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Research article

Hepatitis B and C surveillance and screening programmes in the non-EU/EEA Member States of the WHO European Region: survey findings from 10 countries, 2012

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The hepatitis B virus (HBV) and hepatitis C virus (HCV) epidemics warrant a comprehensive response based on reliable population-level information about transmission, disease progression and disease burden, with national surveillance systems playing a major role. In order to shed light on the status of surveillance in countries of the World Health Organization (WHO) European Region outside of the European Union and European Economic Area (EU/EEA), we surveyed 18 countries in Central and Eastern Europe. Among the 10 countries that responded, the common features of many surveillance systems included mandatory surveillance, passive case-finding and the reporting of both acute and chronic HBV and HCV. Only some countries had surveillance systems that incorporated the tracking of associated conditions and outcomes such as cirrhosis and liver transplantation. Screening programmes for some key populations appeared to be in place in many countries, but there may be gaps in relation to screening programmes for people who inject drugs, prisoners, sex workers and men who have sex with men. Nonetheless, important components of a surveillance structure are in place in the responding study countries. It is advisable to build on this structure to develop harmonised HBV and HCV surveillance for all 53 Member States of the WHO European Region following the example of the system recently instituted in EU/EEA countries.

Introduction

Hepatitis B virus (HBV) and hepatitis C virus (HCV) infections can result in acute and chronic hepatitis and are major public health problems in many parts of the world. Together they are thought to cause more than 1.4 million deaths per year worldwide, mostly due to chronic hepatitis sequelae such as liver cirrhosis and liver cancer [1]. In the World Health Organization (WHO) European Region, an estimated 1.8 per cent of adults are chronically infected with hepatitis B; moreover hepatitis C RNA (HCV RNA) prevalence is estimated to be 2.0 per cent. The eastern part of the Region is disproportionally affected, whereby two-thirds of those infected with HBV or HCV live outside of the European Union (EU) and European Free Trade Association [2]. Throughout the Region, there is evidence of high levels of HCV infection and to a lesser extent HBV infection in populations of people who inject drugs (PWID) [3]. Other notable transmission pathways for hepatitis B in Europe include sexual intercourse, both heterosexual and among men who have sex with men (MSM), and nosocomial transmission in some countries. Available epidemiological evidence on HBV and HCV in migrant populations suggests that in several European countries, many migrant groups are disproportionately affected [4,5]. However, precise data on disease burden and changing trends are lacking in most countries [6], and information on existing viral hepatitis surveillance systems and screening practices is not available at the regional level.

WHO defines public health surveillance as 'the continuous, systematic collection, analysis and interpretation of health-related data needed for the planning, implementation, and evaluation of public health practice' [7]. Surveillance of diseases and health conditions is important for guiding decision-making about how health systems should be configured at the subnational, national, regional and global levels. A key principle of surveillance is that the surveillance system should be designed to address the specific public health objectives at hand [8]. In the case of HBV and HCV, this principle suggests a need for surveillance

Table 1

Respondent and non-respondent countries to a survey on hepatitis B and C surveillance and screening programmes, 2012

Responded to survey (n=10)	Did not respond to survey (n=8)
Armenia; Azerbaijan; Belarus; Croatia; Kyrgyzstan; Moldova; Montenegro; Russia; Tajikistan; Ukraine	Albania; Bosnia and Herzegovina; Bulgaria; Kazakhstan; Serbia; the former Yugoslav Republic of Macedonia; Turkey; Uzbekistan

systems with the capacity to capture and synthesise a complex array of data on disease transmission and progression. This is difficult, since both diseases manifest in ways that can make it highly challenging to track incidence, prevalence, morbidity, mortality and the impact of prevention and treatment interventions [9,10].

In 2006, the European Parliament directed the European Centre for Disease Prevention and Control (ECDC) to prioritise the harmonisation of viral hepatitis surveillance in the EU [11]. In the course of this work, ECDC conducted a survey in 2009 to gather information about various features of national surveillance systems in the EU and European Economic Area (EEA) such as the types of surveillance conducted and the types of data collected [12]. Information of this nature is valuable for interpreting surveillance findings and for assessing how surveillance systems can be further strengthened and harmonised. No comparable studies have been conducted to assess the characteristics of viral hepatitis surveillance systems and screening programmes in European countries outside of the EU/EEA.

The following paper presents findings from a survey conducted by the WHO Regional Office for Europe among mostly non-EU/EEA Member States in Central and Eastern Europe, including Central Asia.

Methods

In 2012, the WHO Regional Office for Europe conducted a survey on viral hepatitis surveillance in 17 non-EU/EEA Member States comprising Albania, Armenia, Azerbaijan, Belarus, Bosnia and Herzegovina*, Croatia, Kazakhstan, Kyrgyzstan, Moldova, Montenegro, Russia, Serbia, Tajikistan, the former Yugoslav Republic of Macedonia, Turkey, Ukraine and Uzbekistan. In addition, Bulgaria was included. These countries were selected because little is known about their current practices. In each country, the person recognised by WHO via the respective ministry of health as the governmental focal point for viral hepatitis surveillance was asked to complete the survey.

The survey consisted of 20 questions relating to HBV and HCV surveillance systems, case definitions and populations screened for hepatitis. Respondents also had the option of providing comments. The survey was developed in English by one of this paper's co-authors who, as a native Russian speaker, ensured correct translation of the questionnaire into Russian. English- and Russian-language versions of the survey were distributed via email to the country focal points. Data collection took place between 1 July and 31 August 2012 and there was one reminder sent midway through the data collection period to increase the response rate.

For the purpose of this study, and with a secondary aim of comparing the findings from non-EU/EEA countries with the findings from the 2009 ECDC survey [12], we selected 10 of the questions, which covered objectives and main features of viral hepatitis surveillance systems; types of data collected through viral hepatitis case reporting; and data linkages with the associated conditions and populations screened for HBV and HCV. For nine of the questions (which are further detailed in a table within the result section), respondents were instructed to tick check-boxes to indicate their answers. The tenth question asked 'Which of the following populations, if any, are screened for hepatitis?' and was followed by check-boxes for 18 options identified. These options and responses are also further detailed in a separate table in the results. One of these options was 'Other groups – please specify', and space was provided for respondents to report this information. Simple definitions of terms such as 'population-based surveillance' and 'active surveillance' were incorporated into survey questions. All survey responses were entered into Microsoft Excel and a descriptive analysis was performed.

Results

Respondents

Completed surveys were received from focal points in 10 of 18 countries as described in Table 1. During the time of this study, all respondent countries were WHO European Region Member States, which were not part of the EU/EEA.

Surveillance objectives

All 10 survey respondents indicated that the objectives of HBV and HCV surveillance systems included monitoring trends and detecting outbreaks. Most stated that the goals were also to monitor changes in disease distribution (n=9 countries) and identify at-risk populations (n=8 for HBV; n=7 for HCV) (Table 2). Fewer respondents reported planning and evaluating control measures (n=7 for HBV; n=6 for HCV), identifying research needs (n=3 for both diseases) and improving epidemiological knowledge as specific surveillance objectives (n=6).

Table 2a

Hepatitis surveillance system features of 10 non-European Union/European Economic Area Member States of the World Health Organization European Region, 2012

Characteristics of the surveillance system	HBV Number of countries responding (names of respondent countries)	HCV Number of countries responding (names of respondent countries)
What are the objectives of the surveillance system?		
Monitor trends	10 (Armenia, Azerbaijan, Belarus, Croatia, Kyrgyzstan, Moldova, Montenegro, Russia, Tajikistan, Ukraine)	10 (Armenia, Azerbaijan, Belarus, Croatia, Kyrgyzstan, Moldova, Montenegro, Russia, Tajikistan, Ukraine)
Detect outbreaks	10 (Armenia, Azerbaijan, Belarus, Croatia, Kyrgyzstan, Moldova, Montenegro, Russia, Tajikistan, Ukraine)	9 (Armenia, Azerbaijan, Belarus, Croatia, Kyrgyzstan, Moldova, Montenegro, Russia, Ukraine)
Monitor changes in disease distribution	9 (Armenia, Azerbaijan, Belarus, Croatia, Kyrgyzstan, Montenegro, Russia, Tajikistan, Ukraine)	9 (Armenia, Azerbaijan, Belarus, Croatia, Kyrgyzstan, Montenegro, Russia, Tajikistan, Ukraine)
Plan and evaluate control measures	7 (Armenia, Kyrgyzstan, Montenegro, Moldova, Russia, Tajikistan, Ukraine)	6 (Armenia, Kyrgyzstan, Montenegro, Moldova, Russia, Ukraine)
Identify research needs and facilitate research	3 (Armenia, Montenegro, Moldova)	3 (Armenia, Montenegro, Moldova)
Improve knowledge of epidemiology	6 (Armenia, Montenegro, Moldova, Russia, Tajikistan, Ukraine)	6 (Armenia, Montenegro, Moldova, Russia, Tajikistan, Ukraine)
Identify at-risk populations	8 (Armenia, Azerbaijan, Belarus, Montenegro, Moldova, Russia, Tajikistan, Ukraine)	7 (Armenia, Belarus, Montenegro, Moldova, Russia, Tajikistan, Ukraine)
Other (please comment)	0	0
In your country, what is the legal basis of hepatitis reporting?		
Mandatory[a]	10	10
Voluntary[a]	0	0
Would you describe your surveillance system as active or passive?		
Active – the proactive searching for case reports from the population	0	0
Passive – the passive receiving of case reports from surveillance structures within the population	9 (Armenia, Azerbaijan, Belarus, Croatia, Kyrgyzstan, Montenegro, Moldova, Tajikistan, Ukraine)	9 (Armenia, Azerbaijan, Belarus, Croatia, Kyrgyzstan, Montenegro, Moldova, Tajikistan, Ukraine)
Depends on type of surveillance	0	0
Which of the following surveillance methods are used?		
Population-based (all cases reported)	10	10
Risk group-based (occupational or behavioural risk group monitoring)	7 (Armenia, Belarus, Croatia, Moldova, Russia, Tajikistan, Ukraine)	6 (Armenia, Belarus, Croatia, Moldova, Russia, Tajikistan)
Sentinel (all cases from selected reporting sites)	0	1 (Tajikistan)
Other (surveys, anonymous sampling, etc.)	3 (Croatia, Russia, Ukraine)	3 (Croatia, Russia, Ukraine)
What types of cases are included in surveillance?		
Acute[a]	10	10
Chronic[a]	8 (Belarus, Croatia, Kyrgyzstan, Montenegro, Moldova, Russia, Tajikistan, Ukraine)	8 (Belarus, Croatia, Kyrgyzstan, Montenegro, Moldova, Russia, Tajikistan, Ukraine)
Asymptomatic	3 (Belarus, Croatia, Russia)	2 (Croatia, Russia)
Suspected[a]	3 (Armenia, Croatia, Russia)	3 (Armenia, Croatia, Russia)
Hepatitis (undefined[a])	5 (Azerbaijan, Belarus, Croatia, Montenegro, Ukraine)	5 (Azerbaijan, Belarus, Croatia, Montenegro, Ukraine)
Other (please comment)	0	0

HBV: hepatitis B virus; HCV: hepatitis C virus; HIV: human immunodeficiency virus.

[a] This term was not defined and its interpretation was at the discretion of the survey respondent.

Table 2b

Hepatitis surveillance system features of 10 non-European Union/European Economic Area Member States of the World Health Organization European Region, 2012

Characteristics of the surveillance system	HBV Number of countries responding (names of respondent countries)	HCV Number of countries responding (names of respondent countries)
What case classifications are included in surveillance?		
Probable[a]	3 (Armenia, Croatia, Russia)	3 (Armenia, Croatia, Russia)
Confirmed[a]	9 (Armenia, Azerbaijan, Belarus, Croatia, Kyrgyzstan, Montenegro, Russia, Tajikistan, Ukraine)	9 (Armenia, Azerbaijan, Belarus, Croatia, Kyrgyzstan, Montenegro, Russia, Tajikistan, Ukraine)
Epidemiologically linked	4 (Azerbaijan, Belarus, Moldova, Russia)	4 (Azerbaijan, Belarus, Moldova, Russia)
Other classification (please comment)	0	0
Which of the following demographic data are collected (if any)?		
Patient identifier, address, sex, occupation, birth date, place of birth	9 (Armenia, Azerbaijan, Belarus, Croatia, Montenegro, Moldova, Russia, Tajikistan, Ukraine)	9 (Armenia, Azerbaijan, Belarus, Croatia, Montenegro, Moldova, Russia, Tajikistan, Ukraine)
Pregnancy status	5 (Armenia, Azerbaijan, Moldova, Russia, Tajikistan)	5 (Armenia, Azerbaijan, Moldova, Russia, Tajikistan)
Ethnic group	2 (Armenia, Moldova)	2 (Armenia, Moldova)
Country in which infection was acquired	4 (Armenia, Azerbaijan, Moldova, Russia)	4 (Armenia, Azerbaijan, Moldova, Russia)
Date of onset	8 (Armenia, Azerbaijan, Belarus, Croatia, Montenegro, Moldova, Russia, Ukraine)	8 (Armenia, Azerbaijan, Belarus, Croatia, Montenegro, Moldova, Russia, Ukraine)
Date of diagnosis	8 (Armenia, Azerbaijan, Belarus, Montenegro, Moldova, Russia, Tajikistan, Ukraine)	8 (Armenia, Azerbaijan, Belarus, Montenegro, Moldova, Russia, Tajikistan, Ukraine)
Date of reporting	8 (Armenia, Azerbaijan, Belarus, Croatia, Montenegro, Moldova, Russia, Tajikistan)	8 (Armenia, Azerbaijan, Belarus, Croatia, Montenegro, Moldova, Russia, Tajikistan)
Which of the following classification data are collected from cases?		
Clinical symptoms	6 (Armenia, Azerbaijan, Belarus, Moldova, Russia, Ukraine)	6 (Armenia, Azerbaijan, Belarus, Moldova, Russia, Ukraine)
Laboratory results	8 (Armenia, Azerbaijan, Belarus, Montenegro, Moldova, Russia, Tajikistan, Ukraine)	8 (Armenia, Azerbaijan, Belarus, Montenegro, Moldova, Russia, Tajikistan, Ukraine)
Epidemiological information[a]	9 (Armenia, Azerbaijan, Belarus, Croatia, Montenegro, Moldova, Russia, Tajikistan, Ukraine)	9 (Armenia, Azerbaijan, Belarus, Croatia, Montenegro, Moldova, Russia, Tajikistan, Ukraine)
For which of the following associated conditions is case data linked to/collected?		
Liver transplant	3 (Armenia, Belarus, Moldova)	3 (Armenia, Belarus, Moldova)
Liver cancer	4 (Armenia, Kyrgyzstan, Moldova, Tajikistan)	4 (Armenia, Kyrgyzstan, Moldova, Tajikistan)
HBV or HCV coinfection	6 (Armenia, Azerbaijan, Belarus, Kyrgyzstan, Moldova, Tajikistan)	6 (Armenia, Azerbaijan, Belarus, Kyrgyzstan, Moldova, Tajikistan)
HIV coinfection	6 (Armenia, Azerbaijan, Belarus, Kyrgyzstan, Moldova, Tajikistan)	6 (Armenia, Azerbaijan, Belarus, Kyrgyzstan, Moldova, Tajikistan)
Hepatitis-associated mortality	5 (Armenia, Belarus, Kyrgyzstan, Moldova, Tajikistan)	5 (Armenia, Belarus, Kyrgyzstan, Moldova, Tajikistan)

HBV: hepatitis B virus; HCV: hepatitis C virus; HIV: human immunodeficiency virus.

[a] This term was not defined and its interpretation was at the discretion of the survey respondent.

Table 3

Populations screened in 10 non-European Union/European Economic Area Member States of the World Health Organization European Region, 2012

Population	HBV Number of countries responding (names of respondent countries)	HCV Number of countries responding (names of respondent countries)
People who inject drugs	5 (Belarus, Croatia, Moldova, Russia, Ukraine)	5 (Belarus, Croatia, Moldova, Russia, Ukraine)
Contacts of infected people who inject drugs	2 (Belarus, Croatia)	2 (Belarus, Croatia)
Sex workers	0	0
Men who have sex with men	0	0
Inmates in closed settings	2 (Croatia, Russia)	2 (Croatia, Russia)
People living with HIV	8 (Armenia, Azerbaijan, Belarus, Croatia, Kyrgyzstan, Montenegro, Moldova, Russia)	9 (Armenia, Azerbaijan, Belarus, Croatia, Kyrgyzstan, Montenegro, Moldova, Russia, Tajikistan)
Transfusion/organ transplant recipients	7 (Armenia, Azerbaijan, Belarus, Croatia, Moldova, Russia, Ukraine)	7 (Armenia, Azerbaijan, Belarus, Croatia, Moldova, Russia, Ukraine)
HBV- or HCV-infected patients	8 (Armenia, Azerbaijan, Belarus, Croatia, Montenegro, Moldova, Russia, Ukraine)	8 (Armenia, Azerbaijan, Belarus, Croatia, Montenegro, Moldova, Russia, Ukraine)
Persons born in endemic areas	1 (Moldova)	1 (Moldova)
Blood and organ donors	10	10
Hospitalised patients/pre-operative patients	7 (Armenia, Kyrgyzstan, Montenegro, Moldova, Russia, Tajikistan, Ukraine)	7 (Armenia, Kyrgyzstan, Montenegro, Moldova, Russia, Tajikistan, Ukraine)
Persons who require immunosuppressive therapy	3 (Armenia, Croatia, Moldova)	3 (Armenia, Croatia, Moldova)
Pregnant women	9 (Armenia, Azerbaijan, Belarus, Croatia, Kyrgyzstan, Montenegro, Moldova, Russia, Ukraine)	6 (Azerbaijan, Belarus, Kyrgyzstan, Montenegro, Moldova, Russia)
Family members	8 (Armenia, Belarus, Croatia, Kyrgyzstan, Montenegro, Moldova, Russia, Ukraine)	6 (Armenia, Belarus, Kyrgyzstan, Montenegro, Moldova, Russia)
Sexual contacts of infected persons	3 (Armenia, Croatia, Moldova)	3 (Armenia, Croatia, Moldova)
Haemodialysis patients	9 (Armenia, Azerbaijan, Belarus, Croatia, Montenegro, Moldova, Russia, Tajikistan, Ukraine)	8 (Armenia, Azerbaijan, Belarus, Croatia, Montenegro, Moldova, Russia, Tajikistan)
Exposed healthcare, public safety, and emergency medical workers	10	8 (Armenia, Belarus, Croatia, Kyrgyzstan, Montenegro, Moldova, Russia, Tajikistan)
Other groups – please specify	3 (Kyrgyzstan, Moldova, Tajikistan)	3 (Kyrgyzstan, Moldova, Tajikistan)

HBV: hepatitis B virus; HCV: hepatitis C virus; HIV: human immunodeficiency virus.

Surveillance system characteristics

All 10 countries reported that their surveillance systems for HBV and HCV were mandatory rather than voluntary, and nine characterised the surveillance systems as passive rather than active (Table 2). Surveillance systems in all 10 countries reportedly employed population-based methods, and in several countries there was risk group-based surveillance as well (n=7 for HBV and n=6 for HCV). The only country that reported carrying out sentinel surveillance was Tajikistan (for hepatitis C but not hepatitis B). All countries reported including acute HBV and HCV cases in surveillance, and all but two countries (Armenia and Azerbaijan) reported including chronic cases as well. Five respondents indicated that 'undefined' hepatitis cases were included in surveillance. Nine countries reported including 'confirmed' cases in surveillance, and three of those countries (Armenia, Croatia and Russia) also included 'probable' cases.

Data collected

Most responding countries (n=9) collected case-based demographic data such as patient identifier, address, sex, occupation, and time and place of birth and epidemiological data (Table 2). Among these, eight countries also collected laboratory results, and six clinical symptoms. Four countries collected data on the country in which infection was acquired, and two countries (Armenia and Moldova) collected information on the ethnic identity of infected individuals. Six countries reported collecting or linking to information about human immunodeficiency virus (HIV) coinfection and HBV/HCV coinfection, while smaller numbers of countries reported doing so for liver transplant (n=3 for both diseases), liver cancer (n=4 for both diseases) and hepatitis-associated mortality (n=5 for both diseases).

Populations screened

All 10 countries reported screening blood and organ donors for both HBV and HCV (Table 3). All countries with the exception of Tajikistan reported screening pregnant women for HBV, and in six countries pregnant women were screened for HCV as well. Other populations screened in most countries included people living with HIV (n=8 for HBV; n=9 for HCV), haemodialysis patients (n=9 for HBV; n=8 for HCV), and exposed healthcare workers, emergency medical workers and public safety workers (n=10 for HBV; n=8 for HCV). Five countries reported screening PWID for both HBV and HCV. Only two countries reported screening inmates in closed settings (Croatia and Russia, for both diseases). One reported screening persons born in endemic areas (Moldova, for both HBV and HCV), and three, screening sexual contacts of infected persons (Armenia, Croatia and Russia, for both diseases). There were no countries in which sex workers or MSM were among the populations screened.

Discussion

This is the first survey that provides an overview of surveillance systems and screening programmes for viral hepatitis B and C in selected countries of the WHO European Region outside of the EU/EEA. Across the 10 countries that responded to our survey, common features of many national surveillance systems included mandatory surveillance, passive case-finding and the reporting of both acute and chronic HBV and HCV. The objectives of the surveillance systems were mostly similar among responding countries. There was considerable variation in the type and amount of data collected across countries; however, basic demographic, epidemiological and clinical data were reportedly collected in the majority of responding countries. Populations screened in most countries included blood and organ donors, pregnant women, people living with HIV, haemodialysis patients and people at risk of occupational exposure.

Similar to the ECDC's 2009 survey of HBV and HCV surveillance in EU/EEA countries, we found that chronic disease surveillance lagged behind acute disease surveillance [12]. This is unsurprising since the traditional focus of surveillance has been newly identified symptomatic patients. When WHO last published guidance on HBV surveillance, in 2003, that document addressed only acute viral hepatitis [13]. The importance of tracking chronic HBV and HCV infections is now more widely recognised due to increased awareness of the burden of disease and to the impact of better treatments on the long-term health of people with chronic HBV and HCV. However, it appears that surveillance systems in some WHO European Region Member States are not reflective of this transition.

Confirming the stage of HBV and HCV infection is known to be challenging. There is no robust marker of acute HCV infection, and for both HBV and HCV, a combination of serological and molecular tests are often required [10]. These can be less accessible in resource-limited settings. The 2009 ECDC survey showed that some EU/EEA countries were not able to differentiate between acute and chronic cases [12], and this has remained an important problem in EU/EEA countries subsequent to the implementation of the ECDC's regional surveillance programme, especially for HCV [14,15]. Our survey did not ask directly if countries are able to distinguish effectively between acute and chronic infections and did not collect information on the case definitions used. The fact that five of 10 responding countries stated that their surveillance systems include undefined hepatitis cases indirectly suggests that countries' capacity to differentiate cases by disease stage may be limited and raises questions about the comparability and robustness of data across different WHO European Region Member States.

Case reporting is the core element of hepatitis surveillance, but it is known to provide a considerable underestimation of the true number of viral hepatitis cases [10]. Because viral hepatitis B and C infections are often asymptomatic, case reporting can be limited by testing practices and by lapses in the implementation of screening programmes. The hepatitis disease burden therefore can be assessed more accurately when case reporting is complemented by other sources of information including death registries, disease registries and serosurveys. Assessing the burden of disease from HBV and HCV is a highly complex undertaking. The most notable sequelae of chronic viral hepatitis – cirrhosis and liver cancer – are not exclusively attributable to HBV and HCV. At the same time, many people with HBV and HCV have comorbidities that might accelerate the development of complications of chronic infection [16,17]. The incorporation of data on conditions and outcomes associated with HBV and HCV into national surveillance allows for better assessment of the burden of disease and of temporal trends at the national level. However, the logistics of pooling data from multiple sources such as cancer registries, other disease registries and death certificate records is challenging even in countries with well-resourced

health systems. It is encouraging to see that HBV and HCV surveillance in some of our study countries appears to encompass the gathering of information on key associated conditions and outcomes. At the same time, however, study findings suggest that associated conditions are not being thoroughly documented in the study countries as a whole.

Despite the major role of injecting drug use as a driver of the ongoing HBV and HCV epidemics in Europe [5], only half of the responding countries in our study reported having screening programmes for PWID. The 2009 ECDC survey indicated that a number of EU/EEA countries also lacked targeted screening programmes for PWID [12]. Across the 10 responding countries in our study, three other high-risk populations appeared to not be taken into account in many countries' screening efforts: inmates in closed settings, sex workers and MSM. A notable commonality among PWID, prison inmates, sex workers and MSM is that health systems often do not reflect their needs, in part because of the stigmatised nature of their activities. Our study findings raise the question of how this pattern might be impacting negatively on the response to HBV and HCV in some countries.

Furthermore, since our study did not ask respondents to provide quantitative information regarding populations screened for HBV and HCV, it is not possible to assess the robustness of screening activities for specific populations. The implication of this data gap can be illustrated by considering responses to the survey question about whether people living with HIV are screened for HBV and HCV. Eight countries reported screening this population for HBV, and nine, for HCV. In any of those countries, a one-time initiative that tested a very small number of HIV-positive people might have been regarded by the survey respondent as 'screening' in that population. Considering the central role of some populations in the HBV and HCV epidemics in many countries, this is an issue that warrants more in-depth research. Future studies might ask countries to report quantitative details about how screening activities for HIV-positive people, PWID and other populations of interest inform national viral hepatitis programmes.

The European Parliament's recognition of specific gaps in individual countries, including any sort of mechanism for coordinated viral hepatitis surveillance, ultimately led to the introduction of 'enhanced surveillance' for HBV and HCV in EU/EEA Member States in 2011, with national governments being requested to submit surveillance data to a common dataset. The first data submission round was completed in September 2013, and ECDC researchers published the findings in late 2014 [14,15]. While it was recognised that the process of harmonising HBV and HCV surveillance across EU/EEA countries was far from complete, the data obtained in the first round still offered valuable insights about the region's HBV and HCV epidemics. The ECDC experience both demonstrates the feasibility of harmonisation across national surveillance systems and provides a functioning regional viral hepatitis surveillance system that might perhaps be expanded through collaboration with other stakeholders. It is thus an opportune time to explore the prospect of instituting harmonised HBV and HCV surveillance for the entire WHO European Region, which currently encompasses the 31 EU/EEA countries and 22 additional countries outside of the EU/EEA. Our study findings, by providing a snapshot of the features of some national surveillance systems outside of the EU/EEA, contributes to the evidence base that would be required to guide such an undertaking.

This study has a number of limitations in addition to those already identified. It was not designed to evaluate the quality of surveillance data collected, nor the extent to which the data collected are reliable and valid for following disease transmission and progression, but instead to describe key characteristics of surveillance systems and screening programmes. Further surveillance data analyses and surveillance system validation studies are needed to assess the utility and reliability of the current surveillance activities in the responding countries. Because of the low response rate, generalisations cannot be made about the study region as a whole, nor is it possible to draw conclusions from comparisons to other regions. Although the survey included brief definitions for many surveillance-related terms, some terms were not defined, and survey questions may have been understood differently by respondents in different settings. Future surveys should define all terms employed.

Being restricted to using either the English or Russian language may have further affected how survey respondents understood and answered questions. Although carefully reviewed, the survey itself was not back-translated from Russian to English. Since there was no verification of information reported by country focal points, the accuracy of the data depends on whether these individuals answered all survey questions correctly. Finally, data for this study come from a survey carried out in 2012. Since that time, it is possible that the passage of the World Health Assembly's second resolution on viral hepatitis in 2014 [18] and the increasing attention given to viral hepatitis by the global public health community may have spurred some countries to make improvements to their viral hepatitis surveillance systems.

In conclusion, as momentum continues to build around the public health response to viral hepatitis at the national, regional and global levels, greater attention to national surveillance systems will be a prerequisite for obtaining suitable data to guide decision-making. This study indicates that some important components of viral hepatitis surveillance such as mandatory surveillance and the reporting of both acute and chronic HBV and HCV are in place in several European countries where little was previously known about the nature of surveillance efforts. At the same time, there is a clear

need for additional research to illuminate key details about how surveillance is carried out in these and other European countries outside of the EU/EEA. The World Health Organization's first-ever global health sector strategy for viral hepatitis, adopted by the World Health Assembly on 28 May 2016, places viral hepatitis surveillance as one of the most important components of the strategic information framework, under the first of its five strategic directions: information for focused action [19]. It seems questionable whether progress under this strategy can be effectively measured in the WHO European Region without a cohesive effort to develop comprehensive and coordinated disease surveillance programmes in the Region. Our study, by providing a baseline overview of viral hepatitis surveillance and screening programmes in some European countries outside of the EU/EEA, contributes to the ECDC's work in this area and informs future plans to harmonise and enhance strategic information activities in the entire WHO European Region.

*Erratum

In the first sentence of the methods section, 'Bulgaria' was removed. This was corrected on 03 June 2016.

Conflict of interest

None declared.

Authors' contributions

All authors contributed extensively to the work presented in this paper. IE conceived of the study, and led in its design and coordination. JVL and KSH prepared the initial analysis of the data and first draft of the article. JVL oversaw the writing process. AM reviewed and revised the draft with input from IE. All authors read and approved the final manuscript.

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Research article

Effects of previous episodes of influenza and vaccination in preventing laboratory-confirmed influenza in Navarre, Spain, 2013/14 season

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We estimated whether previous episodes of influenza and trivalent influenza vaccination prevented laboratory-confirmed influenza in Navarre, Spain, in season 2013/14. Patients with medically-attended influenza-like illness (MA-ILI) in hospitals (n = 645) and primary healthcare (n = 525) were included. We compared 589 influenza cases and 581 negative controls. MA-ILI related to a specific virus subtype in the previous five seasons was defined as a laboratory-confirmed influenza infection with the same virus subtype or MA-ILI during weeks when more than 25% of swabs were positive for this subtype. Persons with previous MA-ILI had 30% (95% confidence interval (CI): −7 to 54) lower risk of MA-ILI, and those with previous MA-ILI related to A(H1N1)pdm09 or A(H3N2) virus, had a, respectively, 63% (95% CI: 16–84) and 65% (95% CI: 13–86) lower risk of new laboratory-confirmed influenza by the same subtype. Overall adjusted vaccine effectiveness in preventing laboratory-confirmed influenza was 31% (95% CI: 5–50): 45% (95% CI: 12–65) for A(H1N1) pdm09 and 20% (95% CI: −16 to 44) for A(H3N2). While a previous influenza episode induced high protection only against the same virus subtype, influenza vaccination provided low to moderate protection against all circulating subtypes. Influenza vaccine remains the main preventive option for high-risk populations.

Introduction

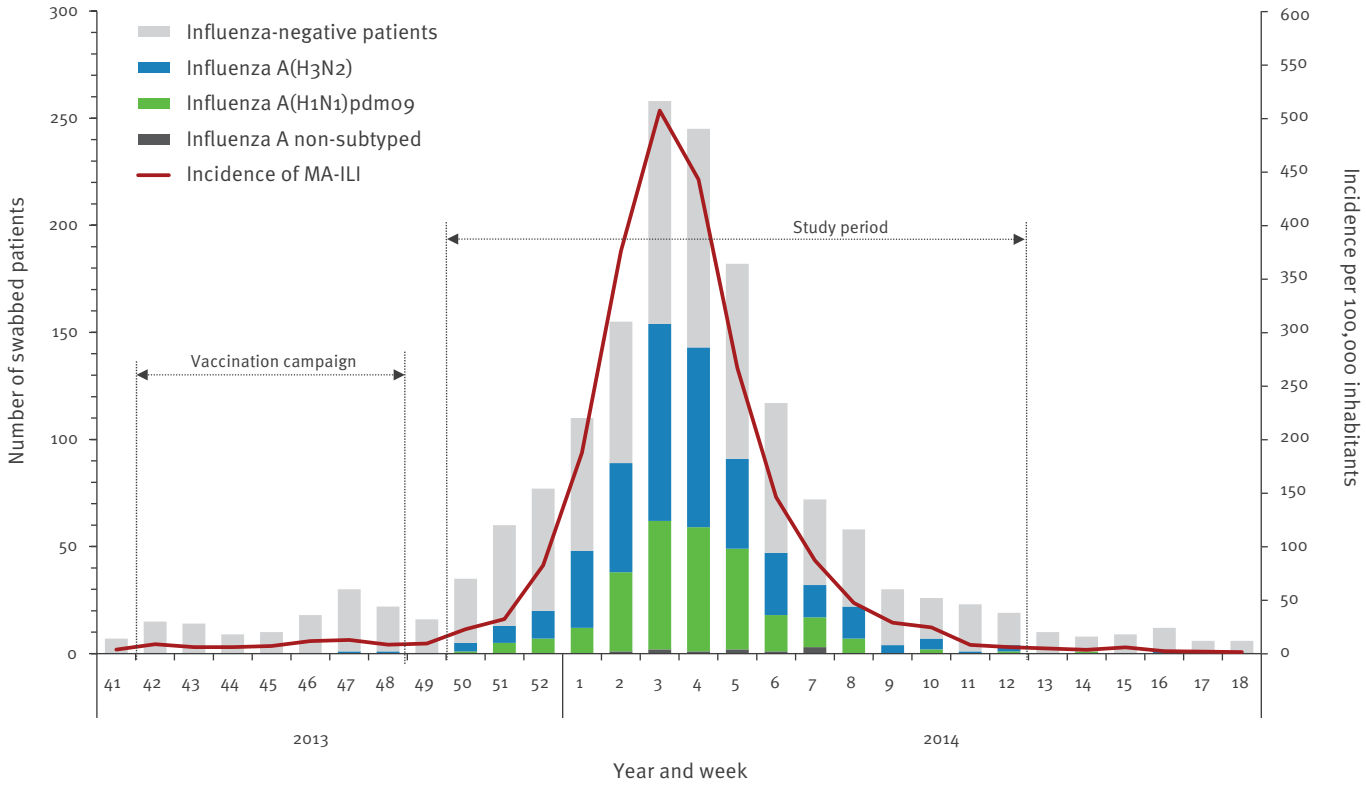
Influenza produces annual epidemics that spread widely in the susceptible population. About 20% of children and 5% of adults worldwide develop symptomatic influenza each year [1]. This exposure could confer immunity that would protect against the same virus type and subtype in subsequent seasons. Since the 2009 pandemic, influenza virus A(H1N1)pdm09, A(H3N2) and B have been alternating, thus part of the population may have acquired natural immunity after exposure to these viruses [2].

In serological surveys, nearly all children aged nine years or older had antibodies against influenza A [3]. However, this does not mean that they are totally protected against this virus type, since antigenic drift of the influenza virus allows it to escape immune control. Differences in protection could not be accounted for by differences in serum haemagglutination inhibition titres, demonstrating that multiple immune mechanisms induced by natural infection confer resistance to influenza [4,5].

Annual influenza vaccination is the primary measure to prevent influenza and its consequences [1]. Trivalent seasonal influenza vaccines include strains of influenza A(H1N1), A(H3N2) and B. In the 2013/14 season, the influenza vaccine composition recommended in the northern hemisphere included an A/California/7/2009(H1N1)pdm09-like virus, an A(H3N2) virus antigenically similar to the cell-propagated prototype virus A/Victoria/361/2011, and a B/Massachusetts/2/2011-like virus [6].

Figure

Weekly incidence of patients with medically attended influenza-like illness and number of swabbed patients by test result, Navarre, Spain, influenza season 2013/14 (n = 1,170 in the study period)



MA-ILI: medically attended influenza-like illness.

During the 2013/14 season, influenza A(H1N1)pdm09 and A(H3N2) viruses co-circulated in Spain and the rest of Europe, and most characterised isolates were A/StPetersburg/27/2011(H1N1)pdm09-like and A/Texas/50/2012(H3N2)-like [7-9].

Although both natural infection and vaccination with inactivated vaccine stimulate serum haemagglutination inhibition antibodies and provide protection against homologous wild-type influenza strains, the protection associated with natural infection lasts longer and is broader than that induced by inactivated vaccine [10,11]. However, the effect of natural immunity and its practical relevance are not generally evaluated. The aim of this study was to estimate the effects of previous influenza episodes and of the trivalent vaccine in preventing inpatient and outpatient cases with laboratory-confirmed influenza in Navarre, Spain, in the 2013/14 season.

Methods

Study population

This study was performed in the region of Navarre, Spain. The Regional Health Service provides healthcare, free at point of service, to 97% of the population. The Navarre Ethical Committee for Medical Research approved the study protocol.

The seasonal vaccination campaign took place from 14 October to 30 November 2013. The trivalent inactivated split non-adjuvanted vaccine was recommended and offered free of charge to people aged 60 years or older and to those with risk factors or major chronic conditions [12]. Other people were also vaccinated if they paid for the vaccine.

In the 2013/14 season and the preceding seasons, influenza surveillance was based on automatic reporting of cases of medically-attended influenza-like illness (MA-ILI) from all primary healthcare centres and hospitals. ILI was considered to be the sudden onset of any general symptom (fever or feverishness, malaise, headache or myalgia) and any respiratory symptom (cough, sore throat or shortness of breath). In addition, a sentinel network composed of a representative sample of primary healthcare physicians, covering 16% of the Navarre population, was asked to take double

Table 1

Predominant circulating influenza virus strains in Navarre, Spain, in the season analysed (2013/14) and the five previous seasons (2008/09–2012/13)

Influenza season	Predominant influenza type/subtype	Predominant genotype	Periods when more than 25% of patients tested positive to the predominant virus type/subtype	Proportion of positive swabs
2008/09	A(H3N2)	A/Brisbane/10/2007(H3N2)	16 Nov 2008 – 1 Feb 2009	70%
2009/10	A(H1N1)pdm09	A/California/7/2009(H1N1)	28 Jun 2009 – 9 Sep 2009 4 Oct 2009 – 20 Dec 2009	51%
2010/11	A(H1N1)pdm09	A/California/07/2009(H1N1)	21 Nov 2010 – 13 Feb 2011	59%
2011/12	A(H3N2)	A/Victoria/361/2011(H3N2) A/England/259/2011(H3N2) A/Iowa/19/2010(H3N2)	23 Dec 2011 – 11 Mar 2012	67%
2012/13	B	B/Estonia/55669/2011 B/Wisconsin/1/2010	31 Dec 2012 – 7 Apr 2013	64%
2013/14	A(H3N2) A(H1N1)pdm09	A/Texas/50/2012(H3N2) A/StPetersburg/27/2011(H1N1)	9 Dec 2013 – 23 Mar 2014	50%

swabs, nasopharyngeal and pharyngeal, after obtaining verbal informed consent, from all their patients diagnosed with ILI whose symptoms had begun less than five days before the consultation. The protocol for influenza cases in hospitals foresees nasopharyngeal and pharyngeal swabbing of all hospitalised patients with ILI.

Swabs were analysed by real-time RT-PCR, using either of two commercial real-time RT-PCR assays: RealCycler FLURSV (Progenie Molecular, Spain) and Real Time Ready Influenza A(H1N1) Detection Set (Roche Diagnostics, Switzerland). Detection of influenza A and B was based on the matrix protein gene and subtyping was based on the haemagglutinin (HA) gene. The internal amplification control was positive in all influenza-negative samples, indicating that failure to detect influenza virus was not due to inhibition.

Strains systematically selected among culture-positive samples by week and virus type/subtype were sent to the National Influenza Centre laboratory in Madrid for genetic characterisation based on partial sequencing of the HA gene (subunit HA1).

Study design and statistical analysis

We carried out a test-negative case–control study in the population covered by the Navarre Health Service. Healthcare workers, persons living in nursing homes and children under six months of age were excluded. The study included the consecutive weeks in which influenza virus was detected, i.e. the period from 9 December 2013 (week 50) to 23 March 2014 (week 12). All information related to patients was linked using a unique identification number.

The cases were MA-ILI patients in primary healthcare or in hospitals for whom influenza virus infection was confirmed by RT-PCR, and the controls were MA-ILI patients who tested negative for influenza virus. Their vaccination status for the trivalent seasonal influenza vaccine was obtained from the regional vaccination register [13]. Subjects were considered to be protected starting 14 days after vaccine administration.

From the electronic records of epidemiological and virological surveillance we obtained information on MA-ILI diagnosis and RT-PCR-positive patients in previous seasons for the study subjects. We defined previous MA-ILI related to a specific virus subtype as a laboratory-confirmed influenza infection with this virus subtype (virological criterion) that had occurred in the seasons from 2008/09 through 2012/13 or as MA-ILI that occurred in these seasons in weeks where more than 25% of swabs were confirmed for this influenza virus subtype (epidemiological criterion). Five previous seasons were considered given the long-lived protection associated with natural infection [10,11] and because no major shift had affected the circulating viruses involved in the analysis. Table 1 shows the periods when more than 25% of patients tested positive to the predominant virus type/subtype and the average percentage of swabbed patients who tested positive for the predominant circulating influenza virus by season. Finally, previous MA-ILI related to any influenza virus included all laboratory-confirmed influenza cases or MA-ILI patients that had occurred in the seasons 2008/09 through 2012/13 in weeks with more than 25% of swabs confirmed for any influenza virus, although on average 64% of swabbed patients tested positive for any influenza virus during these periods.

Percentages were compared by chi-square test. The odds of influenza vaccination and the odds of MA-ILI in the previous five seasons were compared between cases and controls. Logistic regression was used to calculate the odds ratios (OR) with their 95% confidence intervals (CI), adjusting for sex, age group (<5, 5–14, 15–44, 45–64 and ≥65 years), major chronic conditions (heart disease, respiratory disease, renal disease,

Table 2

Characteristics of patients with medically-attended influenza-like illness included in the test negative case–control analysis, by test result, Navarre, Spain, 2013/14 season (n = 1,170)

	Test-negative controls		Influenza cases[a]		p value	A(H1N1)pdm09		A(H3N2)		p value
	n	%	n	%		n	%	n	%	
Age groups (years)					<0.001					<0.001
<5	108	19	29	5		13	6	16	5	
5–14	36	6	34	6		16	7	18	5	
15–44	125	22	196	33		84	36	111	32	
45–64	108	19	163	28		80	34	81	23	
≥65	204	35	167	28		42	18	123	35	
Sex					0.295					0.754
Male	290	50	312	53		127	54	184	53	
Female	291	50	277	47		108	46	165	47	
Month of sample collection					<0.001					0.508
December	99	17	49	8		15	6	34	10	
January	306	53	435	74		179	76	253	72	
February	140	24	96	16		38	16	56	16	
March	36	6	9	2		3	1	6	2	
Residence					0.933					0.970
Rural	167	29	168	29		67	29	99	28	
Urban	414	71	421	71		168	71	250	72	
Major chronic conditions					0.116					0.021
No	285	49	316	54		140	60	174	50	
Yes	296	51	273	46		95	40	175	50	
Healthcare setting [b]					<0.001					0.969
Primary healthcare	182	31	345	59		139	59	205	59	
Hospital	400	69	245	42		97	41	144	41	
Seasonal influenza vaccine 2013/14					<0.001					0.001
No	383	66	445	76		195	83	246	70	
Yes	198	34	144	24		40	17	103	30	
Seasonal influenza vaccine 2012/13					0.006					0.003
No	395	68	443	75		192	82	247	71	
Yes	186	32	146	25		43	18	102	29	
Previous MA-ILI [c]					0.251					0.631
No	523	90	527	89		208	89	314	90	
Virological criteria	13	2	7	1		4	2	3	1	
Epidemiological criteria	45	8	55	9		23	10	32	9	
Previous MA-ILI related to A(H1N1)pdm09 [c]					0.487					0.240
No	546	94	559	95		226	96	328	94	
Yes	35	6	30	5		9	4	21	6	
Previous MA-ILI related to A(H3N2) [c]					0.719					0.022
No	559	96	569	97		222	94	342	98	
Yes	22	4	20	3		13	6	7	2	
Total	**581**	**100**	**589**	**100**		**235**	**100**	**349**	**100**	

MA-ILI: medically attended influenza-like illness.

[a] Includes seven cases of not subtyped influenza A. Two patients had simultaneous positive test results for influenza A(H1N1)pdm09 and influenza A(H3N2).

[b] Two patients were attended in primary healthcare and referred to hospital.

[c] Medically-attended influenza-like illness virologically or epidemiologically related to influenza in the previous five seasons.

Table 3

Characteristics of patients with medically-attended influenza-like illness, by previous influenza diagnosis and influenza vaccination status, Navarre, Spain, 2013/14 season (n = 1,170)

	Total tested	Previous MA-ILI[a]		p value	Influenza vaccination		p value
	n	n	%		n	%	
Age groups (years)				<0.001			<0.001
<5	137	5	4		15	11	
5–14	70	25	36		9	13	
15–44	321	53	17		28	9	
45–64	271	24	9		53	20	
≥65	371	13	4		237	64	
Sex				0.597			0.249
Male	602	59	10		167	28	
Female	568	61	11		175	31	
Residence				0.939			0.896
Rural	835	86	10		245	29	
Urban	335	34	10		97	29	
Major chronic conditions				0.046			<0.001
No	601	72	12		72	12	
Yes	569	48	8		270	47	
Healthcare setting [a]				<0.001			<0.001
Primary healthcare	527	88	17		74	14	
Hospital	645	32	5		269	42	
Previous MA-ILI [b]				NA			0.001
No	1,050	0	0		322	31	
Yes	120	120	100		20	17	
Total	**1,170**	**120**	**10**		**342**	**29**	

MA-ILI: medically attended influenza-like illness; NA: not applicable.

[a] Two patients were attended in primary healthcare and referred to hospital.

[b] Medically-attended influenza-like illness virologically or epidemiologically related to any influenza virus in the previous five seasons.

cancer, diabetes mellitus, liver cirrhosis, dementia, stroke, immunodeficiency, rheumatic disease and body mass index ≥ 40 kg/m^2), month of sample collection and healthcare setting (primary healthcare and hospital). Separate analyses were done by type/subtype of influenza, age group and healthcare setting. The fraction of prevented disease in exposed individuals or vaccine effectiveness (VE) was estimated as (1 – OR) x 100.

Results

During the 2013/14 season in Navarre, the incidence of MA-ILI, the number of swabbed patients and the number of influenza-positive cases followed similar trends, peaking in week 3 of 2014 (Figure).

In the study period, a total of 1,170 MA-ILI patients were swabbed, of whom 525 were attended in primary healthcare and 645 were hospitalised. A total of 589 (50%) were confirmed for influenza virus, all of them for influenza A. Influenza A(H3N2) virus was detected in 349 cases, influenza A(H1N1)pdm09 in 235, and seven remained non-subtyped. Two patients had a simultaneous positive test result for influenza A(H1N1)pdm09 and A(H3N2). Sequence analysis of the amplification product (the HA1 fragment of the haemagglutinin gene) was available for 114 influenza viruses. All 42 A(H1N1) pdm09 viruses were A/StPetersburg/27/2011-like and all 72 A(H3N2) viruses were A/Texas/50/2012-like.

Compared with the test-negative controls (n = 581), confirmed cases of influenza were more frequent among 15 to 64 years-olds (61% vs 40%; p < 0.001) and those attended in primary healthcare (58% vs 31%; p < 0.001). Compared with influenza A(H1N1)pdm09, influenza A(H3N2) was more frequently detected in persons 65 years or older (35% vs 18%; p < 0.001) and in persons with major chronic conditions (50% vs 40%; p = 0.021). The proportion of hospitalised patients was the same for both influenza A(H1N1)pdm09 and A(H3N2) cases (41% vs 41%; p = 0.970) (Table 2).

A similar proportion of laboratory-confirmed cases and influenza-negative controls had had MA-ILI in the previous five seasons (11% vs 10%; p = 0.759), but only 17% of them (20/120) had been laboratory-confirmed for influenza virus in the previous episode. Of the 120 patients who had had any MA-ILI episode in the previous five years, 18 had had more than one episode and only one had had two episodes related to the same virus subtype. Among the 589 cases, 144 (24%) had

Table 4a

Preventive effect of previous episodes of medically-attended influenza-like illness and of the trivalent inactivated influenza vaccine against new cases of laboratory-confirmed influenza in Navarre, Spain, 2013/14 season (n = 1,170)

	Cases; controls	Crude prevented fraction % (95% CI)	p value	Adjusted prevented fraction % (95% CI)[a]	p value
All influenza cases vs controls					
All swabbed patients	589; 581				
Previous MA-ILI related to any influenza[b]	62; 58	−6 (−55 to 27)	0.759	30 (−7 to 54)	0.098
Vaccinated	144; 198	37 (19 to 51)	<0.001	31 (5 to 50)	0.023
Age<65 years	422; 377				
Previous MA-ILI related to any influenza[b]	56; 51	2 (−47 to 35)	0.915	32 (−9 to 57)	0.107
Vaccinated	44; 61	40 (9 to 60)	0.017	35 (−5 to 60)	0.081
Age≥65 years	167; 204				
Previous MA-ILI related to any influenza[b]	6; 7	−5 (−218 to 65)	0.933	21 (−153 to 75)	0.694
Vaccinated	100; 137	27 (−12 to 52)	0.147	28 (−11 to 54)	0.139
Primary healthcare patients[c]	345; 182				
Previous MA-ILI related to any influenza[b]	52; 36	28 (−15 to 55)	0.169	34 (−9 to 60)	0.103
Vaccinated	47; 27	9 (−51 to 46)	0.703	21 (−45 to 57)	0.452
Hospitalised patients[c]	245; 400				
Previous MA-ILI related to any influenza[b]	10; 22	27 (−57 to 66)	0.422	21 (−82 to 65)	0.585
Vaccinated	97; 172	13 (−20 to 37)	0.394	35 (4 to 56)	0.030
Influenza A(H1N1)pdm09 cases vs controls					
All swabbed patients	235; 581				
Previous MA-ILI related to A(H1N1)pdm09[b]	9; 35	38 (−31 to 71)	0.213	63 (16 to 84)	0.017
Vaccinated	40; 198	60 (42 to 73)	<0.001	45 (12 to 65)	0.013
Age<65 years	193; 377				
Previous MA-ILI related to A(H1N1)pdm09[b]	6; 33	67 (19 to 86)	0.016	78 (43 to 91)	0.002
Vaccinated	16; 61	53 (16 to 74)	0.010	52 (8 to 75)	0.028
Age≥65 years	42; 204				
Previous MA-ILI related to A(H1N1)pdm09[b]	3; 2	−677 (−4,700 to −26)	0.027	−613 (−4,470 to −11)	0.038
Vaccinated	24; 137	35 (−28 to 67)	0.216	37 (−27 to 69)	0.193
Primary healthcare patients[c]	139; 181				
Previous MA-ILI related to A(H1N1)pdm09[b]	7; 24	65 (16 to 85)	0.018	70 (26 to 88)	0.010
Vaccinated	13; 27	41 (−20 to 71)	0.144	43 (−28 to 75)	0.171
Hospitalised patients[c]	97; 400				
Previous MA-ILI related to A(H1N1)pdm09[b]	2; 11	25 (−242 to 84)	0.704	−6 (−427 to 79)	0.944
Vaccinated	27; 172	49 (17 to 69)	0.007	45 (1 to 69)	0.047

CI: confidence interval; MA-ILI: medically attended influenza-like illness.

a Results obtained from a logistic regression model adjusted for sex, age group (<5, 5–14, 15–44, 45–64 and≥65 years), month of sample collection, major chronic conditions, healthcare setting (primary healthcare and hospital), medically-attended influenza-like illness virologically or epidemiologically related to the analysed influenza virus in the previous five seasons, and 2013/14 influenza vaccine.

b Medically-attended influenza-like illness virologically or epidemiologically related to influenza in the previous five seasons.

c Patients attended in primary healthcare and referred to hospital were included in both subanalyses.

Table 4b

Preventive effect of previous episodes of medically-attended influenza-like illness and of the trivalent inactivated influenza vaccine against new cases of laboratory-confirmed influenza in Navarre, Spain, 2013/14 season (n = 1,170)

	Cases; controls	Crude prevented fraction % (95% CI)	p value	Adjusted prevented fraction % (95% CI)[a]	p value
All influenza cases vs controls					
Influenza A(H3N2) cases vs controls					
All swabbed patients	349; 581				
Previous MA-ILI related to A(H3N2)[b]	7; 22	48 (−23 to 78)	0.137	65 (13 to 86)	0.024
Vaccinated	103; 198	19 (−8 to 39)	0.150	20 (−15 to 45)	0.228
Age<65 years	226; 377				
Previous MA-ILI related to A(H3N2)[b]	5; 19	57 (−16 to 84)	0.095	70 (15 to 90)	0.024
Vaccinated	28; 61	27 (−19 to 55)	0.205	9 (−59 to 48)	0.727
Age≥65 years	123; 204				
Previous MA-ILI related to A(H3N2)[b]	2; 3	−11 (−573 to 82)	0.911	29 (−400 to 90)	0.731
Vaccinated	75; 137	24 (−22 to 52)	0.257	24 (−24 to 53)	0.269
Primary healthcare patients[c]	205; 182				
Previous MA-ILI related to A(H3N2)[b]	6; 14	64 (7 to 86)	0.042	64 (−1 to 87)	0.051
Vaccinated	34; 27	−14 (−99 to 34)	0.637	0 (−94 to 48)	0.995
Hospitalised patients[c]	144; 400				
Previous MA-ILI related to A(H3N2)[b]	1; 8	66 (−176 to 96)	0.315	65 (−198 to 96)	0.334
Vaccinated	69; 172	−22 (−79 to 17)	0.309	28 (−14 to 54)	0.159

CI: confidence interval; MA-ILI: medically attended influenza-like illness.

a Results obtained from a logistic regression model adjusted for sex, age group (<5, 5–14, 15–44, 45–64 and≥65 years), month of sample collection, major chronic conditions, healthcare setting (primary healthcare and hospital), medically-attended influenza-like illness virologically or epidemiologically related to the analysed influenza virus in the previous five seasons, and 2013/14 influenza vaccine.

b Medically-attended influenza-like illness virologically or epidemiologically related to influenza in the previous five seasons.

c Patients attended in primary healthcare and referred to hospital were included in both subanalyses.

received the 2013/14 seasonal vaccine, vs 198 (34%) of the 581 controls (p<0.001) (Table 2).

The proportion of patients vaccinated in the current season was lower among those with previous MA-ILI than in those without a history of MA-ILI (17% vs 31%; p = 0.001). While previous MA-ILI was more frequent in patients between five and 44 years-old, in those without major chronic conditions and in those attended in primary healthcare, vaccination in the current season was more frequent in patients 65 years and older, in those with major chronic conditions and in patients attended in hospitals (Table 3).

In the analysis adjusted by influenza vaccination and other potential confounders, previous MA-ILI related to any influenza virus showed a 30% (95% CI: −7 to 54) protection against a new episode of laboratory-confirmed influenza, although this did not reach statistical significance. The overall adjusted estimate of the influenza VE was 31% (95% CI: 5–50). The estimate of the VE was 21% (95% CI: −45 to 57) in the analysis restricted to primary healthcare patients, and 35% (95% CI: 4–56) in hospitalised patients (Table 4).

In the comparison between influenza A(H1N1)pdm09 cases and controls, previous episodes of MA-ILI related to A(H1N1)pdm09 virus were 63% (95% CI: 16–84) protective against laboratory-confirmed A(H1N1)pdm09 influenza, even though the natural exposure had in most cases occurred more than two years before. The protective effect was similar in the analysis restricted to patients attended in primary healthcare and to those younger than 65 years. One case without comorbidity that had been confirmed with influenza A(H1N1)pdm09 in the 2009/10 season was again confirmed with influenza from the same virus subtype in the 2013/14 season. In the same models, the overall adjusted VE was

45% (95% CI: 12–65), and similar estimates of the VE were found in the analysis stratified by age group or healthcare setting (Table 4).

The comparison of influenza A(H3N2) cases and controls showed that previous episodes of MA-ILI related to A(H3N2) virus were 65% (95% CI: 13–86) protective against laboratory-confirmed influenza A(H3N2) and 70% (95% CI: 15–90) protective in the analysis restricted to patients younger than 65 years. On the other hand, the overall adjusted VE was 20% (95% CI: –15 to 45), and other estimates of the VE for subgroups of patients were also low and not statistically significant (Table 4). In most cases, the natural exposure had occurred more than a year before.

Minor differences in the VE estimates were seen in the sensitivity analysis performed after excluding the variable of previous MA-ILI from the model. The overall estimate of the influenza VE was 31% (95% CI: 5–50) against any laboratory-confirmed influenza, 45% (95% CI: 12–65) against influenza A(H1N1)pdm09, and 20% (95% CI: –16 to 44) in preventing influenza A(H3N2) cases. The same estimates after excluding from the analysis the patients with previous MA-ILI that was probably related to influenza were 33% (95% CI: 6–52), 48% (95% CI: 27–68) and 19% (95% CI: –18 to 44), respectively.

The sensitivity analysis excluding vaccinated patients also showed similar protective effects of previous episodes of MA-ILI probably related to influenza: 32% (95% CI: –8 to 58) for any influenza, 77% (95% CI: 40–91) for influenza A(H1N1)pdm09 and 63% (95% CI: –3 to 86) for influenza A(H3N2).

Discussion

In this study we estimated at the same time the protection conferred by previous episodes of MA-ILI and by influenza vaccination in a season with intense co-circulation of influenza A(H1N1)pdm09 and A(H3N2). People with a history of MA-ILI attributable to a specific virus subtype in the previous five seasons had a markedly lower risk of disease due to the same subtype. The trivalent inactivated vaccine showed moderate VE in preventing laboratory-confirmed influenza A(H1N1)pdm09 and low effectiveness against influenza A(H3N2). Even though the natural exposure had in most cases occurred more than a year before, it conferred the same or greater protection against the same virus subtype than the vaccine administered a few months previously. In accordance with McLean et al., five previous seasons were considered for natural protection [14] because the protection following natural exposure is stronger and longer-lasting and covers a greater variety of viral strains, which has been related to activation of a more complete immune response that includes mechanisms of cellular immunity [4,15,16]. No major shift had affected the circulating viruses involved in the analysis.

It was possible to define the virus that most probably caused the cases of MA-ILI in the previous five seasons thanks to the fact that one virus clearly predominated in Navarre in each of those five seasons. In seasons with simultaneous co-circulation of various viruses, it would be more difficult to attribute the cases of MA-ILI with certainty to a specific virus subtype.

Since the appearance of the A(H1N1)pdm09 virus in 2009, the circulating strains of this virus have been well matched with the vaccine strain A/California/7/2009(H1N1) [2], which could explain the protection of the vaccine and of influenza episodes in previous seasons.

Although the influenza A(H3N2) virus strains which circulated in the 2013/14 season had a good genetic match with the vaccine strain [2], the observed VE was low. However, this virus showed a high cross protection with the strains circulating in the previous seasons 2008/09 and 2011/12. This difference between natural and vaccine protection with matched strains should encourage the exploration of alternative ways of obtaining better vaccines against influenza.

In the study population, natural and vaccine immunity were distributed in a complementary manner. A history of MA-ILI was more frequent in persons aged five to 44 years, which explains why this protective mechanism was more important in population groups that do not normally get vaccinated against influenza.

Although previous diagnosis of disease from the same virus subtype was associated with high protection, previous MA-ILI related to any influenza virus but not restricted to the same virus subtype conferred only low protection against a new episode of laboratory-confirmed influenza. This is mainly explained by the likelihood of infection by a different type or subtype of influenza virus. Therefore, in persons with risk factors for influenza complications, having had the disease in previous seasons should not be a reason not to get vaccinated. While natural exposure protects specifically against the virus subtype to which one has been exposed, the protection conferred by the trivalent vaccine, although less strong, covers all three virus types/subtypes simultaneously.

Previous episodes of influenza are not usually taken into account as potential confounding factors in studies evaluating influenza VE. To our knowledge, only McLean et al. had adjusted for influenza diagnoses in the prior five seasons in the analysis of influenza vaccine effectiveness [14]. In this and in our study, the estimated VE did not change regardless of whether the models included this history, suggesting that this variable does not act as a confounding factor that needs to be controlled.

Although our end-of-season estimate of VE was additionally adjusted for previous episodes of influenza,

it was consistent with mid-season estimates obtained in Navarre and Spain for this same season [17,18], and with estimates obtained at the end of the season in a European multicentre study and in Greece [19,20]; it was less consistent, however, with estimates from other countries with different distribution of virus types, subtypes and strains detected in the same season [21-23].

Some limitations should be considered in interpreting the results of this study. Previous episodes of MA-ILI reflect the history of exposures to the influenza virus from the healthcare perspective and may be considered a proxy for natural immunity. Some 10% of subjects included in the study had a history of MA-ILI in the previous five seasons. However, the proportion of the population with natural immunity against influenza could be considerably higher, since it is estimated that 30–50% of influenza infections are asymptomatic [24]. In one study conducted in Navarre, 36% of symptomatic cases had not sought medical care [25]. It should also be added that there is possible immunity from exposures occurring more than five years previously. This misclassification in the previous influenza infection is probably non-differential and would bias the estimates towards the null effect. In the absence of this bias, the protection due to previous episodes would have been higher.

Of the patients with a previous episode of MA-ILI, only 17% had a laboratory-confirmed diagnosis, while the rest met only one epidemiological criterion for the disease. Based on the percentage of swabs confirmed for influenza in each season (Table 1), we estimate that this criterion ensures the correct classification of 70% of cases with a history of influenza A(H3N2), of over 50% of cases with a history of influenza A(H1N1) pdm09, and of 64% of cases with a history of any influenza in the previous five years. Accordingly, we cannot totally rule out the possibility of incorrect classification that arose from considering cases that could have been due to another cause such as previous episodes related to a specific virus. If we had had laboratory confirmation of all the cases of influenza in previous years, the protective effect of this history would probably have been greater.

The results presented had limited statistical power for some analyses, mainly because of the low numbers of cases and controls with previous MA-ILI included in the study. Laboratory-confirmed cases were compared with controls recruited in the same healthcare settings before either patient or physician knew the laboratory result, a fact that reduced selection bias [26].

This study included MA-ILI patients recruited from the same population in both primary healthcare centres and hospitals. The healthcare setting could have acted as a confounding factor, therefore the analyses were adjusted for this variable. The possibility that the healthcare setting might have modified the effect or biased the results can be ruled out given the consistency of the estimates obtained in these two patient groups and in the joint analysis. The joint analysis achieved representation of the whole spectrum of patients with influenza in the population.

Conclusion

Our results suggest low to moderate influenza VE in the 2013/14 season, which prevented almost a third of the influenza cases and hospitalisations in the vaccinated population; while not entirely satisfactory, this result is important in terms of individual and public health. Previous influenza episodes were highly effective against new influenza illness by the same virus subtype, and this effect seemed to persist over various seasons, which may point to possible avenues of obtaining better vaccines against influenza. In any case, annual influenza vaccination remains the principal preventive option in persons at high risk of developing complications if they contract influenza.

The members of the Primary Health Care Sentinel Network of Navarra are

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Conflict of interest

None declared.

Authors' contributions

J Castilla, I Martínez-Baz and M Guevara designed the study, coordinated the activities, and undertook the statistical analysis. A Navascués, M Fernández-Alonso, G Reina and C Ezpeleta were responsible of the virological analysis and the interpretation of laboratory results. M García Cenoz, N Álvarez, F Irisarri and I Casado participated in the data collection. E Albéniz coordinated the activities in primary health care. F Pozo was responsible for the virus characterizations. J Castilla, M Guevara and I Martínez-Baz wrote the draft manuscript, and all authors revised and approved the final version.

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Research article

Public preferences for vaccination programmes during pandemics caused by pathogens transmitted through respiratory droplets – a discrete choice experiment in four European countries, 2013

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This study aims to quantify and compare preferences of citizens from different European countries for vaccination programme characteristics during pandemics, caused by pathogens which are transmitted through respiratory droplets. Internet panel members, nationally representative based on age, sex, educational level and region, of four European Union Member States (Netherlands, Poland, Spain, and Sweden, n = 2,068) completed an online discrete choice experiment. These countries, from different geographical areas of Europe, were chosen because of the availability of high-quality Internet panels and because of the cooperation between members of the project entitled Effective Communication in Outbreak Management: development of an evidence-based tool for Europe (ECOM). Data were analysed using panel latent class regression models. In the case of a severe pandemic scenario, vaccine effectiveness was the most important characteristic determining vaccination preference in all countries, followed by the body that advises on vaccination. In Sweden, the advice of family and/or friends and the advice of physicians strongly affected vaccine preferences, in contrast to Poland and Spain, where the advice of (international) health authorities was more decisive. Irrespective of pandemic scenario or vaccination programme characteristics, the predicted vaccination uptakes were lowest in Sweden, and highest in Poland. To increase vaccination uptake during future pandemics, the responsible authorities should align with other important stakeholders in the country and communicate in a coordinated manner.

Introduction

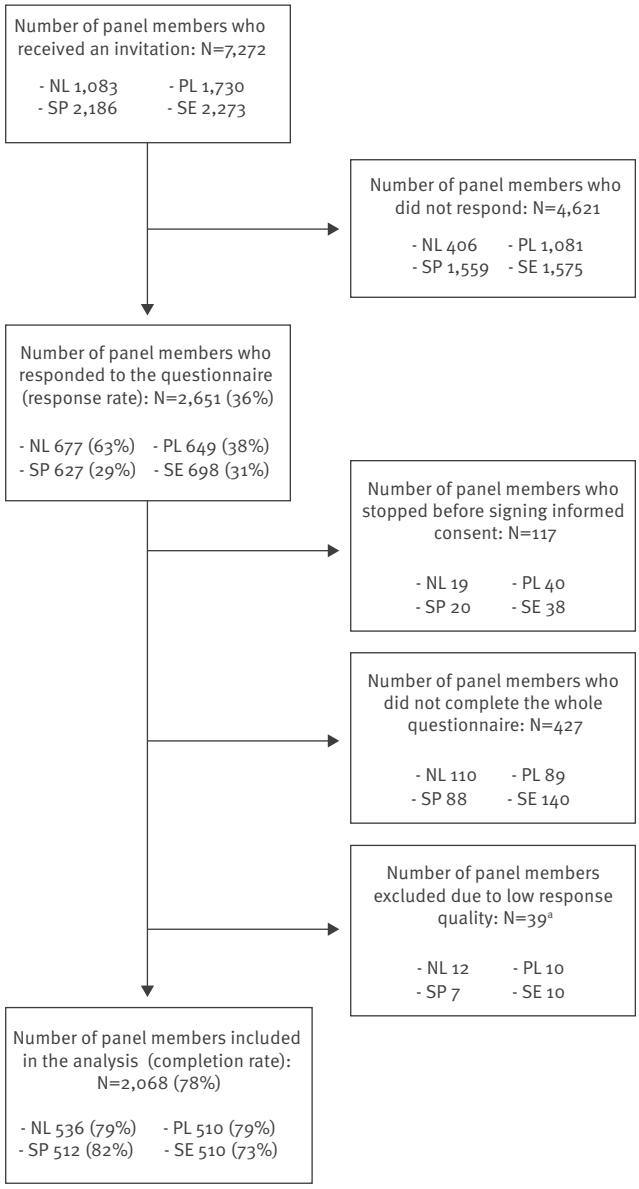
In the past 100 years, there have been several large-scale influenza outbreaks with worldwide impact. These include the 1918 influenza A(H1N1) pandemic that caused between 50 and 100 million deaths particularly in many healthy young adults [1], and more recently the 2009 influenza A(H1N1)pdm09 pandemic [2]. Though characteristics (such as clinical attack rates and pathogenicity) and occurrence of a next influenza pandemic are unpredictable, experts agree there will be future influenza pandemics [2-5].

The World Health Organisation (WHO) urged countries to develop or update national influenza preparedness plans in response to the avian influenza A(H5N1) pandemic threat in 2005 [6]. Such plans subsequently needed to be improved taking into account the lessons learnt from the response to the influenza A(H1N1) pdm09 pandemic [4,7,8]. In addition, countries could learn from each other by sharing information and best practices [9].

Preventive measures are very important in limiting the spread of an influenza pandemic [10-12] and if available, vaccination constitutes the control cornerstone [13,14]. The success of mitigating influenza pandemics depends on many factors, including national public health policies and the availability of vaccines, vaccine effectiveness, and the public's willingness to get vaccinated. Unfortunately, vaccination coverage has proven to be (too) low across Europe during the influenza A(H1N1)pdm09 pandemic. Vaccination coverage among the general public of the European Union, Norway and

Figure 1

Response to the survey to investigate public preferences for vaccination programmes during pandemics caused by pathogens transmitted through respiratory droplets, Netherlands, Poland, Spain, and Sweden, 2013



NL: Netherlands; PL: Poland; SE: Sweden; SP: Spain.

[a] Low response quality was defined as completing the survey in less than 4 min.

Iceland, varied between countries from 0.4% to 59% [15].

Countries within Europe differ from each other with regard to languages, cultures, public trust in health authorities, health system infrastructures, and public health capabilities and capacities. Research has shown that implementing international guidelines at the local level can be a complex process [16]. Having insights into country-specific reasons to accept or decline pandemic influenza vaccination can facilitate the adaptation of preparedness plans, including vaccination strategies, to the local situation [17].

Thus far, only a limited number of reports have focused on the comparison of pandemic influenza vaccination preferences between people of different European countries [18,19], and formal quantitative techniques such as discrete choice experiments (DCEs) [20,21] have not yet been used. The primary aim of this study was to quantify and compare the preferences of European citizens for vaccination programmes for future pandemics. Although we focus on influenza pandemics, we quantified vaccination programme preferences for any emerging or re-emerging large-scale infectious disease outbreak that spreads through respiratory droplets. Our findings might therefore also be applicable to other respiratory infections than influenza, such as, for example, severe acute respiratory syndrome (SARS)-coronavirus (CoV) or Middle East Respiratory Syndrome (MERS)-CoV, should vaccines be available for these viruses in the future. A secondary aim was to calculate the expected uptake of vaccination under different pandemic scenarios. The approach and results might help health policymakers to improve pandemic preparedness plans and communication strategies, in order to make future vaccination programmes more successful.

Methods

Study population

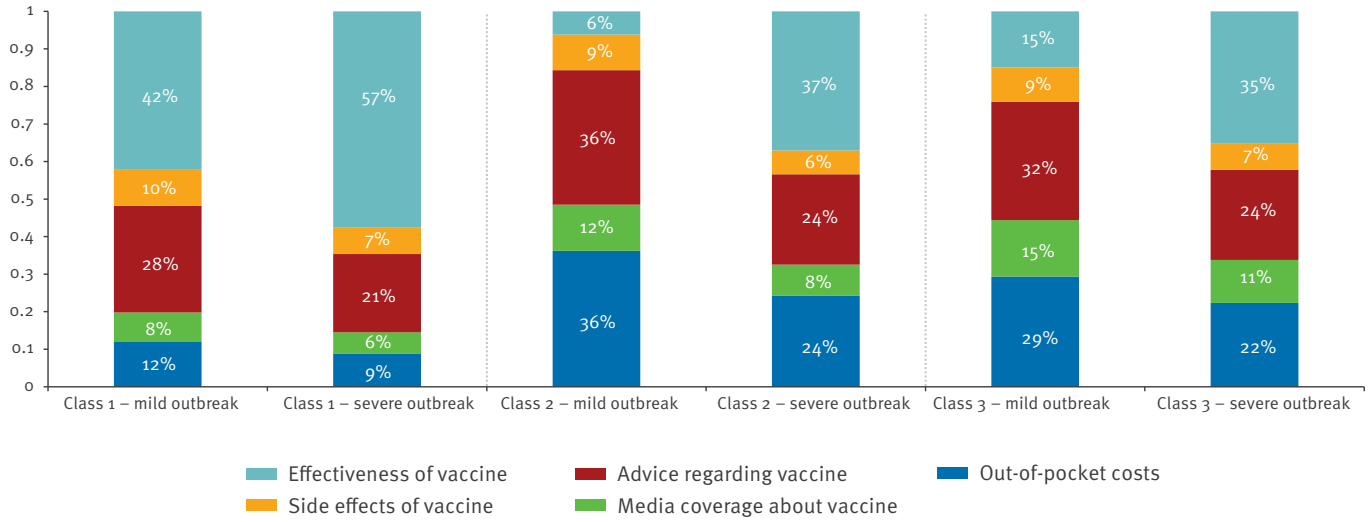
We surveyed a representative sample of the general public (age 18 years and over) of countries from different parts of Europe: eastern Europe (Poland), northern Europe (Sweden), southern Europe (Spain) and western Europe (Netherlands). These countries were chosen because of the availability of high-quality Internet panels (i.e. panels that are ISO certified and/or follow international quality standards for market research) and also because of the cooperation between project members of different work packages within the Effective Communication in Outbreak Management: development of an evidence-based tool for Europe (ECOM) project (www.ecomeu.info). The public health policies of the four included countries with respect to seasonal influenza and influenza A(H1N1)pdm09 are described in Table 1.

Discrete choice experiments

A DCE is a survey-based stated-preference methodology that originates in mathematical psychology [22]. The method has been increasingly used in healthcare, whereby the number of published DCEs has increased from a mean of three per year in the period from 1990 to 2000 to 45 per year between 2009 and 2012 [23]. In a DCE, the relative importance of characteristics (i.e. attributes) of a certain product or intervention is assessed by presenting a series of choice sets to respondents [20,21]. In each choice set, respondents are asked to choose a preferred alternative from a set

Figure 2

Relative importance of vaccination programme attributes for respondents' decision to get vaccinated in the case of mild and severe pandemic scenarios caused by pathogens transmitted through respiratory droplets, Netherlands, Poland, Spain, and Sweden, 2013 (n = 2,068)



The percentages represent the proportion of someone's preference that is based on that attribute (utility). A mild pandemic was defined as a pandemic in which 5% of the population gets the disease (pandemic scenario variable susceptibility), and 5% of the sick people developing severe symptoms (pandemic scenario variable severity). A severe pandemic was defined as a pandemic in which 20% of the population gets the disease (pandemic scenario variable susceptibility), and 75% of the sick people develop severe symptoms scenario variable (pandemic scenario variable severity).

of two or more hypothetical product or intervention alternatives with systematically varying attribute levels [20,21].

Survey

The survey started with an explanation of the DCE exercise. Next, respondents were asked to imagine that a large-scale emerging infectious disease, that started abroad, had spread to the country they lived in. It was stated that the disease spreads through respiratory droplets, that it was vaccine-preventable, and that vaccines were available in their country. Respondents then completed a series of choice sets, followed by questions about socio-demographic characteristics (including previous vaccination experiences), and questions that assessed the perceived difficulty of the survey. The survey ended with an open question in which respondents were given the opportunity to comment on the survey.

In each choice set, a hypothetical pandemic scenario based on two disease variables (susceptibility to the disease (i.e. a number of 1,000 people will get sick) and severity of the disease (i.e. a number of the sick people will develop severe symptoms) was presented. Respondents were then asked to choose between three alternatives: no vaccination, vaccination A, and vaccination B. The vaccination was described by several attributes, and the presented levels differed systematically between vaccination A and vaccination B. In the following choice sets, both the pandemic scenario and the presented attribute levels for vaccination A and B differed. In order to select realistic, relevant and understandable attributes and attributes levels, we conducted a literature study, expert interviews, and focus group discussions. In addition, we closely cooperated with project members when selecting the attributes and levels. PubMed, Embase and Psychinfo were strategically searched for relevant research articles on vaccination preferences. Expert interviews (n = 9) were conducted with both national and international experts (physicians, researchers, policymakers) in the field of infectious diseases, vaccinations, preventive behaviour, and implementation of prevention. We conducted eight focus group discussions with representatives of the general population, of which four in the Netherlands, two in Poland, two with Spanish citizens during their temporary stay in the Netherlands, and two in Sweden. Eligible participants were recruited by research companies and via our network, using purposive sampling to ensure a diverse sample. The focus groups revealed that similar vaccination programme attributes and attribute levels could be included in the DCE for all countries (Table 2). It is not feasible to present a single respondent with all the possible combinations of the included attribute levels. We therefore generated a subset of 48 choice sets by minimizing the D-efficiency criterion using the software programme Ngene (ChoiceMetrics, version 1.1.1). The 48 choice sets were grouped in three different survey versions such that each block has (near) attribute level balance. Each respondent thus needed to answer 16 choice sets. For more information on this part of a discrete choice experiment, see e.g. Reed Johnson et al. [24].

Table 1

Overview of seasonal influenza and influenza A(H1N1)pdm09 policies per country, Netherlands, Poland, Spain, and Sweden, 2009 and 2013

Influenza type and respective policies	Netherlands	Poland	Spain	Sweden
Seasonal influenza [58]				
Groups recommended for vaccination during the 2012/13 influenza season	NA	Children and adolescents, aged ≥ 6 months – < 18 years	NA	NA
	Adults aged ≥ 60 years	Adults aged ≥ 55 years	Adults aged ≥ 65 years[a]	Adults aged ≥ 65 years
	Medical risk groups[b]	Medical risk groups[b]	Medical risk groups[b]	Medical risk groups[b]
	Pregnant women with medical conditions	All pregnant women	All pregnant women	Pregnant women in 2nd or 3rd trimester
	All HCWs	All HCWs	All HCWs	HCWs caring for persons who are severely immunocompromised
Payment scheme vaccine and administration during the 2012/13 influenza season	National health service Employer pays for HCWs	Payment scheme vaccine itself: out-of-pocket; some employers pay for HCWs; local government[c] Payment scheme administration: out-of-pocket; some employers pay for HCWs; local government[c]	Regional health service	Regional health service; out-of-pocket varies with regions[d] Employer pays for HCWs
Vaccination coverage during the 2012/13 influenza season	Overall adults aged ≥ 60 years: 67.8%	Overall adults aged ≥ 65 years: 7.4% HCWs: 9.5%	Overall adults aged ≥ 65 years: 57% HCWs: 22.9%	Overall adults aged ≥ 65 years: 44%
2009 influenza A(H1N1)pdm09 pandemic [19]				
Groups recommended for vaccination during the pandemic period	Children aged ≥ 6 months – 4 years, and household members of babies up to the age of 6 months	Poland did not implement a vaccination programme during the influenza A(H1N1)pdm09	NA	Recommended for all children aged ≥ 6 months – < 18 years
	Adults aged ≥ 60 years	NA	NA	Adults aged ≥ 18 years
	Medical risk groups[b]	NA	Medical risk groups[b]	Medical risk groups[b]
	Pregnant women in 2nd and 3rd trimester	NA	All pregnant women	All pregnant women
	HCWs with close contact with patients	NA	All HCWs	All HCWs
Vaccine brand	Pandemrix, Focetria	NA	Pandemrix, Focetria, and Panenza	Pandemrix
Vaccination sites	GPs, mass vaccination sites in community settings, Municipal Health Services (children and household contacts), and work environment	NA	GPs, hospital settings, and occupational health services	GPs, hospital settings, outpatient care clinics, occupational health services, mass vaccination sites
Payment scheme	Free of charge for all individuals recommended the vaccine	NA	Free of charge for all individuals recommended the vaccine	Free of charge for all individuals recommended the vaccine
Vaccination coverage during the pandemic period	Entire population: 30% Those at risk aged > 6 months: 72% Pregnant women: 58% HCWs: 50%	NA	Entire population: 27.1% Those at risk aged > 6 months: 23.7% Pregnant women: 9% HCWs: 11.6%	Entire population: 59%

GP: general practitioner; HCW: healthcare worker; NA: not applicable.

[a] Recommendation at the national level. However, 10 of 19 regions recommend vaccine for those ≥ 60 years.

[b] Medical risk groups include e.g. patients with chronic pulmonary, cardiovascular and renal diseases, metabolic disorders, and immunosuppression due to disease or treatment (we refer to [1] for more details).

[c] Local government reimbursement of cost of vaccine and administration for those ≥ 65 years of age.

[d] In some regions, the vaccine is charged a symbolic amount (ca 10 euros) for vaccine and vaccination.

Table 2

Attributes and attribute levels included in the survey investigating public preferences for vaccination programmes during pandemics caused by pathogens transmitted through respiratory droplets, Netherlands, Poland, Spain, and Sweden, 2013 (n = 7 attributes)

Scenario variables[a]	Levels
Pandemic scenario variables[a]	
Susceptibility to the disease[b]	5%, 10%, 20%
Severity of the disease[c]	5%, 25%, 50%, 75%
Vaccination programme attributes[d]	
Effectiveness of the vaccine	30%, 50%, 70%, 90%
Safety of the vaccine[e]	Unknown, expected to be safe *(reference level)*
	Unknown, no experience with similar vaccines yet
Advice regarding the vaccine	Family and/or friends recommend vaccination *(reference level)*
	Family and/or friends discourage vaccination
	Your doctor recommends vaccination
	Your doctor discourages vaccination
	Government and national institute of public health recommend vaccination
	International organisations recommend vaccination
Media attention about the vaccine[f]	Traditional media positive *(reference level)*
	Traditional media negative
	Social and interactive media positive
	Social and interactive media negative
Out-of-pocket costs[g]	0 euro, 50 euros, 100 euros

[a] The scenario variables were the same for all alternatives in one choice set.

[b] Defined as the proportion of population affected by the emerging disease, i.e. having symptoms.

[c] Defined as the proportion of the infected population that had severe symptoms or outcomes (death, life-threatening events, hospitalisation and severe or permanent disability).

[d] The attributes safety of the vaccine, advice about the vaccine and media attention about the vaccine were included in the latent class analysis as categorical variables.

[e] Safety of the vaccine with regard to long-term severe side effects (death, life-threatening events, hospitalisation, severe or permanent disability, or side effects leading to birth defects in an unborn fetus).

[f] Traditional media were defined as radio, newspapers and television. Social and interactive media were defined as blogs, Twitter and social network websites.

[g] The levels presented in the Table are the selected levels for the Netherlands. Levels for the out-of-pocket costs attribute were converted to local currency of the other three countries and adapted according to the Organisation for Economic Co-operation and Development (OECD) price levels of May 2013 [26]. Levels of: 0 zloty, 120 zlotys, 240 zlotys for Poland; 0 euro, 45 euros and 90 euros for Spain and 0 kronor, 500 kronor, 1,000 kronor for Sweden.

The survey was first developed in Dutch and subsequently tested using think-a-loud interviews (n = 5) and a pen-and-paper pilot (n = 29). This resulted in some minor changes to the layout and phrasing of the Dutch survey. To be able to use the survey in the other countries, some further changes to the survey were made. For example, we adapted country naming, and currencies for the cost attribute based on Organisation for Economic Cooperation and Development (OECD) comparative price levels [25] of May 2013 [26]. Hereafter, the survey was translated into Polish, Spanish and Swedish. A second translator reviewed each translated survey. To minimise differences between the original Dutch and the translated versions of the survey and to check for inconsistencies, native speakers (speaking Dutch and the respective languages) translated each survey back into Dutch. In Spain, Sweden and Poland, we asked 30 respondents per country to complete the adapted and back-translated survey online and to give their suggestions for improvement. No suggestions were given. More details of the DCE for the current study have been described elsewhere [27].

Data collection

An ISO certified market research company (ISO 26362 [28], ISO 20252 [29], and ISO 14001 [30]), was hired to administer the online survey. This company used their own panel to collect data in the Netherlands, while another company's panels were used to collect data in the other three countries. Both companies follow international quality standards for market research [31]. Panel members were emailed an URL to the survey. Quota sampling was used to ensure that samples were representative for each country based on age, sex, educational level and region. We aimed to have 500 completed surveys per country in order to obtain reliable outcomes [32]. All respondents gave informed consent before participating in the study and received a small financial incentive in local currency for their contribution to the study from the research company. The amount differed per country according to what is

Table 3

Characteristics of respondents who completed the survey per country, Netherlands, Poland, Spain, and Sweden, 2013 (n = 2,068)

Characteristics	Netherlands (n = 536)			Poland (n = 510)			Spain (n = 512)			Sweden (n = 510)		
Age median (IQ range)	50 (35–64)			41 (28–55)			45 (31–57)			50 (35–59)		
	N	%	%[a]	*N*	%	%[a]	*N*	%	%[a]	*N*	%	%[a]
Age groups (years)												
18–24	49	9.1	*11*	95	19	*14*	59	12	*10*	58	11	*11*
25–34	78	15	*16*	95	19	*19*	95	19	*21*	69	14	*16*
35–44	84	16	*19*	101	20	*16*	97	19	*20*	77	15	*18*
45–54	107	20	*19*	90	18	*20*	79	15	*16*	112	22	*16*
≥55	218	41	*35*	129	25	*30*	182	36	*33*	194	38	*39*
Sex *(male)*	289	54	*49*	261	51	*48*	251	49	*49*	245	48	*49*
Country of birth is the country of interest	517	96	*NA*	502	98	*NA*	466	91	*NA*	440	86	*NA*
Educational level[b]												
Lower education	184	34	*34*	224	44	*52*	117	23	*23*	167	33	*33*
Average education	192	36	*40*	199	39	*34*	156	30	*31*	179	35	*34*
Higher education	160	30	*26*	87	17	*14*	239	47	*46*	164	32	*33*
Income[c]												
Low income	106	20	*NA*	133	26	*NA*	93	18	*NA*	120	24	*NA*
Average income	127	24	*NA*	127	25	*NA*	239	47	*NA*	256	50	*NA*
High income	181	34	*NA*	250	49	*NA*	180	35	*NA*	134	26	*NA*
Do not know or do not want to say	122	23	*NA*	0	0	*NA*	0	0	*NA*	0	0	*NA*
Religious *(yes)*	244	46	*NA*	403	79	*NA*	250	49	*NA*	191	37	*NA*
Working in healthcare *(yes)*	56	10	*NA*	20	4	*NA*	33	6	*NA*	48	9	*NA*
Perception of own health												
Worse health than average	41	8	*NA*	40	8	*NA*	36	7	*NA*	44	9	*NA*
Medium health	195	36	*NA*	165	32	*NA*	214	42	*NA*	151	30	*NA*
Better health than average	300	56	*NA*	305	60	*NA*	262	51	*NA*	315	62	*NA*
Seasonal influenza vaccine target group												
Yes	239	45	*NA*	85	17	*NA*	168	33	*NA*	136	27	*NA*
No	270	50	*NA*	382	75	*NA*	300	59	*NA*	321	63	*NA*
No, but receives vaccination via work	27	5	*NA*	43	8	*NA*	44	9	*NA*	53	10	*NA*
Received seasonal influenza vaccination last year *(yes, for persons belonging to target group)*	156	65	*NA*	34	40	*NA*	97	58	*NA*	56	41	*NA*

IQ: interquartile; NA: not applicable.

[a] Census data per country.

[b] Higher education was defined as: college, university, graduate degree; average education as: completed high school; and lower education as: all else, such as only elementary school or vocational education.

[c] Income was defined as: low (‹23,000 euros), average (23,000–34,000 euros), high (›34,000 euros) per year for the Dutch sample; low (‹2,000 zlotys), average (2,000–3,000 zlotys), high (›3,000 zlotys) per month for the Polish sample; low (‹999 euros), average (1,000–2,000 euros),high (›2,000 euros) per month for the Spanish sample; and low (‹175,000 kronor), medium (175,000–500,000 kronor), high (›500,000 kronor) per year for the Swedish sample.

customary in the given country (e.g. Dutch respondents were paid 2.20 euros). Data collection took place between June and September 2013. A declaration of no objection was received from the Medical Ethics Committee of the Erasmus MC, University Medical Center Rotterdam (MEC-2012-263) after they reviewed the study protocol. According to Dutch legislation, the methodology of this study, a survey among volunteers of Internet panels, does not fall within the scope of the Medical Research Involving Human Subjects Act [33]. Although the aim of the study is of medical nature, respondents are not being subjected to any treatment or behavioural adjustments.

Data analysis

The choice observations resulting from the DCE were used to estimate the impact of pandemic scenario variables and vaccination programme attributes (independent variables) on the respondents' choices for vaccination or opting-out (dependent variable). A significant independent variable in this choice model indicates that the attribute or attribute level has a significant impact on vaccination preferences and the

Table 4

Regression coefficients for three latent classes based on responses to a survey investigating public preferences for vaccination programmes during pandemics caused by pathogens transmitted through respiratory droplets, Netherlands, Poland, Spain, and Sweden, 2013 (n = 2,068)[a,b,c]

Parameters	Class 1		Class 2		Class 3	
	Coefficient (p-value)	*SE*	*Coefficient (p-value)*	*SE*	*Coefficient (p-value)*	*SE*
Choice model						
Constant (vaccination)	0.70 (***)	0.04	-0.79 (***)	0.03	-5.02 (***)	0.27
Effectiveness of vaccination (per 10%)	0.18 (***)	0.01	-0.03 (***)	0.01	0.06 (NS)	0.05
Side effects unknown, but expected to be safe *(reference)*	0.16 (Ref)	0.01	0.17 (Ref)	0.01	0.22 (Ref)	0.08
Side effects unknown, no experience yet	-0.16 (***)	0.01	-0.17 (***)	0.01	-0.22 (***)	0.08
Family and/or friends recommend *(reference)*[d]	-0.22 (Ref)	0.02	-0.14 (Ref)	0.02	0.33 (Ref)	0.16
Family and/or friends discourage	-0.34 (***)	0.02	-0.46 (***)	0.03	-0.41 (**)	0.19
Your doctor recommends	0.18(***)	0.02	0.40 (***)	0.02	0.50 (***)	0.15
Your doctor discourages	-0.47 (***)	0.02	-0.75 (***)	0.03	-1.05 (***)	0.28
Government and public health institutions recommend	0.44 (***)	0.02	0.52 (***)	0.02	0.35 (**)	0.17
International organisations recommend	0.40 (***)	0.02	0.42 (***)	0.02	0.27 (*)	0.15
Traditional media is positive *(reference)*	0.03 (Ref)	0.01	0.22 (Ref)	0.02	0.33 (Ref)	0.12
Traditional media is negative	-0.12 (***)	0.02	-0.22 (***)	0.00	-0.41 (***)	0.15
Social / interactive media is positive	0.12 (***)	0.02	0.18 (***)	0.00	0.22 (*)	0.12
Social / interactive media is negative	-0.02 (NS)	0.02	-0.18 (***)	0.00	-0.14 (NS)	0.14
Out-of-pocket costs of the vaccine (per 10 euros)	-0.04 (***)	0.00	-0.13 (***)	0.00	-0.14 (***)	0.02
Interaction: effectiveness of vaccine (per 10%) x susceptibility to the disease (per 100 of 1,000 persons)	0.07 (***)	0.01	0.12 (***)	0.00	0.12 (***)	0.02
Interaction: effectiveness of vaccine (per 10%) x severity of the disease (per 10%)	0.01 (***)	0.00	0.02 (***)	0.00	0.01 (**)	0.00
Class membership model[e]						
Constant	-0.08 (NS)	0.10	0.00 (NA)	0.00	-0.83 (***)	0.13
The Netherlands *(reference)*	0.00 (Ref)	0.00	0.00 (Ref)	0.00	0.00 (Ref)	0.00
Poland	0.64 (***)	0.15	0.00 (NA)	0.00	0.07 (NS)	0.20
Spain	0.60 (***)	0.15	0.00 (NA)	0.00	0.12 (NS)	0.19
Sweden	-0.09 (NS)	0.16	0.00 (NA)	0.00	0.86 (***)	0.17
Class probability[f]	*Proportion (RR)*		*Proportion (RR)*		*Proportion (RR)*	
Average	0.44 (1.00)		0.35 (1.00)		0.21 (1.00)	
Respondents from the Netherlands	0.39 (0.89)		0.42 (1.21)		0.18 (0.86)	
Respondents from Poland	0.55 (1.24)		0.31 (0.89)		0.14 (0.69)	
Respondents from Spain	0.53 (1.20)		0.32 (0.90)		0.16 (0.74)	
Respondents from Sweden	0.30 (0.67)		0.35 (0.99)		0.36 (1.70)	
Model fit[g,h]						
Akaike Information Criterion (AIC)	1.54					
Pseudo-R^2	0.30					

SE: standard error; NA: not applicable; NS: non-significant coefficient; Ref: reference; RR: relative risk.

[a] Effects coded variables used for the safety of the vaccine, advice about the vaccine, media attention about the vaccine.

[b] The values of the vaccination programme attributes' reference levels equals the negative sum of the coefficients of the included attribute.

[c] ***, **, * denotes significance at the 1% and 5% and 10% level respectively.

[d] Note that for class 2 and 3, the recommendation of family and/or friends had a negative effect on utility. However, the utility is still positive compared with discouraging of family and/or friends.

[e] Class 2 does not have parameters in the class membership model as the parameters of class 1 to 3 are relative to class 2.

[f] The relative risks represent the relative probability of someone belonging to that class compared with the average class probability.

[g] Note that the pseudo-R^2 is not the same as the R^2 that is used in a linear regression model. A pseudo-R^2 of 0.3–0.4 is equivalent to a R^2 between 0.6 and 0.8 [21].

[h] A model with 3 classes is presented in the Table. This model had significantly better fit compared with a model with 2 classes (AIC: 1.64, pseudo-R^2: 0.26). Although a latent class model with 4 classes had an improved fit (AIC: 1.50, pseudo-R^2: 0.32), we opted for a model with 3 classes to be able to explain the results to policymakers in a clear manner.

sign of the coefficient reflects whether this impact has a positive or negative effect. Note that pandemic scenario variables could only be included as an interaction effect, as the scenario was the same in the three alternatives presented in each choice set. Several types of discrete choice models can be estimated. We chose a latent class model, since this is a closed form model (i.e. does not rely on complex simulations) that can take the panel nature of the data into account (i.e. dependencies between choice observations by a single respondent) [34].

A latent class analysis assumes the existence of subgroups (i.e. classes) of respondents with homogenous preferences. The researcher pre-specifies the number of classes based on the best model fit using the Akaike Information Criterion (AIC) and sound interpretation of classes. Class membership is latent in that the researcher does not determine who belongs to which class a priori. Instead, class membership is expressed by class probabilities that may depend on the respondent's characteristics. In addition to the choice model, we fitted a class membership model to test whether class membership is dependent on country of residence. Using the output of the class membership model, the class probabilities adjusted for country of residence can be calculated.

Calculation of the relative importance of the attributes enables a direct comparison of preferences between classes. The percentages represent the proportion of someone's preference (utility) that is based on that attribute. The relative importance can be calculated by dividing the difference in coefficient values between the highest and lowest level for a single attribute by the sum of the differences of all attributes for that class, considering interaction effects [35]. The mean expected uptake of a vaccine per class was calculated by taking the exponent of the total utility for vaccination divided by the exponent of utility of both vaccination and no vaccination. We were able to calculate these uptakes per country, by weighing the class-specific uptake with the class probabilities per country. The relative importance of the attributes and the expected vaccination uptake were calculated for two pandemic scenarios: a mild scenario in which 5% of the population gets the disease (susceptibility to the disease), and 5% of the sick people developing severe symptoms (severity of the disease), and a severe scenario in which 20% of the population gets the disease, and 75% of the sick people develops severe symptoms.

We used NLogit 4.0 software to estimate the latent class model and SPSS 21.0 software for all other analyses, such as chi-squared tests to compare proportions between countries.

Results

Study population

In total 7,272 panel members were invited to participate in the study. Of these, 2,651 started the survey (response rates ranged from 29% (627/2,186) for Spanish panel members up to 63% (677/1,083) for Dutch panel members; Figure 1). Of those who started, 2,068 completed the survey, ranging from 73% (510/698) of Swedish panel members up to 82% (512/627) of Spanish panel members. The country samples were approximately representative regarding age, sex, educational level and region (Table 3). However, compared with national census data, lower educated Poles were slightly underrepresented as well as respondents from the western region of Spain.

Respondents took a mean of 19 min (standard deviation: 31 min) to complete the survey. The majority of the respondents indicated that the survey topic was interesting or very interesting (81%; 1,677/2,068), and clear or very clear (74%; 1,528/2,068). A minority of respondents (9%; 179/2,068) found the survey hard or very hard to complete (ranging from 5% (28/510) for Poland to 13% (72/536) for the Netherlands). The proportion of choice sets in which the 'no vaccination' alternative was chosen was highest in the Swedish sample (51%; 4,145/(16*510=8,160)). The proportion of respondents that chose the 'no vaccination' alternative in all 16 choice sets was also higher in the Swedish sample (27% (136/510), $p<0.01$) than elsewhere (10% for Poland (52/510) and Spain (54/512), and 11% (61/536) for the Netherlands). Additionally, the proportion of respondents that always opted for vaccination was lowest in the Swedish sample (16%; 81/510), and highest in the Spanish sample (31%; 161/512).

Latent class analysis

Three latent classes, numbered from one to three, were identified (Table 4). The average class probability was 0.44, 0.35 and 0.21, for class 1, 2, and 3 respectively. The country of residence partly explains class membership, which is an indication for preference heterogeneity between countries. Respondents from Poland and Spain had a significantly higher chance to belong to class 1 (0.55 and 0.53 respectively, $p<0.01$) than respondents from other countries, those from the Netherlands had a significantly higher chance to belong to class 2 (0.42, $p<0.01$), and those from Sweden to class 3 (0.36, $p<0.01$).

Irrespective of the class they belonged to, respondents preferred a more effective vaccine that is expected to be safe, recommended by others, discussed positively in the media and with lower out-of-pocket costs, as can be seen by the positive and negative signs of the coefficients. The significant constant in all three classes indicates that, without considering any vaccination programme attributes, respondents of class 2 and 3 had a rather negative attitude towards vaccination, while respondents belonging to class 1 did

not. Almost all vaccination programme attributes were significant. The positive recommendation of international organisations did not significantly explain preferences of respondents within class 3. The coefficient for social/interactive media attention was not significantly different from positive traditional media attention for respondents of class 3 (both positive and negative social/interactive media attention) and class 1 (only negative social/interactive media attention), meaning that social media only marginally influences respondents' preferences for vaccination. Significant interaction effects between both susceptibility to and severity of the disease, and effectiveness of the vaccine in all classes indicate that the preference for the level of effectiveness of a vaccine is dependent on the seriousness of the pandemic. In other words, the more serious the pandemic, while the effectiveness of a vaccination remains the same, the more the preference for vaccination increases relative to no vaccination.

Relative importance

In the case of a mild scenario, the two most important attributes for class 2 and 3 were advice regarding vaccination and out-of-pocket costs, while effectiveness of the vaccine and advice regarding vaccination were the most important attributes for class 1 (Figure 2). Although advice regarding vaccination was important irrespective of class membership, for respondents belonging to class 3, the advice of friends and/or family and the advice of physicians were most important for vaccination choice (based on differences between coefficients of advice regarding vaccine), while the advice of both national and international health authorities was important for respondents belonging to class 1. Additionally, all respondents were more sensitive to advice against compared with advice in favour of vaccination. The relative importance of attributes varied with the seriousness of the pandemic scenario. Effectiveness was the most important attribute in the case of a severe scenario in all the latent classes and not only for respondents from class 1.

Predicted vaccine uptake

Assuming a realistic vaccination programme (i.e. a vaccination that is 70% effective, expected to be safe, recommended by family and/or friends, positively discussed in traditional media, and without out-of-pocket costs), the mean expected uptake in the case of a mild scenario was lowest for Swedish respondents with 43% (220/510; 95% confidence interval (CI): 40–47%)), followed by 54% (292/536; 95% CI: 51–58%) for Dutch respondents, 62% (318/512; 95% CI: 59–65%) for Spanish respondents, and highest for respondents from Poland with 63% (323/510, 95% CI: 60–66%). In the case of a mild scenario, advice regarding the vaccine and out-of-pocket costs had a relatively large impact on vaccination uptake in all countries, while media attention had little effect on uptake. For example, when out-of-pocket costs increased from 0 to 100 euros, the uptake decreased to 32% (163/510; 95% CI: 29–35%) for Swedish respondents, followed by 41% (222/536; 95% CI: 38–45%) for Dutch respondents, 51% (263/512; 95% CI: 48–55%) for Spanish respondents, and 53% (269/510; 95% CI: 49–56%) for Polish respondents. The uptake rates were expected to increase dramatically in the case of a severe scenario with up to 65% (331/510; 95% CI: 61–69%) for respondents from Sweden, and 82% (419/510; 95% CI: 80–85%) for respondents from Poland.

Discussion

Statement of principal findings

In the case of a severe pandemic scenario, vaccine effectiveness was the most important characteristic determining vaccination preference in all countries. The body that advises a vaccine was found to strongly affect preferences in all countries as well, with respondents being more sensitive to advice against compared with advice in favour of vaccination. Preference heterogeneity between countries was substantial, especially in the case of a mild pandemic scenario; a strong effect on vaccine preferences was found for the advice of family and/or friends and the advice of physicians in Sweden, in contrast to Poland and Spain, where the advice of (international) health authorities was more important. Besides the vaccination advice, out-of-pocket costs were important for Dutch and Swedish respondents, while for respondents from Poland and Spain the effectiveness of the vaccine was important in case of a mild pandemic scenario. Irrespective of pandemic scenario or programme attributes, the predicted vaccination uptakes were lowest in Sweden, and highest in Poland.

Strengths and weaknesses of the study

So far, only a limited number of healthcare-related DCEs have quantitatively compared preferences between respondents from different countries and this is, to our best knowledge, done for the first time in the field of infectious diseases. An additional strength is the advanced analysis technique we used in this study. While already used extensively in the field of transport economics, latent class analysis has been used for only 3% of all health-related DCE analyses conducted between 2009 and 2012 [23]. A possible weakness of our study is that the preferences are stated and based on hypothetical pandemic scenarios. Respondents might have given socially desirable responses. It is not known to what extent the stated preferences differ from preferences during an actual pandemic. However, the external validity of the DCE method has been studied in other health related contexts, and results are encouraging with respect to prediction of preferences on an aggregate level [36,37]. In addition, the hypothetical nature of the study enabled us to compare preferences between different possible future pandemic scenarios. The findings might thus help to prepare for a future pandemic. Additionally, all coefficients had the expected sign, which suggests theoretical validity of the DCE [38]. Another possible weakness is the complexity of the choice sets, due to inclusion of risks

as attributes. However, we thoroughly pilot tested the survey and, during the online survey, only a minority of respondents stated that they experienced problems completing the choice sets.

Results in relation to other studies

Our study showed that the expected vaccination uptake is largely dependent on the seriousness of a pandemic. This was also shown in previous studies, including studies conducted in the Netherlands, Poland, Spain and Sweden [39-45]. During the influenza A(H1N1)pdm09 pandemic, the perceived vulnerability was low and respondents believed that they were less likely to become infected than other people [41,46]. This might have been one of the reasons for the lower than expected uptake during that pandemic with overall, 30%, 27% and 59% of the Dutch, Spanish and Swedish population respectively, having been vaccinated (Table 1). Interestingly, we found that Swedish respondents were least willing to get vaccinated in future influenza pandemics, both in mild and severe scenarios. As previous experiences are likely to influence future vaccination uptake [45], the difference between our study results and actual influenza A(H1N1) pdm09 vaccination coverage might be assigned to the negative experiences Swedish citizens had with vaccination during the 2009 pandemic. In Sweden, the controversy on the association between pandemic vaccines and narcolepsy is still ongoing [47]. In addition, Swedish respondents in the current study less often had received seasonal influenza vaccination in the previous year compared with e.g. Dutch respondents (41% vs 65%, Table 3). Research, conducted in the Netherlands, has shown that trust in health authorities is related to pandemic influenza vaccination uptake [48] and that it is necessary to build up and sustain trust before, during and after an influenza pandemic [16]. Furthermore, during the influenza A(H1N1)pdm09 pandemic Dutch and Swedish participants had more trust in healthcare professionals compared with Polish and Spanish participants [18]. Our research shows the same inter-country differences. Poland did not implement a national vaccination programme during the influenza A(H1N1)pdm09 pandemic [15,44] (Table 1). Seasonal influenza vaccination coverage is reported to be less than 10% for the target population older than 55 years [49]. Reported reasons for the Polish public to reject influenza (both seasonal and pandemic) vaccination include the low level of confidence in the quality and effectiveness of the vaccine [18,50]. Our finding that effectiveness of a pandemic vaccine had by far the strongest effect on vaccination choice of Polish respondents, confirmed this. The lowest seasonal influenza vaccination coverage contrasts with our finding that Polish respondents were more willing to get vaccinated than respondents from other countries. However, in our study, the level of effectiveness of the vaccine was presented to respondents as a known rate, which might explain why we estimated a higher vaccination uptake. Safety of the pandemic vaccine was not as dominant in the current study as in other studies [39,40]. The choice of attribute levels for our DCE might explain this difference in relative importance. We included realistic attribute levels, instead of presenting a certain vaccination risk (e.g. 1 in 100,000) to respondents. We also analysed safety as an interaction with the pandemic scenario variable 'severity of the disease', but with no meaningful outcome. We found almost no effect of social media attention (compared to traditional media) on pandemic vaccination preferences and predicted uptake. The objective framing of this attribute in the DCE survey might explain the finding. However, social media will likely be influential in future pandemics in other ways, e.g. by creating online applications that provide credible health information [51].

Implications for clinicians and policymakers

Our results show that seriousness of a pandemic influences vaccination uptake dramatically. In order to increase pandemic vaccination coverage, it is essential that susceptible people feel susceptible and perceive the pandemic as a serious threat. This can be achieved, for example, by honest and open communication regarding the seriousness of the pandemic, and avoiding conflicting messages and information overload [17,52] and by providing public health messages that include descriptive and injunctive normative information [53,54]. The WHO Regional Office for Europe and the European Centre for Disease Prevention and Control (ECDC) recommend more flexible pandemic preparedness planning, i.e. planning that takes into account different pandemic scenarios [8,9,19]. Findings of our study may facilitate responses to future influenza pandemics with different levels of severity, as our study provides the option to calculate the expected vaccination uptake for different pandemic scenarios, and provides insights into how several vaccination programme attributes influence these uptakes. Additionally, our study also shows that the availability of an effective pandemic vaccine is of paramount importance in order to reach certain coverage levels. Unfortunately, such a highly effective vaccine might not be available due to the crisis situation that is inherent to a pandemic, or proof that the vaccine is effective might be lacking as time is usually limited. In addition, due to contracts or limited availability of vaccines, there are usually only one or two different vaccines available for policymakers to choose from. For all countries, given the high impact of vaccine effectiveness on vaccination preferences, it is therefore important that there is open communication regarding the expected effectiveness, so that the public can make an informed choice whether to get vaccinated or not. The vaccination programme attributes that can be influenced by policymakers directly are out-of-pocket costs and how/what to communicate. As our results show that by whom a vaccine is advised had a different effect on uptake in the included countries, it is important that during future pandemics the responsible authorities align with other important stakeholders in the country and communicate in a coordinated manner.

Unanswered questions and further research

We found differences in preferences for pandemic vaccinations between different European countries. Further research could focus on differences within these countries, e.g. whether preferences of those who previously received seasonal influenza vaccination differ from preferences of those who had not, as previous research shows that the uptake of seasonal influenza vaccination was positively associated with influenza A(H1N1)pdm09 vaccination decision-making [39,55,56]. Additionally, future research could focus on subgroups of the population, such as healthcare workers or under-vaccinated groups. It is unknown whether preferences differ between countries within the same geographical area of Europe. Therefore, it might be useful to conduct the same DCE in other European countries as well. Unfortunately, timely access to vaccinations is not self-evident [57]. It is not known in advance which respiratory pathogen will cause a next pandemic and production capacities might be inadequate. In the case of an influenza pandemic, other preventive measures such as quarantine, and antiviral drugs might be helpful to limit the spread of the virus during the first phase [10]. Further research into preferences for other preventive measures, and differences herein across European countries, using the DCE methodology is thus recommended. Moreover, the DCE methodology could also be used to study motivations and barriers for vaccinations other than pandemic vaccination among different countries.

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Conflict of interest

None declared.

Authors' contributions

All authors made substantial contributions to the acquisition and/or design of the study. DD, IK and EBG collected the data and performed the analysis of the collected data. All authors have contributed to the interpretation of the data. DD, together with EBG, drafted the manuscript. IK, AF, ES, MB, HV, JR, and ML have critically revised the manuscript. All authors read and approved the final manuscript.

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Research article

Clinical and histopathological features of fatal cases with dengue and chikungunya virus co-infection in Colombia, 2014 to 2015

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We report clinical features and histopathological findings in fatal cases with dengue (DENV) and chikungunya (CHIKV) co-infection identified at the Colombian National Institute of Health between September 2014 and October 2015. Seven such cases were documented. Dengue serotype 2 virus was identified in six cases. All patients were adults and comorbidities were present in four. Fever, arthralgia or myalgia was present in all cases. The frequency of rash, haemorrhage, oedema, and gastrointestinal symptoms was variable. Laboratory findings such as thrombocytopenia, renal failure, and leukocyte count were also inconsistent between cases. Post-mortem tissue examination documented focal hepatocellular coagulative necrosis in three cases, incipient acute pericarditis in one and tubulointerstitial nephritis in one. This study provides evidence of mortality in patients with DENV and CHIKV co-infection. Fatal cases were characterised by variable clinical and laboratory features. Evaluation of histopathology of autopsy tissues provided evidence of the pathological consequences of the disease.

Introduction

Arboviral diseases such as dengue and chikungunya infection are among the leading infectious health problems in the world today [1,2]. The majority of dengue virus (DENV) infections occur in Asia, the Pacific, South and Central America and the Caribbean, where they are considered a public health problem [3]. The chikungunya virus (CHIKV) was first isolated in Tanzania in 1953 and has repeatedly been identified in western, central and southern Africa and in many parts of Asia. Imported cases among tourists have been identified in several European countries and the United States [4]. The infection recently appeared in the Americas [5], when autochthonous transmission of the CHIKV was identified in St Martin in 2013. Since then, CHIKV has spread to 33 countries and territories in the Caribbean, South, Central and North America with nearly 2 million cases identified [6].

Patients with DENV or CHIKV infection generally present with a self-limited febrile disease. However, DENV infection has several complications, mainly dengue shock syndrome and haemorrhagic manifestations. CHIKV infection is not regarded as a life-threatening disease [7,8], but mortality due to CHIKV was reported during the Reunion Island outbreak in 2006 [9]. Although the overall proportion of atypical and severe cases was low, mortality in these cases was high. In areas where both viruses co-circulate, both can be transmitted to the same human host and pose a challenge for medical diagnosis. DENV and CHIKV co-infection has been reported in several studies in a non-negligible proportion of cases [10-12]. However, fatalities in patients with DENV and CHIKV co-infection have been rare to date. Mortality has been reported in only one case without other specific information [13].

In this paper we present the clinical and laboratory findings recorded in cases of fatal DENV and CHIKV co-infection occurring in Colombia and correlate them with the histopathological features of post-mortem tissue biopsy.

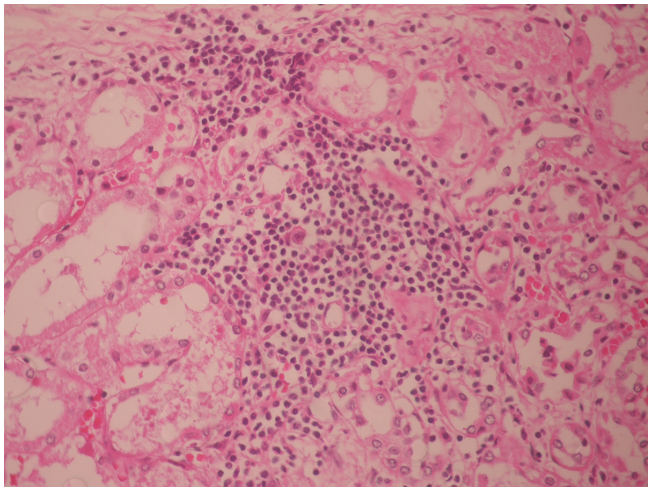
Methods

In accordance with the procedures established in Colombia for the reporting, collection and analysis

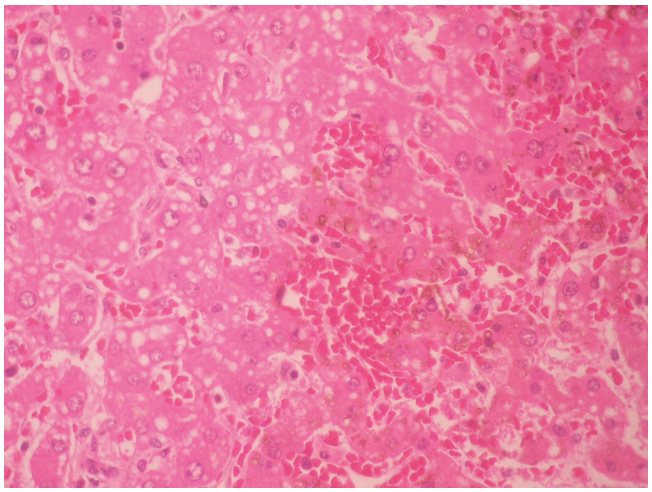
Figure

Histopathological findings of fatal cases of dengue and chikungunya virus co-infection (n = 3)

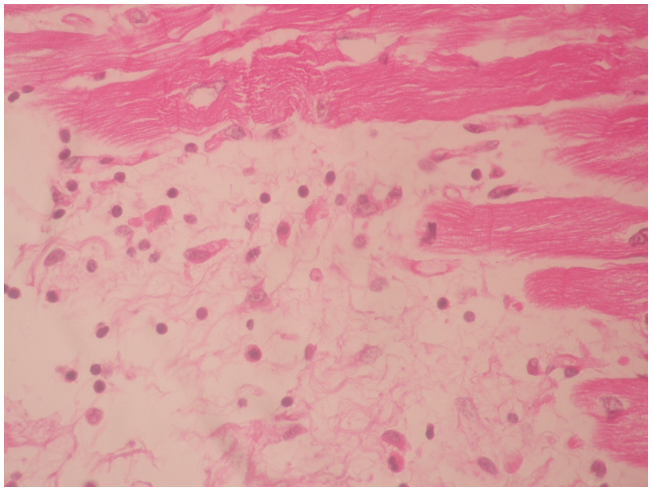
A. Kidney



B. Liver



C. Heart



Haematoxylin- and eosin-stained tissue sections under light microscopy (40X).

(A) Kidney (Case 6): Glomerular oedema, tubular structure with hyaline casts, and small focus of tubulointerstitial nephritis.

(B) Liver (Case 5): Individual areas of coagulative hepatocellular necrosis and mild infiltrate of mixed inflammatory cells.

(C) Heart (Case 7): Epicardium with mild mononuclear inflammatory infiltrate compatible with acute pericarditis, and myocardial tissue without inflammatory infiltrate or remodelling areas.

of clinical data, patients with dengue and chikungunya fever are notifiable and undergo continuous and systematic monitoring. The cases are reported to the National System for Public Health Surveillance (SIVIGILA), which collects all the clinical information of cases of public health interest from around the country. Cases with initial diagnoses of 'probable' are then confirmed by the National Institute of Health through laboratory tests or histopathological findings and clinical features [14-16]. The guidelines of the Ministry of Health and Social Protection stipulate that post-mortem biopsies are required for fatal events due to DENV or CHIKV. The case definitions for dengue and chikungunya fatal cases are described elsewhere [14-16]. A fatal case of dengue is defined as a patient with severe dengue with laboratory-confirmed diagnosis by anti-DENV IgM, viral isolation or PCR (PCR), and compatible histopathological findings. A fatal case of chikungunya is defined as a patient with an acute illness consistent with the disease who developed severe or atypical clinical manifestations, and with laboratory-confirmed diagnosis by anti-CHIKV IgM, viral isolation or PCR.

This retrospective study included all fatal cases that occurred from September 2014 through October 2015 reported to SIVIGILA and that were laboratory-confirmed for DENV and CHIKV co-infection by the National Institute of Health. We collected detailed, serial clinical findings including history, physical examination, and haematological, biochemical, radiological and virological results, and entered them into a predesigned database. Histopathological examinations were performed when tissue autopsy was available.

Serum samples obtained at hospital admission and tissues from autopsies were processed in the Arbovirus Laboratory at the National Institute of Health. For the determination of anti-DENV IgM in serum, a commercial capture ELISA kit was used. DENV and CHIKV were identified by PCR on serum or tissue. On tissue sections, cell lysis was performed and the viral RNA was extracted using a commercial QIAamp viral RNA mini kit. DENV was identified and characterised by conventional PCR as described elsewhere [17]. The CHIKV identification test was conducted with qRT-PCR protocol according to Lanciotti et al. [18].

Histopathological features were reported for four cases. Formalin-fixed tissues from fatal cases were processed, embedded in paraffin, and cut in 5 µm sections. Histopathological changes were examined on haematoxylin- and eosin-stained tissue sections under light microscopy.

Results

During the study period, seven fatal cases of DENV and CHIKV co-infection were identified among 58 CHIKV deaths documented by the National Institute of Health. Clinical features were reported for all cases, but in one patient, laboratory findings were not available (Table). Co-infection was diagnosed by positive CHIKV and DENV PCR on post-mortem tissue in Cases 2, 3 and 6 (CHIKV PCR was also positive in serum in Cases 2 and 6), by positive CHIKV and DENV PCR on serum in Cases 1, 5 and 7 (DENV PCR was also positive in post-mortem tissue in Case 7), and by positive CHIKV PCR on serum and positive DENV PCR on post-mortem tissue in Case 4. The cycle threshold values for CHIKV PCR were 16.8–32.5. Anti-DENV IgM in serum was analysed in two cases and the results were negative. Dengue serotype 2 virus (DENV-2) was identified in six cases and DENV-3 in the remaining case.

All patients were adults (four were older than 60 years) and four were female. One case was a pregnant woman in the 37th week of gestation. Two days after hospital admission, this patient gave birth to a live child with normal physical examination. Four of the seven cases presented comorbidities. Hypertension was the most frequent underlying disease (three cases) and one case had hypothyroidism. Time from symptom onset to hospital admission was shorter than four days in all cases (range: 1–4 days). Although all patients reported to have had fever at home, axillary temperature was high (›38 °C) in only two cases at hospital admission. Arthralgia or myalgia at hospital admission was reported for all patients. Haemorrhagic manifestations were documented in four cases (mucosal bleeding in three cases and haemorrhagic stroke that occurred six days after hospital admission in one case) and oedema of the lower limb in two patients. Two cases reported gastrointestinal symptoms (diarrhoea, nausea or vomiting). However, no ascites or pleural effusion were reported.

As regards laboratory findings at admission (Table), one case had leucopenia (‹4 × 10^9/L) and three had leukocytosis (›12 × 10^9/L). Haematocrit and haemoglobin index were below 3.2 in all cases. Thrombocytopenia (‹100 × 10^9/L) was documented in two cases at admission, and Cases 2, 4 and 7 developed the condition during hospitalisation (range: 22 to 55 × 10^9/L). Renal failure (creatinine›2 mg/dL) was reported in three patients (Case 1 developed this complication after admission). Four patients presented elevated transaminases, mainly aspartate aminotransferase (›34 U/L), but their values were not higher than 1,000 U/L.

Six patients died within three days of hospital admission, and the last died after 16 days (Case 4). Causes of mortality were multiorgan dysfunction syndrome, shock in one case, and sepsis associated with nosocomial infection in the pregnant woman (Case 4). For Case 4, blood and respiratory cultures yielded *Acinetobacter baumannii*.

Post-mortem tissue examination was performed for four cases. The histopathological findings in Case 4 were related with septic shock. The other three cases (Cases 5, 6 and 7) presented coagulative hepatocellular necrosis; Case 5 presented incipient acute pericarditis and Case 6 tubulointerstitial nephritis (Figure). Mild oedema was observed in the lung of all four patients but there was no evidence of inflammation. Similarly, no inflammatory infiltrate was found in the myocardial or brain tissues.

Discussion

Our data provide evidence of mortality associated with DENV and CHIKV co-infection. DENV-2 was the predominant serotype in our study. The clinical picture of DENV and CHIKV infection regularly presented fever, arthralgia or myalgia, and rash. Other clinical and laboratory characteristics presented variations. Histopathological examinations were consistent with arbovirus infection.

Some studies using serological assays or PCR tests have reported that co-infection of DENV and CHIKV is not uncommon [10-13,19]. In a study performed in India in 2010, Taraphdar et al. [10] found that 68 (12.4%) of 550 blood samples of febrile cases had IgM antibodies against both DENV and CHIKV. In another study, 16 (2.8%) of 1,502 suspected cases of CHIKV infection were confirmed to be DENV and CHIKV co-infections in the Caribbean island of St Martin [11]. Moreover, a study carried out in Gabon documented 37 co-infected patients with DENV serotype 2 and CHIKV among 4,287 febrile patients (1,567 with CHIKV infection) [12]. Importantly, in all previous studies, dual infected patients were not severely ill and recovered quickly. Mortality has been reported in only one case without other specific information [13]. However, these observations should be interpreted with caution in view of the limited number of clinical and biological investigations available [19].

Moreover, DENV or CHIKV may be underdiagnosed in areas where both viruses circulate. Multiple infections in a single patient may change the spectrum of clinical manifestations or overlapping clinical symptoms and thus complicate the diagnosis [10,20,21]. In addition, the recent emergency of Zika virus has led to the co-circulation of these three arboviruses in many countries and there is the possibility of co-infections [22].

In our study, we found that the main classical clinical manifestations associated with DENV or CHIKV infection [23] were present at hospital admission, but the incidence of other manifestations such as haemorrhage, oedema and gastrointestinal symptoms tended to vary. Other laboratory findings such as thrombocytopenia, renal failure, and leukocyte count were also inconsistent among our cases. Moreover, none of our cases had systemic vascular leak syndrome (haemoconcentration, pleural effusion and ascites). In previous studies, thrombocytopenia and bleeding were rare complications in patients mono-infected with CHIKV,

Table

Clinical features and laboratory findings in fatal cases with dengue and chikungunya virus co-infection (n = 7)

	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Case 7
Clinical features							
Age (› 60 years-old)	Yes	Yes	Yes	No	No	Yes	No
Comorbidities	Hypertension	Hypertension	Hypothyroidism	Pregnancy	None	Hypertension	None
Time from symptom onset (days)	3	3	4	1	3	2	3
Fever	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Arthralgia	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Rash	Yes	Yes	Yes	Yes	No	No	Yes
Haemorrhagic manifestations	No	Petechiae	Haemorrhagic blisters	Haemorrhagic stroke	No	Upper gastrointestinal bleeding	No
Oedema	Yes	No	Yes	No	No	No	No
Time from admission to mortality (days)	3	1	2	16	1	1	2
Laboratory findings at admission							
Haemoglobin (g/dL)	15.4	13.9	19.2	14	14.6	NA	11.5
Haematocrit (%)	44	42	58	42	44	NA	35.7
Total white cells (10^9/L)	12.6	15.1	26.7	7.4	5.4	NA	3.1
Platelets (10^9/L)	210,000	161,000	60,000	120,000	99,000	NA	151.000
Creatinine (mg/dL)	0.98	5.5	2.4	0.49	0.65	NA	0.67
Aspartate aminotransferase (U/L)	NA	419	252	13	72.4	NA	186
Alanine aminotransferase (U/L)	NA	69	37	8	50.2	NA	137

NA: not available.

Normal ranges: Haemoglobin: 12–17 g/dL; haematocrit: 40–50%; platelets: 150–600 × 10^9/L; total white cells: 4–12 × 10^9/L; creatinine: 0.75–1.2 mg/dl; aspartate aminotransferase 10–34 U/L; alanine aminotransferase: 5–59 U/L.

but were more frequent in patients infected with DENV. Similarly, recent studies have reported renal failure in patients who died of CHIKV infection. Finally, leukopenia has frequently been described in patients with DENV, but leukocytosis has commonly been documented in fatal cases of CHIKV [21,24,25]. These data emphasise the need for a multidimensional diagnostic approach in these clinical situations. In countries where both diseases are endemic, the differential diagnosis between severe DENV and CHIKV infections or co-infection may be a challenge.

The critical period of development of complications or mortality in patients with DENV infection is between four and six days after symptom onset [26], and from four to eight days in patients with CHIKV infection [24,25]. Similar time frames were documented in the present study of co-infected DENV and CHIKV fatal cases. Moreover, the assessment of histopathology of autopsy tissues in the present study provided evidence of the pathological consequences of the disease. It is important to note that in one of our cases, mortality did not seem to be directly related to virus infection: this patient died several days after hospital admission due to a sepsis associated with nosocomial infection, and the histopathological findings were compatible with a septic process. Conversely, in the other six cases, laboratory data coincided with those found in histopathological studies. Renal failure with high creatinine values and elevated transaminases concurred with the pathology findings of tubular interstitial nephritis or tubular necrosis and hepatocellular necrosis. Moreover, the histopathological results in the liver coincided with those described in fatal DENV infections [27]. However, these liver pathology results have also been found in fatal cases of CHIV mono-infection in Colombia (data not shown). Interestingly, although no histopathological evidence of myocarditis or encephalitis was observed in any of the case-patients in the present study, these complications have been reported previously in patients with DENV or CHIKV infection [14,15]. A study has documented DENV in cerebrospinal fluid, in macrophage-like cells and neurons in the central nervous system [28]. Similarly, experimental infection in animal models has documented the capacity of CHIKV to infect leptomeningeal tissue and glial cells [29].

Most of our cases were co-infected with DENV serotype 2. Along with serotypes 1, 3 and 4, this serotype has been associated with CHIKV co-infection in other studies [11-13,19,30,31]. Moreover, the genome sequence of the CHIKV strain circulating in America was shows that it belongs to the Asian genotype, suggesting Asia as

the probable origin of the circulating virus [32,33]. In Colombia, the National Health Institute reported similar data. By contrast, lethal cases of CHIKV infection have previously been associated with infections by the east/central/south African (ECSA) genotype, which was responsible for the large epidemics on islands in the Indian Ocean and the Indian subcontinent. However, in a recent study performed in Bahia State in east-central Brazil, the ECSA genotype was also found [34].

It is difficult to establish the possible effect of both CHIKV and DENV on mortality in our cases. A previous study documented that CHIKV and DENV serotype 2 loads in co-infected patients were always significantly lower than those in DENV and CHIKV mono-infected patients. However, the co-infected patients might have high loads of CHIKV or DENV, or both [12]. The authors of that study suggest that interaction between viruses or the timing of a bite from an infected mosquito could explain these findings. Unfortunately, in our study we did not analyse viral load and immune response. Moreover, it has been documented that when shock sets in, dengue virus is no longer detectable in blood, and it has therefore been suggested that the host response should play a key role in pathogenesis. But there is evidence suggesting that DENV replication may occur in some organs, while viraemia is no longer detectable [28]. It is interesting to note that in the present study, time from symptom onset to mortality was shorter than six days in most cases (except Case 4) and RT-PCR on serum (6 cases) or post-mortem tissues (5 cases) was positive while the serological results were negative, suggesting viraemia or viral replication in tissues.

There are several limitations to our study. The detection of suspected cases of mortality due to DENV or CHIKV infection depends on the reports made by physicians in different areas of the country. In addition, serological tests and PCR for DENV and CHIKV are not available in most Colombian hospitals. Therefore, it is likely that certain cases were not detected. Moreover, anti-DENV and anti-CHIKV IgM were not determined in all cases. However, it can be expected that serology would have been negative because most of the cases had an acute infection (≤ 6 days since symptom onset). Finally, post-mortem examination was not performed in one case and immunohistochemical studies were not done.

Conclusion

Our data provide evidence of mortality associated with DENV and CHIKV co-infection. Post-mortem histopathological findings were consistent with arbovirus infection. The variations in the clinical and laboratory findings make an accurate diagnosis difficult and highlight the need for sensitive and rapid tests. It is important to differentiate between them as their management, especially for dengue, is different. Prospective studies evaluating the immune response and virological aspects of co-infection are now required.

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This study was approved by the Ethics Committee of the Universidad del Norte in Barranquilla, Colombia.

Conflict of interest

All authors have no conflicts of interest to disclose.

Authors' contributions

All authors had full access to the study data. Study concept and design: M.M, J.A, and D.V. Collection, analysis and interpretation data: E.P, L.P, A.C, and A.R. Drafting of the manuscript: M.M, E.G, and D.V. Critical revision of the manuscript for important intellectual content: J.A, E.P; L.P, and A.C. Obtained funding: D,V. All authors have seen and approved the final manuscript.

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2016 European guideline on the management of non-gonococcal urethritis published

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On 4 May 2016, the evidence-based '2016 European Guideline on the management of non-gonococcal urethritis' was published online [1]. This guideline is a comprehensive updated version of the European guideline from 2009 [2] and provides up-to-date and detailed guidance regarding aetiology, clinical features, diagnosis, testing, treatment and general management of symptomatic non-gonococcal urethritis in Europe.

The most common organisms implicated are *Chlamydia trachomatis* and *Mycoplasma genitalium,* with the latter perhaps causing more symptoms [3]. Testing male patients with urethritis for *M. genitalium*, preferably with screening for macrolide resistance, is highly likely to improve clinical outcomes [4]. Testing symptomatic patients for *M. genitalium* is therefore recommended.

Of major concern is the increasing azithromycin resistance of *M. genitalium* [5]. Azithromycin, especially single dosage of 1 g, is associated with the development of macrolide resistance in *M. genitalium*, and is likely to increase the circulation of macrolide-resistant strains in the population [5]. Consequently, single dose azithromycin is no longer recommended as first-line treatment for non-gonococcal urethritis.

Updates to the guideline include recommendations on the diagnosis, testing and treatment of non-gonococcal urethritis:

Urethritis should be confirmed by urethral smear microscopy in symptomatic patients.

- Symptoms and negative urethral smear: No empirical treatment. Re-attend for early morning smear if nucleic acid amplification testing (NAAT) was negative and symptoms do not settle.

- All men assessed for sexually transmitted infections, regardless of symptoms, should be tested for *C. trachomatis* from a first-void urine specimen and for *Neisseria gonorrhoeae* if they have urethritis. If a NAAT is positive for gonorrhoea, a culture should be performed before treatment.

- All men who have sex with men should be tested for both *C. trachomatis* and *N. gonorrhoeae* from any potentially exposed site.

- Recommended syndromic regimen: doxycycline 100 mg twice daily for seven days. Azithromycin 1 g immediately should not be used routinely because of the increased risk of inducing macrolide antimicrobial resistance with *M. genitalium*.

- If *M. genitalium*-positive: azithromycin 500 mg immediately, followed by a 250 mg oral dose for four days. A test of cure three to five weeks after treatment in those who tested positive for *M. genitalium* should be performed.

In case of persistent and/or recurrent non-gonococcal urethritis, testing for *M. genitalium* using a NAAT, preferably with screening for macrolide resistance, should be considered, as well as testing for *Trichomonas vaginalis* using a NAAT if it is prevalent in the local population. Recurrent non-gonococcal urethritis should only be treated if the patient has definite symptoms of urethritis, or if there are physical signs and microscopic evidence of urethritis on examination.

The guidelines are available here

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European Medicines Agency publishes draft advice on the use of colistin products in animals, for consultation

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On 26 May, the European Medicines Agency (EMA) published a draft for consultation updating advice on the use of colistin products in animals in the European Union, the development of colistin resistance and the possible impact on human and animal health [1]. The consultation ends on 26 June 2016.

Following the recent discovery of the *mcr-1* gene which causes bacteria to become resistant to colistin, the European Commission requested the EMA to update its previous 2013 advice on the use of colistin in animals. The draft updated advice published on 26 May provides an analysis of colistin toxicity, susceptibility testing, activity and resistance mechanisms, risk profile, and risk management options.

As reported by Skov et al., 'the *mcr-1* gene (i) has spread to most continents (ii) has been found in bacteria isolated from various food animals, from the environment including river water, from various types of meat and vegetables, and from infected patients and asymptomatic human carriers including international travellers, (iii) has been found in various bacterial species, mostly *E. coli*, and on several different plasmids, and (iv) is highly transferrable with in vitro transfer rates as high as 10^{-1}' [2].

As antimicrobial resistance is generally on the increase the recent developments are especially alarming as colistin is a drug of last resort in the treatment against multidrug-resistant *Pseudomonas aeruginosa, Acinetobacter baumannii* and Enterobacteriaceae such as *Escherichia coli* and *Klebsiella pneumoniae*.

Comments on the draft can be submitted to the following address vet-guidelines@ema.europa.eu. Read more here

References

1. The European Medicines Agency (EMA). Updated advice on the use of colistin products in animals within the European Union: development of resistance and possible impact on human and animal health EMA/231573/2016. London: EMA; 26 May 2016. Available from: http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2016/05/WC500207233.pdf
2. Skov RL, Monnet DL. Plasmid-mediated colistin resistance (mcr-1 gene): three months later, the story unfolds. Euro Surveill. 2016;21(9):30155. DOI: 10.2807/1560-7917.ES.2016.21.9.30155

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