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Middle East Respiratory Syndrome coronavirus – two years into the epidemic

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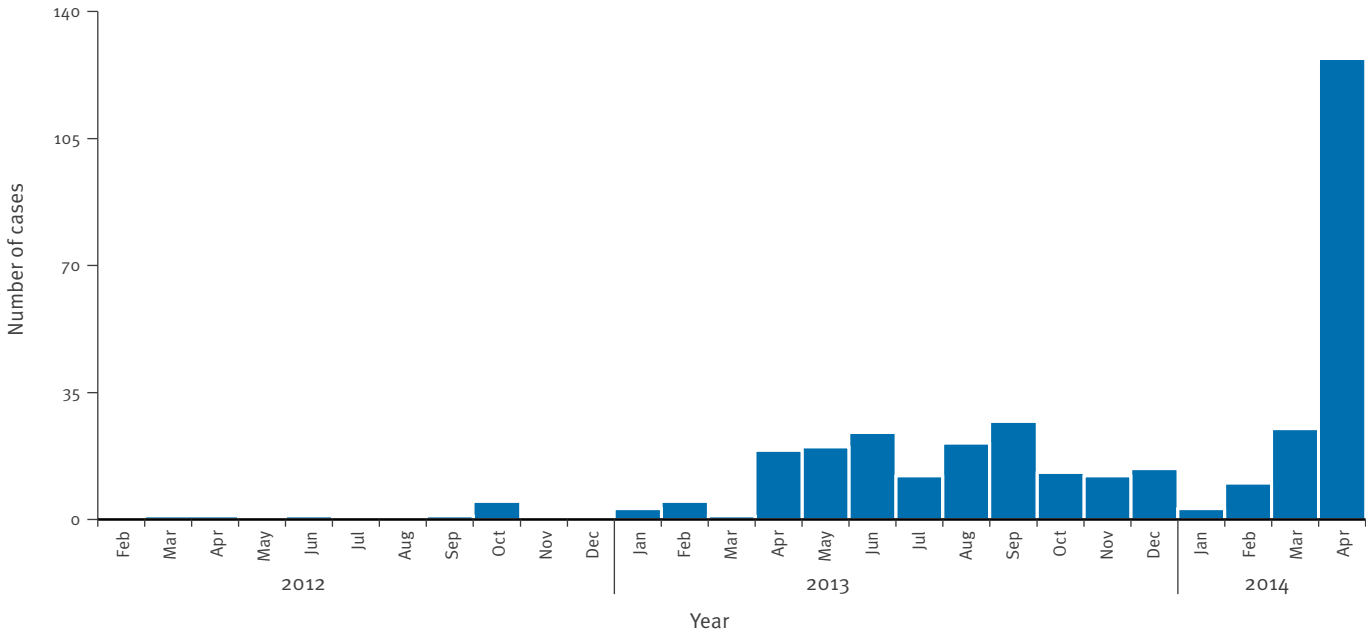
Two years ago, on 23 April 2012, media reported a cluster of severe respiratory infection in a hospital in Jordan [1]. Only several months later did it become evident that this was the first known occurrence of the new Middle East Respiratory Syndrome coronavirus (MERS-CoV) that since then continues to puzzle scientists and public health experts alike.

As of 23 April 2014, 345 people have been reported infected, and of those 107 have died [2]. Most cases occurred in Saudi Arabia (SA) and to a lesser extent in the United Arab Emirates (UAE), still further 11 countries in Europe, Asia and North Africa have reported cases linked to the Arabian peninsula. Few clusters and cases were noted in the second half of 2012, and the epidemic has been stable at low levels in 2013, with about 15 cases notified monthly (Figure). This has changed dramatically over the past weeks when we faced an unprecedented increase in cases and community transmission as well as transmission in hospital settings.

In the past weeks, MERS-CoV cases imported to Jordan, Malaysia and the Philippines, have reminded us of the risk of seeing cases among expatriate residents in the Arabian Peninsula visiting their home countries or among travellers returning from SA. In this issue of *Eurosurveillance*, Tsiodras et al. report about the public health response to a MERS-CoV infection in a Greek national residing in SA who was diagnosed in Greece upon returning from SA [3]. The patient initially presented with fever and diarrhoea, possibly indicating

Figure*

Distribution of Middle East Respiratory Syndrome coronavirus cases by month of disease onset, February 2012–23 April 2014 (n=345)



Note: Where the month of disease onset is unknown, the month of reporting has been used.

that the presentation of cases at early onset of the disease may not include prominent respiratory symptoms; this was already reported in a French case-patient with immunosuppressive condition in 2013 [4]. The World Health Organization (WHO) MERS-CoV Research Group reviewed 161 patients in November 2013, and indicated the French patient as the only one admitted for fever and diarrhoea in without initial respiratory symptoms. The group mentioned however, that at least one-third of patients also had gastrointestinal symptoms, such as vomiting and diarrhoea [5].

A second paper in this issue by Nowotny and Kolodziejek provides further evidence that the MERS-CoV is likely a zoonosis with camels playing a role as a reservoir for the virus as well as a possible source for transmission to humans, potentially through the respiratory route [6]. Still other transmission routes, e.g. foodborne, or a combination of routes cannot be excluded according to van Doremalen et al. [7].

In the past, emerging zoonoses have been triggered by changes in the interface between humans and animals. An example is the Q-fever outbreak in the Netherlands in 2009, where intensive goat farming in the vicinity of populated areas resulted in widespread transmission of the disease within the community [8]. Progressive changes have taken place in the farming of camels in SA in recent years, with a large increase in camel population and camel farms, in the proximity of the cities. While a Washington post editorial last week [9], pointed-out that 'after all, camels are not sitting in hospital waiting rooms' to support the fact that the recent hospital clusters in SA are likely to result from failure of the infection control procedures, camels are becoming more 'urbanised', as periurban camel farming is developing in SA [10].

The change in the epidemiology of MERS-CoV over the past weeks is of concern as stated in a facebook post by WHO's Eastern Mediterranean Regional Office on 23 April 2014 [11]. Interestingly, over the past two years, voices on social media have been increasingly important for reports about the MERS-CoV situation as they have kept the topic high on the agenda of by raising pertinent questions, curating content on blogs, and reporting on cases in near-real time via Twitter. We have seen the MERS CoV debate on Twitter engage bloggers and journalists along with public health organisations, epidemiologists and doctors alike, often resulting in faster reporting and better understanding of the situation. This debate relates to a new phenomenon called 'crowd epidemic intelligence' [12] and is particularly important given the many unknowns about the MERS epidemic.

Recent MERS-CoV cases comprise a significant proportion of healthcare workers and asymptomatic cases or cases presenting with mild symptoms. While this could partly result from a more aggressive screening of contacts in the context of two hospital clusters, it is unlikely to fully account for the observed increase as community-acquired cases have increased in parallel as well [2]. The increase of community-acquired reported cases noted since March this year may correspond to a seasonal factor in community-based transmission, potentially related to exposure to camels. It mimics the increase noted in April 2013 and the first occurrence observed in March 2012. Nowotny and Kolodziejek describe the high viral load in young camels. It is interesting to note that the possible seasonal increase of human MERS-CoV cases corresponds to the end of the calving season for camels in Saudi Arabia. [13] This temporal correlation has still to be explored.

Secondary person-to-person transmission in close family as well as in healthcare settings has occurred in the past and WHO issued recommendations for infection control measures for handling cases of MERS-CoV [14]. Yet it is unclear if the recent hospital clusters in SA and UAE, resulted from a failure to adhere to these recommendations, or from the failure of these measures themselves!

Previous publications in *Eurosurveillance* already in September 2012, September and December 2013 raised questions and called for collaborative international public health efforts to mitigate and possibly contain the MERS-CoV outbreak [15-17]. Still, two years in the epidemic, many of the essential epidemiological parameters remain unknown, e.g. the source, the reservoir and the mode of transmission for primary cases. The role of camels in the emergence and transmission of the disease is further unexplained, even though the evidence for their implication is growing from veterinary and virology studies.

MERS-CoV infections present with a high case-fatality ratio, multiple transmission routes are suspected, cases are reported among healthcare workers, multiple disease foci are affecting SA, and cases have been exported. All these facts are criteria for considering declaring a public health event of international concern listed in annex II of the WHO international health regulations [18]. Two years and 345 cases after the start of this epidemic, we remain with many unanswered questions and lack serological studies and sequences from human cases.

Currently, SA bears the main burden of managing the MERS-CoV epidemic and lately also the UAE. So far, cases detected outside the Arabian Peninsula have not resulted in sustained onward transmission. However, the recent rapid change in the epidemiological pattern of the disease should call for a change of approach to ensure a rapid understanding of the determinants of this emerging epidemic and its effective control, which will require a joint intervention from veterinary as well as human health authorities worldwide.

* Authors' correction:

On request of the authors, in the Figure the title was updated and a footnote added. This correction was made on 25 April 2014.

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A case of imported Middle East Respiratory Syndrome coronavirus infection and public health response, Greece, April 2014

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On 18 April 2014, a case of Middle East Respiratory Syndrome coronavirus (MERS-CoV) infection was laboratory confirmed in Athens, Greece in a patient returning from Jeddah, Saudi Arabia. Main symptoms upon initial presentation were protracted fever and diarrhoea, during hospitalisation he developed bilateral pneumonia and his condition worsened. During 14 days prior to onset of illness, he had extensive contact with the healthcare environment in Jeddah. Contact tracing revealed 73 contacts, no secondary cases had occurred by 22 April.

On 17 April 2014, the Hellenic Center for Disease Control and Prevention (HCDCP) in Athens Greece was notified about a suspected case of Middle East Respiratory Syndrome coronavirus (MERS-CoV) infection in a tertiary care hospital in Athens. The patient had presented a few hours after returning to Greece from Jeddah, Saudi Arabia. Herein we report preliminary findings of an extensive public health investigation conducted from 18 April 2014 (Good Friday) when the case was laboratory confirmed, through 22 April, when contact tracing was nearly complete.

Case report

A 69-year-old Greek national, who resides permanently in Jeddah, Saudi Arabia, presented to a tertiary care center with prolonged fever and diarrhoea, a few hours after arriving in Athens, Greece on 17 April 2014. He had travelled to Greece on the same day on two consecutive flights, one from Jeddah to Amman, Jordan and the other from Amman to Athens. Due to fear of contracting another disease during his travel, the patient was wearing a face mask for most of his journey to Athens up until reaching the hospital.

Clinical presentation and laboratory findings in Athens

He presented at the hospital in Athens with a fever of 38.3 °C and low oxygen saturation (92%). A chest x-ray depicted bilateral lung infiltrates consistent with viral pneumonia. The patient was immediately placed under isolation because of suspicion of MERS-CoV infection and treated by his physicians as community acquired pneumonia.

Laboratory tests were performed on the same day at the National Reference Laboratory for Influenza at the Hellenic Pasteur Institute, Athens. On 18 April, the MERS-CoV infection was confirmed,* in an oropharyngeal sample, based on real-time RT- PCR using primers for the upstream of envelope gene (upE) as a screening test and for the open reading frame (ORF) 1A gene as a confirmatory assay [1]. The same day, the diagnosis was confirmed at the Department of Microbiology of the University of Athens Medical School. Sequencing of the viral genome is ongoing. Laboratory testing for influenza virus, legionella and pneumococcus was negative as well as a stool culture for salmonella.*

After notification of the positive result for MERS-CoV, the patient was transferred to a specialised respiratory disease unit in the Chest Diseases Hospital of Athens where he was treated in a negative pressure room. His respiratory function gradually worsened and since 20

April 2014, he is intubated and ventilated in critical condition in intensive care.

Patient history in Saudi Arabia

The case investigation was conducted through interviews with the patient (before his intubation) and his wife and was based on a standard form available by WHO [2]. Patient history revealed that on 8 April, while still in Jeddah, he had developed a fever and visited a local hospital, the Al-Jedaani Group of Hospitals. He returned to the hospital two days later, on 10 April, when he additionally developed diarrhoea and again on 14 April because of persisting symptoms. He was prescribed ciprofloxacin for a presumptive diagnosis of typhoid fever based on a single positive serology by Widal test (H-antigen test titer of 1:160). Tests for dengue fever and brucellosis were negative, no tests for MERS–CoV were performed. A chest x-ray was negative for infiltrates.

The patient had additional extensive contact with the nosocomial environment in Jeddah from 31 March until 5 April, when his wife was hospitalised in the same hospital as above, with the laboratory confirmed diagnosis of typhoid fever (positive stool culture mentioned in the history of the patient without further documentation). The patient had continuous contact with his wife during her hospital stay. No MERS-CoV diagnostic was performed in the wife during her stay in the hospital in Jeddah.* Laboratory tests in blood, urine, and faeces for MERS-CoV in Greece, several days after her symptoms of infection had resolved, were negative.

The patient had further contact with the healthcare system between 25 and 29 March, when he accompanied his wife to her daily physiotherapy sessions at a rehabilitation center prior to her illness. A timeline of events is depicted in the figure.

The patient had no contact with camels, a known virus reservoir [3, 4] but reported indirect contact with bats, another possible reservoir of MERS-CoV [5], on 15 March outside of the incubation period for MERS-CoV, at an outdoor dinner. Moreover, both the patient and his wife had contact with several people suffering from upper respiratory tract illness with cough as their main symptom, during festivities for the Greek National day on 25 March, four days prior to illness onset for the wife and 14 days prior to illness onset for the patient and during one outdoor dinner on 27 March.

Case definition

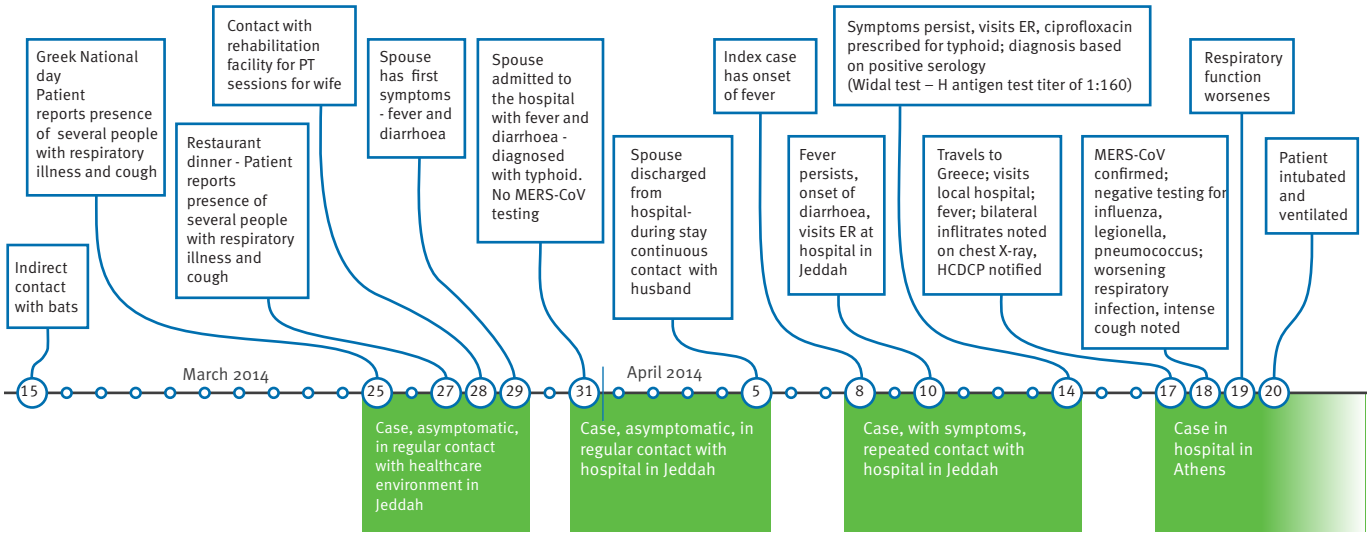
In Greece a *possible case of MERS-CoV* infection is defined as follows:

Any patient with an acute respiratory infection, that may include history of fever (≥38 °C) and cough and indications of pulmonary parenchymal disease (i.e. clinical, radiological or histopathological evidence of pneumonia or acute respiratory distress syndrome (ARDS)), AND at least one of the following:

- history of travel to or residence in affected areas (in the Middle East), during the 14 days before symptom onset;
- close contact, during the 14 days before symptom onset, with a symptomatic confirmed case of MERS-CoV infection;

Figure

Timeline of possible exposure and clinical course of Middle East Respiratory Syndrome coronavirus infection case, Greece March-April 2014



ER: emergency room; HCDCP: Hellenic Center for Disease Control and Prevention; MERS-CoV: Middle East Respiratory Syndrome coronavirus; PT: physiotherapy

- being a healthcare worker that cared for a possible or confirmed case of severe respiratory infection attributed to the new coronavirus;
- belonging to a cluster of severe lower respiratory tract respiratory infections;
- respiratory illness not responding to therapeutic measures instituted by the treating physician.

A *probable case of MERS-CoV* infection in Greece is defined as follows:

A person with a febrile acute respiratory illness with clinical, radiological or histopathological evidence of pulmonary parenchymal disease (e.g. pneumonia or ARDS), AND

- for whom MERS-CoV infection has not been laboratory confirmed, AND
- who has a direct epidemiological link to a confirmed MERS-CoV case.

A *confirmed case of MERS-CoV* is defined as a person with laboratory confirmation of MERS-CoV infection.

According to the Hellenic Center for Disease Control and Prevention (HCDCP) guidelines, clinical samples from the upper respiratory tract (nasopharyngeal swabs), as well as lower respiratory tract specimens (sputum or bronchoalveolar lavage fluid, when possible) have to be collected and tested for MERS-CoV in an initial screening by one of the National Reference Laboratories (Hellenic Pasteur Institute, Microbiology Department University of Athens Medical School, Microbiology Department Aristotle University of Thessaloniki Medical School).

Contact tracing

As soon as the diagnosis was confirmed, contact tracing was initiated by the national public health authorities. Despite the reported lack of respiratory symptoms in the patient, Greek authorities decided to aggressively trace all contacts since: (i) the exact mode of transmission of the MERS-CoV virus is not known; (ii) the patient had fever and diarrhoea and; (iii) he was not continuously using his face mask and there was the possibility that he had mild respiratory symptoms that he ignored.

Contacts were defined according to the scheme proposed in a previous publication [6] as all people who either had close contact with the confirmed case during (i) the air travel, (ii) his 'community stay' between the landing of the airplane and his visit to the hospital, (community contacts were defined as i.e. contact with the patient at the household setting, any person who had prolonged (>15 minutes) face-to-face contact with the confirmed case any time during the illness in a household setting or in any other enclosed setting), or (iii) his hospital stay (i.e. a healthcare worker (HCW) who provided direct clinical or personal care to, or examined the symptomatic confirmed case, or was within close vicinity of an aerosol-generating procedure). HCW contacts were further sub-classified according to whether they wore full personal protective equipment at the time of contact i.e. correctly fitted high filtration mask, gown, gloves and eye protection or not.

Surveillance of contacts

Active follow-up of all cases was initiated by the command center of the HCDCP for all outpatient contacts and from the hospital infection control committee for all HCW. Contacts were contacted by phone and were asked to report to HCDCP any fever ≥38°C (via regular temperature checking preferably twice a day) and/or respiratory tract symptoms and/or digestive tract symptoms during a 14-day period, equal to the maximum incubation period for MERS-CoV according to WHO guidance [7], after their last contact with the confirmed case. Contacts were also provided with a hotline number (the number of the HCDCP command center which operates on a 24/7 basis) and instructed to call anytime in case of any symptoms or other questions.

For all contacts voluntary baseline and follow-up (15 days) serological sampling was offered for future testing for MERS-CoV antibodies. Oropharyngeal swabbing was offered free of charge to all contacts especially those experiencing symptoms as well as immediate prolonged face-to-face contact with the case.

Results from contract tracing

Seventy-three contacts were identified and placed under clinical surveillance; 12 from the two flights i.e. passengers who were on the same flight as the MERS-CoV case with an assigned seat in the same row and in the two rows in front and behind him, nine community contacts and 52 HCW at the two hospitals (six not using personal protective equipment). Prolonged face-to-face contact with the index case, defined as duration of at least 15 minutes within one meter from the confirmed case, was identified in two HCW.

Five contacts chose to undergo oropharyngeal swab testing, two relatives, one HCW and two people exhibiting mild respiratory or gastrointestinal symptoms within three days after contact. All tested negative for MERS-CoV and were offered repeated testing after seven days or earlier if symptoms worsened.

Further public health measures

Revised guidance to HCW for early recognition and response including the institution of the necessary screening, prevention and infection control measures for all suspected cases presenting to Greek Hospitals was sent by email to all hospital administrations to be further distributed to the Infection Control Committee of each hospital and from there to all HCWs, on 19 April. A triage procedure for MERS-CoV using clinical and epidemiological criteria is recommended in all Greek hospitals according to the existing national guidance [8].

The HCDCP travel office has issued extensive guidance for points of entry and especially airports, regarding information to all outgoing and incoming travelers from affected areas, as well as guidance for healthcare services at the airport and air flight crew members. Special posters and leaflets have been distributed throughout the main airports of the country, especially in Athens.

The HCDCP is in continuous communication with the Greek Foreign Ministry, Ministry of Defense, the Civil Aviation service, and consulate offices in Saudi Arabia. Appropriate communication regarding the case was initiated at the European level, though the European Early Warning and Response System (EWRS) on 18 April 2014. In addition, the International Health Regulation (IHR) focal points in Saudi Arabia and Jordan were notified about the case and possible exposure of people in their countries.

A press release was issued on 18 April 2014 by the HCDCP to inform the general public about MERS-CoV and preventive measures.

Discussion

As of 20 April 2014, 250 laboratory confirmed cases of MERS-CoV have been confirmed worldwide since the first detection of this novel virus in Saudi Arabia, including 93 deaths [9]. Herein, we report the fourth imported and tenth laboratory confirmed case in an otherwise healthy adult, in the European Union. Other cases have been reported in the United Kingdom, Germany, France and Italy [10-13].

The source of infection of our patient although unclear, is most likely respiratory transmission from a patient, in the healthcare environment, in Saudi Arabia, as previously reported for other cases [14-17]. Backward investigation revealed close contact with the healthcare environment in Jeddah over several weeks. Regarding other possible sources of infection we doubt that his brief exposure to bats during dinner with friends in a neighborhood of Jeddah, outside the incubation period for MERS-CoV could have constituted a significant risk for infection. However, a zoonotic hypothesis should be always considered in similar cases: because the patient did not report any direct contact with bats or camels, this does not mean he did not have any exposure to an animal source of virus that he might have forgotten or not been aware of.

The information about several cases of respiratory illness in the community, deserves attention and should be further investigated. Both the patient and his wife described extensive spread of a 'respiratory illness' in the community during the incubation period. Alternatively, he could have contracted the infection from his wife if her febrile diarrhoeal episode was associated with MERS-CoV. Although she tested negative for MERS-CoV, she did not have an x-ray, furthermore she could have stopped shedding virus by the time she arrived to Greece and only serological testing will prove whether she had a past infection. The National Reference Center will perform serological tests as soon as an assay becomes available locally. Family clusters of MERS-CoV infections have been described even with asymptomatic or mild cases [18]. If the patient's wife would have been the source of infection, then she would also be a case without respiratory signs and with a very short shedding period; however, this is highly improbable unless she also is a secondary case. If not acquired from his wife, the infection could have been acquired during one of the patient's visits on 10 or 14 April at the local hospital in Jeddah. At this moment, we do not have any official confirmation if there had been cases of MERS-CoV in the hospital.

We are not fully convinced by the diagnosis of typhoid fever in this patient because of the negative laboratory findings despite continued diarrhoea, upon presentation in Greece and the low specificity of a single Widal test [19]. Even though the diarrhoea could have been due to some persistent bowel irritation from a previous typhoid episode, we rather believe that his diarrhoea might be related to the MERS-CoV infection.

Our case together with another recent case of MERS-CoV infection in a traveler from Jeddah to Malaysia [20] as well as the increasing number of newly reported cases by the Ministry of Health of Saudi Arabia in the previous days [21], highlight the need for enhanced awareness regarding the presence of the virus in (i) all persons with fever especially when hospitalised in Saudi Arabia, possibly even when only gastrointestinal symptoms are present and in (ii) travelers coming from the Arabian Peninsula with symptoms compatible with MERS-CoV infection even when atypical, especially if fever is present. In support of the latter we like to point out that our patient had a negative chest x-ray during his hospital evaluation in Saudi Arabia. Moreover, we emphasise the need for vigilance and institution of appropriate, strict infection control measures at the hospital environment in Saudi Arabia.

The absence of respiratory symptoms early in the course of his disease is puzzling in our case. The possibility of mild respiratory symptoms gone unnoticed exists. Thus, we used a rather sensitive case definition for close contacts that included diarrhoea in order to identify new infections presenting in a similar manner to the index patient and prevent further spread.

Since large numbers of Greeks reside in the Arabian peninsula and there is ongoing travel to the affected areas, Greek authorities are considering to issue specific guidance to avoid travel for travelers at risk for developing severe disease similar to the one issued by the Saudi Arabia Ministry of Health for the pilgrimages of Umrah and Hajj [22]. European level action to this effect is anxiously awaited.

In conclusion, every new MERS-CoV case, independent of whether it occurs in the Arabian peninsula, Europe

or elsewhere, and the response to it, is associated with a high work load and investment of resources for the public health sector. However, it is at the same time a step forward and an opportunity to realise the gaps in knowledge associated with this relatively new worldwide threat.

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Conflict of Interest

None declared

Authors' contributions

Sotirios Tsiodras together with George Saroglou, Panos Efstathiou, Athanassios Tsakris , Jenny Kremastinou and Theodoros Papadimitriou coordinated the response to the case at the national level and at the two hospitals and wrote the first draft of manuscript together with Agoritsa Baka, Dimitrios Eliopoulos and Andreas Mentis.

Sotirios Tsiodras, Agoritsa Baka, Dimitris Eliopoulos, Xanthi Dedoukou, Georgia Papamavrou, Spyridoula Karadima, Androula Pavli, Georgia Spala and Helena Maltezou, performed the contact tracing activities.

Maria Emmanouil, Thanos Kossyvakis, Nikolaos Spanakis, Vasiliki Pitiriga, Andreas Mentis, and Athanassios Tsakris performed the laboratory investigation.

Aikaterini Karageorgou and Panos Efstathiou, coordinated the response at the hospital level both locally and nationally

Sotirios Tsiodras, Epaninodnas Kosmas, Stavros Tsiagklis, Spyridon Gkatzias, Nikolaos G. Koulouris, Antonia Koutsoukou, Petros Bakakos, Evangelos, Markozanhs, George Dionelis, Kostantinos Pontikis, Nikoletta Rovina, Magdalene Kyriakopoulou and George Saroglou provided care for the patient.

All co-authors critically read and revised the drafts of the manuscript.

* Authors' correction:

On request of the authors, the words 'in an oropharyngeal sample' were added to the sentence "On 18 April, the MERS-CoV infection was confirmed, in an oropharyngeal sample, based on real-time RT- PCR using primers for the upstream of envelope gene (upE) as a screening test and for the open reading frame (ORF) 1A gene as a confirmatory assay [1]." and the following sentence was deleted: "Tests for MERS-CoV using real-time RT-PCR in an oropharyngeal and a rectal swab as well as in a blood and a urine sample were negative". This correction was made on 28 April.

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Middle East respiratory syndrome coronavirus (MERS-CoV) in dromedary camels, Oman, 2013

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A countrywide survey in Oman revealed Middle East respiratory syndrome coronavirus (MERS-CoV) nucleic acid in five of 76 dromedary camels. Camel-derived MERS-CoV sequences (3,754 nucleotides assembled from partial sequences of the open reading frame (ORF)1a, spike, and ORF4b genes) from Oman and Qatar were slightly different from each other, but closely related to human MERS-CoV sequences from the same geographical areas, suggesting local zoonotic transmission. High viral loads in nasal and conjunctival swabs suggest possible transmission by the respiratory route.

Background

In June 2012 a novel betacoronavirus, subsequently named Middle East respiratory syndrome coronavirus (MERS-CoV), was isolated from a patient with fever and respiratory symptoms who had been admitted to a hospital in Jeddah, Saudi Arabia [1]. As of 22 April 2014, the number of reported laboratory-confirmed cases of MERS-CoV worldwide amounts to 333, with 107 deaths [2]. To date, only fifteen cases, including ten in Europe, have been reported outside of the Middle East [2] and the vast majority of cases were reported from Saudi Arabia. Thus, it seems that the virus may originate from the Arabian Peninsula. Family, healthcare associated and community case clusters of MERS-CoV infections have been reported (e.g. [3]).

Besides limited human-to-human transmission, however, epidemiological data point towards an animal reservoir of MERS-CoV. First evidence of such a reservoir host was provided when all 50 investigated sera of dromedary camels (*Camelus dromedaries*) from Oman exhibited high-titre neutralising antibodies against MERS-CoV [4]. This observation has meanwhile been confirmed by several studies from other countries on the Arabian Peninsula and beyond (e.g. Egypt [5]). Other important farm animals on the Arabian Peninsula such as cattle, goats, sheep and chickens were also investigated but were found negative [6,7]. Although different species of bats carry a variety of coronaviruses and have been suggested as the most likely primary animal reservoir for MERS-CoV, so far only a short (190 nucleotides (nt)) sequence in a conserved region of the MERS-CoV genome was amplified from a faecal pellet of a bat (*Taphozous perforates*) in Saudi Arabia [8]. The present study was initiated in order to identify the virus in camels and to compare it genetically with human-derived MERS-CoV.

Sample collection

In December 2013, nasal and conjunctival swabs were taken from 76 dromedary camels of different age, breed and sex from all over Oman. All swabs were taken in duplicates, one stored in virus isolation medium and the other in the virus-inactivating buffer DNA/RNA Shield (Zymo Research, Irvine, USA). The former samples were frozen at -80°C at the Veterinary Research Center of the Ministry of Agriculture and Fisheries, Oman, for later use, while the latter samples were shipped to the University of Veterinary Medicine Vienna for analysis. DNA/RNA Shield effectively lyses cells and inactivates nucleases and infectious agents. In addition it ensures nucleic acid stability during sample storage and transport at ambient temperatures.

Nucleic acid extraction and polymerase chain reactions

RNA was extracted employing a Quick-RNA MiniPrep Kit (Zymo Research, Irvine, USA), following the manufacturer's instructions.

For screening, two published MERS-CoV reverse transcription-quantitative polymerase chain reaction (RT-qPCR) assays were performed, one in the open reading frame (ORF)1a gene region [9] and the other in the ORF1b gene region [10], and both were optimised for SuperScript III Platinium One-Step RT-qPCR System (Invitrogen/LifeTechnologies, CA, USA). For confirmation, the positive samples were retested by reverse transcription-polymerase chain reactions (RT-PCRs) in the RNA-dependent RNA polymerase (RdRp)- and nucleocapsid (N) gene regions [9] as well as in the spike

Table

Primers used for amplification of Middle East respiratory syndrome coronavirus genetic sequences and subsequent phylogenetic analysis, 2013

Primer name, position[a] and sequence	Target sequence	Expected size
MERS_1767_F: 5'-CTCGCAATTCTCTCTGGAAC-3' MERS_2615_R: 5'-GTCAGTAGGTTGGAGCAGTC-3'	ORF1a	848 nt
MERS_11419_F: 5'-CAA GCC CCA TTG CCT ATC TG-3' MERS_12064_R: 5'-GCT TGA AGT ACG CTA GGA GTG-3'	ORF1a	645 nt
MERS_22074_F: 5'-CGTAATGCCAGTCTGAACTC-3' MERS_23127_R: 5'-CAGGGTGAGTATTGATTAGCG-3′	Spike	958 nt
MERS_24156_F: 5'-GCTGATCCTGGTTATATGCAAGG-3' MERS_24903_R: 5'-CAACCTCAATGTGGTTGCTAGG-3'	Spike	747 nt
MERS_26042_F: 5'-CTT TGG CCA AAC AGG ACG CA-3' MERS_26856_R: 5'-GAC GCC GAG AAA GCC ATA GTT C-3'	ORF4b	814 nt

F: forward; MERS: Middle East respiratory syndrome; nt: nucleotide; ORF: open reading frame; R: reverse.

[a] Referring to the sequence of the MERS-coronavirus strain HCoV-EMC/2012 with the GenBank accession number: JX869059.

gene region [11] and by five other RT-PCRs designed for subsequent phylogenetic analysis (Table). The MERS-CoV genome organisation and the location of the five RT-PCR amplicons used to generate a concatenated sequence for phylogenetic analysis are displayed in Figure 1.

All conventional RT-PCRs were conducted using OneStep RT-PCR kit (Qiagen, Hilden, Germany). Primer synthesis and sequencing in both directions were carried out by Microsynth (Balgach, Switzerland). The obtained sequences were verified by Basic Local Alignment Search Tool (BLAST) search, aligned using the ALIGN PLUS programme (Scientific & Educational Software), and compiled to one concatenated sequence.

Phylogenetic analysis

For phylogenetic analysis, three camel-derived (from Oman, Qatar, and Egypt) and 33 human-derived MERS-CoV sequences were included. A multiple sequence alignment was performed using BioEdit Sequence Alignment Editor version 7.0.9.0 and verified using the CLUSTAL_X programme (version 1.8). Phylogenetic neighbour-joining and maximum likelihood analyses were conducted with the help of the Molecular Evolutionary Genetics Analysis (MEGA) 5 programme [12]. The evolutionary distances were computed using the Kimura 2-parameter model. Bootstrap resampling analysis with 1,000 replicates was employed. The sequences used for phylogenetic analysis were deposited at GenBank under the accession numbers KJ573789–KJ573793 (MERS-CoV sequences derived from camel Oman_30_2013), and KJ598493–KJ598496 and KF933384 (sequences derived from camel Qatar_1_2013).

Results

Nasal and conjunctival swabs of five of a total of 76 camels (6.6%) proved positive in all applied RT-qPCR and RT-PCR assays. The cycle threshold (Ct) values ranged from 15.74 to 36.29. Concatenated sequences of a total of 3,754 nts were obtained from all five positive Omani camels by compiling the sequences derived from the five different PCR amplification products covering the ORF1a, spike and ORF4b gene regions (Table, Figure 1). The five concatenated sequences derived from the Omani camels were 100% identical to each other, and they exhibited 99% identity (differing in 12 of 3,754 nts) to the sequence derived from the Qatari

Figure 1

Genome organisation of Middle East respiratory syndrome coronavirus and location of five reverse transcription-polymerase chain reaction amplification products used to generate a concatenated sequence for phylogenetic analysis, 2013

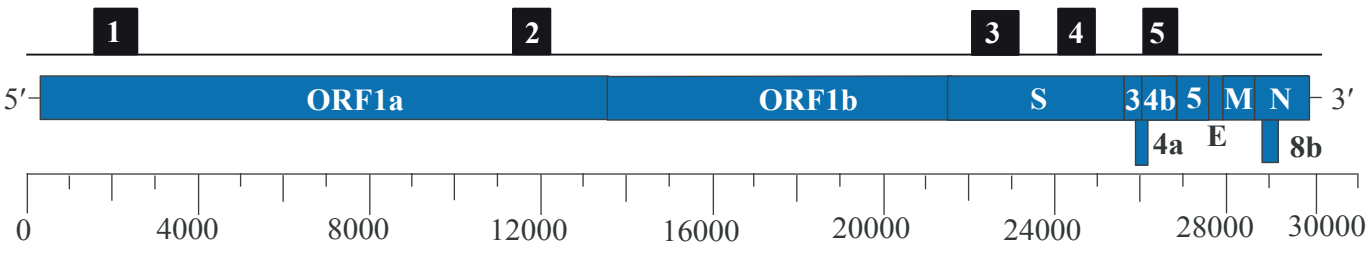
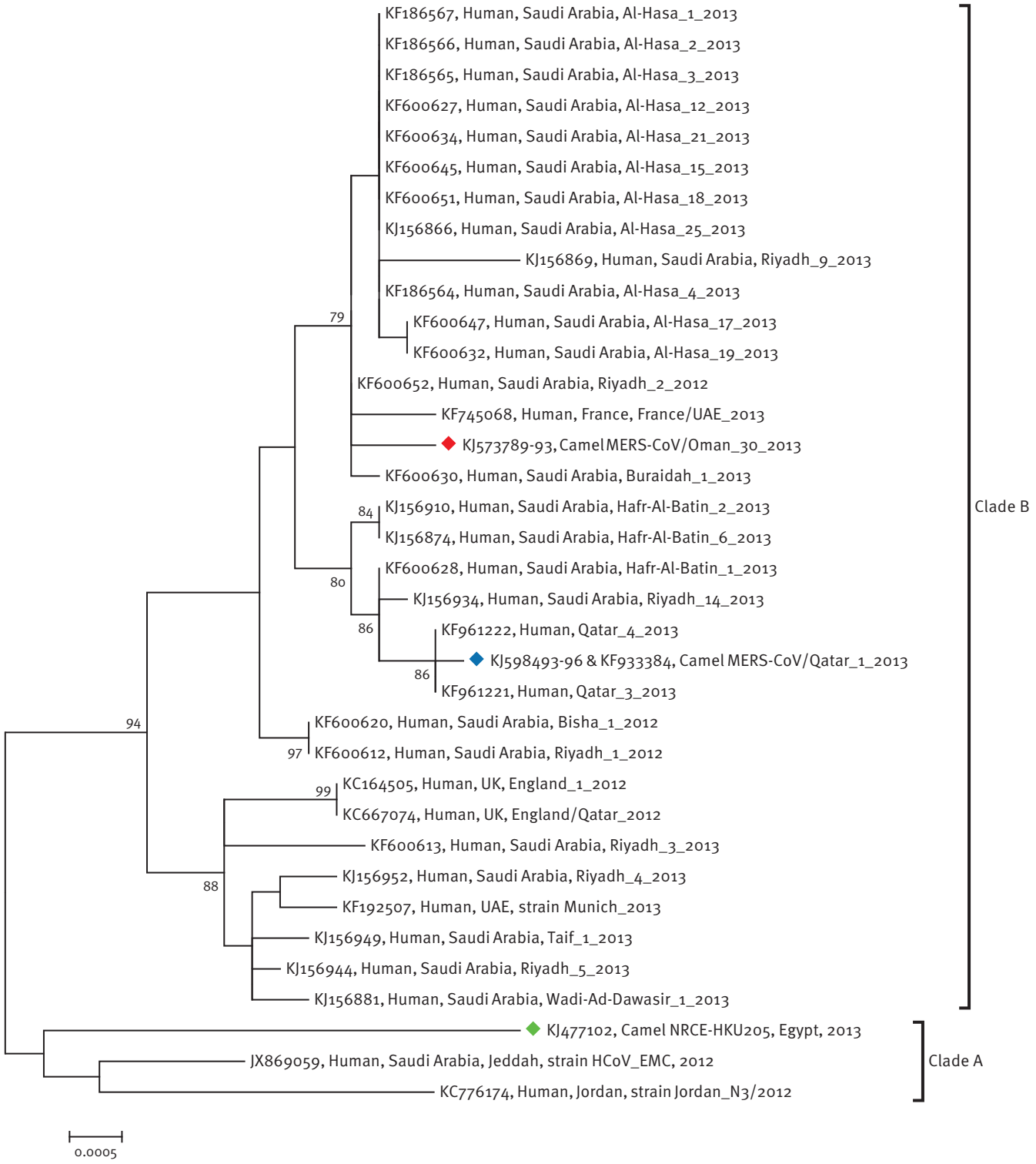


Figure 2

Phylogenetic analysis of three camel- and 33 human-derived Middle East respiratory syndrome coronavirus (MERS-CoV) nucleotide sequences, 2013



UAE: United Arab Emirates; UK: United Kingdom.

Each 3,754 nucleotide long sequence used to generate the tree was obtained from concatenating partial sequences of the open reading frame (ORF)1a, spike and ORF4b gene regions. Of note the different clustering of the camel-derived sequences originating from Oman (marked with a red diamond), Qatar (blue diamond) and Egypt (green diamond). The Qatari and Omani camel-derived MERS-CoV sequences cluster close to the human-derived sequences originating from the same areas.

camel and also 99% identity (31 nts difference) to the known respective sequence derived from an Egyptian camel (Figure 2).

The phylogenetic analysis involving all three currently available camel-derived MERS-CoV sequences and all 33 available human-derived sequences is shown in Figure 2. It clearly indicates that the camel-derived MERS-CoV sequences are clustering independently from each other, but together with the human-derived MERS-CoV sequences from the same geographical areas. This could be demonstrated unambiguously for the Qatari samples (exhibiting only 1 nt difference between the camel-derived and the human-derived MERS-CoV sequences), but also for the camel-derived Omani sequence, which is clustering close to a human MERS-CoV sequence from neighbouring United Arab Emirates (GenBank accession number: KF745068, with only 5 of 3,754 nts being different; no human Omani MERS-CoV sequences are available so far). For the Egyptian camel-derived MERS-CoV sequence [13] no human-derived sequence with as high relative sequence identity and/or from a respectively close geographical area has yet been detected.

Discussion

The results of our study and similar studies from Qatar [11] and Saudi Arabia [14-16] show a close genetic relationship between camel-derived and human-derived MERS-CoV from the same geographical areas, suggesting local zoonotic transmission. A proof of cross-species transmission of MERS-CoV from dromedary camels to humans was reported in a publication on human infection with MERS-CoV after exposure to infected camels in Saudi Arabia recently [15]. The authors concluded that camels may act as a direct source of human MERS-CoV infection. For the implementation of effective, but reasonable precautions, however, further pieces of the puzzle must be put together: a Ct-value of 15.74, corresponding to approximately 33 million RNA copies, in the nasal swab of one of the Omani camels (this study) indicates a very high virus load and the probability of the transmission of high numbers of virus particles by the nasal route. Data from another recent publication suggest that neither passively acquired maternal antibodies nor prior infections seem to lead to full immunity in the camels, and viral nucleic acid was detected in such animals, though at a significantly lower level [16]; these and other recent results, however, require confirmation.

Evidence was provided that MERS-CoV has been circulating in camels at least since 1992 [14], but probably much longer, while strains in humans emerged only recently [17]. Initially, dromedary camels have been considered possible intermediate hosts of MERS-CoV, while bats were suggested as primary reservoirs – in analogy to severe acute respiratory syndrome-coronavirus (SARS-CoV). According to our current knowledge, however, dromedary camels might even be considered the primary reservoir hosts of MERS-CoV. Comparative analyses of camel- and human-derived MERS-CoV sequences exhibit >99% nt identities to each other with so far no striking genetic differences observed. Given the high prevalence of MERS-CoV-specific antibodies in dromedary camels [4,5], infection of camels with this virus seems to be frequent on the Arabian Peninsula. This is in sharp contrast to the comparatively low number of recorded human infections. Since currently no other obvious explanation for this discrepancy exists, it is postulated that a high infectious dose through very close contact between an infected camel and a human being is required for initiation of human MERS-CoV infection by camels. Younger camels may play a particular role in zoonotic transmission since they seem to be more frequently infected and seem to shed more virus than older ones [14-16]. Although the respiratory route is, in our opinion, the most likely route of transmission, a recent paper demonstrated that MERS-CoV survived in raw camel milk slightly longer than in milk of other species [18], suggesting further investigations on a possible food-borne route of transmission. Also, it is not clear yet whether the course of MERS-CoV infection in camels is generally asymptomatic or associated with mild respiratory symptoms, as suggested recently [16].

Conclusions

Phylogenetic analysis and high MERS-CoV viral load in nasal swabs of dromedary camels suggest local zoonotic transmission through the respiratory route.

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Conflict of interest

None declared.

Authors' contributions

NN designed, coordinated and supervised the study and wrote the manuscript; JK performed laboratory testing, analysed data, read and revised manuscript.

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West Nile virus surveillance in mosquitoes, April to October 2013, Vojvodina province, Serbia: implications for the 2014 season

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After the West Nile virus (WNV) outbreak in 2012, we collected mosquito samples from Vojvodina province, Serbia, in 2013. We found high WNV infection rate in two species, *Culex pipiens* and *Anopheles maculipennis*. Phylogenetic analysis showed that Serbian WNV strains from 2013 were most closely related to Italian and Greek strains isolated in 2012 and 2010, respectively. Public health authorities should be aware of a potentially increased risk of WNV activity during the 2014 season.

In this report we provide evidence for an unprecedented detection rate of West Nile virus (WNV) in competent mosquito vectors during the 2013 season. Given that these arthropods overwinter, our WNV surveillance of mosquitoes collected during the 2013 season of high mosquito activity (April to October) from Vojvodina province, Serbia, may have implications for the preparation for the WNV season in 2014.

WNV has been documented in Serbia since the 1970s by seroepidemiological surveys and was detected in *Culex pipiens* mosquitoes during a mosquito surveillance programme conducted in 2010 [1,2]. After the 2012 epidemic, which was associated with WNV lineage 2 and caused 58 human cases in Serbia (eight of them fatal), the virus was successfully detected and characterised in migratory bird samples [3]. Based on phylogenetic analyses it appeared likely that WNV lineage 2 was introduced on two independent occasions [3,4]. The 2012 season was followed by a more severe epidemic season in 2013 with over 300 human cases. In the absence of information about strains causing the WNV outbreak in Serbia during 2013, it was not possible to elucidate whether the 2012 strains circulated and overwintered to cause epidemics in the consecutive year. [5]. The aim of the present study was to detect and characterise WNV strains circulating in the 2013 mosquito activity season in Vojvodina.

Mosquito collection

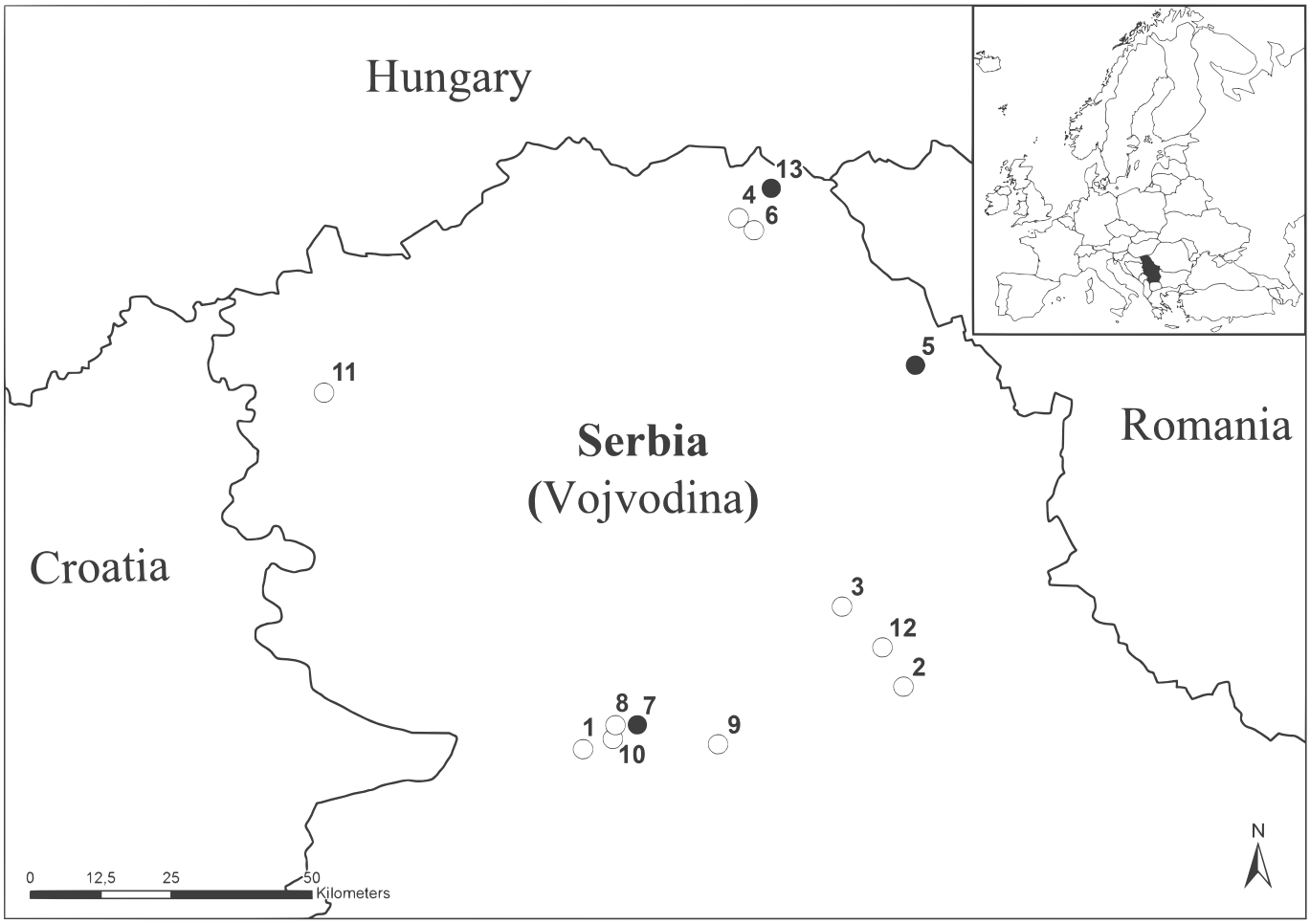
Center for Disease Control and Prevention (CDC) light traps baited with dry ice were placed at 13 sampling sites in Vojvodina province, Serbia, between April and October, 2013. Between four and six traps were used at each sampling site and operated overnight (from 19:00 to 07:00). Altogether, 25 overnight sampling events were conducted during the study period. Sites were sampled as part of the Serbian seasonal mosquito control activities in Vojvodina. Site selection and the number of sampling events directly correlated with the human-inhabited areas or mosquito breeding sites. Sampling sites and collection dates are shown in Figure 1. To obtain the most reliable virological results, traps were emptied immediately after each 12-hour period, and collected specimens were placed on dry ice. Mosquitoes were kept frozen at –80 °C until processing in the laboratory.

Laboratory analysis

Mosquitoes were determined by species according to their taxonomic keys [6] and female mosquitoes were finally pooled by collection site, date and species, with a maximum of 50 individuals per pool. Mosquitoes were homogenised in sterile 600 µL phosphate-buffered saline, viral RNA was extracted from 200 µL of supernatants using DiaExtract Total RNA Isolation Kit (DIAGON Ltd., Hungary). Samples were tested with a TaqMan real-time RT-PCR targeting the NS3 region of WNV, using reagents of the OneStep RT-PCR Kit (Qiagen) [7]. Representative strains from each sampling location were re-amplified with primers targeting a longer fragment of the NS3 genomic region [8]. The amplicons were directly sequenced (BigDye Terminator v1.1 Cycle Sequencing Kit in ABI Prism 310 DNA Sequencer

Figure 1

Location of mosquito sampling sites (n=13) and collection dates for West Nile virus surveillance, Vojvodina province, Serbia, April–October 2013



WNV: West Nile virus.

Black dots represent WNV-positive mosquito collection sites.

5: Kikinda, August 13 August;

7: Novi Sad, 16 June, 29 June, 9 July, 22 July, 1 August, 12 August, 17 September, 2 October;

13: Srpski Krstur, 20 July.

White dots show the negative sampling sites.

1: Beočin, 3 July;

2: Ečka, 9 May;

3: Elemir, 9 May, 4 June, 13 July;

4: Kanjiža, 20 July;

6: Novi Kneževac, 20 July;

8: Novi Sad rural territory. 1 August, 15 August;

9: Kovilj, 22 July;

10: Novi Sad urban territory, 2 October;

11: Sombor, 16 April;

12: Zrenjanin, 9 May, 14 June, 12 July.

instrument, Applied Biosystems) and the obtained sequences were used in phylogenetic analysis.

Vector detection

A total of 6,369 female mosquitoes (combined in 180 pools) representing 11 species were tested in this study (Table). Ten (5.5%) of 180 pools (minimal infection rate (MIR) value: 1.57) sampled were positive for WNV RNA. WNV was detected in nine pools of *C. pipiens* (MIR value: 1.61; 95% confidence interval: 0.70–3.10) and a single pool of *Anopheles maculipennis* (MIR value: 45; 95% confidence interval: 1.20–228.40), suggesting that these two mosquito species may play an important role of WNV transmission in the study area.

Mosquitoes tested positive for WNV were collected in July and August, at sampling sites in Kikinda (four positive pools) (45°48'56.51"N; 20°25'18.24"E), Novi Sad (five positive pools) (45°13'36.10"N; 19°49'30.41"E) and Srpski Krstur (one positive pool) (46° 6'39.68"N;

20° 7'51.76"E). The number of collected *C. pipiens* mosquitoes and the monthly distribution of WNV-positive pools followed a similar profile over time as the mean temperature (Figure 2).

Of the 10 WNV positive pools, four representative strains were selected for further phylogenetic analyses: two strains from Kikinda and one strain each from Srpski Krstur and Novi Sad. In the phylogenetic tree, the Serbian WNV strains (KJ652314–KJ652317) clustered with other WNV lineage 2 sequences, but unambiguously separated from other lineages (Figure 3). The analysis based on the NS3 gene showed that Serbian strains were distinct from each other according to geographic locations, i.e. Kikinda, Srpski Krstur and Novi Sad. WNV sequences from 2013 showed a longer phylogenetic distance from those isolates derived from the same region in 2010 and 2012. The 2013 Serbian WNV strains were most closely related to sequences from Italy and Greece with the greatest nucleotide similarity 98–100% and 98–99%, respectively.

Discussion

In the present mosquito-based surveillance of WNV we found a high infection rate in two mosquito species, *C. pipiens* and *A. maculipennis* in the Vojvodina province, Serbia. These two species have previously been identified as competent vectors of WNV in Europe [9]. The overall 5.5% positivity are consistent with an MIR of 1.61 for *C. pipiens* and of 45 for *A. maculipennis*, which is higher than described in many other European mosquito-based surveillance studies [10,11]. Detection of WNV in *A. maculipennis* in Serbia is a valuable observation, although not unprecedented in Europe [12]. It is important to investigate the role of this species in WNV transmission in the future. The numerous cases of confirmed human infections in 2013 along with the high infection rate of mosquitoes in the same year well demonstrate remarkable activity of the virus [5]. Two WNV-positive sampling sites, Kikinda and Srpski Krstur, are close to the borders with Hungary and Romania. The situation may therefore represent a threat to human health not only in Serbia but also in neighbouring countries, if the ecological conditions in those areas are favourable for mosquito populations.

Phylogenetic analysis showed that Serbian WNV strains from 2013 were most closely related to Italian and Greek WNV strains isolated in 2012 and 2010 and were more diverse than strains identified in 2010 (KC496016) and 2012 (KC407673) in Serbia. Previous studies found at least two different genetic clusters of lineage 2 WNV that circulated simultaneously in Serbia in 2012, suggesting that WNVs were introduced to the country by at least two different events [4]. Phylogenetic analyses with the WNV strains detected from mosquitoes in the present study point to a potential third independent introduction event of the virus.

WNV is typically a seasonal disease strongly associated with mosquito activity. Surveillance of WNV relies on multiple pillars. Laboratory testing of dead birds and of horses with central nervous system disease, together with monitoring of mosquito pools before the late summer–early autumn season provide relevant information to public health authorities about the risk of human infections with WNV. Here we report a greater than expected prevalence of WNV in *C. pipiens* and *A. maculipennis* mosquitoes collected during 2013 in parts of Serbia. Our findings were consistent with the great number of human cases in Serbia during 2013 and provide a possible explanation for the explosive spread of WNV in the affected area. This high detection rate together with the mild winter and the capability of the primary mosquito vectors to overwinter in Serbia may be alarming for the upcoming WNV season.

Public health authorities should be aware of a potentially increased risk, and should educate local inhabitants and inform the public health authorities of neighbouring countries if evidence about enhanced WNV activity emerges over time.

Table

West Nile virus surveillance in mosquitoes in Vojvodina province, Serbia, April–October 2013 (n=6,369)

Species	Number of collected mosquitoes	Number of tested (positive pools)
Culex pipiens	5,568	115 (9)
Aedes vexans	405	19
Ochlerotatus caspius	195	13
Ochlerotatus sticticus	120	7
Coquillettidia richiardii	34	7
Anopheles maculipennis	22	11 (1)
Ochlerotatus geniculatus	18	3
Anopheles hyrcanus	3	1
Culiseta annulata	2	2
Aedes rossicus	1	1
Culiseta longiareolata	1	1
Total	**6,369**	**180 (10)**

Acknowledgments

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Conflict of interest

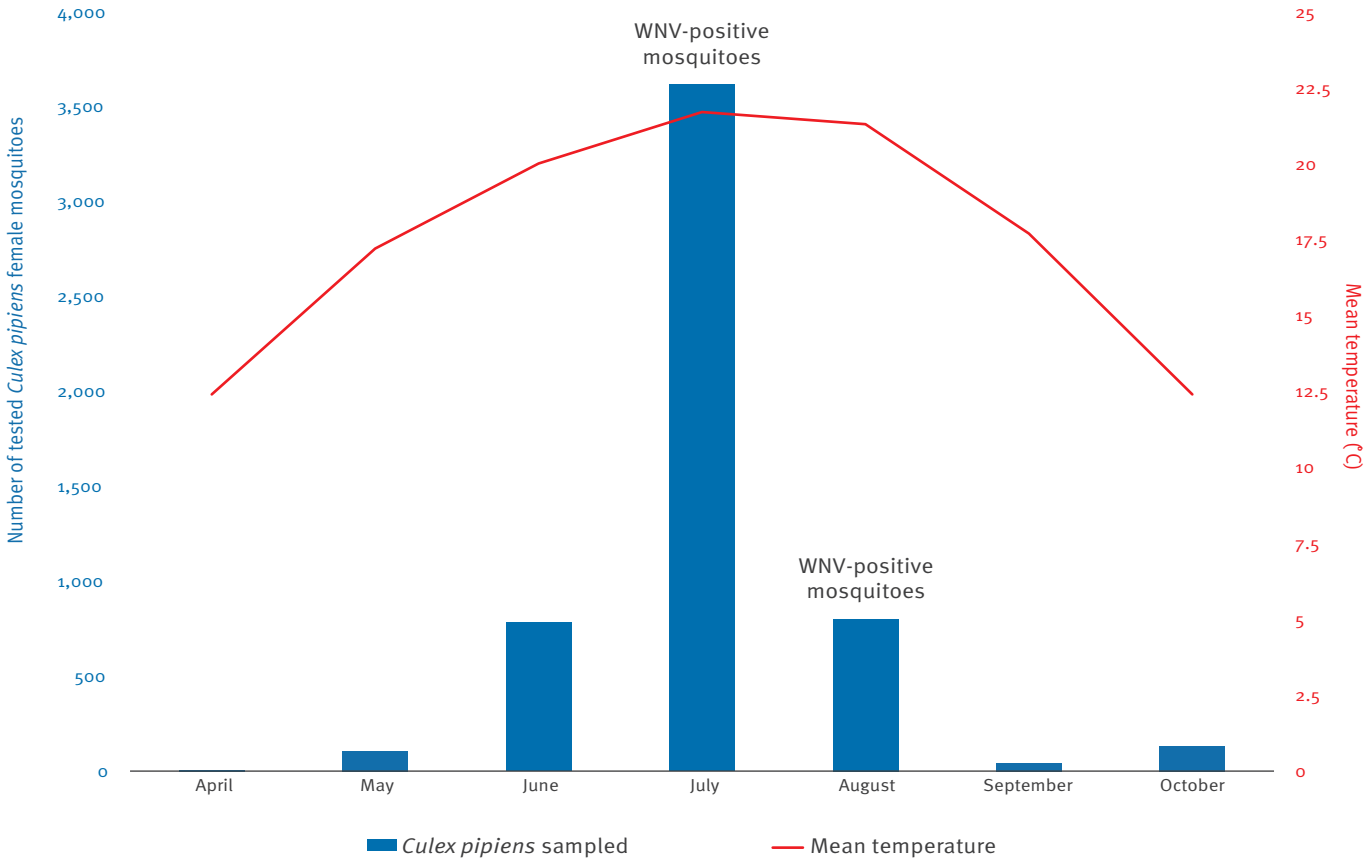
None declared.

Authors' contributions

Gábor Kemenesi: wrote the manuscript, coordinated the study, identified mosquitoes, coordinated laboratory processes. Vesna Milankov, Bosiljka Krtinić: field sampling of

Figure 2

Abundance of female *Culex pipiens* mosquitoes in the sampling sites versus mean temperature, Vojvodina province, Serbia, April–October 2013 (n=6,369)



mosquitoes, interpretation of the study. Anna Kutas, Bianka Dallos, Viktória Németh: perform the virological tests. Miklós Oldal: sequencing, bioinformatics and phylogenetic analyses, contributed to the revision of the draft manuscript. Nóri Somogyi: mosquito identification, entomological verification. Krisztán Bányai: coordination and interpretation of the study, contribution to the revision of the draft manuscript. Ferenc Jakab: supervised the study and co-writer of the manuscript, contribution to the revision of the draft manuscript.

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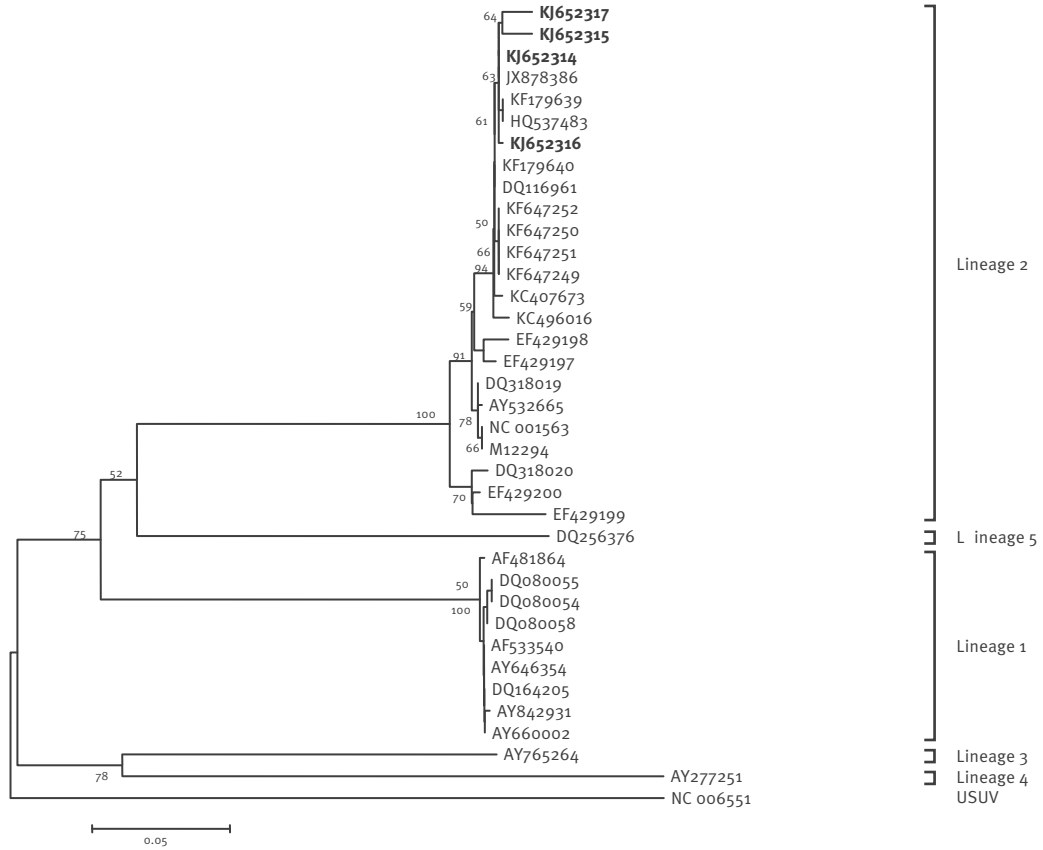
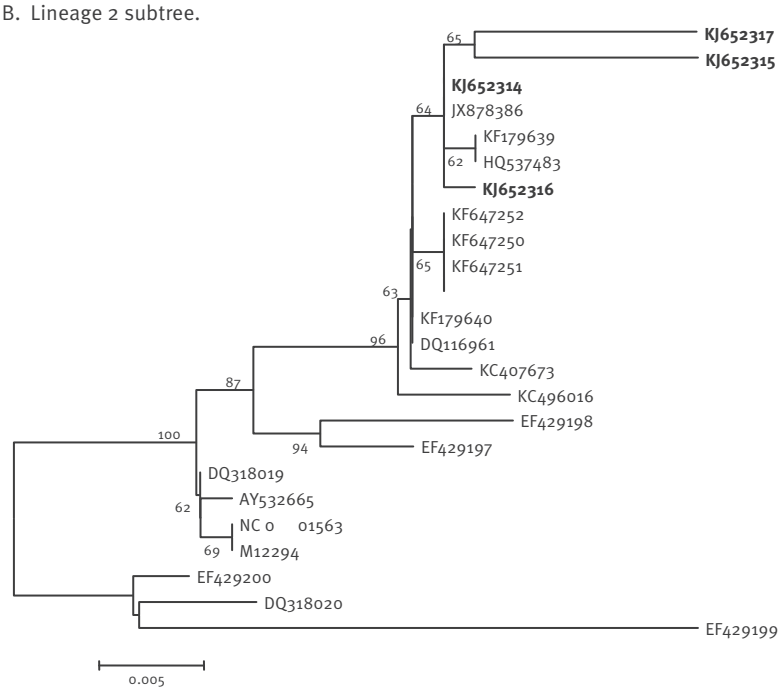
Figure 3

Phylogenetic analyses of West Nile virus strains detected in *Culex pipiens* mosquitoes in Vojvodina province, Serbia, April–October 2013 (n=4)

A. Sequences from the five lineages of West Nile virus.



B. Lineage 2 subtree.



"WNV/95Cp/HUN/2013"	**Serbia /Kikinda /2013**
WNV/60Cp/HUN/2013	**Serbia /Kikinda /2013**
"WNV/35Cp/HUN/2013"	**Serbia /Srpski Krstur /2013**
"2436/12"	Italy / 2012
"Greece/2012/Kavala/39".1	Greece / 2012
"Nea SantaGreece2010"	Greece / 2010
"WNV/71 Cp/HUN/2013"	**Serbia / Novi Sad / 2013**
"Italy/2013/Rovigo/35.1"	Italy / 2013
"Italy/2013/Rovigo/33.1"	Italy / 2013
"Italy/2013/Padova/34.1"	Italy / 2013
"Italy/2013/Rovigo/33.2"	Italy / 2013
"Austria/2008_gh"	Austria / 2008
"goshawkHungary/04"	Hungary / 2004
"Sad/12"	Serbia / 202
"Novi Sad2010"	Serbia / 2010
"SA93/01"	South Africa / 2001
"SPU116/89"	South Africa / 1989
"ArD76104"	Senegal/-
"B956"	-
"Strain 956"	-
"33/G8; 34/F6	-
"H442"	South Africa / 1958
"ArB3573/82"	Central African Republic/-
"SA381/00"	South Africa / 2000

Phylogenetic trees were constructed with MEGA v5.0 software using neighbour-joining algorithm with maximum composite likelihood parameter model based on nucleic acid sequences of a 670 bp long region of the NS3 gene. Number of bootstrap replications was 1,000, and the analysis was performed rooted using Usutu virus as outgroup.

Smoking and older age associated with mumps in an outbreak in a group of highly-vaccinated individuals attending a youth club party, the Netherlands, 2012

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We describe a mumps outbreak in a highly-vaccinated population attending a party at a youth club. In a retrospective cohort study with 60 of approximately 100 participants responding, vaccination status was verified for 58/59 respondents, of whom 54 were vaccinated twice and four once. The attack rate was 22% (13 cases, all vaccinated), with smoking at the party (risk ratio (RR) 3.1; 95% confidence interval (CI): 1.6–6.0, p=0.001) and age ≥21 years (RR 4.7; 95% CI: 2.1–10.2, p<0.0001) as risk factors for disease in the binominal regression analysis. Mild upper respiratory illness was also highly prevalent in those who did not meet the mumps case definition (n=46) after the party, suggesting that mumps virus infection may cause mild disease in vaccinated individuals. Our investigation adds to evidence that crowded social events and smoking may facilitate spread of mumps virus among vaccinated populations, with waning immunity playing a role. The suggestion that mumps virus infection in vaccinated individuals may manifest as mild upper respiratory illness could have implications for transmission and warrants further investigation.

Introduction

Mumps is caused by a paramyxovirus infection and is characterised by acute swelling of the parotid and other salivary glands. Although usually mild, complications such as orchitis, pancreatitis, meningitis and deafness can occur. Routine mumps vaccination has been implemented in the Netherlands since a measles-mumps-rubella (MMR) vaccine containing the Jeryl Lynn virus strain was introduced into the National Immunisation Programme in 1987. This vaccine is offered as a two-dose schedule at 14 months and nine years of age. Although vaccination coverage with two doses has consistently exceeded 93% [1], several outbreaks in highly vaccinated populations have occurred recently, particularly in students [2-4]. These incidents contribute to growing evidence that high vaccine coverage may not suffice to prevent outbreaks [5,6].

In spring 2012, a mumps outbreak occurred in a Dutch village with 25 cases notified to the municipal health service (MHS). Dates of onset for the notified cases ranged from 17 February to 2 April 2012, and three of these cases were laboratory-confirmed as infected with mumps virus genotype G5. Of 23 cases who could be contacted by the MHS, 22 were confirmed to have been vaccinated twice (the remaining case was born outside the Netherlands and had no accessible vaccination record). Eighteen of the 23 cases reported attending a party with approximately 100 guests at a youth club on 9 March 2012. We conducted a retrospective cohort study to investigate attack rates (AR) and risk factors for mumps disease at the party, and to explore the hypothesis that infection of vaccinated individuals may manifest as mild upper respiratory illness (URI).

Methods

We used an online questionnaire (Questback), publicised largely through social media, and active from 4 May to 4 June 2012, to collect information from party attendees regarding demographics, vaccination status, party-related activities (see Table 1), mumps history, and symptoms of mild upper respiratory illness/mumps-like illness within 25 days of the party (the maximum incubation period) and also at the time they completed the questionnaire [7]. We defined cases as respondents with self-reported mumps (swelling of one/both cheeks with symptoms lasting ≥ two days) within 12 to 25 days after the party (the minimum and maximum incubation period), i.e. between 21 March and 3 April 2012. Vaccination status was verified using the national register. We explored associations between risk factors and mumps using univariable analysis and then binomial regression, entering all variables with p<0.20 into the model. To investigate the prevalence of mild respiratory illness around the time of the outbreak, we used McNemar's test to compare the prevalence of URI-specific (runny nose, sore throat, cough, and swollen cervical lymph nodes) and other

Table 1

Questions about party-related activities included in questionnaire sent to people who had attended a youth club party in a village in the Netherlands on 9 March 2012

Activity	Possible response
Time of arrival at party	HH:MM (24h clock)
Time of departure from party	HH:MM (24h clock)
Number of people you spoke for >5 minutes at the party	<10 10-20 21-30 >30
Did you spend time with friends before going to the party?	Y/N
Did you go to another party/bar after leaving the youth club party?	Y/N
During the party, did you do any of the following things:	
Smoke a cigarette	Y/N/Don't know or prefer not to say
Share a cigarette/cannabis joint	Y/N/Don't know or prefer not to say
Share a drink (e.g drink from a glass or bottle that another person had used)	Y/N/Don't know or prefer not to say
Share food with someone (e.g. use a fork or plate that another person had used)	Y/N/Don't know or prefer not to say
Kiss someone	Y/N/Don't know or prefer not to say

N: no; Y:yes.

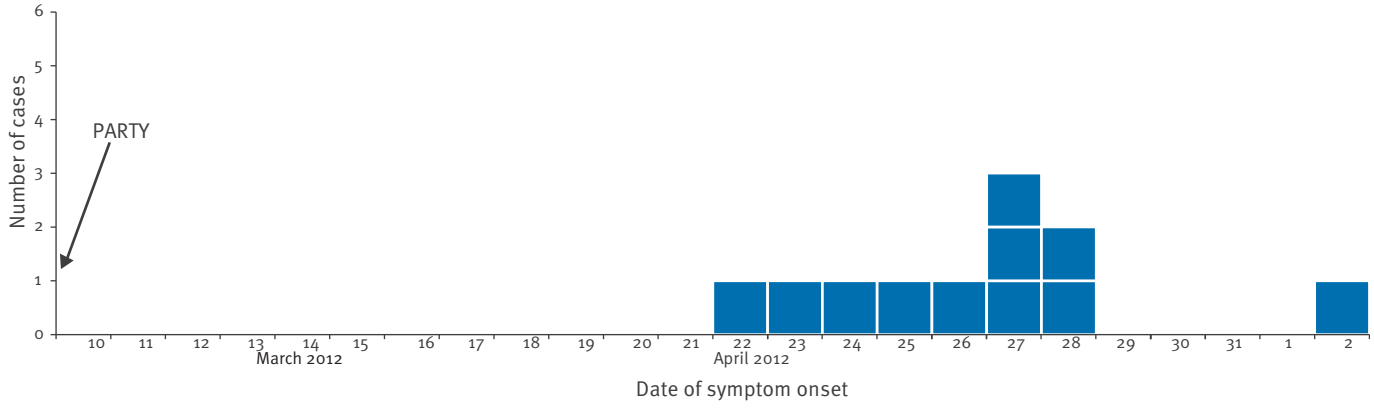
symptoms (stomach ache, myalgia, fever and loss of appetite) within 25 days of the party to the point prevalence of these symptoms at time of questionnaire completion, excluding mumps cases from this analysis. We performed analysis using Stata 11. The study adhered to national ethical guidelines for health research [8-10].

Results

In total, 60 eligible questionnaires were returned. The exact number of people who attended the party is not known, but was estimated to be about 100. We do not know how many people saw the questionnaire, but the approximated response rate is 60%. One individual with confirmed mumps with date of onset before January 2012 was excluded from analyses. The age range of the respondents was 15–25 years old (median 18), and 51% were male (n=30). Vaccination status was verified for 58/59 (98%) respondents, of whom 54 were known to have been vaccinated twice and four at least once. The remaining respondent's vaccination status was unknown. Thirteen respondents met our case definition for mumps, equivalent to an AR of 22%. Nine of these cases had been notified to the MHS. One case had been laboratory-confirmed and eight reported confirmation by a physician. Incubation period ranged from 13 to 24 days (i.e. date of onset between 22 March and 2 April 2012), with a median of 18 days and a peak at 17–18 days (27–28 March 2012, see Figure). All 13 cases had been vaccinated twice. None of the

Figure

Number of cases of mumps associated with attending a village youth club party on 9 March 2012, by date of symptom onset, the Netherlands, March–April 2012 (n=11)



Thirteen cases were reported by questionnaire respondents, but dates of onset were not available for two cases.

Table 2

Characteristics of and risk factors for mumps disease among questionnaire respondents after a youth club party in a village, the Netherlands, 9 March 2012 (n=59)

Variable		N	Cases (n)	Attack rate (%)	Univariable analysis		Binomial regression	
					Risk ratio (95% CI)	p value	Risk ratio (95% CI)	p value
Sex	Male	30	8	27	1.5 (0.5-4.0)	0.534	NA	NA
	Female	29	5	18	Reference	-	-	-
Age group	<21 years	48	7	15	Reference	0.005	4.7 (2.1-10.2)	<0.0001
	21+ years	11	6	55	3.7 (1.5-8.7)	-	-	-
Vaccination status against mumps	Two doses	54	13	24	-	-	-	-
	Vaccinated but number of doses unknown	4	0	0	-	-	NA	NA
	Unknown status	1	0	0	-	-	-	-
Education	Full time	45	8	18	0.5 (0.1-2.0)	0.385	-	-
	Part time	8	3	38	1.1 (0.3-4.8)	-	NA	NA
	None	6	2	33	Reference	-	-	-
Smoked cigarette at party	No	44	7	16	Reference	0.050	3.1 (1.6-6.0)	0.001
	Yes	13	5	42	2.6 (1.0-6.8)	-	-	-

CI: confidence interval; NA: not applicable; Reference: reference group; -: denotes a result that cannot be calculated

For one respondent, information on self-reported mumps symptoms was missing, therefore ARs and RRs are calculated with n=58 as denominator

respondents reported complications (meningitis, orchitis, pancreatitis or deafness) or hospitalisation.

Table 2 shows the results of the univariable and multivariable analyses. Respondents aged ≥21 years had a significantly higher AR (54.6%) than those under 21 (14.9%), (risk ratio (RR) 3.7; 95% CI: 1.5–8.7, p=0.005). Respondents who smoked at the party also had a higher AR (41.7%) than non-smokers (15.9%); this result approached significance (RR 2.6, 95% CI 1.0–6.8, p=0.05). No other variables had p <0.20 in univariable analysis. Both factors remained significant in binomial regression: RR for age ≥21 years was 4.7 (95% CI: 2.1–10.2, p<0.0001), and for smoking at the party 3.1 (95% CI: 1.6–6.0, p=0.001).

Table 3 shows the results of the symptoms analyses. Symptoms that were significantly more prevalent in the 25 days after the party compared to the time of questionnaire completion were all URI-specific, namely sore throat (p=0.0016), cough (p=0.0047) and swollen cervical lymph nodes (p=0.0253).

Table 3

Prevalence of mild upper respiratory and non-respiratory symptoms in non-mumps cases after a youth club party in a village on 9 March 2012, and at time of questionnaire completion (May–June 2012), the Netherlands (n=46)

	9 March – 3 April 2012 (up to 25 days after the party)	4 May – 4 June 2012 (at time of questionnaire completion)	McNemar chi-squared
	n (%)	n (%)	p value
Upper respiratory illness symptoms			
Runny nose	8 (17)	4 (9)	0.2059
Sore throat	11 (24)	1(2)	0.0016
Cough	8 (17)	1 (2)	0.0082
Swollen cervical lymph nodes	4 (9)	0 (0)	0.0455
Other symptoms			
Stomach ache	2 (4)	1 (2)	0.5637
Myalgia	2 (4)	1 (2)	0.5637
Fever	3 (7)	1 (2)	0.3137
Loss of appetite	0 (0)	1 (2)	0.3137

NA: not applicable

Discussion

We describe a mumps outbreak with a 22% AR following a party at a youth club where over 90% of outbreak investigation participants had received two doses of MMR vaccine. Smoking at the party and age ≥21 years were independent risk factors for mumps: smokers were three times more likely to become ill than non-smokers, and individuals aged ≥21 years were almost five times more likely to become ill than individuals under 21. In addition to classic mumps disease, our results suggest that prevalence of mild URI was significantly higher around the time of the outbreak compared to a baseline prevalence at the time of questionnaire completion.

The observation that older age was a risk factor for mumps adds to previous evidence suggesting that waning of vaccine-derived immunity may prompt outbreaks [11–14]. As our investigation was conducted online and several weeks after the outbreak, it was not possible to use serology to explore the role of primary versus secondary vaccine failure in more detail through avidity studies; however, IgG avidity testing following a mumps outbreak in a class of highly vaccinated 17–18 year-olds at a Korean school demonstrated that 73.3% of the cases had secondary vaccine failure [15]. Together with the previous studies that also found older age groups to be at increased risk in mumps outbreaks, we conclude it is likely that waning immunity was the most likely explanation for older individuals being at higher risk of mumps in our study. A possible explanation for smoking being associated with increased risk could be that the practice of sharing cigarettes may transmit mumps virus via saliva; however, this behaviour was not commonly reported by study participants (data not shown). Alternative explanations could be that smoke may act as a vehicle for inhalation of droplets carrying mumps virus, putting anyone who breathed the contaminated air at increased risk, or simply that smokers were in contact with each other more frequently than were non-smokers. As smoking indoors at the party was prohibited, it is likely that smokers congregated together outside the youth club to smoke, which would support the two latter explanations. Nonetheless, smoking was not identified as a risk factor in similar outbreaks investigated previously [3,4].

Our AR of 22% seems high in comparison to other studies that found ARs of 2.2–3.6% in populations vaccinated with the Jeryl Lynn virus strain [5]. It is possible that our study overestimated AR for two reasons: firstly, mumps was self-reported and not confirmed serologically, allowing misclassification. However, in an outbreak context it can be expected that persons experiencing mumps-like symptoms within the incubation period are highly likely to be true cases. Secondly, mumps cases may have been more likely to participate, introducing bias. However, of the 16 cases notified to the MHS who did not respond to the survey and whose date of onset fell within the incubation period, ten reported attending the party. If these ten cases are included in the numerator and all other non-responders are assumed to be non-mumps cases (i.e. making the denominator all the people at the party, estimated to be 100), the estimated AR remains similar at 23%. Two studies in the Netherlands which investigated mumps outbreaks in highly-vaccinated populations following parties found comparable ARs in attendees of 16% [3] and 23% [4]. It is likely that intense crowding and perhaps environmental factors at parties contribute to high ARs.

The finding of a significantly higher prevalence of mild URI in non-mumps cases after the party may be suggestive that some infected individuals may present with mild disease and perhaps contribute to further transmission. This hypothesis is further supported by no similar apparent pattern for non-respiratory symptoms. However, care must be taken in interpretation, as numbers were small and mild URI can be expected to be more common in early spring than in summer. Indeed, routine surveillance data suggest that in 2012, more upper respiratory pathogens were circulating in the Netherlands in weeks 10–14 than in weeks 18–23 (personal communication, Rianne van Gageldonk, September 2012), and unfortunately it was not possible to confirm or refute mumps virus infection serologically.

In summary, our study suggests that intense social mixing, waning immunity and smoking contributed to an outbreak of mumps in a highly-vaccinated population attending a party. Crowded social events appear to facilitate high attack rates among vaccinated populations, especially among age groups where there is no natural immunity and where several years have passed since vaccination. Our finding that mumps virus infection of vaccinated individuals may manifest as mild URI may have implications for transmission and warrants further investigation in future studies where serological confirmation is a possibility. Ongoing studies in the Netherlands will study the role of asymptomatic or mild mumps infections in onwards transmission.

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Conflict of interest

None declared.

Authors' contributions

GL wrote the outbreak investigation study protocol, participated in the outbreak investigation, analysed the data, and wrote the manuscript SO and TW led the outbreak investigation at the local level and contributed to and reviewed the manuscript RB and HB led the laboratory investigations and contributed to and reviewed the manuscript SH supervised the overall project, contributed to the outbreak investigation study protocol, and contributed to and reviewed the manuscript.

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Seasonal influenza immunisation in Europe. Overview of recommendations and vaccination coverage for three seasons: pre-pandemic (2008/09), pandemic (2009/10) and post-pandemic (2010/11)

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Since 2008, annual surveys of influenza vaccination policies, practices and coverage have been undertaken in 29 European Union (EU)/ European Economic Area (EEA) countries. After 2009, this monitored the impact of European Council recommendation to increase vaccination coverage to 75% among risk groups. This paper summarises the results of three seasonal influenza seasons: 2008/09, 2009/10 and 2010/11. In 2008/09, 27/29 countries completed the survey; in 2009/10 and 2010/11, 28/29 completed it. All or almost all countries recommended vaccination of older people (defined as those aged ≥50, ≥55, ≥59, ≥60 or ≥65 years), and people aged ≥6 months with clinical risk and healthcare workers. A total of 23 countries provided vaccination coverage data for older people, but only 7 and 10 had data for the clinical risk groups and healthcare workers, respectively. The number of countries recommending vaccination for some or all pregnant women increased from 10 in 2008/09 to 22 in 2010/11. Only three countries could report coverage among pregnant women. Seasonal influenza vaccination coverage during and after the pandemic season in older people and clinical groups remained unchanged in countries with higher coverage. However, small decreases were seen in most countries during this period. The results of the surveys indicate that most EU/EEA countries recommend influenza vaccination for the main target groups; however, only a few countries have achieved the target of 75% coverage among risk groups. Coverage among healthcare workers remained low.

Introduction

Influenza is a contagious viral respiratory infection, which typically occurs as epidemics during the winter months in temperate zones. Although the illness caused by influenza is usually self-limiting, even in those outside recognised risk groups, it can cause considerable impact on an individual's daily life. At a population level, large numbers of cases with mild to moderate severity of illness increase demands on health services and decrease productivity in the workforce, with associated economic cost and social disruption [1-3]. The number of people affected varies from year to year among countries, making it hard to predict the annual number of deaths or economic impact.

Annual influenza epidemics are associated with high morbidity and mortality. The European Centre for Disease Prevention and Control (ECDC) estimates that on average nearly 40,000 people die prematurely each year from influenza in countries of the European Union (EU)/European Economic Area (EEA) covered by Vaccine European New Integrated Collaboration Efforts (VENICE). VENICE covers all EU/EEA countries except Lichtenstein [4]. Death has been reported in 0.5–1 per 1,000 cases of influenza, with the highest hospitalisation rates occurring among children less than two years of age and individuals ≥65 years in United States [5]. The most effective single public health intervention to mitigate and prevent seasonal influenza is vaccination [6]. Unlike the situation for most childhood vaccines, the European policy for influenza is protection of

those at higher risk either directly by vaccinating them or indirectly by vaccinating those who are likely to infect them (healthcare workers (HCWs) and pregnant women). Vaccination of pregnant women protects the women during and immediately after pregnancy and also decreases the risk to their infant [7].

The primary indicators of success in implementation of vaccination programmes are the group coverages, i.e. the proportion of specific target populations who have been vaccinated. In December 2009, the European Council unanimously recommended that EU countries adopt and implement national action plans to achieve 75% influenza vaccination coverage in all at-risk groups by the influenza season of 2014/15 [8]. The selection of risk groups followed guidance from ECDC and recommendations of the World Health Organization (WHO): 'older' individuals (often defined as aged ≥65 years) and people of all ages above six months with underlying medical conditions [9-11], referred to in this article as clinical risk groups. This EU recommendation encouraged countries to adopt and implement national, regional or local action plans or policies to improve seasonal influenza vaccination including among HCWs and to measure coverage in all risk groups. Countries were also encouraged to report on a voluntary basis to the European Commission on the implementation of the recommendation. ECDC-supported VENICE surveys have been to be the most effective way of doing this without placing additional reporting burdens on countries [12,13].

The overall aim of this paper is to document progress towards achieving the 75% coverage target in risk groups in the EU/EEA Member States since the 2009 recommendation. More specific objectives are to provide an overview of data collected for pre-pandemic (2008/09), pandemic (2009/10) and post-pandemic (2010/11) influenza seasons in order to monitor the progress of specific items in the recommendation and to identify changes in country-specific vaccination recommendations for the targeted age and risk groups during this period and also to report on vaccination coverage in the first season after the 2009/10 pandemic across EU/EEA countries.

Methods

The methodology of the VENICE project influenza surveys has been previously described [14-16]. In November 2011, VENICE conducted the fourth seasonal influenza vaccination survey and collected data for the 2010/11 influenza season. This survey was a collaborative study between EU/EEA countries, ECDC and the VENICE project group.

A standard questionnaire (similar to those used in previous years) was amended to reflect additional information needs for the 2010/11 season. This can be seen in the full survey report [12,17]. Following a pilot phase, the questionnaire was placed on a restricted-access web platform. The questionnaire contained prefilled data from the previous survey relating to the 2009/10 season. Experts (gatekeepers) of all 27 EU Member countries plus Norway and Iceland identified in each country at the beginning of the VENICE project in 2006 were asked to update information on vaccination policies and action plans and were requested to provide the available vaccination coverage rates for the 2010/11 influenza season.

We sought accurate and validated information on population groups that were targeted for influenza vaccination (age, occupation, clinical risk or other groups, e.g. contacts of infants less than six months of age or immunosuppressed individuals), most recent (at the time of survey) vaccination coverage results by population group for the 2010/11 influenza season (or most recent season if not available) and planned policy or operational changes across countries expected in forthcoming years. National survey returns were validated by the gatekeepers with authorities in their ministries of health.

We present and compare vaccination coverage data for the older population, clinical risk groups, pregnant women and HCWs obtained from the three latest consecutive VENICE surveys. All data provided in this paper for the 2009/10 influenza season refer to seasonal influenza vaccination during the 2009/10 pandemic (coverage with the pandemic vaccines have already been reported by VENICE [17]). Influenza vaccination recommendations that are detailed by age group for the 2010/11 influenza season refer to vaccination regardless of other clinical risk indications. Vaccination coverage data in the countries covered by VENICE were provided for one, two or all three influenza seasons, depending on data availability in each country. The methods used (administrative or survey) to calculate vaccination coverage for people in clinical risk groups and HCWs [18] are recorded in this paper. For comparison of vaccination coverage, we did not use any statistical test.

Vaccination coverage data for the United Kingdom (UK) were provided separately for Northern Ireland, Wales, England and Scotland. In our analysis, the UK is counted as one country, but coverage data are presented for each part. Vaccination coverage for pregnant women in the UK was calculated separately for those who were healthy and those with a clinical risk indication.

Results

Response rate

Of the 29 EU/EEA countries participating in the VENICE project, 27 provided data for 2008/09 influenza season (Bulgaria and Luxembourg did not respond to the survey); 28 countries reported data for 2009/10 season (the UK did not respond to the survey, but provided vaccination coverage data); 28 countries responded to the survey that collected data for the 2010/11 influenza

Table 1

Age groups recommended for seasonal influenza vaccination by EU/EEA country[a] (n=29) in the 2010/11 influenza season

Country	Age group										
	Children					Adults (years)					
	≥6 months –2 years	≥6 months –3 years	≥6months –4 years	≥6months –12 years	≥6months –<18 years	≥18 –64	≥50	≥55	≥59	≥60	≥65
Austria					X		X				
Belgium											X
Bulgaria											X
Cyprus											X
Czech Republic											X
Denmark											X
Estonia					X	X					X
Finland		X									X
France											X
Germany										X	
Greece										X	
Hungary											X
Iceland										X	
Ireland[b]							X				
Italy											X
Latvia	X										X
Lithuania											X
Luxembourg											X
Malta			X					X			
The Netherlands										X	
Norway											X
Poland					X			X			
Portugal											X
Romania											X
Slovakia				X					X		
Slovenia	X										X
Spain											X
Sweden											X
United Kingdom											X

EEA: European Economic Area; EU: European Union.

[a] All EU/EEA countries except Lichtenstein, surveyed by the Vaccine European New Integrated Collaboration Effort (VENICE) seasonal influenza survey, November 2011.

[b] Vaccination was recommended for individuals aged >50 years but only those aged >65 years were vaccinated free of charge. Vaccination coverage was calculated for those aged >65 years.

season (Finland did not respond to the survey, but provided clarifying information regarding age groups recommended for vaccination for the 2010/11 season at the time of writing. Consequently, the number of countries in some parts of the results section in this paper was 29).

Policy initiatives

At the time of completion of the 2010/11 influenza seasonal survey (November 2011), it was reported that seven countries had updated a previous action plan and two had developed plans after the Council recommendation to improve seasonal influenza vaccination coverage by 2014/15. The Netherlands had already achieved the target coverage. There was no report of any action plan for 18 countries.

Vaccination recommendations

Age groups targeted for seasonal influenza vaccination

All 29 countries recommended seasonal influenza vaccination for the older-age population in 2010/11; however, the specified age differed between countries. Of the 29 countries, 20 recommended vaccination for individuals ≥65 years. In four countries (Germany, Greece, Iceland and the Netherlands), vaccination was recommended for those aged ≥60 years. Two countries (Malta and Poland) recommended vaccination for individuals ≥55 years; Slovakia recommended vaccination for individuals aged ≥59 years. The remaining two countries (Austria and Ireland) recommended vaccination for those ≥50 years. In Ireland, however, vaccination is only provided free of charge and vaccination coverage

Table 2

Population groups recommended for seasonal influenza vaccination in EU/EEA countries[a] during three influenza seasons

Recommendation for target population groups	Number of countries where vaccination was recommended by influenza season		
	2008/09 (n=27)	2009/10[b] (n=28)	2010/11 (n=28)
Clinical risk groups, disorders			
Chronic pulmonary diseases	27	28	28
Cardiovascular diseases	27	28	28
Renal diseases	25	28	28
Haematological/metabolic disorders	26	28	28
Immunosuppression due to disease or treatment	25	28	28
HIV/AIDS	24	24	25
Any condition compromising respiratory function[c]	12	18	19
Hepatic diseases	15	17	19
Children on long-term aspirin therapy	18	17	16
Morbid obesity (body mass index >40 kg/m2)	–	–	9
Pregnancy-related recommendations			
Vaccination recommended during pregnancy	10	16	22[d]
Any trimester	–	–	9
Either 2nd or 3rd trimester	–	–	13
Postpartum if not vaccinated during pregnancy	–	–	1
Occupational setting			
Healthcare workers	22	23	25
People in essential services (police and fire service)	5	8	8
Military personnel	6	9	10
Poultry industry workers	13	11	12
Families that raise poultry, pigs or waterfowl	4	9	9
Pig industry workers	–	–	8
Educational sector workers	–	–	5
Public transport workers	–	–	6
Energy sector workers	–	–	3
Finance/banking sector workers	–	–	4
Border control/Immigration/customs staff	–	–	4
Other settings/groups			
Residents of long-term care facilities	22	24	25
Household contacts of:			
Individuals belonging to the clinical risk groups	–	10	14
Children <6 months of age	–	6	11
Immunosupressed individuals	–	9	16
Older people (e.g. aged ≥65 years)	–	4	10

AIDS: acquired immunodeficiency syndrome; EEA: European Economic Area; EU: European Union; HIV: human immunodeficiency virus.
Dashes in cells mean that this information was not previously collected, nor specifically asked.

[a] A total of 27 or 28 EU/EEA countries except Lichtenstein surveyed by the Vaccine European New Integrated Collaboration Effort (VENICE) seasonal influenza surveys.

[b] The data refer to seasonal influenza vaccine recommendations in the 2009/10 pandemic influenza season.

[c] Any condition (e.g. cognitive dysfunction, spinal cord injuries, seizure disorders or other neuromuscular disorders) that can compromise respiratory function or the handling of respiratory secretions or that can increase the risk of aspiration.

[d] Recommended for all pregnant women in 19 countries; for those with additional clinical risk in three countries.

monitored for individuals aged ≥65 years. Detailed information on age groups targeted for the 2010/11 influenza season is presented in Table 1.

Of the 29 responding countries, eight (Austria, Estonia, Finland, Latvia, Malta, Poland, Slovakia and Slovenia) reported recommending seasonal influenza vaccination for various age groups of healthy children aged <18 years in the 2010/11 influenza season. In Latvia and Slovenia, vaccination was recommended for children aged ≥6 months to 2 years; in Finland, vaccination was recommended for children aged ≥6 months to 3 years; in Malta, vaccination was recommended for children aged ≥6 months to 4 years; in Slovakia, vaccination was recommended for children aged <12 years. Austria, Estonia and Poland recommended vaccination for children aged ≥6 months to <18 years.

Only two countries reported changes in the age groups recommended for vaccination in the 2010/11 season compared with the 2009/10 season. Poland recommended vaccination for those <18 years in 2010/11, which had not been recommended in previous seasons. Hungary recommended vaccination for those aged ≥65 in 2010/11 instead of those aged ≥60 years as in 2009/10.

Clinical risk groups targeted for seasonal influenza vaccination in the 2010/11 season

All 28 responding countries in 2010/11 recommended vaccination for individuals with chronic pulmonary, cardiovascular and renal disease, those who were immunosuppressed due to disease or treatment and those with haematological and metabolic disorders. A total of 19 countries recommended vaccination for individuals with any condition compromising respiratory function. Nine countries recommended vaccination for individuals with morbid obesity (body mass index ≥40 kg/m^2).

In comparison with previous VENICE surveys and since the Council recommendation, a number of countries had made changes to their seasonal influenza vaccination recommendations and policies compared with previous seasons, specifically related to risk groups. The number of countries that recommended vaccination for pregnant women increased (16 countries in 2009/10 vs 22 countries in 2010/11). Of the 22 countries in 2010/11, 19 recommended vaccination for all pregnant women; three recommended vaccination for pregnant women with an additional clinical risk condition. A total of 13 countries recommended vaccination during the second or third trimester and nine countries recommended vaccination at any stage during pregnancy.

From 2009/10 to 2010/11, more countries included a recommendation that household contacts of people in clinical risk groups, older individuals or children less than 6 months of age should be vaccinated (e.g. 10 countries in 2009/10 vs 14 countries in 2010/11 for household contacts of individuals belonging to clinical risk groups; six countries in 2009/10 vs 11 countries in 2010/11 for household contacts of children less than 6 months of age) (Table 2). There were no substantial changes relating to recommendations regarding vaccination of members of occupational groups. Of the 28 responding countries, 20 recommended vaccination for all HCWs and five only to some HCWs in 2010/11 (the recommendations differed in these five countries: e.g. staff with close contact with patients; or staff with no contact with patients, but contact with potentially contaminated material; or social care staff directly involved in frontline patient care). Three countries did not recommend vaccination for HCWs.

Vaccination coverage rates

Overall, 23 countries provided vaccination coverage data. This is very similar to the situation before the Council recommendation (22 vs 23 countries for 2008/09 and 2010/11, respectively). Six countries (Austria, Belgium, Bulgaria, Cyprus, Czech Republic and Greece) were unable to provide any group-specific coverage data in any of three influenza seasons surveyed.

Table 3

Vaccination coverage for seasonal influenza for children in nine European Union countries[a]

Method for coverage calculation by country	Vaccination coverage (%) by influenza season by age group		
	2008/09	2009/10[b]	2010/11
Administrative method			
≥6 months–<2 years			
Latvia	0.3	0.1	0.1
≥6 months–<3 years			
Finland	–	32	–
≥6 months–<5 years			
Estonia	1	1	–
Poland	2	1	1
Italy	–	6.1	–
Slovenia	0.7	0.8	0.5
≥6 months–<10 years			
France	–	–	13.8
≥6 months–14 years			
Estonia	–	–	0.9
≥6 months–15 years			
Slovakia	8.6	7.5	4.3
5–14 years			
Estonia	2	1	–
Italy	–	5.1	–
Poland	2.9	1.7	1.8
5–18 years			
Slovenia	1.1	1.2	0.5
10–19 years			
France	–	–	16.7
Survey method			
≥6 months–4 years			
France	–	9.9	–
5–14 years			
France	–	6.5	–
≥6 months–15 years			
Portugal	–	13	9.6

EEA: European Economic Area; EU: European Union.

Dashes in cells mean that vaccination coverage was not provided.

[a] Nine of all the EU/EEA countries except Lichtenstein surveyed by the Vaccine European New Integrated Collaboration Effort (VENICE) seasonal influenza survey.

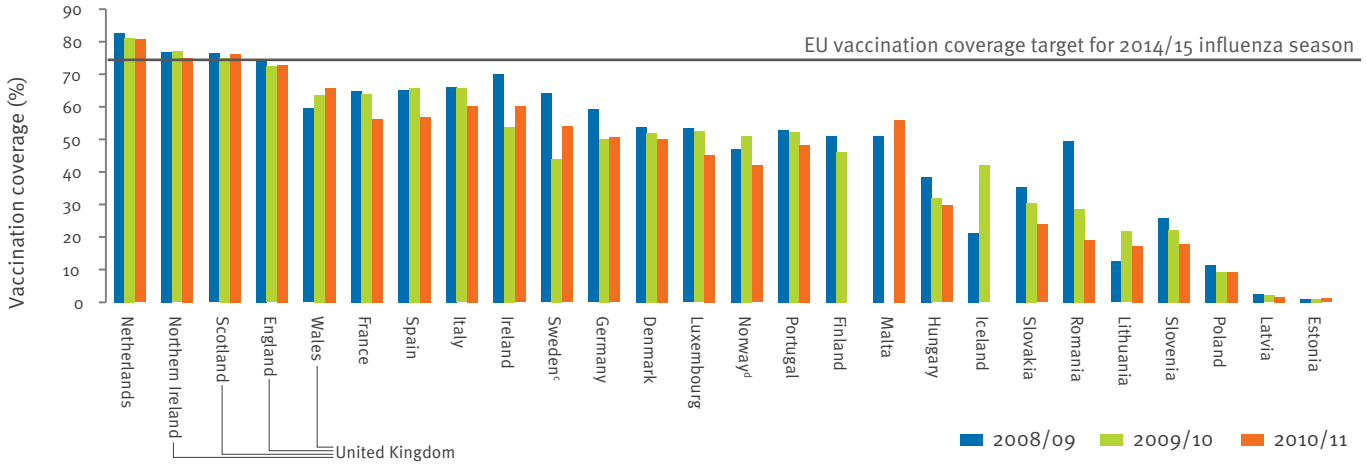
[b] The data refer to seasonal influenza vaccine recommendations in the 2009/10 pandemic influenza season.

Healthy children and adolescents

Nine countries reported vaccination coverage data for a variety of age groups of children and adolescents calculated by administrative or survey methods for at least one of the three influenza seasons (Table 3). Six of these countries (Estonia, Finland, Latvia, Poland, Slovakia and Slovenia) recommended vaccination of children or adolescents, while three other countries (France, Italy and Portugal) provided vaccination

Figure 1

Reported seasonal influenza vaccination coverage in older[a] population in 23 EU/EEA countries[b] during three influenza seasons



EEA: European Economic Area; EU: European Union.

[a] Defined as those aged >55, >59, >60 or ≥65 years in the responding countries.

[b] All EU/EEA countries except Lichtenstein, surveyed by the Vaccine European New Integrated Collaboration Effort (VENICE) seasonal influenza survey. The United Kingdom is counted as one country here.

[c] Reports for Sweden were received for only around 60% of the population for the 2009/10 influenza season.

[d] Coverage results for Norway were calculated for those aged ≥65 years and clinical risk groups together.

coverage for some age groups although vaccination was not recommended for healthy children and adolescents in these countries. Two of the countries that recommended influenza vaccination for children did not provide vaccination coverage data (Austria and Malta).

Older population groups

A total of 23 countries were able to provide vaccination coverage rates of their older population groups targeted for vaccination for two or three influenza seasons (2008/09, 2009/10 or 2010/11), i.e. notwithstanding the recommendations of the European Council and WHO, six countries were not gathering any age group-specific data on vaccination coverage. The data provided for each country refer to the specific age group defined by each country as constituting the older population (≥50, ≥55, ≥59, ≥60 or ≥65 years).

Vaccination coverage among older age groups ranged from 1% (Estonia) to 82% (the Netherlands) in 2008/09 influenza season. The highest reported vaccination coverage rates were in the Netherlands and some parts of the UK (England, Northern Ireland and Scotland) that achieved or almost achieved EU 2014/15 target. Five countries (France, Germany, Ireland, Italy and Spain) reported vaccination coverage around 60% for this specific age group. Denmark, Finland, Luxembourg, Malta, Norway, Portugal and Sweden reported vaccination coverage around 50%. In six countries (Hungary, Iceland, Lithuania, Romania, Slovakia and Slovenia) vaccination coverage was below 50%. In the remaining three countries (Estonia, Latvia and Poland), vaccination coverage was about 10% or less.

Comparing pre-pandemic, pandemic and post-pandemic influenza seasons, there were small decreases in vaccination coverage in half of the countries. In contrast, Ireland, Scotland and Wales reported coverage that was slightly higher in the post-pandemic influenza season in comparison with that during the pandemic (Figure 1).

Clinical risk groups

Of 28 countries surveyed, seven were able to provide vaccination coverage rates for one, two or three influenza seasons for people in clinical risk groups. The coverage varied, ranging from approximately 29% in Ireland (2009/10) to 70% in the Netherlands (2010/11) and 80% in Northern Ireland (2009/10). In all countries that reported vaccination coverage rates, except the Netherlands and Northern Ireland, vaccination coverage was well below the 2014/15 EU target. The Netherlands almost achieved and Northern Ireland had already achieved the target.

Comparing pandemic and post-pandemic influenza seasons in some countries, there was a decrease in coverage of these risk groups (e.g. in Netherlands and Portugal); however, in others (e.g. Scotland), an increase in vaccination coverage was reported.

Overall, three Member States (Romania, Slovenia and the UK) were able to report vaccination coverage rates among pregnant women. The coverage was low in Romania and Slovenia (3.7% and 2.4%, respectively). In the UK, there was variation in reported coverage,

Table 4

Vaccination coverage for seasonal influenza for clinical risk groups, pregnant women, residents of long-term healthcare facilities and healthcare workers[a]

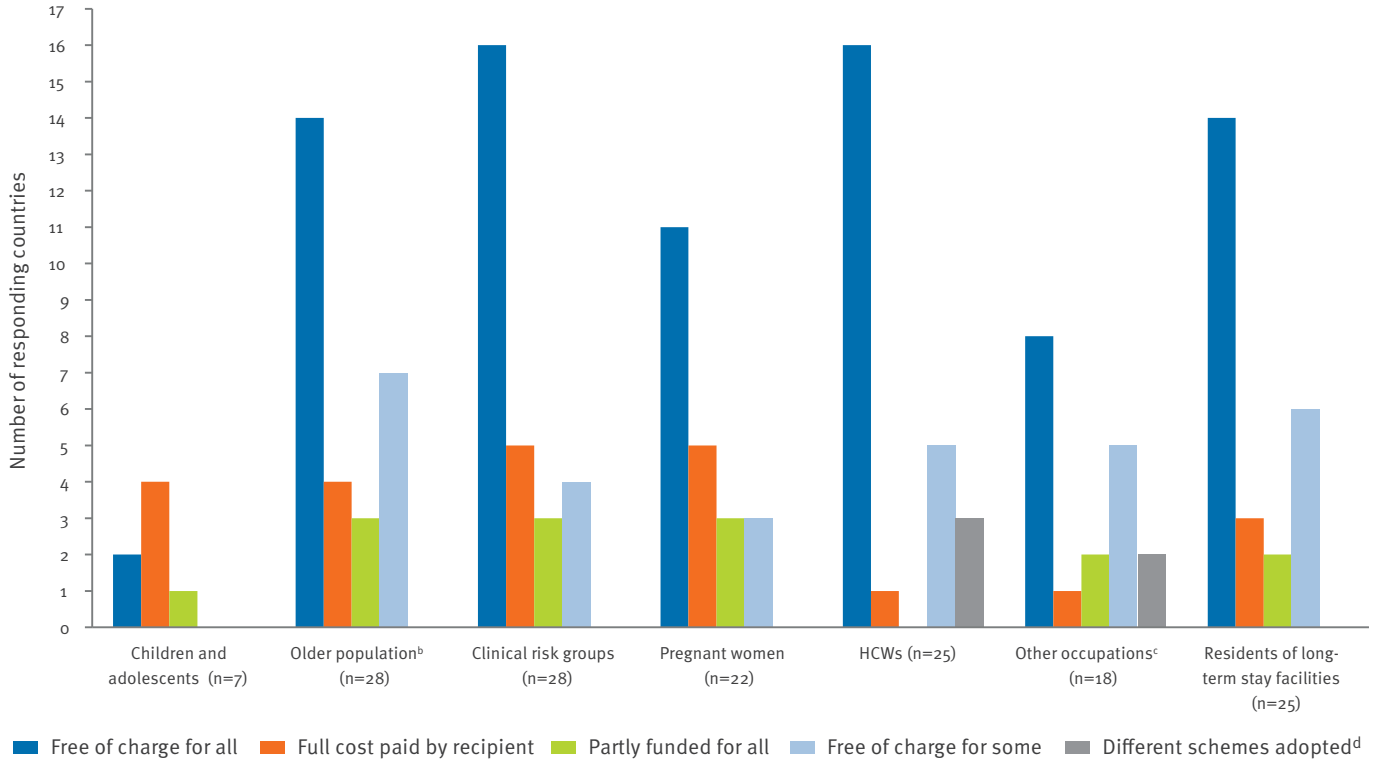
Influenza season	Country and method for calculation of influenza vaccination coverage[b]																			
	France		Germany	Hungary	Ireland	Netherlands	Norway		Portugal		Romania	Slovakia	Slovenia	Spain	United Kingdom					
															England		Northern Ireland	Scotland		Wales
	A	S	S	A	S	A	A[c]	S	A	S	A	A	A	A	A		A	A		A
Clinical risk groups (n=7)[d]																				
2008/09	39.4	–	43.3	–	–	71.5	47	–	–	36	–	–	–	–	47.1		74	47.8		40.8
2009/10[h]	47.2	–	39.8	–	28.9	70.4	51	33	–	32	–	–	–	–	51.6		80	51.1		49.1
2010/11	37.2	–	41	–	–	68.9	47	38	–	29	–	–	–	–	50.4		78.7	56.1		48.5
Pregnant women (n=3)[d]																				
2010/11	–	–	–	–	–	–	–	–	–	–	3.7	–	2.4	–	36.6[e]	56.6[f]	–	64.9[e]	74.8[f]	–
Healthcare workers (n=10 countries)[d]																				
2008/09	–	25.6	30.5	44	–	–	–	–	32	–	97.8	–	–	32.4	–	–	–	–	–	–
2009/10[h]	–	33.9	27.3	53.6	26.5	–	–	12	44	–	–	–	–	34.8	–	26.4	18	–	–	11.6
2010/11	–	27.6	25.8	41.2	–	–	–	14	34	–	63.9	–	16.6	21.1	–	34.7	–	–	30.4	18.5
Residents of long-term care facilities (n=2)[d]																				
2008/09	–	–	–	–	–	–	–	–	80[g]	–	–	82.6	–	–	–	–	–	–	–	–
2009/10[h]	–	–	–	–	–	–	–	–	87	–	–	82.9	–	–	–	–	–	–	–	–
2010/11	–	–	–	–	–	–	–	–	85	–	–	82.9	–	–	–	–	–	–	–	–
Staff of long-term care facilities (n=1)[d]																				
2009/10[h]	–	–	–	–	–	–	–	–	36	–	–	–	–	–	–	–	–	–	–	–
2010/11	–	–	–	–	–	–	–	–	27	–	–	–	–	–	–	–	–	–	–	–

EEA: European Economic Area; EU: European Union.
Dashes in cells mean that data were not provided.

[a] All EU/EEA countries except Lichtenstein, surveyed by the Vaccine European New Integrated Collaboration Effort (VENICE) seasonal influenza survey, November 2011.
[b] A: by administrative method; S: by survey method.
[c] Coverage results calculated for those aged >65 years and clinical risk groups together.
[d] Numbers in parentheses are the number of EU countries that provided vaccination coverage for the particular population group. UK calculated as one country here.
[e] Refers to healthy women
[f] Refers to women with an additional clinical risk factor.
[g] Data were not reported from one region in 2008/09.
[h] The data refer to seasonal influenza vaccine recommendations in the 2009/10 pandemic influenza season.

Figure 2

Payment scheme for influenza vaccine for different age, risk or target groups in EU/EEA countries[a] in the 2010/11 influenza season



EEA: European Economic Area; EU: European Union; HCW: healthcare worker.

[a] EU/EEA countries except Lichtenstein, surveyed by the Vaccine European New Integrated Collaboration Effort (VENICE) seasonal influenza survey, November 2011.

[b] Older population defined as those aged ≥55, ≥59, ≥60 or ≥65 years in the responding countries.

[c] Occupations, clinical risk groups, pregnant women, HCWs and residents of long-stay care facilities as specified in Table 2, according to national recommendations.

[d] Full cost paid by recipient or paid by employer; free of charge for some, paid by recipient for others.

which was calculated separately for healthy pregnant women (37% and 65% in England and Scotland, respectively) and for those with additional clinical risk factors (57% and 65% in England and Scotland, respectively) (Table 4).

Healthcare workers

A total of 10 of the countries were able to report vaccination coverage for one, two or three influenza seasons for HCWs. The reported vaccination coverage varied, ranging from 12% (Norway and Wales in 2009/10) to 98% (Romania in 2008/09). In England, Hungary, Portugal and Scotland, coverage was between 30% and 50% in 2010/11. The remaining countries (France, Germany Norway, Slovenia, Spain and Wales), with exception of Romania, reported vaccination coverage ranged between 14% and 28% in 2010/11. When comparing the pandemic and post-pandemic influenza seasons, there was decrease in vaccination coverage in France, Germany, Hungary, Portugal and Spain, while increased vaccination coverage was reported in England, Wales and Norway. Detailed information is presented in Table 4.

Payment scheme for influenza vaccine

Older individuals (aged ≥50, ≥55, ≥59, ≥60 or ≥65 years, depending on the recommendation in specific countries) received influenza vaccine free of charge in 14 countries in 2010/11; seven of these countries reported vaccination coverage around 50% in older individuals.

Of seven countries that recommended vaccination for children in the 2010/11 influenza season, only two offered the vaccine free of charge (Malta and Slovakia). In four of them (Austria, Estonia, Poland and Slovenia), the full cost was paid by the recipient and in Latvia, the vaccine was partly funded.

The vaccine for members of clinical risk groups and HCWs was free of charge in 16 countries; for pregnant women and residents of long-stay care facilities, the vaccine was free of charge in 11 and 14 countries, respectively, in 2010/11 (Figure 2).

Discussion

The analyses presented in this paper summarise information obtained from annual surveys implemented by VENICE among EU/EEA Member States. The results provide part of the data used to monitor progress following the 2009 Council recommendation [8]. Other relevant data were collected by the European Commission for an interim report which was prepared in 2013 [19]. The same data can also be used to monitor WHO recommendations for groups to be targeted for vaccination (revised in 2012)[20].

Interpretation of results for the period 2008/09 to 2010/11 is complicated as there was both the introduction of the seasonal influenza recommendation and the very varied experience of the pandemic and its vaccination campaigns across European countries [13,21,22]. Given the difficulties experienced with pandemic vaccination in some European countries, it is reassuring that coverage in the older age groups held up as well as it did in 2010/11. However, there has been little improvement in seasonal vaccination coverage in other risk groups despite national and the Council recommendations; in some countries, coverage has decreased. Since only nine countries in November 2011 reported having action plans to implement the Council recommendation, it may be that countries delayed implementing the recommendation, given their pandemic experience.

The challenges that countries face implementing national and Council recommendation varied and may be related to different knowledge, attitudes and practices, risk perception, health systems and related cost issues that differ by country across the region. In addition, media coverage and public debate about vaccine effectiveness, which depends on the match with circulating vaccine strains, can negatively impact vaccination coverage [23,24]. The experience of narcolepsy following use of pandemic vaccines in some EU/EEA countries undoubtedly had a negative impact on public perception of vaccine safety, which may also have led to subsequent decrease in coverage in some countries [25,26]. Anti-vaccination groups and media coverage may also have contributed to this decrease [27].

Many countries appear to have had difficulties monitoring coverage in target groups other than older people. This may be related to differences in health system delivery, how vaccination is implemented in the country and data collection or information systems available for capturing such data. What is possible in one country may not be easily adopted in another.

During and after the pandemic, a number of countries made changes to national recommendations regarding additional risk groups who would benefit from vaccination, influenced by collected epidemiological data during pandemic. More countries recommended vaccination of pregnant women and individuals with morbid obesity. Morbid obesity was recognised as an independent risk factor for hospitalisation and death due to pandemic influenza [28-30]. Before the pandemic, no EU/EEA country had included this group in recommendations for influenza vaccination.

There is currently no consensus within European countries regarding routine seasonal influenza vaccination of children, although such recommendation is now standard in the United States [31] and WHO is recommending vaccination of children ≥6 to 59 months of age [20]. Since the pandemic, more countries are adopting such recommendations [32]. The reluctance of countries to recommend routine seasonal influenza vaccination of children may reflect a lack of evidence regarding cost-effectiveness and risk perception of this measure [32]. Partially, this reflects that there are so few data from Europe. Even in those countries that have recommended seasonal vaccination of children for a number of years, the reasons for low coverage have not been explored in our study but it may reflect low risk perception among the public and the medical community. Live intranasal vaccines that do not require injection were licensed by the European Medicines Agency in 2010 and may increase acceptance and delivery of annual vaccination among those EU/EEA countries recommending vaccination for children [33].

The 2010/11 survey found an increase in the number of countries recommending seasonal influenza vaccination for pregnant women. This increase may reflect better awareness of influenza morbidity among pregnant women that was notably evident during the pandemic [34-36]. A body of literature has demonstrated the safety and effectiveness of vaccine in this group and there may also be benefits for the fetus and newborn child [37,38]. It is disappointing that only three of the 22 countries recommending vaccination of pregnant women were able to report coverage data for this high-risk population. In line with a growing consensus on the importance of vaccination for pregnant women, it is clear that this is an area in which countries should seek to improve information on programme implementation.

In operational terms, HCWs are a crucial group involved in influenza vaccination. They should be vaccinated to protect their patients; they have to give the vaccine and to advocate the vaccination to their patients. Repeated surveys have indicated that it is the opinion of the doctor or nurse that is most important in determining whether or not a person is immunised [39-41]. While most countries have long-standing recommendations to immunise HCWs with seasonal influenza vaccine, only a third could report vaccination coverage rates for any season. In addition, in most of these countries, coverage among HCWs is still low (with Romania and Hungary being the exceptions) and does not show signs of improvement. Moreover, it is surprising that coverage data for staff working in long-term care facilities were provided by only one country and coverage data for residents of such facilities was known in only two countries.

Costs associated with vaccine can be a deterrent or barrier for vaccination, particularly if the costs are borne by the individual [27]. We found that half of the countries surveyed have adopted a policy of provision of vaccine free of charge, in total or in part, predominantly for elderly people, individuals with chronic disease, pregnant women and HCWs. However, four of seven countries reported that the full cost is paid for vaccination of children.

Survey limitations

The survey data presented here have limitations. Comparison of vaccination coverage data is difficult across European countries as different methods of estimating coverage are often used; within a given country, comparisons between years may be difficult if methods or response rate differ by year. How countries enumerate the denominator data (numbers eligible for vaccination) is often difficult to determine, especially when it comes to less specific groups, such as the clinical risk groups and HCWs. The enumeration of numbers vaccinated (numerator data) also has limitations as countries may use either data provided from administrative records or immunisation registries or from others surveys, both of which may have their own limitations. While the surveys report exact details on how numerator and denominator data are calculated, the surveys do not explore or report the specific limitations. Denominator data for clinical risk groups are particularly difficult to estimate accurately for most EU/EEA countries, reflecting the lack of information systems (disease registers) or other standardised methodologies for collecting these data in the countries. Some countries have used population surveys to estimate the number of individuals at risk. But even this may not be comparable between countries as a variety of methodologies have been used (e.g. household surveys, mail, face to face, telephone interviews). The reasons for low or high uptake across EU/EEA countries were not collected in these surveys: future studies are needed.

Recommendations

Additional efforts are needed to increase vaccination coverage among older population groups, individuals with a clinical risk indication, pregnant women and HCWs in order to achieve the target of 75% by the winter of 2014/15. The continued low vaccination coverage levels reported for HCWs are of concern and highlight the need for more focused and intensive health promotion and implementation of vaccination campaigns.

Some countries have achieved coverage higher than the target and there is value in sharing information between countries on how this has been achieved. Additional country-level research is required to identify the reasons for non-vaccination so that specific issues can be addressed through more targeted promotion campaigns. All countries should strive to collect information on vaccination coverage for older age groups as well as those in other risk groups, without which monitoring progress is not possible.

VENICE gatekeepers

Austria: Christina Kral, Jean Paul Klein; Belgium: Pierre Van Damme, Martine Sabbe, Françoise Wuillaume; Bulgaria: Mira Kojouharova; Czech Republic: Bohumir Kriz, Jan Kyncl; Cyprus: Chrystalla Hadjianastassiou, Soteroulla Soteriou; Denmark: Palle Valentiner-Branth, Tyra Grove Krause, Hanne-Dorte Emborg; England: Richard Pebody; Estonia: Natalia Kerbo, Irina Filippova; Finland: Tuija Leino; France: Daniel Levy-Bruhl, Isabelle Bonmarin; Germany: Sabine Reiter, Ole Wichmann; Greece: Theodora Stavrou; Hungary:-Zsuzsanna Molnàr; Iceland: Thorolfur Gudnason; Ireland: Suzanne Cotter; Italy: Fortunato D'Ancona, Caterina Rizzo; Latvia: Jurijs Perevoscikovs; Lithuania: Egle Savickiene; Luxembourg: Berthet Francoise; Malta-Tanya Melillo; the Netherlands: Bianca Snijders, Hester de Melker; Northern Ireland: Brian Smyth; Norway: Berit Feiring; Poland: Iwona Stankiewicz; Portugal: Paula Valente, Teresa Fernandes; Romania: Rodica Popescu; Scotland: Jim McMenamin ; Slovakia: Helena Hudecova; Slovenia: Alenka Kraigher, Veronika Učakar; Spain: Aurora Limia, Isabel Pachon del Amo; Sweden: Annika Linde; Wales: Simon Cottrell.

The gatekeepers are also listed in the 2010/11 report on the VENICE website [17].

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Conflict of interest

None declared.

Authors' contributions

The work presented here was carried out in collaboration between all authors. AN, DOF, PL and TN defined the research theme. JM, SC, DA and TW worked on designing methods for survey and developing survey tool, interpreted results. JM analysed data, interpreted results and wrote the draft manuscript. AN, DLB, CG, PVB, IS and EA provided their comments, participated in discussions, writing the manuscript. LD contributed providing IT support. Gatekeepers completed a questionnaire in each EU/EEA Member State. All authors have contributed to, seen and approved the manuscript.

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The European Medicines Agency publishes interim guidance on enhanced safety surveillance for seasonal influenza vaccines in the European Union

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Article published on 24 April 2014

On 15 April, the European Medicines Agency (EMA) published an interim guidance on enhanced safety surveillance for seasonal influenza vaccines in the European Union. The interim guidance focuses on enhanced safety surveillance and outlines principles to be followed for improved continuous routine surveillance for influenza vaccines [1].

More details can be found **here**.

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ECDC public consultation on working document on potential introduction of varicella vaccination

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Article published on 24 April 2014

The European Centre for Disease Prevention and Control (ECDC) has prepared a preliminary guidance on 'Varicella vaccine in the European Union [1] and has opened a public consultation on the document and the issue.

Evidence points to varicella vaccines being highly immunogenic, efficacious and safe but the recommendation to vaccinate varies across Europe. Those countries that have universal varicella vaccination have seen a reduction in varicella cases, complications, associated hospitalisation and deaths in all age groups, both in vaccinated and in unvaccinated individuals.

The deadline for submissions is 23 May 2014 and further details can be found **here**.

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