and 2, sapovirus, astrovirus, influenza viruses A and B and rhinovirus.

CIRMF then received 15 rectal swabs, 14 throat swabs, and five cerebrospinal fluid (CSF) samples on 2 November of which thirteen rectal swab specimens (86.7%), five throat specimens (35.7%) and one CSF specimen (20.0%) were positive for enterovirus in a real-time RT-PCR targeting the 5'-noncoding region [4]. The faecal and throat samples had threshold cycles ranging from 24 to 38 in real-time RT-PCR, indicating medium to high virus concentrations, while the concentration in the positive CSF sample was low (cycle threshold (CT) 38). The genome was studied by the Institute of Virology at the University of Bonn Medical Centre in Germany, based on partial VP1 sequencing [5], 3D sequencing (unpublished in-house assay) and 5'-UTR sequencing [6].

Poliovirus type 1 was identified in one sample (100% amino acid identity to recent poliovirus strains of Indian genotype in 'typing' VP1 PCR). The amplified 327 nt sequence of this sample (corresponding to genome positions 2,631 to 2,957 in WPV1 strain Brunhilde) shared 94.8-96.3% identity with poliovirus type 1 sampled in Africa (Angola and Democratic Republic of the Congo) in 2006 and 2007 (isolates ANG-LUA-KIL-07-003 and RDC-BCG-SEK-06-004) and 95.1% identity with a strain recovered during a polio outbreak in Tajikistan in 2010 (GenBank accession number HQ317702). A more sensitive strain-specific nested PCR assay amplifying a 201 bp VP1 fragment was developed from the initial sequencing data, and all but two of the 19 samples positive in the enterovirus real-time RT-PCR were typed as wildtype 1 polio virus. Partial sequences of the 3D genomic region encoding the viral polymerase (181 nt corresponding to genome positions 6869-7049 in WPV1 strain Brunhilde) were retrieved from five samples. The maximum nucleotide identity of all these samples was 96.1% with two poliovirus type 1 strains recovered in Russia and the Philippines after the year 2000 (isolates P1W/Bar65 and Mindanao-01-1, respectively). Partial 5'-UTR sequencing yielded positive results for all the specimens tested. The 115 nt thus obtained (corresponding to genome positions 466 to 580 in WPV1 strain Brunhilde) were 96.5% identical to recent WPV1 strains from China (isolate CHN-Hainan/93-2). Therefore, all analysed genomic regions were identified as WPV1, indicating absence of putative intra- or interspecies recombination. An additional enterovirus C strain distantly related to enterovirus 109 (EV109) in the VP1 region was retrieved from a rectal swab of a deceased patient. In the 322 nt VP1 sequence fragment that could be retrieved, the virus showed 75% to 77% nt sequence identity with the five EV109 sequences available in GenBank, and 90.5% nt identity in the 3D genome region with EV109 isolate NICA08-4327 recovered in Nicaragua in 2010 [7]. Of note, the corresponding sample contained one of the highest enterovirus RNA copy numbers (CT value 24). Polio virus was not detected in this sample with the broad-range typing assays described above, nor with strain-specific VP1 nested and 3D real-time PCR assays. No other sample was positive for the EV109-related virus in a strain-specific 3D real-time PCR assay. The presence of other enteric viruses (adenovirus, astrovirus, enterovirus, rotavirus A, sapovirus and norovirus genogroups 1 and 2) was ruled out in all the faecal samples except for one WPV-positive sample which also contained norovirus RNA. Genome sequencing and further typing of samples containing poliovirus and enterovirus, targeting the complete VP1 genomic region, are ongoing. Additional samples have been sent to CIRMF for analysis.

Conclusions

The preliminary sequencing data and clinical picture are compatible with a wildtype poliovirus outbreak. Further epidemiological and serological studies are required to explain the unusually high case fatality rate and the patients' relatively advanced age. One possible explanation is that only severe cases may be reported. Alternatively, the population may be immunologically naïve and highly susceptible, although this is unlikely given the claimed success of vaccination campaigns. Epidemiological investigations have just begun. At this time, no direct contact between cases has been observed, and no apparent spatial pattern was identified, with neither domestic dissemination nor within the same subdivisions. Together, these observations suggest a diffuse source of contamination, e.g. from water drawn from wells in the poor neighbourhoods of the city. Involvement of a more virulent virus, or potentially other viruses, is another possibility. More information on non-hospitalised patients and mild cases is needed. Information on predisposing conditions of the fatal cases as well as full-length sequencing of VP1 and the full genome are currently ongoing.

The WHO Country Office, Regional Office, and Headquarters are supporting the Ministry of Health in Pointe-Noire, and the WHO Country Office is supporting the operational costs of the investigation and response teams. At least 1.1 million people are to be vaccinated in the epicentre of the outbreak (Pointe-Noire region), and further 600,000 people will be vaccinated simultaneously in the neighbouring regions of Congo near Angola, where the last cases of WPV1 occurred in 2010.

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Two cases of verified clinical failures using internationally recommended first-line cefixime for gonorrhoea treatment, Norway, 2010

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Neisseria gonorrhoeae **has developed resistance to most of the available therapeutic antimicrobials. The susceptibility to extended-spectrum cephalosporins, the last remaining first-line treatment option, is decreasing globally. This report describes the first two cases outside Japan of verified gonorrhoea clinical failures using internationally recommended first-line cefixime treatment. Enhanced awareness and more frequent follow-up examination, test-of-cure and appropriate verification/falsification of presumed clinical treatment failures, involving several clinical and laboratory parameters should be strongly emphasised worldwide.**

Introduction

Gonorrhoea remains a public health problem worldwide. The prevalence of antimicrobial resistance (AMR) in *Neisseria gonorrhoeae* is high to all antimicrobials previously used as first-line treatments [1,2]. The susceptibility to the extended-spectrum cephalosporins (ESCs) ceftriaxone (injectable) and cefixime (oral), i.e. to the current internationally recommended first-line and last remaining treatment options, is decreasing worldwide [1,2]. Using ceftriaxone, no treatment failure has yet been reported. However, cefixime standard treatment (one oral dose of 400 mg) is the most common treatment in many countries due to the simple oral, single-dose regimen and its affordability. Verified treatment failures have so far only been reported in Japan [3]. Nevertheless in many cases, these failures may not be recognised because many of the gonorrhoea patients are also treated with azithromycin for suspected concomitant chlamydial infection [1,4], and/or because follow-up examinations and test-of-cure are rare [1]. In a recent review describing the latest World Health Organization (WHO) global initiatives to meet the public health challenge posed by the emergence of untreatable gonorrhoea, among others the ideal criteria for verification of gonorrhoea treatment failures are described. These involve several clinical and laboratory parameters [1].

This report describes the first two cases outside Japan of verified treatment failures of gonorrhoea using internationally recommended first-line treatment cefixime.

Case reports

Case 1

In July 2010 a Norwegian heterosexual man in his 30s presented to a hospital in Norway with purulent urethral discharge and dysuria (day 1). Urethritis and the presence of intracellular diplococci in polymorphonuclear lymphocytes were confirmed after methylene-blue staining of a urethral smear, and the patient was given one oral dose of cefixime (400 mg). *N. gonorrhoeae* was also cultured from an additional urethral sample, and *N. gonorrhoeae*–specific DNA was detected in a urine sample using an in house PCR for the *porA* pseudogene [5]. On day 21, the patient returned with persisting symptoms, and microscopy, culture (urethral sample) and PCR (urine sample) remained positive for *N. gonorrhoeae*. The patient reported no sexual contacts between treatment and test-of-cure. He was then administered one dose of ceftriaxone (500 mg) intramuscularly (IM). On day 29, follow-up examination showed that the symptoms had resolved, and microscopy, culture (urethral sample), and PCR (urine sample) were negative for *N. gonorrhoeae*.

Case 2

In August 2010 another Norwegian heterosexual in his 30s presented to the same hospital in Norway with similar symptoms and initial laboratory findings as case one and was treated with one oral dose of cefixime (400 mg). On day 11, he returned with persisting, although milder, symptoms. Microscopy, culture (urethral sample) and PCR (urine sample) were still positive for *N. gonorrhoeae*. The patient reported no sexual contacts between treatment and test-of-cure. He was

administered one dose of ceftriaxone 500 mg IM. On day 26, follow-up examination showed that the symptoms had resolved, and PCR (urine sample) was negative for *N. gonorrhoeae*.

Characterisation of *N. gonorrhoeae* isolates (before and after treatment)

All *N. gonorrhoeae* isolates were species–confirmed by sugar utilisation test, Phadebact Monoclonal GC Test (Pharmacia Diagnostics), and a *porA* pseudogene [5]. The characterisation of the isolates is summarised in the Table.

In both cases the pre- and post-treatment isolates were indistinguishable using full-length *porB* gene sequencing (identical sequence) and *N. gonorrhoeae* multiantigen sequence typing (NG-MAST; ST1407), performed as previously described [6]. Using Etest, the paired isolates displayed a cefixime minimum inhibitory concentration (MIC) of 0.5 mg/L and 0.25/0.5 mg/L in case 1 and 2, respectively, and indistinguishable antibiograms (beta-lactamase negative, ceftriaxone: 0.125 mg/L, ciprofloxacin: ›32 mg/L, azithromycin: 0.5 mg/L, spectinomycin: 12 mg/L, and ampicillin: 1-4 mg/L). According to the breakpoints stated by the Committee on Antimicrobial Susceptibility Testing (EUCAST), these isolates were considered resistant to cefixime (MIC›0.12 mg/L). Sequencing of resistance determinants for ESCs (*penA, mtrR* and *porB1b* alterations) was performed as previously described [7]. All four isolates from the two cases contained an identical *penA* mosaic allele, which had previously been correlated to treatment failures in Japan, and *mtrR* and *penB* resistance determinants that enhance the MICs for ESC further (Table) [8,9].

Discussion and conclusion

This report describes the first two cases outside Japan – with no known links to Japan – of verified clinical treatment failures of urogenital gonorrhoea using standard first-line cefixime treatment, i.e. one dose of 400 mg. The treatment failures were strictly verified using several clinical and laboratory parameters in full accordance to WHO criteria [1,10], i.e. a detailed clinical history was recorded, re-exposure and reinfection was ruled out, the pre-treatment and post-treatment isolates were phenotypically and genetically indistinguishable by highly discriminatory molecular epidemiological typing methods, the MICs of cefixime were substantially enhanced (in vitro–resistance according to current breakpoints), and the isolates contained genetic resistance determinants causing the enhanced cefixime MICs. According to Monte Carlo simulations, a 400 mg dose of cefixime results in median times of free cefixime above the MIC ($fT_{>MIC}$) of only 14.9 h (12.3-18.3 h) and 11.4 h (9.0-14.2 h) for the detected MICs of 0.25 mg/L and 0.5 mg/L, respectively [4]. Accordingly, these levels of cefixime MICs are high enough to cause treatment failures. Most worrying, the gonococcal isolates from the present treatment failures were identified as ST1407, which is a multi-resistant strain that, together with its evolving subtypes, has been shown to circulate in many countries worldwide [11].

In conclusion, cases of clinical failures of urogenital gonorrhoea using the internationally recommended first-line treatment cefixime have now also occurred outside Japan. Besides the two cases identified in Norway (with places of exposure in the Philippines and Spain/Norway), similar rare cases are likely to be identified elsewhere. Accordingly, an enhanced awareness of the existence of failures using cefixime for gonorrhoea treatment needs to be strongly emphasised worldwide as well as the need for more frequent follow-up examination, test-of-cure and appropriate verification/falsification of presumed clinical treatment

Table

Characteristics of two *Neisseria gonorrhoeae* cases of verified clinical failure using internationally recommended first-line cefixime treatment, Norway, 2010

Age (years) / Sex	Possible place of exposure	Treatment	Diagnostics (pre-treatment/ post-treatment isolate)	MIC (mg/L)		NG-MAST	penA allele[a]	mtrR[b]	penB[c]	
				Cefixime	Ceftriaxone					
37/male	Philippines	Cefixime 400 mg´1 oral dose	Microscopy, culture, PCR	0.5	0.125	ST1407	Mosaic	A-del in promoter	G120K	A121N
			Microscopy, culture, PCR	0.5	0.125	ST1407	Mosaic	A-del in promoter	G120K	A121N
31/male	Spain/ Norway	Cefixime 400 mg´1 oral dose	Microscopy, culture, PCR	0.25	0.125	ST1407	Mosaic	A-del in promoter	G120K	A121N
			Microscopy, culture, PCR	0.5	0.125	ST1407	Mosaic	A-del in promoter	G120K	A121N

MIC: minimum inhibitory concentration; NG-MAST: Neisseria gonorrhoeae multiantigen sequence typing; PCR: polymerase chain reaction; ST: sequence type.

[a] penA mosaic allele encodes the mosaic penicillin-binding protein 2 (PBP2), which causes decreased susceptibility to extended-spectrum cephalosporins.

[b] A-del in promoter: characteristic single nucleotide (A) deletion in the inverted repeat of the promoter region of mtrR that causes overexpression of the MtrCDE efflux pump, which results in a further decreased susceptibility to extended-spectrum cephalosporins.

[c] penB: alterations of amino acids 120 and 121 in the porin PorB1b that cause a decreased intake of extended-spectrum cephalosporins and, accordingly, a further decreased susceptibility to extended-spectrum cephalosporins.

failures. Pharyngeal gonorrhoea poses an additional challenge as it is often asymptomatic, more difficult to eradicate, and may act as a reservoir for emergence of resistance [1]. Accordingly, it is important to collect information regarding the patients' sexual practices and to take also extragenital samples.

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Seasonal influenza vaccination and the risk of infection with pandemic influenza: a possible illustration of non-specific temporary immunity following infection

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Four Canadian studies have suggested that receipt of seasonal influenza vaccine increased the risk of laboratory-confirmed infection with 2009 pandemic influenza A(H1N1). During the influenza season of 2009 in Victoria, Australia, this virus comprised 97% of all circulating influenza viruses for which sub-typing was available. We found no evidence that seasonal influenza vaccine increased the risk of, or provided protection against, infection with the pandemic virus. Ferret experiments have suggested protection against pandemic influenza A(H1N1) 2009 from multiple prior seasonal influenza infections but not from prior seasonal vaccination. Modelling studies suggest that influenza infection leads to heterosubtypic temporary immunity which is initially almost complete. We suggest these observations together can explain the apparent discrepant findings in Canada and Victoria. In Victoria there was no recent prior circulation of seasonal influenza and thus no temporary immunity to pandemic influenza. There was no association of seasonal influenza vaccine with pandemic influenza infection. In Canada seasonal influenza preceded circulation of the pandemic virus. An unvaccinated proportion of the population developed temporary immunity to pandemic influenza from seasonal infection but a proportion of vaccinated members of the population did not get seasonal infection and hence did not develop temporary immunity to pandemic influenza. It may therefore have appeared as if seasonal vaccination increased the risk of infection with pandemic influenza A(H1N1) virus.

Introduction

Four Canadian studies have recently suggested that receipt of seasonal influenza vaccine increased the risk of infection with 2009 pandemic influenza A(H1N1) 1.4–2.5-fold [1]. The authors of these studies advanced two hypotheses to explain these unexpected observations. Firstly they suggested that the seasonal influenza vaccine effectively blocked cumulative infections with seasonal influenza viruses, thus preventing the induction of cross-reactive immunity which may have protected from pandemic influenza A(H1N1) infection. They referred to this as the 'cross-protection block hypothesis' but argued that unrealistically high values for seasonal influenza vaccine effectiveness and seasonal influenza attack rates needed to be assumed for this hypothesis to produce odds ratios consistent with their observations (Appendix G in reference [1]). Secondly, based on the concept of antibody-dependent enhancement (ADE) of virus replication, they argued that vaccination against one or more influenza strains actually increased the risk of infection with a subsequent heterosubtypic strain that was antigenically remote from any of the vaccine strains [1]. According to this hypothesis, low levels of pre-existing weakly heterotypic or heterosubtypic antibodies promoted virus endocytosis and subsequent replication, increasing viral load production. In ferrets challenged with a lethal virus following immunisation with a non-adjuvanted human vaccine known not to provide protection to naïve animals, the previous vaccination with a heterosubtypic strain appeared harmful [2]. This observation supported the hypothesis of possible ADE, although we believe these studies might not be transferable to humans.

In Victoria, Australia, we found no evidence of increased risk of laboratory proven pandemic influenza A(H1N1) infection in a population that had previously received only seasonal influenza vaccine [3]. Pandemic influenza occurred during the southern hemisphere influenza season of 2009 and, in Victoria, comprised more than 97% of all circulating influenza viruses for which sub-typing was available [4]. This would have been the perfect setting to have demonstrated ADE of viral replication if it were a general phenomenon, given that pandemic influenza A(H1N1) is a quadruple reassortant virus that is antigenically distant from recently circulating strains and that there was a significant antigenic distance between the vaccine and circulating strains of influenza [5]. However we found no evidence of increased risk of seasonal influenza

amongst recipients of the seasonal influenza vaccine, with an estimated age adjusted odds ratio of 0.97 (95% confidence interval (CI): 0.6–1.56) [3]. A household study in Western Australia found a similar result with an adjusted odds ratio of 1.0 (95% CI: 0.7–1.5), although this study used influenza-like illness rather than laboratory-confirmed influenza as its outcome [6]. Laboratory-confirmed influenza was the outcome assessed in both Canada [1] and Victoria [3].

Based on a series of ferret experiments we have found that experimental infection(s) with seasonal influenza decreased the risk of infection subsequent to challenge with pandemic influenza, but that vaccination with adjuvanted seasonal influenza vaccine provided neither protection or enhancement, even with high levels of antibodies [7]. On the other hand, vaccination with adjuvanted human seasonal influenza vaccine protected the ferrets from seasonal influenza infection.

The temporary immunity hypothesis

We would like to suggest an alternative hypothesis to explain the findings from both Canada and Victoria. In our hypothesis recent infection with any strain of influenza would confer temporary immunity to infection with any other strain, independent of antigenic distance, but vaccination would not confer protection unless the vaccine and circulating strains were antigenically similar.

Although it is not an accepted immune phenomenon, the concept of temporary immunity has been explored in the modelling literature for a number of years. In 2003 Ferguson *et al*. reported that 'short-lived strain-transcending immunity' was necessary to reproduce what was known from 'epidemiological dynamics and viral evolution at the sequence level' in order to restrict viral diversity and produce influenza drift dynamics with seasonal patterns [8]. Without non-specific temporary immunity, simulations produce more divergent strains than are demonstrated by surveillance. The concept has subsequently been supported by other investigators, including Tria *et al*. [9], Omori *et al*. [10]; and our own modelling [11]. It has been further elaborated in a series of papers by Minayev and Ferguson [12,13].

The biology of temporary immunity

Type I interferons and other cytokines have been suggested as mediators of this phenomenon by inducing an antiviral state in infected, and neighbouring, epithelial cells [14]. In a guinea pig model of influenza infection, daily intranasal administration of recombinant human interferon alpha completely blocked transmission of pandemic influenza A(H1N1) both from and to treated animals [15]. Further, prophylaxis with interferon alpha reduced virus shedding of ferrets and guinea pigs infected with highly pathogenic influenza strains and morbidity following seasonal influenza challenge [16,17].

Protection from symptomatic infection may also be mediated by T-cell immunity, specific for conserved epitopes in heterosubtypic influenza strains [7,15,18], and B-cell immunity through antibodies specific for common epitopes, generally between homosubtypic strains [19,20]. Animal models of influenza have shown that prior infection(s) with seasonal influenza strains reduce transmission and virus shedding of pandemic influenza A(H1N1) virus [7,15].

Temporary immunity induced by previous experimental infection has also been demonstrated to reduce morbidity and mortality following highly pathogenic influenza challenge [18]. We suggest temporary immunity may be mediated by a combination of innate and adaptive immune responses. Protection provided by temporary immunity may vary with age but would be expected to be immediately higher than that provided by antibody-mediated cross-protection alone.

The duration of temporary immunity is a critical issue in this hypothesis but is not yet clearly defined. On theoretical grounds Ferguson *et al*. suggested that following infection, temporary immunity would initially protect any infected person against all influenza strains before waning with time (supplementary material in reference [8]). Most models simulate temporary immunity using rate equations decaying with an exponential half life. A mean duration of temporary immunity of three months is credible in modelling with seasonality, since immunity needs only to last as long as the influenza season. If it lasts too long it does not allow the next season epidemic to start; if it is too short, too many strains proliferate.

Evidence for temporary immunity in humans

In a re-analysis of data from school surveys following the 1918-19 pandemic in England and Wales, Mathews *et al*. have suggested that temporary immunity is one of the mechanisms that need to be invoked to explain the observed wave phenomenon of that pandemic [21]. The authors also calculated odds ratios for infection with a second influenza sub-type following infection with a different primary sub-type from published studies of the influenza A(H2N2) pandemic of 1957-8 and the re-emergence of influenza A(H1N1) in 1977 [21]. Slepushkin studied a group of factory workers and reported that workers with symptoms during the seasonal influenza A(H1N1) outbreak in the spring of 1957 were less likely to be symptomatic when the new influenza A(H2N2) appeared [22]. The calculated odds ratio was 0.4 (95% CI: 0.3–0.6) [21]. Sonoguchi *et al*. reported protection from influenza A(H1N1) infection in Japanese school children who had previously been infected with influenza A(H3N2) when influenza A(H1N1) re-emerged in 1997-8 [23]. When exposure was separated only by days or weeks, the calculated odds ratios for a second infection, given primary infection with a different subtype, was calculated as 0.06 (95%

CI: 0.02–0.13) for high school students and 0.2 (95% CI: 0.08–0.3) for primary school students [21].

Extending this hypothesis, and further suggesting that temporary immunity may be conferred not only by influenza infections but also by other viral infections, is a contemporary report from Sweden that rhinovirus infection may have decreased the risk of infection with pandemic influenza A(H1N1) by what was suggested to be a cytokine-mediated phenomenon [24].

Temporary immunity following seasonal influenza virus circulation in Canada and Australia

If the temporary immunity hypothesis is valid, and if the seasonal influenza vaccine provided no protection against pandemic influenza, we would have expected no protection against pandemic influenza A(H1N1) in Victoria in 2009, when there was minimal circulation of any seasonal influenza virus prior to the introduction of pandemic influenza A(H1N1) [3]. In Victoria there was no opportunity for temporary immunity to develop.

However our expectations in Canada would have been different, given that seasonal influenza circulated with peak incidence eleven weeks before the first notified cases of pandemic influenza A(H1N1). Canadians who had received seasonal influenza vaccine had approximately 50% protection against seasonal infection [1] and would have foregone the potential temporary immunity induced by seasonal infection. However temporary immunity to pandemic influenza A(H1N1) would have been induced in unvaccinated Canadians who had a seasonal influenza infection. This may have resulted in an *apparent* increased risk of pandemic influenza A(H1N1) infection in people vaccinated against seasonal influenza.

We explored the temporary immunity hypothesis, making a range of assumptions about seasonal influenza vaccine effectiveness (VE) and seasonal and pandemic influenza infection rates (the cumulative incidence of infection). Our primary assumption was that there was no protection from pandemic influenza following receipt of the seasonal influenza vaccine. We also assumed there was no risk associated with vaccination, implying that for seasonal influenza vaccine VE=0 against pandemic influenza A(H1N1) infection.

In a case-control study VE is estimated as 1-OR, where OR is the odds ratio. We considered a theoretical case-control study and calculated the OR for the outcome of pandemic infection given the exposure to seasonal vaccination. We used a method similar to that outlined in Skowronski *et al.* (Appendix G in reference [1]). We let θ_S be the seasonal infection rate, θ_P the pandemic infection rate and α the proportion of temporary immunity afforded by a recent seasonal influenza infection.

The probability of a vaccinated individual being infected with pandemic influenza A(H1N1) can be expressed as

$$p_V = [\theta_S*(1\text{-}VE)*(1\text{-}\alpha) + \theta_S*VE + (1\text{-}\theta_S)]*\theta_P.$$

This formula acknowledges there are three ways a vaccinated individual could have become infected with pandemic influenza A(H1N1): first, the individual was exposed to seasonal influenza and infected (θ_S), vaccination was not effective (1-VE) and there was no temporary immunity (1- α); second, the individual was exposed to seasonal influenza but the vaccine was effective (VE), so that the individual was not infected with seasonal influenza and therefore had no temporary immunity; third, the individual was not exposed to seasonal influenza and therefore not infected (1- θ_S), and hence had no temporary immunity. In each case the individual had θP chance of infection with pandemic influenza.

Similarly the probability for an unvaccinated individual being infected with pandemic influenza A(H1N1) (p_U) is given by

$$p_U=(\theta_S*(1\text{-}\alpha) + (1\text{-}\theta_S))*\theta_P.$$

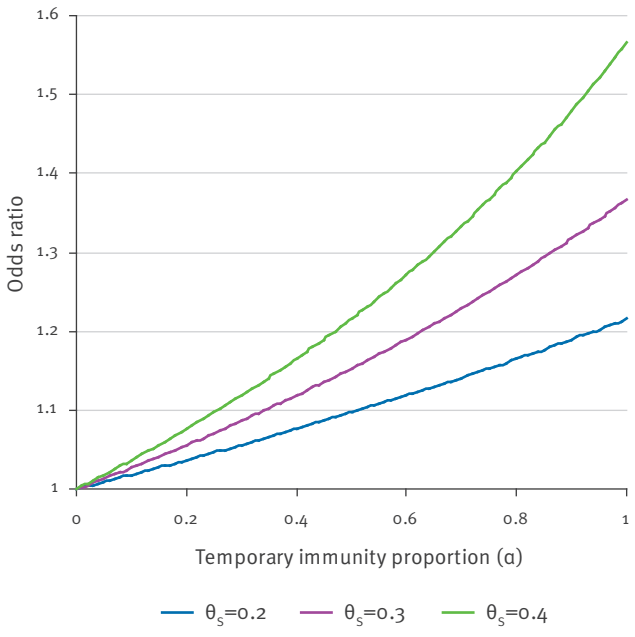
The odds ratio is calculated as

$$p_V\,(1\text{-}\,p_U)/((1\text{-}\,p_V)*p_U).$$

We assumed a seasonal influenza VE of 70%, consistent with estimates from the Cochrane review of healthy adults for matched circulating and vaccine strains [25], and seasonal and pandemic infection rates (that is,

Figure

Odds ratio of seasonal vaccination comparing patients with and without pandemic influenza infection and proportion of patients with temporary immunity to pandemic influenza following seasonal influenza infection



θ_S: seasonal influenza infection rate.
Seasonal influenza vaccine effectiveness was set at 70%.

seasonal cumulative incidence of serologically confirmed infection) between 20 and 40%, broadly consistent with community studies of seasonal influenza [26] and serological studies of pandemic influenza [27]. For these parameters, we found an odds ratio between approximately 1.2 and 1.5 comparing vaccinated and unvaccinated people infected with pandemic influenza, if temporary immunity from recent previous infection was assumed to be of the order of 80-90% (Figure). As previously noted, temporary immunity was modelled to be 100% initially. The odds ratio increased for higher seasonal VE, higher cumulative incidence of infection and a higher population proportion of temporary immunity. All calculations assumed that there was no protection and no risk from seasonal vaccination against pandemic infection.

Discussion

We suggest that temporary immunity following infection may explain the apparently conflicting findings that the seasonal influenza vaccine provided no protection from pandemic infection in Victoria but increased the risk of pandemic infection in Canada. Assuming that the seasonal influenza vaccine had no effect on the risk of pandemic infection, we have shown that temporary immunity from seasonal infection will result in an *apparent* increase in risk, due to a proportion of vaccinated individuals being infected with pandemic influenza having foregone the temporary protection from seasonal infection. Using a simple susceptible–infected–recovered (SIR) model we have extended our analysis to include the effect of time, and have confirmed that the temporary immunity hypothesis can account for the apparently discrepant findings [11].

This phenomenon would only be observed where pandemic virus circulation occurred within two to three months of seasonal virus circulation, allowing sufficient time for temporary immunity to develop and to wane. This was seen in parts of the northern, but not the southern, hemisphere. Because of the timing of seasonal and pandemic virus circulation, we might also have expected to see the *apparent* harmful effect of seasonal influenza vaccine in the United States and the United Kingdom. We would not have expected to see the effect in any northern hemisphere country that experienced only one wave of pandemic influenza A(H1N1) virus circulation at the time that other countries experienced a second wave, because temporary immunity from prior seasonal influenza infection would have decayed by the time the second wave commenced. Assuming that people infected in the first wave were not infected again in the second wave, neither would we expect to see the apparently harmful effect of seasonal vaccination during a second wave in a country that experienced a first wave, again because temporary immunity from prior seasonal influenza infection would have decayed. The absence of an apparently harmful (or protective) effect of seasonal influenza vaccine during the second pandemic wave in Canada supports this expectation [28].

However, a range of VE results for seasonal vaccination against pandemic influenza infection have now been reported, only some of which can be explained by the temporary immunity hypothesis (Table). The studies from Mexico [29,30] showing protection from pandemic infection following seasonal vaccination are not consistent with one of the assumptions in our model,

Table

Reported odds ratios and vaccine effectiveness for the association of seasonal influenza vaccine and pandemic influenza infection, by study site, 2009

Location	2009	Odds ratio (95% confidence interval)	Vaccine effectiveness (%) (95% confidence interval)
United States, military [33]	Apr-May	2.90 (1.84, 4.57)	-190 (-357, -84)
Canada [1] x 4	Apr-July	1.4 – 2.5 (1.03, 2.74)	-68 (-174, -3)
Castellon, Spain [34]	Oct –Jan 2010	1.20 (0.62-2.34) 2008-9 seasonal influenza vaccine	-20 (-134, 38)
United States [35]	May-Jun	1.10 (0.85, 1.46)	-10 (-46, 15)
United States, university [36]	Mar-May	1.10 (0.90, 1.40)	-10 (-40, 10)
United States, school [37]	Apr	1.05 (0.91, 1.20)	-5 (-20, 9)
Castellon, Spain [34]	Oct –Jan 2010	0.96 (0.50, 1.86) 2009-10 seasonal influenza vaccine	+4 (-86, 50)
Victoria [3]	Apr-Dec	0.97 (0.60, 1.56)	+3 (-56, 40)
Western Australia [6]	May-Aug	1.00 (0.70, 1.50)	0 (-50, 30)
Mexico [30]	Apr-July	0.65 (0.55, 0.77)	+35 (23, 45)
Mexico, hospital [29]	Mar-May	0.27 (0.11, 0.66)	+73 (34, 89)

that seasonal vaccine provided no protection against pandemic infection. However both these studies have been reasonably criticised on methodological grounds [31,32]. While some protection is biologically plausible, based on the demonstration of cross-reacting antibodies to pandemic influenza A(H1N1) virus, it would be expected mostly in older people. The findings from the other studies listed in the Table are consistent with the temporary immunity hypothesis.

As described above, the temporary immunity hypothesis suggests that one would only find an *apparent* increase in the risk of pandemic influenza infection associated with the receipt of seasonal influenza vaccination where the circulation of pandemic influenza virus followed that of seasonal influenza virus within a few weeks to months. Recognising this phenomenon would depend on infection rates with both viruses and seasonal influenza vaccine coverage, with the association more evident where seasonal and pandemic influenza infection rates and seasonal influenza vaccine coverage were higher. We suggest that odds ratios up to 1.5 can be explained by this hypothesis using plausible values for infection rates and seasonal influenza vaccine effectiveness. However we agree with Skowronski *et al*. that a high level of immunity, due to temporary immunity in our hypothesis or cross protection in their hypothesis, is needed to completely explain the apparent harmful association of seasonal influenza vaccination with pandemic influenza infection [1].

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Phylogenetic analysis in a recent controlled outbreak of Crimean-Congo haemorrhagic fever in the south of Iran, December 2008

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Crimean-Congo haemorrhagic fever (CCHF) is a viral zoonotic disease with a high mortality rate in humans. The CCHF virus is transmitted to humans through the bite of *Ixodid* ticks or contact with blood or tissues of CCHF patients or infected livestock. In December 2008, a re-emerging outbreak of CCHF occurred in the southern part of Iran. Five people were hospitalised with sudden fever and haemorrhaging, and CCHF was confirmed by RT-PCR and serological assays. One of the cases had a fulminant course and died. Livestock was identified as the source of infection; all animals in the incriminated herd were serologically analysed and more than half of them were positive for CCHFV. We demonstrated that two routes of transmission played a role in this outbreak: contact with tissue and blood of infected livestock, and nosocomial transmission. Phylogenetic analyses helped to identify the origin of this transmission. This outbreak should be considered as a warning for the national CCHF surveillance system to avoid further outbreaks through robust prevention and control programmes.

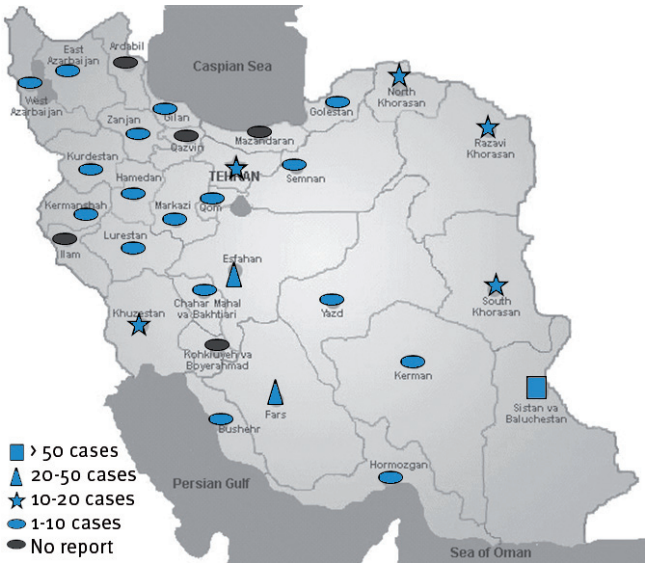
Introduction

Crimean-Congo haemorrhagic fever (CCHF) is a viral zoonotic haemorrhagic fever with up to 13-50% mortality rate in humans. Infected animals are unsymptomatic. The disease is caused by Crimean-Congo haemorrhagic fever virus (CCHFV) that belongs to the family *Bunyaviridae*, genus *Nairovirus*. The of negative single-stranded RNA genome consists of three segments, large (L), medium (M) and small (S), coding for the viral polymerase (L), the envelope glycoproteins (M) and the viral nucleoprotein (S) [1-5]. The typical course of CCHF progresses through four distinct phases: incubation, pre-haemorrhagic phase, haemorrhagic phase and convalescence [6-8]. After a incubation period of one to three days, the patient has sudden onset of fever, myalgia, nausea and severe headache. Within three to six days of the onset of illness, a petechial rash and haemorrhagic symptoms such as epistaxis, haematemesis, and melaena may occur. The most severely ill patients develop multiorgan failure characterised by shock, haemorrhaging and coma [9-11]. The virus is transmitted to humans through the bite of *Ixodid* ticks or by contact with blood or tissues from infected livestock [12-14]. In addition to zoonotic transmission, CCHFV can be spread from person to person and is one of the rare haemorrhagic fever viruses able to cause nosocomial outbreaks in hospitals [15-20].

In the period from 1 January 2000 to 12 September 2010, 738 confirmed cases of CCHF and 108 associated fatalities were notified in Iran [15,21]. The province reporting most infections was Sistan-va-Baluchistan, Isfahan and Fars (Figure 1).

Figure 1

Geographical distribution of Crimean-Congo haemorrhagic fever in Iran, 1 January 2000-12 September 2010 (n=738)



Table

History and laboratory results of probable cases of Crimean-Congo haemorrhagic fever, Fars province, Iran, November–December 2008 (n=5)

Patient	Profession	Age (years)/sex	Contact with livestock	Contact with suspected patient	Date of fever	Date of sampling		Death	Clinical and paraclinical signs						Ig M		Ig G		RT-PCR
						First sample	Second sample		Fever	Petechia	Haemorrhage	Leukocytopenia	Thrombocytopenia	Proteinuria	First sample	Second sample	First sample	Second sample	
A[a]	Housewife	46/F	Yes	No	18 Dec 2008	21 Dec 2008	Not taken	Yes	Yes	No	Yes	Yes	Yes	No report	Negative	Not taken	Negative	Not taken	Positive
B	Butcher	21/M	Yes	No	18 Dec 2008	20 Dec 2008	25 Dec 2008	No	Yes	No	Yes	Yes	Yes	Yes	Positive	Positive	Negative	Negative	Negative
C	Butcher	24/M	Yes	Yes	19 Dec 2008	20 Dec 2008	25 Dec 2008	No	Yes	No	Yes	No	Yes	Yes	Negative	Positive	Negative	Positive	Positive
D	Self-employed	28/M	Yes	No	21 Dec 2008	22 Dec 2008	Not taken	No	Yes	No	No	Yes	Yes	Yes	Positive	Not taken	Negative	Not taken	Negative
E	Nurse	26/F	No	Yes	27 Dec 2008	30 Dec 2008	Not taken	No	Yes	Yes	Yes	No	Yes	No	Positive	Not taken	Negative	Not taken	Positive

F: female; M: male.

[a] Index case.

Outbreak description

Here, we report a CCHF outbreak in Fars province, Iran, caused by contact of humans with blood or tissues of infected livestock, with additional nosocomial transmission. In total, five patients (A-E) were admitted to the regional hospital with similar presentations of a haemorrhagic condition in the period from 18 December to 21 December 2008. This period coincides with the Muslim ceremony Eid-al-Adha (the ceremony of sacrificing livestock) which is celebrated in Islamic countries on 9 December.

Patients A and D (who are part of the same family) bought a calf from a butchery run by two brothers, patients B and C, and hired them to sacrifice the animal. On the morning of 18 December 2008, Patient A, the index case of this outbreak, was admitted to hospital and died after a fulminant course of CCHF. In the evening of the same day, Patients B and C were admitted to the same hospital with fever and chill, severe headache, dizziness, photophobia. Patient D developed similar clinical signs on 21 December and was hospitalised. Nine days after the index case, the nurse caring of these four patients was also hospitalised with haemorrhagic symptoms (Patient E).

Materials and methods

Case definition

The case definition for probable cases included patients admitted between 18 December and 27 December 2008 in the regional hospital and presenting with a clinical picture compatible with CCHF, or contact with tissues or blood from a possibly infected animal, or a healthcare worker with a history of contact with a CCHF case. Probable cases with positive IgM serology and/or positive RT-PCR were considered as CCHF confirmed cases.

Laboratory analysis

Human and animal sera were analysed by ELISA for anti-CCHFV IgM and IgG as described [15,22]. Viral RNA was extracted from patient's serum using QIAamp RNA Mini kit (QIAgen GmbH, Hilden, Germany) and analysed by gel-based and real-time RT-PCR with a one-step RT-PCR kit (QIAgen GmbH, Hilden, Germany). A 536 bp fragment of the S segment of the CCHFV genome was amplified [4,8,12] and sequenced.

Phylogenetic analysis was performed with the neighbour-joining method based on Kimura two-parameter distances by using Mega 4 software. Bootstrap confidence limits were based on 500 replicates. Evolutionary divergence, distance matrix and subsequently sets of phylogenetic trees were calculated by the software [23].

Results and discussion

As summarised in the Table, five probable CCHF cases in this outbreak were confirmed by serological and molecular methods. It is worth mentioning that no immunological response was detected in the fatal case that had a fulminant course, Patient A, and CCHF

in this case was only confirmed by a strongly positive RT-PCR. There is evidence of other fatal cases lacking an immune response to CCHFV [6,24]. Patients C and E were positive both for viral RNA and antibodies against CCHFV, while Patients B and D were negative in the PCR and only confirmed by serological assay. Notably, ribavirin was administered to the patients in hospital.

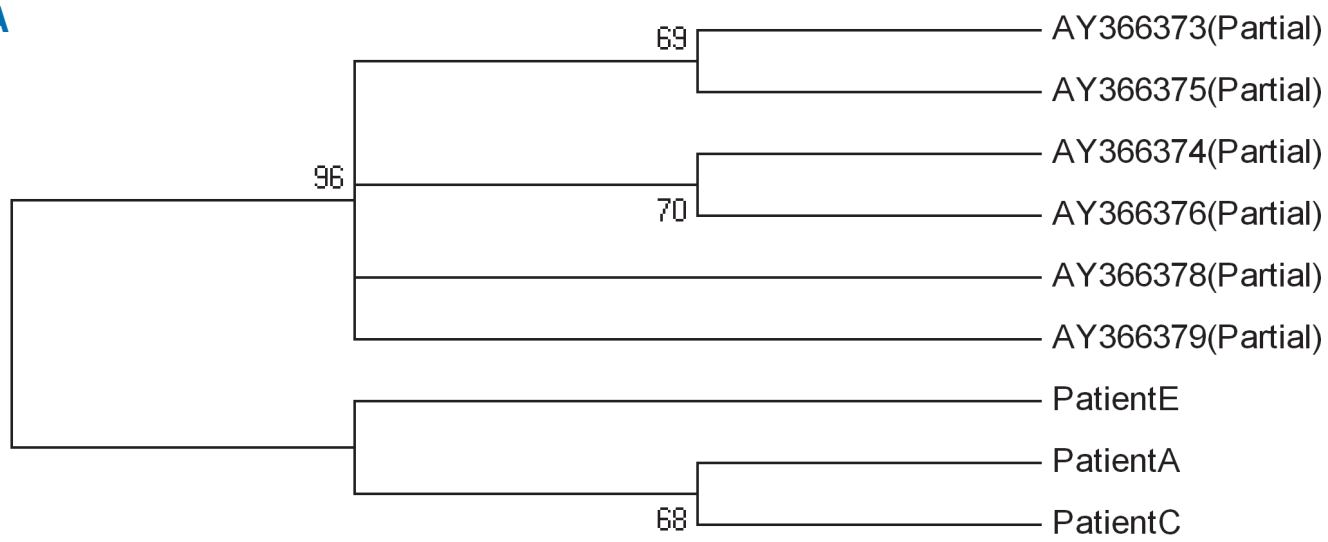
At the same time, serum samples were collected from 50 animals in the herd from which the calf had been bought. In 30 of these samples antibodies to CCHFV were detected. Although CCHF is an asymptomatic disease in livestock that does not kill the animals, seroepidemiological surveys of animal populations in endemic areas and high risk regions could be useful in that they may complement the national surveillance system and serve as an early warning of CCHF in the area.

In this outbreak, it was demonstrated that the main transmission route of CCHF was through handling blood or tissues of infected livestock (for patients A, B, C and D), while patient E had had no contact to livestock and was infected nosocomially. It is unclear why Patient A had such a fulminant course of disease and died. Patients B, C and D were infected through the same route, by direct contact with tissue and blood of the same animal, but had a milder course of disease and recovered. It is important in infectious disease outbreaks to investigate what factors determine the severity of the disease in different individuals [6]. There are published reports on the influence of cytokine levels on the immune response to CCHFV in different patients [24,25]. It has been shown that patients infected with a higher dose of virus develop more severe disease symptoms and outcomes [26-28]. Although we did not use quantitative RT-PCR, the band density of the PCR product obtained from patient A was much higher than that of the other patients. On the other hand, it seems likely that patients B and C presented a mild form of the disease because they may already have had antibodies against CCHFV due to their professional exposure. Moreover, no anti-CCHFV IgG antibody response was detected for the patient B, whereas a normal serological and molecular pattern was seen in patient C, which

Figure 2

Phylogenetic comparison of Crimean-Congo haemorrhagic fever virus isolates from Iran with isolates form patients in the recent outbreak in Fars province, Iran, December 2008

A



B

	Percent identity										
Divergence		1	2	3	4	5	6	7	8	9	
	1		99.9	99.9	99.9	99.9	99.8	97.6	97.6	97.7	1	AY366373(partial)
	2	0.2		100.0	99.9	99.9	99.9	97.6	97.6	97.7	2	AY366379(partial)
	3	0.2	0.0		99.9	99.9	99.9	97.6	97.6	97.7	3	AY366378(partial)
	4	0.2	0.4	0.4		99.8	99.8	97.5	97.6	97.6	4	AY366375(partial)
	5	0.4	0.2	0.2	0.6		99.9	97.6	97.6	97.7	5	AY366374(partial)
	6	0.6	0.4	0.4	0.8	0.2		97.5	97.5	97.6	6	AY366376(partial)
	7	1.0	0.8	0.8	1.2	1.0	1.2		99.9	99.8	7	Patient A
	8	1.0	0.8	0.8	1.3	1.0	1.3	0.2		99.9	8	Patient C
	9	0.8	0.6	0.6	1.0	0.8	1.0	0.4	0.2		9	Patient E
		1	2	3	4	5	6	7	8	9		

A. The phylogeny tree of nucleotide sequences spanning described regions of the S-segment of CCHF virus genome which is detected in the outbreak. B. Nucleotide identity and divergence of CCHF virus genomes isolated from patients of the outbreak.

might be interpreted to indicate that patient B was infected with a very low viral dose. Recent investigations have concluded a relationship linking the severity and outcome of CCHFV infections with the strength of the host immune response and the initial viral load [24,25,27].

Phylogenetic analysis of alignments of three partial genomic sequences (536 bp) of the CCHFV S segment indicates that the viruses isolated from patients A, C and E can be differentiated into two distinct branches, with a slightly lower identity between patients A and E. As illustrated in Figure 2, the sequences obtained in this outbreak are not clustered with other CCHFV sequences isolated in Iran (about 97.5% identity). It is possible that a new strain occurred in the outbreak region, and further phylogenetic analyses are required to identify the precise origin of this genetic variant. However, comparison of the isolates from our patients with isolates from other areas may give some indications as to the origin of this outbreak [12].

One of the factors that contributed to the control of this outbreak was the well-coordinated and efficient surveillance system for CCHF and other viral haemorrhagic fevers that is in place in Iran. The system is not only responsible for continuous monitoring of these diseases but also deals with outbreaks. Rapid and precise laboratory diagnosis of CCHF allowed controlling this outbreak. Nevertheless, a higher level of training and precautionary measures for healthcare workers (such as use of isolation chambers in hospital wards, mask and other medical shields during contact to CCHF patients) and other high risk professions could help to decrease the outbreak rate in the endemic areas. In conclusion, with Iran being an endemic country for CCHF in the Middle East and neighbouring Turkey an endemic country in Europe, efficient surveillance and control programmes on CCHF in Iran could prove beneficial also for the European region.

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New research results on EU consumers' perceptions on food-related risks

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On 17 November 2010, the European Food Safety Authority (EFSA) published the results of a recent Eurobarometer survey report on consumers' risk perception in the European Union (EU) [1]. The report, commissioned by EFSA, is based on interviews with nearly 27,000 European citizens from 27 Member States. It highlights consumers' perceptions in the following areas (i) perceptions of food and food-related risks, (ii) concerns about food-related risks, (iii) sources of information: public confidence and response, and (iv) the role and effectiveness of public authorities. Some of the findings are presented below.

- A majority of respondents associates food and eating with pleasure, and with enjoyment of meals with friends and family. The economic crisis and environmental pollution are viewed by more respondents as risks very likely to affect their lives than food-related problems.
- There is no single, widespread concern about food-related risks mentioned spontaneously by a majority of respondents. Nearly 20% of citizens spontaneously cite chemicals, pesticides and other substances as the major concerns. Fewer citizens are concerned about health and nutrition risks such as putting on weight or not having a healthy balanced diet.
- When asked to indicate the extent to which they feel confident about various information sources, citizens express the highest levels of confidence in information obtained from doctors and other health professionals (84%) and family and friends (82%). Other trusted information sources comprise consumer organisations, scientists and environmental protection groups.
- A majority of respondents (›80%) believes that public authorities in the EU should do more to ensure that food is healthy and to inform people about healthy diets and lifestyles. The majority of EU citizens thinks that public authorities in the EU are doing a good job in protecting them from specific food-related risks, but the survey also shows that there is room for improvement.

The Eurobarometer report was the second such study to be carried out in five years. The research objectives were to gain insights into consumer concerns relating to food and risks associated with the food chain and to establish the level of consumer confidence in public authorities on food safety-related issues.

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