



Impact factor **5.9**

Eurosurveillance

Europe's journal on infectious disease epidemiology, prevention and control

Vol. 22 | Weekly issue 4 | 26 January 2017

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Steep rise in norovirus cases and emergence of a new recombinant strain GII.P16-GII.2, Germany, winter 2016

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Citation style for this article:

Niendorf S, Jacobsen S, Faber M, Eis-Hübinger AM, Hofmann J, Zimmermann O, Höhne M, Bock CT. Steep rise in norovirus cases and emergence of a new recombinant strain GII.P16-GII.2, Germany, winter 2016. *Euro Surveill.* 2017;22(4):pii=30447. DOI: <http://dx.doi.org/10.2807/1560-7917.ES.2017.22.4.304472>

Article submitted on 20 December 2016 / accepted on 25 January 2017 / published on 26 January 2017

Since early November 2016, the number of laboratory-confirmed norovirus infections reported in Germany has been increasing steeply. Here, we report the detection and genetic characterisation of an emerging norovirus recombinant, GII.P16-GII.2. This strain was frequently identified as the cause of sporadic cases as well as outbreaks in nine federal states of Germany. Our findings suggest that the emergence of GII.P16-GII.2 contributed to rising case numbers of norovirus gastroenteritis in Germany.

In 2016, the increase of notified norovirus cases in the winter season was unexpectedly strong and early (Figure 1) in Germany. In November 2016, 14,872 laboratory-confirmed cases were reported to the national public health authority compared with a median of 7,810 cases in the same month of the previous five years. This may be due to a new variant's ability to escape herd immunity to the previously circulating strains. In this study, we conducted a phylogenetic analysis of the currently circulating norovirus strains in order to assess whether one or several new strains could be responsible for the current steep rise in norovirus cases.

Sample collection and molecular characterisation

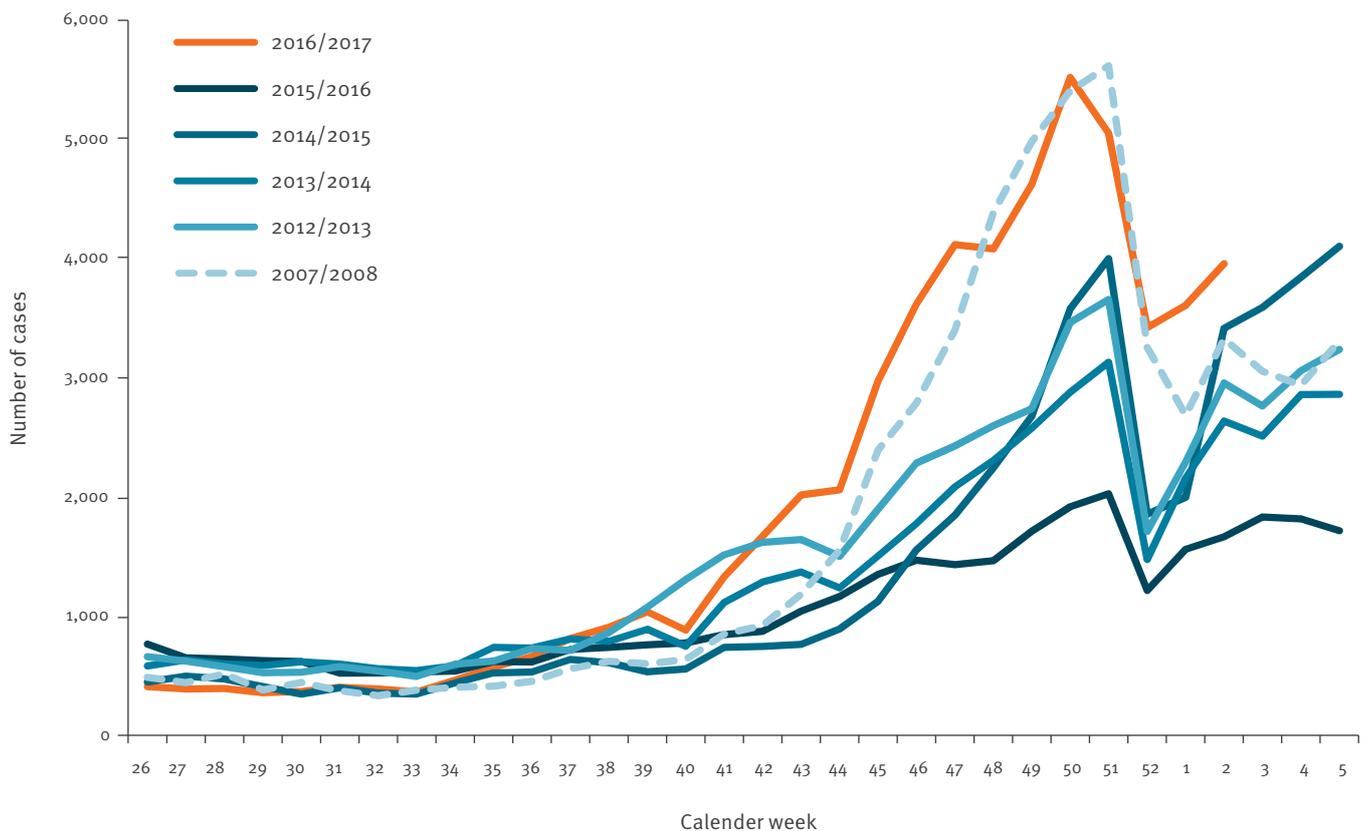
The Consultant Laboratories (CL) and National Reference centre (NRC) are officially appointed and funded by the German Federal Ministry of Health and play a central role in detection and prevention of infection disease in Germany. The coordination of the CL and NRC is hosted by the Robert Koch-Institute. The CL for norovirus at the Robert Koch-Institute is focused on the molecular

surveillance of viral gastroenteritis pathogens, especially noroviruses. For genotyping analysis, stool specimens from norovirus-positive outbreaks were sent to the CL by diagnostic laboratories, physicians and local public health authorities. Between September and December 2016, 240 norovirus positive stool samples from patients with norovirus-associated AGE from 13 federal states of Germany were analysed at the CL for noroviruses. Altogether 175 samples were associated with 69 outbreaks, mainly in childcare facilities (n=39 outbreaks) and nursing homes (n=12 outbreaks) in 11 of 16 federal states (Baden-Wuerttemberg, Bavaria, Berlin, Hesse, Lower Saxony, Mecklenburg-Western Pomerania, North Rhine-Westphalia, Rhineland-Palatinate, Saxony, Schleswig-Holstein and Thuringia). Altogether 65 samples were from sporadic AGE and were sent by hospitals and diagnostics laboratories from six federal states (Baden-Wuerttemberg, Berlin, Brandenburg, Hamburg, Lower Saxony and North Rhine-Westphalia).

Samples were genotyped as previously described [6] by phylogenetic analysis of ORF1 and ORF2 sequences. To determine the recombination breakpoint, 14 samples of the new norovirus recombinant were analysed in addition, using a newly established semi-nested RT-PCR spanning the 3' end of the ORF1 and the P2 domain. In brief, RT-PCR reactions were performed using SuperScriptIII One-Step RT-PCR system Platinum TAQ DNA Polymerase (Thermo Fisher, Waltham MA, US) and primer sets NV1a (5'-ATGAATATGAATGAAGATGG-3'), NV1b (5'-ATGAACACAATAGAAGATGG-3'), NV348a (5'-GGTTRACCCARGAATCAAA-3'), NV348b (5'-GRTTMACCCAAGAITCAAA-3') and NV348c

FIGURE 1

Laboratory-confirmed norovirus infections by calendar week and year of notification, compared previous seasons, Germany, week 26 2016–week 2 2017 (n = 56,384)



Data as on 24 Jan 2017.

(5'-GRTRACCCAIACCTCAAAA-3') for the first PCR (2328 bp fragment). The second PCR reaction was carried out using the HotStarTaq Master Mix Kit (Qiagen, Hildesheim, Germany) and primers NV6 (5'-TACCACTATGATGCAGATTA-3'), NV6a (5'-TATCACTATGATGCTGACTA-3'), NV348a, NV348b, NV348c. PCR conditions were 5 min at 55°C, 55 min at 45°C, 2 min at 94°C, followed by 40 cycles of 15 s at 94°C, 30 s at 45°C, 3 min at 68°C and finally 5 min at 68°C. The resulting 2,274 bp amplicons were subjected to direct sequencing. Nucleotide sequences of these samples were submitted to the GenBank database with the accession numbers KY357449 to KY357462.

Molecular genetic results

We identified emerging recombinant norovirus strains previously not described in Germany in outbreaks or in sporadic cases of AGE. Typing results of all 240 analysed samples are shown in the Table.

The phylogenetic analysis revealed a recombination of GII.P16 (ORF1) and GII.2 (ORF2) strains (Figures 2 and 3). Using SimPlot analysis, the recombination point could be mapped to the ORF1/ORF2 junction region at nucleotide positions 732–734 (data

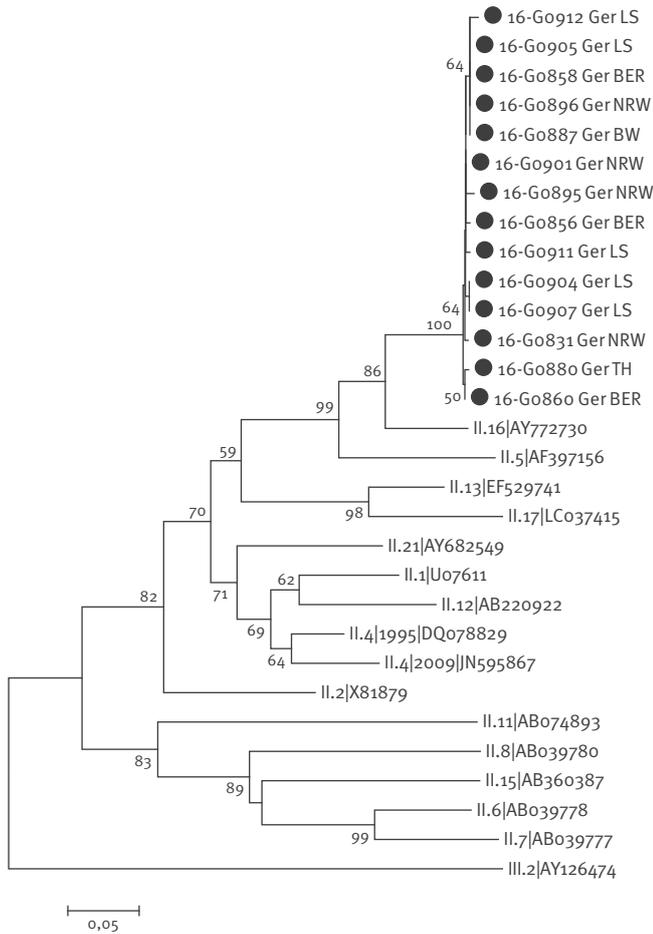
not shown). The recombinant strain GII.P16-GII.2 was detected in 29 of 69 investigated outbreaks, in nine of the 11 federal states of Germany that had outbreaks (Baden-Wuerttemberg, Bavaria, Berlin, Hesse, Lower Saxony, Mecklenburg-Western Pomerania, North Rhine-Westphalia, Rhineland-Palatinate and Thuringia) and was considered as the aetiological agents in 31 of 65 cases of sporadic AGE. The new recombinant was detected in specimens obtained from the sporadic cases in four hospitals in Berlin, North Rhine-Westphalia, Baden-Wuerttemberg and Lower Saxony. Besides the new recombinant strain, the well-known norovirus genotypes GI.P3-GI.3 and GII.P17-GII.17 and the recombinant strains GII.Pe-GII.4 2012 and GII.P4 2009-GII.4 2012 were found co-circulating, were but less frequently detected in the current season.

Discussion

We found a new norovirus strain GII.P16-GII.2 in samples from sporadic AGE and from norovirus outbreaks derived from nine federal states of Germany. It was recently shown that the emergence of new GII.4 norovirus variants can result in an increasing number of reported norovirus infection [5]. This has already been observed in Germany in the season 2007/08 which was

FIGURE 2

Phylogenetic analysis based on the nucleotide sequence of a 357 bp region (ORF1) of genogroup II norovirus, Germany, 2016/17 (n = 14 representative samples)



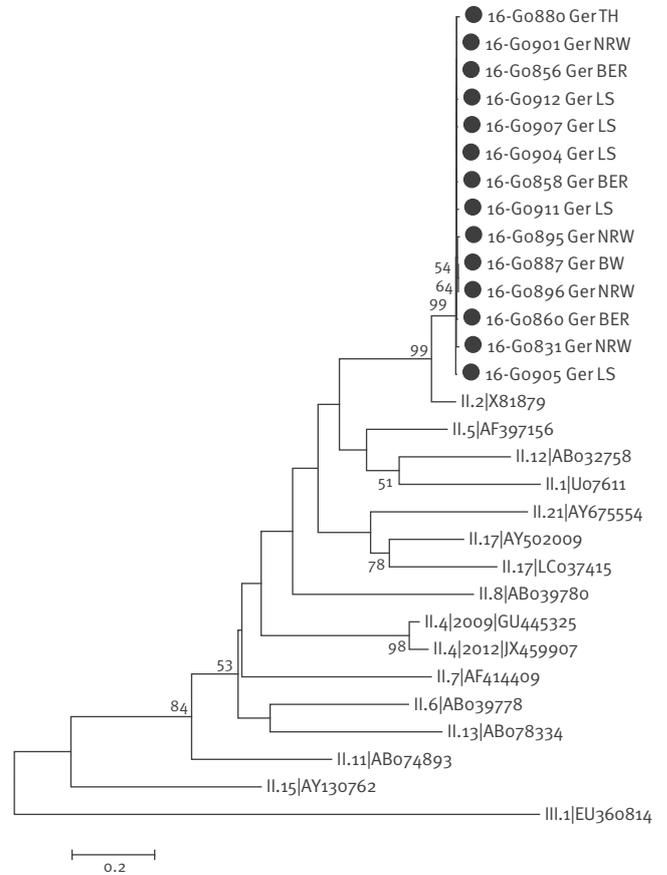
BER: Berlin; BW: Baden-Wuerttemberg; Ger: Germany; LS: Lower Saxony; NRW: North Rhine-Westphalia; TH: Thuringia.

Analysed sequence: nucleotide positions 4,332–4,689 according to accession no: AY772730. Strains analysed in this study are denoted with a black bullet (●). Scale bar indicates nucleotide substitution per site. Sequence alignments were performed with the ClustalW algorithm. Neighbour-joining phylogenetic tree was produced using the MEGA 7 software with bootstrap test (1,000 replicates). Bootstrap values (1,000 replicates) above 50 are shown. The evolutionary distances were computed using the Kimura-2 parameter method.

also characterised by an early rise and high total number of notified norovirus infections, with most of the analysed outbreaks caused by the new epidemic variant GII.4 2006b [7]. Another example is the emergence of a novel variant GII.P17-GII.17 in the season 2014/15, which was first genotyped in China and Japan and replaced the previously dominant genotype GII.Pe-GII.4 2012 with an increased outbreak activity [8,9]. The new 2016 GII.P16-GII.2 recombinant has sporadically been reported to the international molecular surveillance database NoroNet from Australia, Finland, France and Russia, and previously from Japan and China [10,11], suggesting a worldwide distribution.

FIGURE 3

Phylogenetic analysis based on the nucleotide sequence of a 628 bp region of the P2 domain (ORF2) of genogroup II noroviruses, Germany, 2016/17 (n = 14 representative samples)



BER: Berlin; BW: Baden-Wuerttemberg; Ger: Germany; LS: Lower Saxony; NRW: North Rhine-Westphalia; TH: Thuringia.

Analysed sequence: nucleotide positions 5,871–6,509 according to accession no: AY772730. Strains analysed in this study are denoted with a black bullet (●). Scale bar indicates nucleotide substitution per site. Sequence alignments were performed with the ClustalW algorithm. Neighbour-joining phylogenetic tree was produced using the MEGA 7 software with bootstrap test (1,000 replicates). Bootstrap values (1,000 replicates) above 50 are shown. The evolutionary distances were computed using the Kimura-2 parameter method.

So far, it is unclear whether the new recombinant is associated with more severe symptoms. Further molecular and epidemiological investigations are needed to assess whether the emerging new recombinant norovirus strain GII.P16-GII.2 can replace the predominant GII.Pe-GII.4 2012 strain and how this will affect outbreak sizes, course of disease and herd immunity of the population, not only in Germany but also in other countries in Europe.

TABLE

Distribution of norovirus genotypes detected in samples sent to the Consultant Laboratory (outbreaks vs. sporadic cases of acute gastroenteritis), Germany, September–December 2016 (n = 65 samples from sporadic cases, n = 175 samples from 69 outbreaks)

Norovirus genotype	Sporadic cases		Outbreaks	
	n	%	n	%
GI.P1-GI.1	0	0	1	1.4
GI.P3-GI.3	3	4.6	2	2.9
GI.P4-GI.4	1	1.5	3	4.3
GI.P5-GI.5	0	0	1	1.4
GI.Pb-GI.6	0	0	2	2.9
GII.P2-GII.2	1	1.5	0	0
GII.P4 2009-GII.4 2012	5	7.7	7	10.1
GII.P7-GII.6	1	1.5	4	5.8
GII.P7-GII.7	0	0	3	4.3
GII.P8-GII.8	0	0	1	1.4
GII.P16-GII.2	31	47.7	29	42.0
GII.P16-GII.4 2012	7	10.8	7	10.1
GII.P17-GII.17	0	0	6	8.7
GII.P21-GII.3	2	3.1	1	1.4
GII.P21-GII.13	1	1.5	0	0
GII.Pe-GII.4 2012	12	18.5	2	2.9
GII.Pg-GII.1	1	1.5	0	0
Total	65	100	69	100

Acknowledgements

We thank the staff of local public health authorities for collecting samples and data and Kathrin Stanossek, Roswitha Lorenz, Sonja Zimmermann and Ute Obst for excellent technical support.

Conflict of interests

None declared.

Authors' contributions

Wrote the paper: SN, MF. Analysed the data: SN, SJ, MF. Collected samples and data: AMEH, JH, OZ. Critical review the manuscript: SJ, MF, CTB, MH. Conceived and designed the experiments: SN, MH.

References

- Ahmed SM, Hall AJ, Robinson AE, Verhoef L, Premkumar P, Parashar UD, et al. Global prevalence of norovirus in cases of gastroenteritis: a systematic review and meta-analysis. *Lancet Infect Dis.* 2014;14(8):725-30. DOI: 10.1016/S1473-3099(14)70767-4 PMID: 24981041
- Kroneman A, Vega E, Vennema H, Vinjé J, White PA, Hansman G, et al. Proposal for a unified norovirus nomenclature and genotyping. *Arch Virol.* 2013;158(10):2059-68. DOI: 10.1007/s00705-013-1708-5 PMID: 23615870
- de Graaf M, van Beek J, Vennema H, Podkolzin AT, Hewitt J, Bucardo F, et al. Emergence of a novel GII.17 norovirus – End of the GII.4 era? *Euro Surveill.* 2015;20(26):21178. DOI: 10.2807/1560-7917.ES2015.20.26.21178 PMID: 26159308
- Bull RA, Eden JS, Rawlinson WD, White PA. Rapid evolution of pandemic noroviruses of the GII.4 lineage. *PLoS Pathog.* 2010;6(3):e1000831. DOI: 10.1371/journal.ppat.1000831 PMID: 20360972
- Bruggink L, Catton M, Marshall J. A norovirus intervariant GII.4 recombinant in Victoria, Australia, June 2016: the next epidemic variant? *Euro Surveill.* 2016;21(39):30353. DOI: 10.2807/1560-7917.ES.2016.21.39.30353 PMID: 27719750
- Höhne M, Niendorf S, Mas Marques A, Bock CT. Use of sequence analysis of the P2 domain for characterization of norovirus strains causing a large multistate outbreak of norovirus gastroenteritis in Germany 2012. *Int J Med Microbiol.* 2015;305(7):612-8. DOI: 10.1016/j.ijmm.2015.08.010 PMID: 26341330
- Bernard H, Höhne M, Niendorf S, Altmann D, Stark K. Epidemiology of norovirus gastroenteritis in Germany 2001-2009: eight seasons of routine surveillance. *Epidemiol Infect.* 2013;142(1):63-74. DOI: 10.1017/S0950268813000435 PMID: 23517686
- Han J, Ji L, Shen Y, Wu X, Xu D, Chen L. Emergence and predominance of norovirus GII.17 in Huzhou, China, 2014-2015. *Virol J.* 2015;12(1):139. DOI: 10.1186/s12985-015-0370-9 PMID: 26362650
- Matsushima Y, Ishikawa M, Shimizu T, Komane A, Kasuo S, Shinohara M, et al. Genetic analyses of GII.17 norovirus strains in diarrheal disease outbreaks from December 2014 to March 2015 in Japan reveal a novel polymerase sequence and amino acid substitutions in the capsid region. *Euro Surveill.* 2015;20(26):21173. DOI: 10.2807/1560-7917.ES2015.20.26.21173 PMID: 26159307
- Iritani N, Kaida A, Abe N, Kubo H, Sekiguchi J, Yamamoto SP, et al. Detection and genetic characterization of human enteric viruses in oyster-associated gastroenteritis outbreaks between 2001 and 2012 in Osaka City, Japan. *J Med Virol.* 2014;86(12):2019-25. DOI: 10.1002/jmv.23883 PMID: 24415518
- Wang YH, Zhou DJ, Zhou X, Yang T, Ghosh S, Pang BB, et al. Molecular epidemiology of noroviruses in children and adults with acute gastroenteritis in Wuhan, China, 2007-2010. *Arch Virol.* 2012;157(12):2417-24. DOI: 10.1007/s00705-012-1437-1 PMID: 22886184

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Hepatitis A among refugees, asylum seekers and migrants living in hosting facilities, Greece, April to December 2016

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Citation style for this article:

Mellou K, Chrisostomou A, Sideroglou T, Georgakopoulou T, Kyritsi M, Hadjichristodoulou C, Tsiodras S. Hepatitis A among refugees, asylum seekers and migrants living in hosting facilities, Greece, April to December 2016. *Euro Surveill.* 2017;22(4):pii=30448. DOI: <http://dx.doi.org/10.2807/1560-7917.ES.2017.22.4.30448>

Article submitted on 06 December 2016 / accepted on 26 January 2017 / published on 26 January 2017

An increased number of hepatitis A cases among refugees, asylum seekers and migrants residing in hosting facilities in Greece were recorded between April and December 2016. In total, 177 laboratory-confirmed symptomatic cases were reported; of these, 149 (84%) occurred in hosting camps mostly among Syrian children under 15 years. All cases reported symptom onset after their entry into the country. Public health interventions focused on hygiene measures and vaccination.

In this report, we present the epidemiological data for hepatitis A (HA) cases among refugees, asylum seekers and migrants in hosting facilities in Greece between April and December 2016. We also describe the public health response, the main challenges in the management of the cases and the data from the most affected hosting facilities. For the purpose of this manuscript, we refer to refugees, asylum seekers and migrants, as refugees.

Case definition

A HA case was defined as any symptomatic case of acute illness with a discrete onset of any sign or symptom, consistent with acute viral hepatitis (e.g. fever, headache, malaise, anorexia, nausea, vomiting, diarrhoea, and abdominal pain), and either (i) jaundice, or (ii) elevated serum alanine aminotransferase (ALT or SGPT) or aspartate aminotransferase (AST or SGOT) levels, and (iii) confirmed by testing for anti-IgM hepatitis A virus (HAV), from 1 April to 31 December 2016, in the population of refugees residing at hosting facilities (camps and others) in Greece.

Information on data collection

Any HA case presenting at a local health facility was reported to the Hellenic Centre for Disease Control

and Prevention (HCDCP) and recorded in a specific database kept by the Department of Epidemiological Surveillance and Response of the HCDCP.

To calculate notification rates, we used as denominators the information available on population estimates: (i) the mean total population residing in hosting facilities between 1 April 2016 and 31 December 2016, according to daily official estimates [1]; (ii) the distribution of the population by age group and country of origin recorded at preregistration of 28,000 refugees between June and July 2016 [2]. We assumed this distribution of population by age group and country of origin to apply to all refugees residing in hosting facilities during the reporting period. The estimates derived were compatible with information on arrivals to Greece [3].

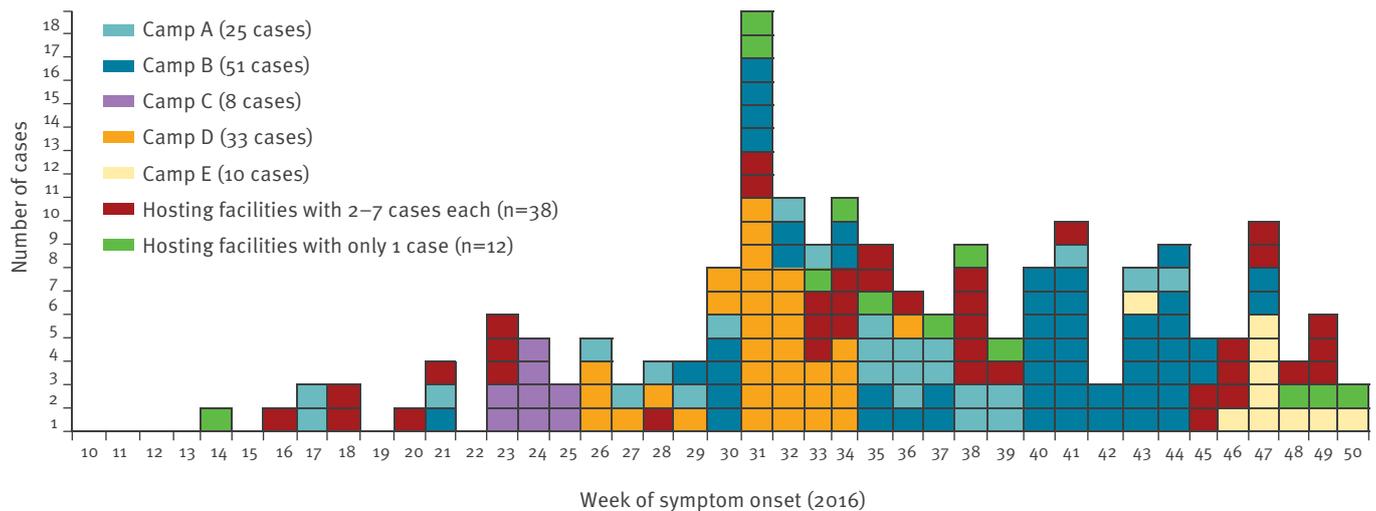
Laboratory tests and molecular typing

Blood samples from clinically-suspected cases were confirmed by testing for anti-IgM HAV with the locally available method (usually an ELISA test).

The Regional Laboratory of Public Health of Thessaly analysed seven stool samples. All had been collected from Syrian children, aged from 4 to 9 years, and the infection was confirmed by serology. Viral nucleic acids were extracted with the iPrep Invitrogen device using the iPrep Virus Kit. We performed molecular detection and typing of the VP1–2A region of the virus according to the HAV NET typing protocol [4]. The initial reverse transcriptase PCR was performed with the SuperScript One-Step RT-PCR System with Platinum Taq DNA Polymerase, Invitrogen, whereas the nested PCR was performed with KAPA Taq HotStart PCR kit, Kapa Biosystems, according to manufacturers' instructions. Sequencing followed to an ABI 3730xl Analyser.

FIGURE

Cases of hepatitis A among refugees by week of symptom onset, Greece, April–December, 2016 (n=177)



Results

Epidemiological investigation

In total, 177 HA cases were recorded from 1 April to 31 December 2016.

Cases were reported in 29 different locations: 16 hosting camps (149 cases), 10 hotels (23 cases) and three apartments (5 cases). Of these, 150 cases were hospitalised (85%) after referral by the medical services in the hosting facilities. Triage, laboratory investigation and hospital care were provided free of charge to all cases by the National Health Care System hospitals in Greece.

One hundred and forty two (80%) of the recorded cases presented with jaundice; the average alanine and aspartate aminotransferase values (norm: 7 to 56 and 5 to 40 units per liter (IU/L), respectively) were 1,294 IU/L (standard deviation (SD): 799) and 1,085 IU/L (SD: 866). All cases fully recovered; no complications or cases of fulminant HA or acute liver failure were recorded.

The distribution of notified cases by week of symptom onset is presented in the Figure.

All cases reported onset of symptoms at least 50 days after their entry in the country (i.e. after the maximum incubation period for HA). Ninety-six cases were male (54%), the median age of cases was 7 years (range: 8 months–29 years), and 86% (n=152) were children under 15 years.

The majority of cases were from Syria (152 cases) followed by Iraq (9 cases), Afghanistan (8 cases), while in eight cases the country of origin was not recorded. The notification rate among Syrians was almost seven times higher than that among the refugees from Afghanistan

and Iraq. The distribution of cases and the notification rate per 1,000 estimated population by age group and country of origin is presented in Table 1.

One hundred and fifteen cases (65%) were notified from northern Greece, 44 (25%) from central Greece, 16 (9%) from Attica and 2 (1%) from eastern Aegean. In five camps the occurrence of HA cases lasted for several weeks and mass childhood vaccination was undertaken. Data regarding these camps are summarised in Table 2.

During this period, four HA cases were reported via the mandatory notification system among staff responsible for cleaning the lavatories and other common areas in two hosting camps. These cases were hospitalised with jaundice and fever and were discharged from the hospital 8 to 10 days after full recovery. No other cases among the members of the non-governmental organisations or people working at or visiting the hosting facilities have been identified. No secondary community cases related to the cases in the hosting facilities have been recorded.

Based on the results from the Regional Laboratory of Public Health of Thessaly all seven samples tested were HAV genotype I subtype B.

Management and control measures

A protocol for the management and response to the occurrence of HA cases at refugee hosting facilities was developed by the epidemiologists of HCDCP in early April as an adaptation of the 'Hepatitis A management protocol for sporadic cases and outbreaks', already available at HCDCP [5].

During intervention, focus was placed on hygiene measures and vaccination of close contacts of sporadic cases, within 14 days after their last contact with the

TABLE 1

Number of notified cases and notification rate of hepatitis A in hosting facilities, by age group and country of origin, Greece, April–December 2016 (n=169^a)

Age group (years)	Country of origin		
	Syria n (rate per 1,000) ^b	Afghanistan n (rate per 1,000) ^b	Iraq n (rate per 1,000) ^b
0–4	43 (8.8)	4 (2.1)	2 (1.8)
5–9	55 (11.9)	1 (0.5)	3 (2.5)
10–14	32 (9.5)	2 (1.2)	4 (4.2)
15–29	22 (2.2)	1 (0.1)	0 (0.0)
Total	152 (5.0)	8 (0.5)	9 (1.2)

^a For eight cases the country of origin was not known.

^b Notification rate per 1,000 estimated population as described in the text.

case (ring vaccination). Priority was given to vaccination of children aged 1–14 years. For contacts aged 15 years old or older, serological testing for anti-HAV IgG and consequent vaccination according to result was recommended, given that the cost of testing was lower than the vaccination cost and that testing could be performed in time, without cancelling out the benefit from vaccination. If serological testing was not possible, vaccination was recommended instead. It should be noted that even though serological testing was included in the protocol, in practice, most adult contacts (aged 15 years or older) were vaccinated without prior serological testing because of time constraints.

Refugees were specifically advised on hygiene measures and the need for thorough hand washing with soap and water. In addition, brochures as well as posters with instructions on personal hygiene translated into Arabic, Urdu and Farsi were distributed to the population at hosting facilities. Hygiene rules regarding drinking water, food preparation and waste disposal were promoted in cooperation with the local public health authorities.

When cases occurred in a camp, staff and volunteers were also informed about the disease, the modes of transmission and the necessary hygiene measures.

According to the National Immunisation Programme, all cleaning staff and people working in waste and sewage management are advised to get vaccinated against HAV [6].

Vaccination of the entire childhood population was decided in five camps during this period, and performed with the co-operation of the non-governmental organisations (NGOs) (Table 1).

In total, 1,681 refugees were vaccinated from April to December 2016, with 1,082 (64.4%) vaccinations being part of the mass child vaccination at the five camps, and 599 vaccinations (of 309 and 290 contacts aged 1–14 years and 15 years old or older, respectively)

performed during ring vaccination of the 177 reported cases.

Hepatitis A notifications and situation of refugees in Greece

HA is a mandatory notifiable disease in Greece. Incidence in the general population, as well as the number of travel-related cases has been quite stable in recent years [7]. The mean notification rate for 2010–2015 was 0.72 per 10,000 population (SD: 0.39). In 2015 and the first trimester of 2016, 15 and 10 cases respectively were reported among refugees travelling via Greece to other European countries. During this time, it was estimated by the United Nations High Commission for Refugees (UNHCR) that there were 857,000 and 151,000 arrivals to Greece respectively, and most of these persons left the country after a few days' stay [3].

In March 2016, the northern borders of Greece closed and Greece, from a previously mainly transit country, turned into one of medium-term stay and a large number of refugees were 'stranded' in the country [3]. The population residing in hosting facilities was estimated to be around 52,000 on 1 April; by 31 December, this number had increased to 62,700 people [1]. At the end of December, the hosting facilities included 51 camps (including first reception centres in the Aegean islands), and several hotels and apartments, under an UNHCR initiative [1]. The size and the demographic characteristics of the population residing in each of the hosting facilities have been changing in the period mentioned above because of the arrival of new refugees and their mobility inside the country. The majority of refugees who arrived to Greece during 2016 originated from Syria, Afghanistan, and Iraq [3].

Discussion

HAV was frequently reported among refugees residing in hosting facilities in Greece from April to December 2016. Most cases were reported in children aged 1–14 years. Overcrowding and poor personal hygiene at hosting facilities are among the main predisposing factors for HAV infection in refugees. Children are the main pool of susceptible population, since adult refugees from HAV endemic countries are expected to be immune due to prior infection. In most instances, children are not vaccinated and often experience asymptomatic infection; thus, the disease can easily spread among them.

Genotyping of the virus infecting the reported cases showed that the virus belonged to hepatitis A subgenotype IB. Based on the literature, genotype I is more prevalent than other genotypes worldwide, and subtype A is more common than subtype B [8]. However, worldwide, in most regions, there is co-circulation of IA and IB strains but the predominant strain usually accounts for more than 95% of HAV strains. Based on the recorded data, in Turkey and Middle East, 95% of HAV strains belong to genotype I subtype B [9,10],

TABLE 2

Summary of data regarding the most affected camps and results of the mass and ring vaccination efforts, Greece, April–December 2016

Hosting camp	Number of recorded cases	Population ^a	Time ^b (weeks)	Median age (years) (range: min–max)	Number of children aged 1–14 years vaccinated during mass vaccination	Number of close contacts aged 1–14 years vaccinated during ring vaccination	Number of close contacts aged 15 years or older vaccinated during ring vaccination	Proportion of hosted population vaccinated (%)
A	25	1,300	17–44	8 (1–29)	440	45	25	39
B	51	1,900	21–47	8 (2–27)	274	51	66	20
C	8	361	23–25	7.5 (3–17)	126	0	49	48
D	33	700	26–36	9 (1–28)	139	36	63	34
E	10	233	43–50	7 (2–12)	103	2	0	45
Total	127	4,494	NA	7 (1–29)	1,082	134	203	32

NA: not applicable.

^a Estimated average population living in the camps during reported HAV transmission.

^b Time between the first and the last reported case in the hosting camp.

whereas in Europe the predominant strain is genotype I subtype A [11]. In Greece, the available data are in accordance with the other European countries [12]. The findings presented may suggest the possible introduction of IB strains in Greece by refugees, and highlight the importance of molecular testing in mobile populations. Results, if provided in a timely fashion, would help understand transmission patterns and document introduction of possibly new hepatitis A strains in the European Union (EU). Molecular surveillance of cases in both refugees and the local population will continue.

The occurrence of HA mostly among Syrian children, implies higher susceptibility among them, compared with children from Afghanistan and Iraq, probably reflecting the epidemiology of the disease in the countries of origin.

In a 2000 study, anti-HAV IgG were present in 89% of the Syrian population with 50% in the 1–5 years age group and 95% in the 11–15 years age group [13]. Seroprevalence studies in the following years are not available and it is unknown whether there was a shift in HAV infection to an older age as in other neighbouring developing countries [14–16]. The notification rates by age group in the Syrian refugee population indicate that the seroprevalence of Syrian children and young adults might be lower than described in the literature and seroprevalence data for these age groups are needed. High incidence of HA among Syrian refugees has also been reported from Turkey [17], while a HA outbreak among Syrian refugees in northern Iraq has been documented [18]. Moreover, most children have asymptomatic HAV infection; thus, the number of infections that occurred during the study period was probably much higher than reported. An overestimated HAV seroprevalence contributed to the lack of routine HAV vaccination in Syrian refugee children, not only in Greece, but also in other countries hosting Syrian refugees.

The risk of occurrence of secondary cases in the community appears low based on the epidemiological data available so far.

Finally, even though the risk of disease transmission to volunteers and staff at the hosting facilities is small, we identified four cases among the cleaners at the most affected camps so advice has been given to the aforementioned population group.

Limitations of the case management varied widely depending on the characteristics and the living conditions at each hosting centre. Accommodation type (organised centre, hotel, apartment), size and demographics of the hosted population, nationalities, degree of organisation and activities of NGOs, substantially vary from one facility to another and management had to be tailored based on these specific characteristics.

Other factors, such as the proximity of the facility to the local hospital and the ability of the local authorities to take action to ensure the implementation of the hygiene measures at the camp that largely depends on the availability of human and other resources of the local authority, were also taken into account during response.

Response was challenging at prefectures with more than one established camp because of the potential competing priorities at a given time (e.g. in the event of HAV cases in more than one camps). Surge capacity issues were faced by local hospitals due to either: (i) hospitalisation of all HAV cases in order to reduce transmission rather than severity of disease and (ii) serologically testing of contacts before vaccination. Thus, other healthcare facilities were asked to support management of cases.

An additional challenge was the exact identification of close contacts, which was not easy in most of the

cases. Family members and people living in the same room/tent were always offered vaccination, but it was particularly difficult to list all the other possible close contacts inside the hosting camp; the help of the medical staff/volunteers working in the area was of great importance in the early identification of contacts or additional cases.

Another obstacle in contact tracing was population mobility. There was constant movement of refugees from one facility to another and tracking down some of the contacts proved impossible in some occasions.

Finally, making vaccines available was time consuming, especially during national holidays; this led to delays in timely intervention in some instances.

At the moment, enhanced surveillance and timely vaccination of contacts is our main priority. Hygiene standards are necessary for preventing further occurrence of the disease. The ultimate goal is to have the entire refugee child population (1–14 years) fully follow the routine national childhood immunisation programme in Greece according to which, all children older than 12 months are vaccinated against HAV. The improvement of living conditions in hosting facilities will further limit the occurrence of HAV in this susceptible population.

Acknowledgements

We would like to thank the members of the non-governmental organisations and volunteers working at the camps for their support during response (recording of close contacts and vaccination) and the local public health authorities for their continuous efforts to maintain a high level of hygiene inside the camps.

Conflict of interest

None declared.

Authors' contributions

Kassiani Mellou, Theano Georgakopoulou, Sotirios Tsiodras: had the concept, analysed the data, prepared the first draft, participated in critical revision and final draft preparation;

Anthi Chrisostomou, Theologia Sideroglou: participation in surveillance and response, collection of data, critical review of the first and all subsequent drafts;

Maria Kyritsi, Christos Hadjichristodoulou: laboratory investigation, critical review of the first and all subsequent drafts.

References

1. Greek Ministry of Health – National Health Operations Center. Situation report on refugee flows (daily). 01/04/2016–31/10/2016. [Accessed 30 Dec 2016]. [Greek]. Available from: <http://geochoros.survey.ntua.gr/ekepy/>
2. Hellenic Ministry of Interior. UNHCR. Analysis of preregistration data, 9 June to 30 July 2016. [Accessed 23 Nov 2016]. [Greek]. Available from: <http://asylo.gov.gr/wp-content/>

- uploads/2016/08/Preregistration-data_template_5_GR_EXTERNAL.pdf
3. United Nations High Commissioner for Refugees (UNHCR). Refugees/Migrants Emergency Response – Mediterranean / Greece (data in excel file). [Internet]. [Accessed 30 Dec 2016]. Available from: <http://data.unhcr.org/mediterranean/country.php?id=83>
 4. National Institute for Public Health and the Environment (RIVM). Ministry of Health, Welfare and Sport. Protocol. Molecular detection and typing of VP1-2A region of Hepatitis A Virus (HAV). Bilthoven: RIVM. Available from: http://www.rivm.nl/en/Topics/H/HAVNET/Protocols/Typing_protocol_HAVNET_VP1P2A.org
 5. Sideroglou T, Mellou K. Hepatitis A management protocol for sporadic cases and outbreaks. [Accessed 26 Dec 2016]. [Greek]. Available from: http://www.keelpno.gr/Portals/0/Αρχεία/Ηπατίτιδες/Ηπατίτιδα%20Α/protokolo_apokrisis_ipatitida_a.pdf
 6. Greek Ministry of Health. National Immunisation Schedule for Children and Adolescents 2015. Athens: Greek Ministry of Health. 22 Jan 2015. Greek. Available from: <http://www.moh.gov.gr/articles/health/dieythynsh-dhmosias-ygieinhs/3207-ethniko-programma-emboliasmwn-efhbwn-amp-paidiwn-2015>
 7. Mellou K, Sideroglou T, Papaevangelou V, Katsiaflaka A, Bitsolas N, Verykoui E, et al. Considerations on the current universal vaccination policy against hepatitis A in Greece after recent outbreaks. *PLoS One*. 2015;10(1):e0116939. DOI: 10.1371/journal.pone.0116939 PMID: 25590132
 8. Costa-Mattioli M, Di Napoli A, Ferré V, Billaudel S, Perez-Bercoff R, Cristina J. Genetic variability of hepatitis A virus. *J Gen Virol*. 2003;84(Pt 12):3191–201. DOI: 10.1099/vir.0.19532-0 PMID: 14645901
 9. Normann A, Badur S, Onel D, Kilic A, Sidal M, Larouze B, et al. Acute hepatitis A virus infection in Turkey. *J Med Virol*. 2008;80(5):785–90. DOI: 10.1002/jmv.21137 PMID: 18360891
 10. Nejati A, Makvandi M, Samarbafzadeh A, Neisi N, Moradzadegan H. Molecular epidemiology of hepatitis A virus in patients in the Ahwaz region of Iran. *J Med Virol*. 2012;84(4):582–6. DOI: 10.1002/jmv.23238 PMID: 22337296
 11. Nainan OV, Xia G, Vaughan G, Margolis HS. Diagnosis of hepatitis a virus infection: a molecular approach. *Clin Microbiol Rev*. 2006;19(1):63–79. DOI: 10.1128/CMR.19.1.63–79.2006 PMID: 16418523
 12. Mellou K, Sideroglou T, Papaevangelou V, Katsiaflaka A, Bitsolas N, Verykoui E, et al. Considerations on the current universal vaccination policy against hepatitis A in Greece after recent outbreaks. *PLoS One*. 2015;10(1):e0116939. DOI: 10.1371/journal.pone.0116939 PMID: 25590132
 13. Antaki N, Kebbewar MK. Hepatitis A seroprevalence rate in Syria. *Trop Doct*. 2000;30(2):99–101. DOI: 10.1177/004947550003000215 PMID: 10842558
 14. Habboub G, Alhalabi SM, Mousabasha G, Al-Faham Z. Hepatitis A vaccination in developing countries: Is Syria next? *Avicenna J Med*. 2012;2(3):77–8. DOI: 10.4103/2231-0770.102284 PMID: 23826552
 15. Jacobsen KH, Koopman JS. The effects of socioeconomic development on worldwide hepatitis A virus seroprevalence patterns. *Int J Epidemiol*. 2005;34(3):600–9. DOI: 10.1093/ije/dyio62 PMID: 15831565
 16. Sacy RG, Haddad M, Baasiri G, Khoriaty A, Gerbaka BJ, Abu-Elyazeed R. Hepatitis a in Lebanon: a changing epidemiological pattern. *Am J Trop Med Hyg*. 2005;73(2):453–6. PMID: 16103621
 17. Doganay M, Demiraslan H. Refugees of the Syrian Civil War: Impact on Reemerging Infections, Health Services, and Biosecurity in Turkey. *Health Secur*. 2016;14(4):220–5. DOI: 10.1089/hs.2016.0054 PMID: 27362427
 18. World Health Organization (WHO) Regional Office for the Eastern Mediterranean. WHO responds effectively to an outbreak of hepatitis A among Syrian refugees in northern Iraq. 17 Nov 2013. Nasr City: WHO EMRO. Available from: <http://www.emro.who.int/irq/iraq-news/hepatitis-outbreak-2013.html>

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Widespread activity of multiple lineages of Usutu virus, western Europe, 2016

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Citation style for this article:

Cadar D, Lühken R, van der Jeugd H, Garigliany M, Ziegler U, Keller M, Lahoreau J, Lachmann L, Becker N, Kik M, Oude Munnink BB, Bosch S, Tannich E, Linden A, Schmidt V, Koopmans MP, Rijks J, Desmecht D, Groschup MH, Reusken C, Schmidt-Chanasit J. Widespread activity of multiple lineages of Usutu virus, western Europe, 2016. *Euro Surveill.* 2017;22(4):pii=30452. DOI: <http://dx.doi.org/10.2807/1560-7917.ES.2017.22.4.30452>

Article submitted on 25 November 2016 / accepted on 11 January 2017 / published on 26 January 2017

In the summer of 2016, Belgium, France, Germany and the Netherlands reported widespread Usutu virus (USUV) activity based on live and dead bird surveillance. The causative USUV strains represented four lineages, of which two putative novel lineages were most likely recently introduced into Germany and spread to other western European countries. The spatial extent of the outbreak area corresponded with R_0 values > 1 . The occurrence of the outbreak, the largest USUV epizootic registered so far in Europe, allowed us to gain insight in how a recently introduced arbovirus with potential public health implications can spread and become a resident pathogen in a naïve environment. Understanding the ecological and epidemiological factors that drive the emergence or re-emergence of USUV is critical to develop and implement timely surveillance strategies for adequate preventive and control measures. Public health authorities, blood transfusion services and clinicians in countries where USUV was detected should be aware of the risk of possible USUV infection in humans, including in patients with unexplained encephalitis or other neurological impairments, especially during late summer when mosquito densities peak.

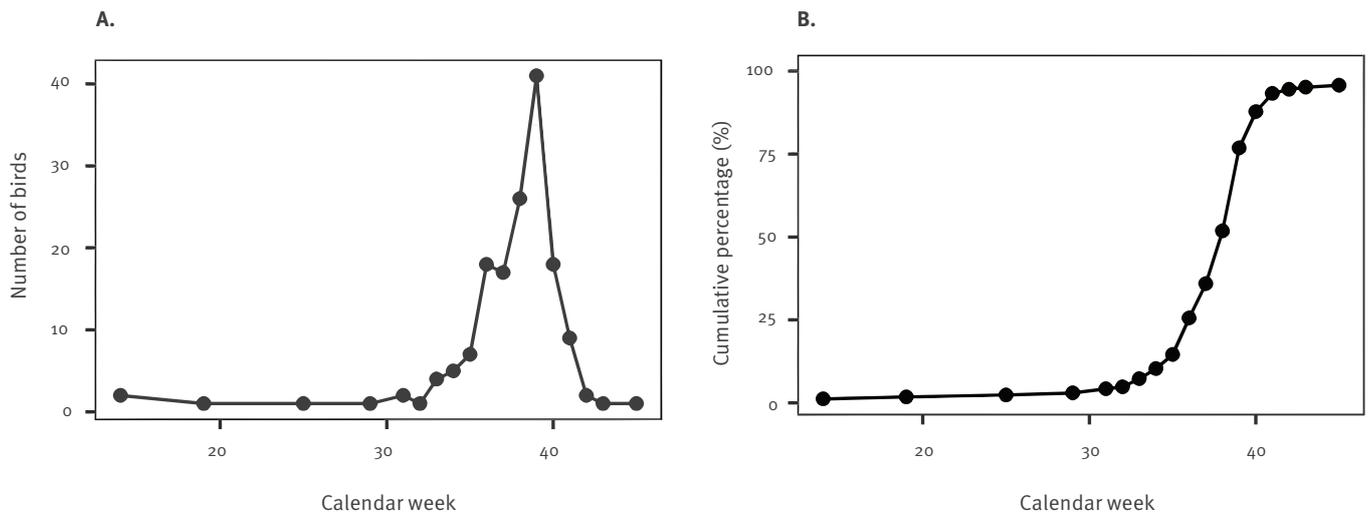
Introduction

Usutu virus (USUV) is a mosquito-borne flavivirus that was first isolated from a *Culex neavei* mosquito in South Africa in 1959 [1] and emerged for the first time in Europe in 1996 causing deaths among Eurasian blackbirds (*Turdus merula*) in Italy [2]. Since then, USUV has been the causative agent of epizootics and smaller outbreaks among wild and/or captive birds in Austria, Belgium, Czech Republic, France, Germany, Hungary, Spain and Switzerland, with its first emergence in the Netherlands in 2016 [3-7], often resulting in a massive die-off of blackbirds and captive great grey owls (*Strix nebulosa*) [4]. The transmission pattern seems predominantly determined by temperature conditions influencing both the developmental rate of the mosquito vectors and the extrinsic incubation period of the virus in its mosquito hosts i.e. the time required for the development of the virus in its mosquito vector, from the time of uptake of the virus by the mosquito to the time when the mosquito is infective [8].

In 2009, the first human cases of severe encephalitis due to USUV infection were reported from Italy in two immunocompromised persons, demonstrating the zoonotic potential of USUV [9,10]. Recently, a study in the Emilia-Romagna Region in northern Italy, indicated

FIGURE 1

Number (panel A) and cumulative percentage (panel B) of outbreak-related USUV-positive live and dead birds, western Europe^a, 2016 (n=164)^b



USUV: Usutu virus.

^a Belgium, France, Germany and the Netherlands.

^b No collection date information was available for seven of the 164 USUV-positive birds which were excluded from this figure.

that human USUV infection may not be a sporadic event. In this study, USUV infections in patients with or without neurological impairments occurred more frequently than West Nile virus (WNV) infections in a four-year period [11], highlighting the need for vigilance towards the public health implications of USUV circulation in large parts of Europe.

Here, we describe from a multi-country perspective, patterns of the 2016 USUV epizootic in western Europe and highlight the need for a cross-border analysis in order to gain a proper understanding of USUV spread and evolution and its potential impact on public health.

Methods

Bird collection and sampling

Birds from Belgium, France, Germany and the Netherlands were included in this investigation. Dead and live birds were sampled; sampling was performed according to national animal ethics regulations in all countries. Location and date of sampling of live birds as well as the location and date of finding of dead birds were registered.

After signals of USUV outbreaks in the Netherlands and Germany and media reports of blackbirds with neurological illness in Belgium, a dead bird surveillance was started on 3 October 2016 in the Belgian capital, Brussels, and Walloon regions, using information media to request citizens to submit found dead blackbirds. The bird that yielded the Opglabbeek sequence

(see phylogenetic tree in the Results) was actively trapped for sampling.

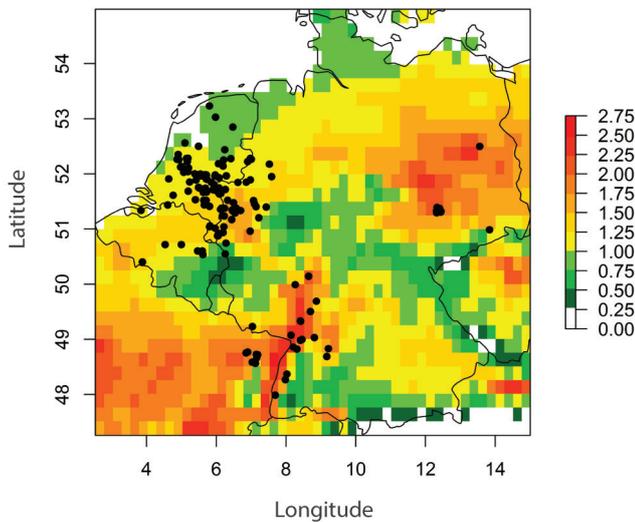
In France, samples of captive birds that had died of unknown causes from 1 August to 20 September 2016 in an animal park in the Lorraine region were submitted to Erasmus Medical Center, Rotterdam, the Netherlands, where USUV infection was determined as cause of death.

Since the first outbreak of USUV in Germany in 2011–12, dead birds sent to the national reference laboratories have been regularly screened for USUV. In addition, active surveillance of living birds has been conducted at selected locations. After the first indication of a new USUV outbreak in Germany at the end of September 2016, German citizens were requested to send in dead birds for USUV screening; this request was made via press releases of involved institutes and subsequent dissemination of the information by different kinds of media.

In the Netherlands, live and dead wild birds and dead captive birds were collected, sampled and analysed as described in [3], in the period from 2 April 2016 to 5 November 2016. In brief, live wild bird samples were obtained through an existing zoonosis-targeted surveillance project; dead wild birds were obtained through the national wildlife disease scanning surveillance programme which relies on post-mortem investigation of carcasses submitted by citizens; dead captive birds were submitted by owners for post-mortem investigation.

FIGURE 2

Mean daily basic reproduction number and distribution of outbreak-related USUV-positive birds, western Europe^a, 2016 (n=164 birds)



USUV: Usutu virus.

^a Belgium, France, Germany and the Netherlands.

The map represents the spatial distribution of the potential risk for a USUV outbreak between June and September 2016 and the laboratory-confirmed USUV-positive live and dead birds (the black dots) in western Europe. The temperature-dependent transmission model by Rubel et al. [8] was used on the base of daily mean temperature data on a 0.25° regular latitude-longitude grid downloaded from <http://www.ecad.eu>.

Modelling the basic reproduction number

The daily basic reproduction number (R_0) is an indicator for the potential spread of an infectious disease through a naïve population. R_0 was calculated with the temperature-dependent transmission model by Rubel [8] taking various drivers of disease emergence such as host immunity, extrinsic incubation period and vector reproduction rate, into consideration. Daily mean temperature data on a 0.25° regular latitude-longitude grid (E-OBS dataset, January 2009–September 2016) were downloaded from <http://www.ecad.eu> [12]. For each grid cell, R_0 values were averaged for the period from June to September 2016. The averaged R_0 could be interpreted as average number of secondary infections arising from the introduction of a single infected individual into a completely susceptible population during this period [8]. Data analysis and visualisation was conducted with the programme R [13] using the packages `plyr` [14], `lubridate` [15], `raster` [16], `colourRamps` [17], `rworldmap` [18], `ggplot2` [19] and `gridExtra` [20].

Detection and phylogenetic analysis of USUV

Birds found in Belgium were sequenced at the Faculty of Veterinary Medicine, University Liège; in Germany, birds were sequenced at the Bernhard Nocht Institute

for Tropical Medicine, Hamburg; French and Dutch birds were sequenced at Erasmus Medical Center (EMC), Rotterdam.

Total RNA from homogenised tissue samples (brain, liver, lung, and heart) was extracted and analysed for the presence of flavivirus RNA by using a modified pan-flavivirus reverse transcription PCR [21]. Direct sequencing of the pan-flavivirus PCR amplicons showed USUV nucleic acid sequences in each positive sample. In Germany (BNI) these were further subjected to PCRs to amplify and sequence a partial region of the USUV non-structural (NS) 5 gene. The complete coding sequences of the Dutch and French bird USUVs were obtained using random-primed 454-based NGS at the EMC in Rotterdam [22] while full genomes of the Belgian bird USUVs were obtained using random-primed sequencing with Ion Torrent PGM technology at UL exactly as described in [23].

To investigate the genetic relationship between the USUV strains responsible for the 2016 European outbreaks and those available in databases, phylogenetic reconstructions were performed using Bayesian Monte Carlo Markov Chain (MCMC) sampling method implemented in BEAST v.1.8.3 [24] and in parallel a maximum likelihood inference in PhyML v3.1 [25] based on partial NS5 gene coding sequences. The MCMC and PhyML trees (data not shown) were reconstructed using Tamura Nei (TN93) model (TN)93 plus Gamma evolutionary model placed by JModeltest2 [26] as the nt substitution model best fit the data. Sequences were aligned using the MAFFT algorithm and then visually inspected in Geneious v9.1.4.

Results

Spatial distribution of the epizootics and epidemic modelling

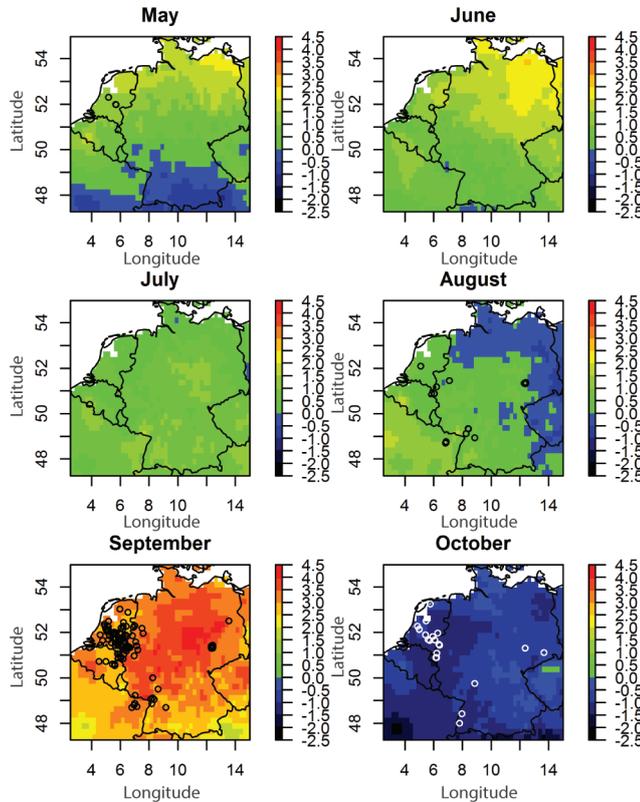
In 2016, there were a total of 17 live and 147 dead USUV-positive birds reported in the four countries. In Germany, besides recurrent circulation in known affected areas, USUV expanded its geographical distribution. In the Netherlands, USUV RNA was detected for the first time in two healthy blackbirds in the beginning of April 2016 [3].

Since early August (week 31), there was an increasing number of reported disease-associated mortality in blackbirds and captive great grey owls from Belgium, France, Germany and the Netherlands, that peaked in September (weeks 35–39) (Figure 1).

Of the 17 live and 147 dead USUV-positive birds reported in 2016, 120 were detected in the tristate area of Belgium, Germany and the Netherlands. The spatial distribution of the majority of positive cases in 2016 fell in an area with a mean basic reproduction number larger than one ($R_0 > 1$) (Figure 2).

FIGURE 3

Monthly temperature anomalies and distribution of outbreak-related USUV-positive birds, western Europe^a, 2016 (n=164)^b



USUV: Usutu virus.

^a Belgium, France, Germany and the Netherlands.

^b No collection date information was available for seven of the 164 USUV-positive birds which were excluded from this figure.

The maps represent the monthly temperature anomalies, i.e. deviation from the 30-year mean temperatures (E-OBS dataset, <http://www.ecad.eu>), in degree Celsius for the months from May to October 2016 and the laboratory-confirmed USUV-positive live and dead birds (black or white circles) in western Europe, per month. Circles for the month of May include two USUV-positive birds from April 2016 and circles for the month of October include one USUV-positive bird from November 2016.

This R_0 was driven by extraordinary high temperatures during September 2016, with values exceeding the long-term mean (1986–2015) by more than 3°C (E-OBS dataset, <http://www.ecad.eu>) (Figure 3).

Genetic characterisation of epizootic strains

In total, 28 positive samples (22 from Germany, 4 from Belgium, 1 from the Netherlands, 1 from France) were characterised based on partial sequences of the NS5 coding region (Figure 4).

Previous studies showed that this partial NS5 sequence exhibits a phylogenetic signal similar to the complete genome [5,27–29] allowing a rapid characterisation of the circulating virus strains. The 2016 USUV strains

represented four lineages (Figure 4). The viruses detected in Belgium, France and the Netherlands clustered with viruses that previously circulated in mosquito vectors, wild birds and/or bats in Germany between 2011 and 2014 [5,6,27,29].

In Germany, a putative novel USUV lineage, called Europe 5, was identified and this was constituted of strains found in birds in west-central North Rhine-Westphalia while lineage Europe 3 USUV emerged outside the previously known endemic areas [6]. The Africa 2 strain that killed two great grey owls in the Berlin Zoo in 2015 [28] was found in 2016 outside the zoo, in a blackbird.

Discussion

Since the first large outbreaks in the 2000s [7], USUV has become a potential public health concern given the increasing number of reported human infections [9–11,30]. Arbovirus surveillance programmes based on birds and mosquitoes have been conducted in western Europe in recent years and allowed us to elucidate the possible origin, pattern of spatial dynamics, and eco-epidemiological factors that contributed to the 2016 epizootic. It can be speculated that the USUV lineages detected in Belgium, France and the Netherlands were most likely imported from Germany via infected semi-resident wild birds. Introduction via active/passive mosquito dispersal is another possible scenario that was contemplated for WNV, a closely related flavivirus with a similar avian-mosquito life-cycle, as well [31–33]. However, independent long-distance introductions via migratory birds cannot be excluded and geo-phylogenetic analysis of USUV genomes in more birds with a wider geographic coverage, especially in France and the Netherlands, will increase our understanding of the dispersal of USUV across Europe.

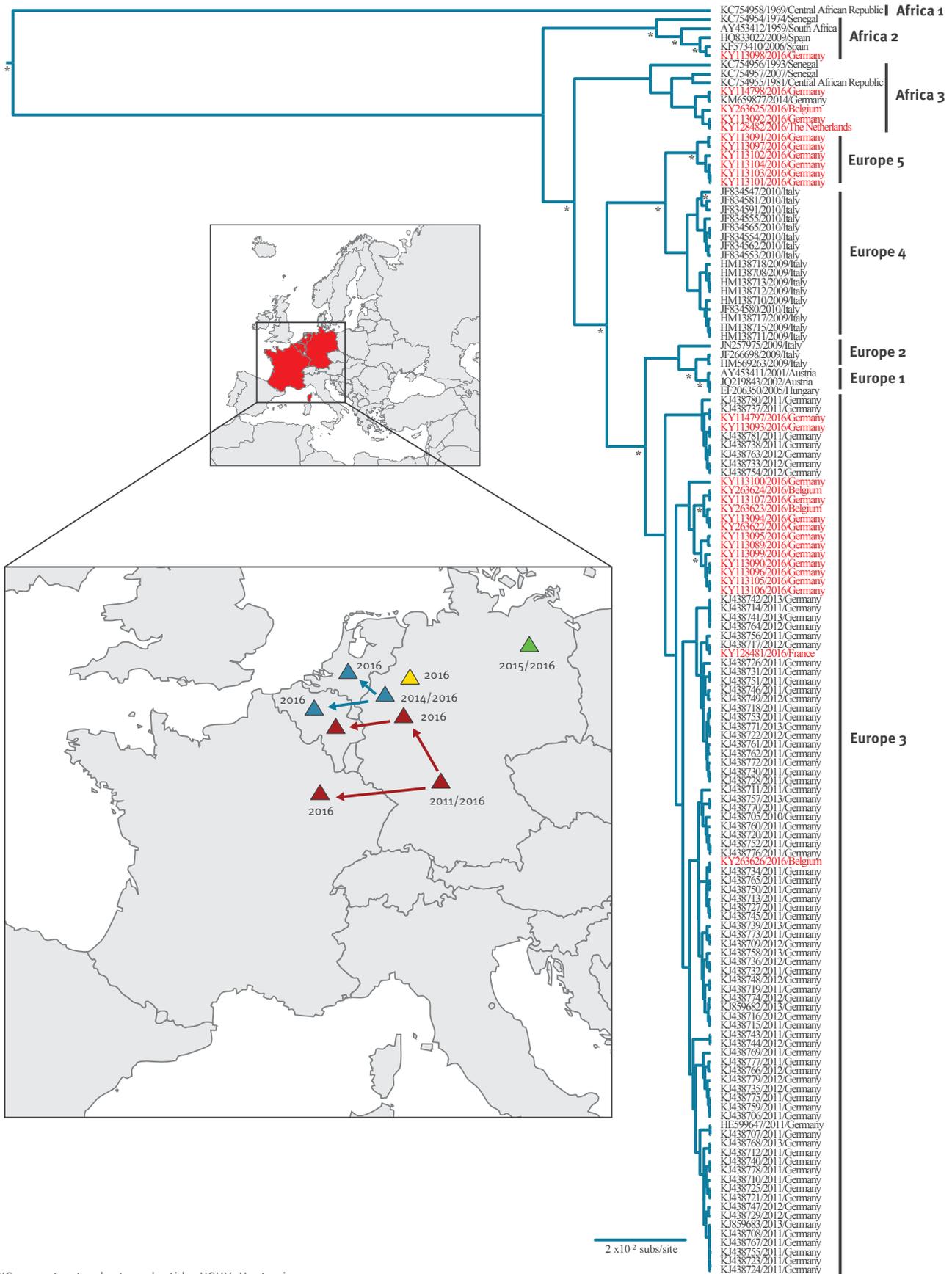
The presence of a Europe 3 lineage strain in France and an Africa 3 strain in the Netherlands could each represent a single introduction event with Germany as possible source (Figure 4). In contrast, the USUV epizootic in Belgium was linked to both lineage Africa 3 and Europe 3, indicative for at least two distinct introductions.

The USUV Africa 2 strain found in Berlin seemed to be restricted to this city, thereby supporting the observation that the adaptation of USUV to naïve vector and host populations can lead to the emergence of local virus variants [5]. The geographically distinct lineages occurring in Europe are separated from each other by barriers such as climate, vegetation, different host species, and other unknown ecological conditions [5]. Nevertheless, the synchronous emergence of different USUV lineages in western Europe and their co-circulation in the same regions indicate similar basic ecological parameters driving the transmission of the different lineages involved in the recent outbreak.

The high activity of USUV in the late summer-beginning of autumn of 2016 could be linked to temperature

FIGURE 4

Phylogenetic tree of USUV variants responsible for outbreaks in captive and wild birds and the possible origin and spread pattern, western Europe^a, 2016



NS: non-structural; nt: nucleotide; USUV: Usutu virus.

^a Belgium, France, Germany and the Netherlands.

The tree is based on the partial NS5 gene and it shows placement of the USUV variants that were detected during the ongoing outbreaks in comparison to all available USUV sequences from GenBank. To improve visualisation, phylogenetic positions of the USUV detected in 2016 are bold and red. Taxon information includes the GenBank accession number, isolation/detection year and country in which the virus was isolated/detected. Scale bar indicates mean number of nt substitutions per site. The small map indicates the European countries which have reported USUV outbreaks (red) in 2016, while the magnified map shows the possible origin of different USUV lineages (coloured triangles) involved in the recent European outbreaks. Arrows indicate the likely trend of spatial diffusion pattern of the USUV (coloured based on USUV lineages).

anomalies in September, i.e. significant positive deviation from the 30-year mean temperatures, which will have shortened the extrinsic incubation period, and caused an increase in the vector abundance and therefore the associated vector-host contact rate, at the same time [8]. Based on the known epidemiology of USUV in Europe and given the expected increasing temperatures due to climate change, there could be a risk that the already established USUV loci will expand and further large outbreaks will occur in naïve regions resulting in an increased infection pressure on humans. The current USUV outbreak exhibited similar patterns to the outbreak of the closely related WNV lineage 2 in central Europe in 2008–2009 when, after a few years of limited local circulation, the virus subsequently spread to Balkan states and northern Greece, where it caused a neuroinvasive disease outbreak among humans with 197 cases [34,35].

Early detection of enzootic circulation based on mosquito and avian surveillance can ensure timely implementation of prevention and control measures. Data from the Dutch USUV outbreak showed that signalling based on live bird surveillance can precede signals from dead bird surveillance up to five months [3]. Enhanced surveillance and monitoring of the densities and infection level of the vector should support the timeliness of bird surveillance. Based on the availability of near real-time temperature data, surveillance sites and time periods with high risk for virus activity can be determined by continuing spatial-temporal analysis. Our findings in the context of what is known about the USUV ecology, emphasise the need for a transboundary strengthening of collaboration and coordination across different research, veterinary and public health sectors, for an effective control and implementation of specific preventive measures.

The adaptation of USUV to naïve vector and vertebrate host populations by introduction/reintroduction of the virus and migratory bird flyways are considered key determinants in the spatial dispersal and establishment of USUV. Thus, multiple complete genome analyses are clearly necessary to fully understand the impact of ecological/immunological/virological factors on USUV epidemiology and evolution of different virus lineages [5]. The recent observations on human USUV infections in northern Italy [11] and the continuous geographic expansion of USUV in Europe should raise awareness among physicians to include USUV in the differential diagnosis of encephalitis cases of unknown aetiology, and among policymakers to address putative issues with blood safety and wildlife conservation alike.

Acknowledgements

We acknowledge the E-OBS dataset from the EU-FP6 project ENSEMBLES (<http://ensembles-eu.metoffice.com>) and the data providers in the ECA&D project (<http://www.ecad.eu>). We thank Heike Baum, Alexandra Bialonski, Anne van der Linde, Robert Kohl and Claudia Schapendonk for assistance

in the laboratory work. We acknowledge the valuable contributions of the Dutch bird ringers.

Funding statement: This work was financially supported by the German Federal Ministry of Food and Agriculture (BMEL) through the Federal Office for Agriculture and Food (BLE) with the grant numbers 2819104315. The WBA-Zoo wild bird pathogen surveillance and sequencing project is supported by the German Centre for Infection Research (DZIF). Scanning surveillance of dead wild birds is funded by the Dutch Ministry of Economic Affairs grant number 1300022600. The Eco-Alert project (live bird surveillance) is funded by ZonMW grant number 50-52200-98-026. Sequencing of Usutu virus was funded in part by the EU Horizon 2020 program COMPARE (EC Grant Agreement No. 643476). Approval of the work by an ethics committee is not required for scanning surveillance of dead wild birds in the Netherlands. Surveillance of live birds in the Netherlands within EcoAlert is performed under license AVD/263002015342 to Vogeltrekstation NIOO-KNAW.

Conflict of interest

None declared.

Authors' contributions

Wrote the manuscript: CR, MPK, RL, DC, JSC, NB, MG, MMG, ET

Performed laboratory or epidemiological investigations: BBOM, JL, HVJ, JR, UZ, MK, DD, RL, DC, MMG, VS, SB, LL, AL

Performed data analysis: BBOM, CR, JR, MK, RL, DC, JSC

References

1. McIntosh BM. Usutu (SAAR 1776), nouvel arbovirus du groupe B.Int Cat Arboviruses.1985;3:1059-60.
2. Weissenböck H, Bakonyi T, Rossi G, Mani P, Nowotny N. Usutu virus, Italy, 1996.Emerg Infect Dis. 2013;19(2):274-7. DOI: 10.3201/eid1902.121191 PMID: 23347844
3. Rijks JM, Kik ML, Slaterus R, Foppen R, Stroo A, IJzer J, et al. Widespread Usutu virus outbreak in birds in the Netherlands, 2016. Euro Surveill. 2016;21(45):30391. DOI: 10.2807/1560-7917.ES.2016.21.45.30391 PMID: 27918257
4. Nikolay B. A review of West Nile and Usutu virus co-circulation in Europe: how much do transmission cycles overlap?Trans R Soc Trop Med Hyg. 2015;109(10):609-18. DOI: 10.1093/trstmh/trv066 PMID: 26286946
5. Engel D, Jöst H, Wink M, Börstler J, Bosch S, Garigliani MM, et al. Reconstruction of the Evolutionary History and Dispersal of Usutu Virus, a Neglected Emerging Arbovirus in Europe and Africa. MBio. 2016;7(1):e01938-15. DOI: 10.1128/mBio.01938-15 PMID: 26838717
6. Becker N, Jöst H, Ziegler U, Eiden M, Höper D, Emmerich P, et al. Epizootic emergence of Usutu virus in wild and captive birds in Germany. PLoS One. 2012;7(2):e32604. DOI: 10.1371/journal.pone.0032604 PMID: 22389712
7. Weissenböck H, Kolodziejek J, Url A, Lussy H, Rebel-Bauder B, Nowotny N. Emergence of Usutu virus, an African mosquito-borne flavivirus of the Japanese encephalitis virus group, central Europe.Emerg Infect Dis. 2002;8(7):652-6. DOI: 10.3201/eid0807.020094 PMID: 12095429
8. Rubel F, Brugger K, Hantel M, Chvala-Mannsberger S, Bakonyi T, Weissenböck H, et al. Explaining Usutu virus dynamics in Austria: model development and calibration. Prev Vet Med. 2008;85(3-4):166-86. DOI: 10.1016/j.prevetmed.2008.01.006 PMID: 18314208
9. Cavrini F, Gaibani P, Longo G, Pierro AM, Rossini G, Bonilauri P, et al. Usutu virus infection in a patient who underwent orthotopic liver transplantation, Italy, August-September 2009. Euro Surveill. 2009;14(50):19448.PMID: 20070935
10. Pecorari M, Longo G, Gennari W, Grottola A, Sabbatini A, Tagliacuzzi S, et al. First human case of Usutu virus neuroinvasive infection, Italy, August-September 2009. Euro Surveill. 2009;14(50):19446.PMID: 20070936

11. Grottole A, Marcacci M, Tagliacucchi S, Gennari W, Di Gennaro A, Orsini M, Monaco F, Marchegiano P, Marini V, Meacci M, Rumpianesi F, Lorusso A, Pecorari M, Savini G. Usutu virus infections in humans: a retrospective analysis in the municipality of Modena, Italy. *Clin Microbiol Infect*. 2016 Sep 24. pii: S1198-743X(16)30425-6.
12. Haylock MR, Hofstra N, Klein Tank AMG, Klok EJ, Jones PD, New M. A European daily high-resolution gridded dataset of surface temperature and precipitation for 1950-2006. *J Geophys Res Atmos*. 2008;113(D20):D20119. DOI: 10.1029/2008JD010201
13. R Core Team. R: A Language and Environment for Statistical Computing [Internet]. Vienna, Austria: R Foundation for Statistical Computing. [Accessed 31 Oct 2016]. Available from: <https://www.R-project.org/>
14. Wickham H. The split-apply-combine strategy for data analysis. *J Stat Softw*. 2011;40(1):1-29. DOI: 10.18637/jss.v040.i01 PMID: 27818617
15. Grolemond G, Wickham H. Dates and Times Made Easy with lubridate. *J Stat Softw*. 2011;40(3):1-25. DOI: 10.18637/jss.v040.i03
16. Hijmans RJ. raster: Geographic Data Analysis and Modeling. [Accessed 31 Oct 2016]. Available from: <http://CRAN.R-project.org/package=raster>
17. Keitt T. colorRamps: Builds color tables. 29 Oct 2012. Available from: <http://CRAN.R-project.org/package=colorRamps>
18. South A. rworldmap: A New R package for Mapping Global Data. *R J*. 2011;3(1):35-43.
19. Wickham H. ggplot2: elegant graphics for data analysis. New York: Springer; 2009. 213 p. Available from: <http://had.co.nz/ggplot2.org/book>
20. Auguie B. gridExtra: functions in Grid graphics. 2016. Available from: <http://CRAN.R-project.org/package=gridExtra>
21. Becker N, Jöst H, Ziegler U, Eiden M, Höper D, Emmerich P, et al. Epizootic emergence of Usutu virus in wild and captive birds in Germany. *PLoS One*. 2012;7(2):e32604. DOI: 10.1371/journal.pone.0032604 PMID: 22389712
22. van den Brand JM, van Leeuwen M, Schapendonk CME, Simon JH, Haagmans BL, Osterhaus AD, et al. Metagenomic analysis of the viral flora of pine marten and European badger feces. *J Virol*. 2012;86(4):2360-5. DOI: 10.1128/JVI.06373-11 PMID: 22171250
23. Garigliany M, Linden A, Gilliau G, Levy E, Sarlet M, Franssen M, et al. Usutu virus, Belgium, 2016. *Infect Genet Evol*. 2016;48:116-9. DOI: 10.1016/j.meegid.2016.12.023 PMID: 28017913
24. Drummond AJ, Suchard MA, Xie D, Rambaut A. Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Mol Biol Evol*. 2012;29(8):1969-73. DOI: 10.1093/molbev/mss075 PMID: 22367748
25. Guindon S, Dufayard JF, Lefort V, Anisimova M, Hordijk W, Gascuel O. New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. *Syst Biol*. 2010;59(3):307-21. DOI: 10.1093/sysbio/syq010 PMID: 20525638
26. Durraba D, Taboada GL, Doallo R, Posada D. jModelTest 2: more models, new heuristics and parallel computing. *Nat Methods*. 2012;9(8):772. DOI: 10.1038/nmeth.2109 PMID: 22847109
27. Cadar D, Bosch S, Jöst H, Börstler J, Garigliany MM, Becker N, et al. Putative Lineage of Novel African Usutu Virus, Central Europe. *Emerg Infect Dis*. 2015;21(9):1647-50. DOI: 10.3201/eid2109.142026 PMID: 26291923
28. Ziegler U, Fast C, Eiden M, Bock S, Schulze C, Hoepfer D, et al. Evidence for an independent third Usutu virus introduction into Germany. *Vet Microbiol*. 2016;192(192):60-6. DOI: 10.1016/j.vetmic.2016.06.007 PMID: 27527765
29. Cadar D, Becker N, Campos RM, Börstler J, Jöst H, Schmidt-Chanasit J. Usutu virus in bats, Germany, 2013. *Emerg Infect Dis*. 2014;20(10):1771-3. DOI: 10.3201/eid2010.140909 PMID: 25271769
30. Vilibic-Cavlek T, Kaic B, Barbic L, Pem-Novosel I, Slavic-Vrzic V, Lesnikar V, et al. First evidence of simultaneous occurrence of West Nile virus and Usutu virus neuroinvasive disease in humans in Croatia during the 2013 outbreak. *Infection*. 2014;42(4):689-95. DOI: 10.1007/s15010-014-0625-1 PMID: 24793998
31. Peterson AT, Vieglais DA, Andreasen JK. Migratory birds modeled as critical transport agents for West Nile Virus in North America. *Vector Borne Zoonotic Dis*. 2003;3(1):27-37. DOI: 10.1089/153036603765627433 PMID: 12804378
32. Rappole JH, Derrickson SR, Hubálek Z. Migratory birds and spread of West Nile virus in the Western Hemisphere. *Emerg Infect Dis*. 2000;6(4):319-28. DOI: 10.3201/eid0604.000401 PMID: 10905964
33. Goldberg TL, Anderson TK, Hamer GL. West Nile virus may have hitched a ride across the Western United States on *Culex tarsalis* mosquitoes. *Mol Ecol*. 2010;19(8):1518-9. DOI: 10.1111/j.1365-294X.2010.04578.x PMID: 20456236
34. Bakonyi T, Ferenczi E, Erdélyi K, Kutasi O, Csörgő T, Seidel B, et al. Explosive spread of a neuroinvasive lineage 2 West Nile virus in Central Europe, 2008/2009. *Vet Microbiol*. 2013;165(1-2):61-70. DOI: 10.1016/j.vetmic.2013.03.005 PMID: 23570864
35. Danis K, Papa A, Theocharopoulos G, Dougas G, Athanasiou M, Detsis, et al. Outbreak of West Nile virus infection in Greece, 2010. *Emerg Infect Dis* 2010; 17, 1868-1872. <http://dx.doi.org/> DOI: 10.3201/eid1710.110525 PMID: 22000357

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Live attenuated influenza vaccine effectiveness against hospitalisation due to laboratory-confirmed influenza in children two to six years of age in England in the 2015/16 season

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Citation style for this article:

Pebody R, Sile B, Warburton F, Sinnathamby M, Tsang C, Zhao H, Ellis J, Andrews N. Live attenuated influenza vaccine effectiveness against hospitalisation due to laboratory-confirmed influenza in children two to six years of age in England in the 2015/16 season. *Euro Surveill.* 2017;22(4):pii=30450. DOI: <http://dx.doi.org/10.2807/1560-7917.ES.2017.22.4.30450>

Article submitted on 09 December 2016 / accepted on 18 January 2017 / published on 26 January 2017

The United Kingdom is introducing a universal annual influenza vaccination programme for children. Live attenuated influenza vaccine (LAIV) effectiveness (VE) against laboratory-confirmed influenza hospitalisation in 2 to 6 year-olds in England was measured in 2015/16 using the screening method. VE adjusted for age, geography and month was 54.5% (95% confidence interval (CI): 31.5% to 68.4%) for all influenza types combined; 48.3% (95% CI: 16.9% to 67.8%) for A(H1N1)pdm09 and 70.6% (95% CI: 33.2% to 87.1%) for B. The findings support on-going programme roll-out.

Introduction

The United Kingdom (UK) started the phased introduction of a universal paediatric influenza vaccination programme in 2013/14, following recommendations from the UK Joint Committee on Vaccination and Immunisation (JCVI) [1]. The programme will ultimately be targeted at all children 2 to 16 years of age, with the offer of a single dose of a newly licensed live attenuated influenza vaccine (LAIV) to healthy children. The programme aims to both directly protect the children themselves, but also by reducing their ability to spread influenza, protect other vulnerable members of the population. The programme initially targeted all 2 and 3 year-olds across the UK with trivalent LAIV, and by 2015/16, had extended to all children aged 2 to 4 years of age plus school years 1 and 2 (5 and 6 years of age) in England with quadrivalent LAIV [2].

The UK has published a series of papers demonstrating that the programme has provided direct protection against influenza-confirmed infection in primary care over the first three seasons [3,4]. The UK has published evidence that LAIV provided significant protection against influenza for children consulting in primary care in 2015/16 [4], however, to date no data have

been published on the potential effectiveness of this vaccine against more severe disease. The UK Severe Influenza Surveillance System (USISS) was established after the 2009 influenza pandemic and collects information on laboratory-confirmed influenza hospitalisations through a sentinel network of acute hospital trusts in England [5]. This surveillance system provides an opportunity to measure whether the new paediatric influenza vaccination programme also provides direct protection against more severe infection in children.

Methods

We used the screening method to estimate vaccine effectiveness (VE) in vaccine-eligible children aged 2 to 6 years in England in the 2015/16 season, comparing vaccination coverage in children hospitalised with laboratory-confirmed influenza infection to vaccination coverage in children in the general population. This approach has been described elsewhere [6,7].

A case was defined as a child aged 2 to 6 years on 1 September 2015, and thus eligible for influenza vaccination, and reported to be hospitalised with laboratory-confirmed influenza infection by reverse transcription real-time PCR (RT-PCR) in the period between week 40 2015 and week 20 2016.

Cases were identified from the USISS, a national surveillance system which collects individual level reports on laboratory-confirmed hospitalisations of influenza in children from a sentinel laboratory network in England [5]. Cases' general practitioners (GP) were sent a postal questionnaire to identify whether the cases had received influenza vaccination during the 2015/16 campaign and if so, the vaccination date and whether the vaccine was administered by injection or

intranasally. Finally phone contact was made with non-responding practices.

A child was classified as vaccinated if they received at least one dose of influenza vaccine at least 14 days before the child's date of reported symptom onset, as this was considered the minimum time period for the child to achieve maximum protection. If the child was vaccinated less than 14 days before onset or had an unknown vaccination record then the child was not considered in the analysis. Cases vaccinated by injection (i.e. by injected inactivated vaccine; IIV) were also excluded. This information was used to determine the proportion of cases vaccinated (PCV).

Seasonal influenza vaccination coverage (PPV) for the population of children 2 to 6 years of age on 1 September 2015 in England was identified through a national electronic reporting system (Immform). This is a web-based system developed to collect data on influenza vaccine uptake in near real time during the influenza season. Data are collected from all GP practices on a monthly basis online using almost entirely fully automated data extraction methods. These include seasonal influenza vaccination for children 2 to 4 years of age [8]. Data were extracted from Immform each month on the number of children registered in primary care, and number of children who received seasonal influenza vaccination between 1 September 2015 and 31 January 2016. Immform does not distinguish whether LAIV or IIV was administered (a small number of children will have received IIV if they are contraindicated because of severe asthma or egg allergy or immunosuppression). Immform data were extracted at the end of each month from GP information systems and were available by year of age.

In addition, cumulative monthly uptake in children of school year 1 and 2 (5 and 6 years of age) was available across England through a separate manual reporting system into Immform. Local teams undertaking school-based campaigns report the number of eligible registered children and number of children who received influenza vaccine to Immform. Monthly data were also available for this collection for the period between 1 September 2015 and 31 January 2016 [9].

Coverage data for all age groups were available each month at the Local Authority (LA) and Regional level.

Cases included in the analysis were described by age at September 2015 (2–4 and 5–6 years), month of infection (September 2015 to April 2016), LA of residence (unless this information was missing, in which case the LA of their GP practice was used), influenza A(H3N2), A(H1N1)pdm09 and B and influenza vaccine status (intranasal vaccine, unvaccinated) in 2015/16. Information was not available on risk group status for cases.

Crude VE for hospitalised influenza cases were estimated as:

$$1 - \frac{PCV_{all}}{(1 - PCV_{all})} \\ PPV_{all} / (1 - PPV_{all})$$

Where PCV_{all} is the overall proportion of cases vaccinated and PPV_{all} the overall end of season population coverage.

Adjusted VE for hospitalised influenza cases was estimated by obtaining the PPV that matched to each case according to LA, age at 1 September 2015 and the end of the month closest to two weeks before hospital admission (the 2 weeks is to allow time for protection following vaccination). This was undertaken for all circulating influenza: influenza A(H1N1)pdm09 and influenza B (the dominant circulating strains). Adjusted VE was then estimated from a logistic regression model where the matched log of (PPV/(1-PPV)) was used as an offset and the outcome was vaccination status for each case, an approach described previously [6,7].

This work was undertaken as a routine public health function to monitor vaccination programmes; Public Health England (PHE) holds permissions to collect data under Section 251 of the National Health Service Act 2006 and the 2002 Health Service (Control of Patient Information) regulations as part of monitoring the performance of the national vaccination programme.

Results

There were a total of 176 children 2 to 6 years of age on 1 September 2015 with laboratory-confirmed influenza infection reported to USISS, who were hospitalised between week 40 2015 and week 20 2016. Response was received from GPs for all the cases. Nineteen cases were excluded (11%), five due to unknown vaccination status in the returned questionnaire; one with no hospital admission date; one that was vaccinated within 14 days of admission; 11 that had received IIV and one that had vaccine type unknown. This left 157 cases for analysis. There were 10 cases where vaccination date was unknown but they were assumed vaccinated at more than 14 days before onset as all were hospitalised after mid-January, when the vast majority of vaccinations had been completed.

Of these 157 included cases, overall 99 (63.1%) tested positive for influenza A(H1N1)pdm09, 14 (8.9%) for influenza A (subtype unspecified) and 44 (28.0%) for influenza B. Median age at time of influenza infection was 4 years.

Overall 34 cases (21.7%) had received LAIV in 2015/16; the median interval between vaccination and date of onset of illness for those with information available was 120 days (range: 16–173 days).

TABLE 1

Cumulative live attenuated influenza vaccine^a uptake by age group and month, England, 1 September 2015–31 January 2016 (n=3,431,319 persons)

Age group in years	Per cent (N children vaccinated/N children registered)			
	1 Sep 2015–31 Oct 2015	1 Sep 2015–30 Nov 2015	1 Sep 2015–31 Dec 2015	1 Sep 2015–31 Jan 2016
2–4	16.7% (320,013/1,920,171)	26.9% (550,382/2,048,535)	30.9% (642,106/2,077,665)	31.5% (661,423/2,098,909)
5–6	11.7% (148,480/1,264,339)	40.3% (530,610/1,317,526)	50.2% (668,072/1,331,241)	53.0% (706,534/1,332,410)
Total 2–6	14.7% (468,493/3,184,510)	32.1% (1,080,992/3,366,061)	38.4% (1,310,178/3,408,906)	39.9% (1,367,957/3,431,319)

^a The paediatric influenza vaccination programme in England offers a single dose of live attenuated influenza vaccine (LAIV) to all healthy children. A small number of children will have received inactivated influenza vaccine (IIV) if they are contraindicated because of severe asthma or egg allergy or immunosuppression, Information on IIV uptake is unavailable.

TABLE 2

Crude and adjusted live attenuated influenza vaccine effectiveness by age group, England, 1 September 2015–22 May 2016

Age group in years	Influenza type	PCV	Crude VE (95%CI)	Adjusted VE ^a (95%CI)
2–4	Any influenza	29/133 (21.8%)	39.4% (7.7% to 61.3%)	49.6% (23.6% to 66.7%)
	(H1N1)pdm09	19/84 (22.6%)	36.5% (-7.6% to 64.0%)	46.7% (10.7% to 68.2%)
	B	6/36 (16.7%)	56.4% (-6.1% to 85.1%)	66.0% (17.9% to 85.9%)
5–6	Any influenza	5/24 (20.8%)	76.7% (35.3% to 93.2%)	69.6% (15.9% to 86.4%)
	(H1N1)pdm09	4/15 (26.7%)	67.7% (-8.8% to 92.5%)	55.6% (-45.2% to 86.4%)
	B	1/8 (12.5%)	87.3% (1.2% to 99.7%)	84.9% (-30.1% to 98.3%)
Total 2–6	Any influenza	34/157 (21.7%)	58.3% (38.8% to 72.4%)	54.5% (31.5% to 68.4%)
	(H1N1)pdm09	23/99 (23.2%)	54.5% (26.5% to 72.8%)	48.3% (16.9% to 67.8%)
	B	7/44 (15.9%)	71.5% (35.1% to 89.4%)	70.6% (33.2 to 87.1%)

CI: confidence interval; PVC: proportion of cases vaccinated; VE: vaccine effectiveness.

^a Adjusted VE by local authority, month of infection and age in years.

Nationally in 2015/16, 1,367,957 of 3,431,319 (39.9%) children 2 to 6 years had received seasonal influenza vaccination. Coverage is shown in Table 1 by age group and month.

The crude and adjusted VE for preventing influenza hospitalised cases in healthy children by age group and by influenza type is shown in Table 2. Crude overall VE was 58.3% for all influenza types, which decreased to 54.5% after adjusting for geography, month and age. Results after stratifying by influenza A(H1N1)pdm09 and B gave an adjusted VE in children 2 to 6 years of age of 48.3% for influenza A(H1N1)pdm09 and 70.6% for influenza B. There was no significant difference on stratifying by age group.

Discussion

Our study finds evidence that quadrivalent LAIV administered to children 2 to 6 years of age in England in 2015/16 was effective in preventing laboratory-confirmed influenza hospitalisation. We demonstrate good overall protection, including against both A(H1N1)pdm09 and influenza B.

There are a number of potential strengths and weaknesses to our study. The screening method is a well-recognised observational study design which has the potential to provide rapid and economical estimates of VE. However, it is recognised to have a number of potential limitations: firstly, VE estimates can be

biased, if the cases arise from a population that differs from the population used to determine coverage rates; secondly if important confounders remain unadjusted and finally if vaccine information is incomplete. In this study, we have attempted to minimise the potential bias highlighted in the first point by comparing the vaccine coverage among cases to the uptake in the general population of the same age in the local area where cases lived. For the second point, we have adjusted for the main confounders identified in other influenza VE studies, namely age, time of infection and place of residence. Although we did not have information on risk-groups status for the cases and were not able to adjust for this potential confounder, we have not found it to be an important confounder previously for studies in primary care [3]. It is also important to note that influenza vaccine was offered to all children in these age groups. Nevertheless if cases belonged to risk groups and coverage was higher in those in risk groups, then we may have underestimated effectiveness. We assessed this further by increasing matched coverage by 5% which in turn increased VE estimates by ca 8%. For the final point on vaccine status, information on vaccination status of cases was ascertained from the patients' records by their GPs and was almost complete. Although population information on type of vaccine administered was not available, the proportion of vaccinated children in the general population who received IIV was small, as this would only be children with severe asthma, egg allergy or immunosuppression who were contraindicated LAIV.

Our findings of quadrivalent LAIV effectiveness for protection against influenza-related hospitalisation in 2015/16 are consistent with recently published findings from the UK which found that LAIV also provided significant protection against laboratory-confirmed influenza in primary care in 2 to 17 year-olds, with a similar effectiveness of 57.6% (95% confidence interval (CI): 25.1% to 76.0%) [4]. It is also encouraging that our findings of protection against severe disease with the screening method are congruent with results of a study reported from Scotland also for the 2015/16 season, but which rather used linked hospitalisation data and a cohort design [10]. In addition, the results are also consistent with those from Finland, where LAIV was also offered to children in 2015/16 and evidence of effectiveness was found in preventing laboratory-confirmed infection [11]. On the other hand, our results of significant protection are at odds with those reported from North America, where recent studies have suggested no evidence of significant effectiveness of LAIV in children against medically attended laboratory-confirmed influenza over the same period [12].

The VE findings for influenza A(H1N1)pdm09 demonstrate significant protection against influenza A(H1N1)pdm09 confirmed hospitalisation. Again these results are congruent with the UK study undertaken in primary care using the test-negative case-control approach [4]. The LAIV VE finding for influenza A(H1N1)pdm09 in

this paper of 48.3% (95% CI: 16.9% to 67.8%) is consistent with previous hospital based studies. A study undertaken using the test-negative design in 2013/14 found an effectiveness of influenza vaccine against A(H1N1)pdm09 confirmed hospitalisation of 42.8% (95% CI: 6.3% to 65.0%) [13]. The findings presented here are encouraging particularly in light of the very poor vaccine effectiveness of LAIV against influenza A(H1N1)pdm09 noted recently in the US. The reasons for this apparent discordance between the UK, and other countries such as Finland using LAIV, and the US remain under investigation. It could relate to the vaccine itself, the circulating viruses or the population and their prior exposure [14,15]. The 2015/16 season in the UK, as in North America, was dominated by circulation of influenza A(H1N1)pdm09 strain, which was antigenically well matched to the A/Bolivia/559/2013 (A/California/7/2009-like) vaccine strain. Others authors have suggested that the US results and the relative reduction in A(H1N1)pdm09 effectiveness for LAIV compared with IIV in a range of settings, including the UK, is related to reduced replicative fitness of the A(H1N1)pdm09 LAIV A/Bolivia/559/2013 strain [15], although that factor alone would not explain the discordance of the US CDC with the UK and other results in both primary and secondary care. This may relate to country specific issues such as prior vaccination, or how the vaccine is handled. Further work is required to understand, what role each of these factors might be playing in contributing to the current observations. Nonetheless, the vaccine manufacturer has acknowledged these findings and is working to identify a more effective A(H1N1)pdm09 LAIV strain for potential incorporation to the 2017/18 LAIV vaccine [15].

Finally the LAIV VE influenza B finding in this paper is also consistent with the 2015/16 UK study of LAIV in primary care [4]. Although the UK experienced influenza B circulation mainly of the B/Victoria lineage, there were also some circulating viruses of the B/Yamagata-lineage [16]. As LAIV in 2015/16 was a quadrivalent vaccine containing both a B/Phuket/3073/2013-like virus and B/Brisbane/60/2008-like virus, both well matched to the circulating strains, this presumably explains the relatively high VE.

In summary, we have demonstrated that in 2015/16, LAIV provided moderate protection against laboratory-confirmed influenza infection resulting in hospitalisation in England, including against A(H1N1)pdm09 infection and also influenza B. The findings support the recommendation of the JCVI for the on-going roll-out of the UK paediatric programme [17]. Close ongoing monitoring will be critical to provide assurance that these positive findings are maintained, particularly in the light of the recent US observations.

Acknowledgements

We would like to thank all the hospital trusts and primary care practices that contributed to this surveillance.

Conflict of interest

None declared.

Authors' contributions

RP conceived the study and drafted the article; BS, CT and HZ collected and collated the data; FW and NA undertook the analysis; all co-authors interpreted the data, commented on and approved the article.

References

1. Hakin B, Cosford P, Harvey F. The flu immunisation programme 2013/14 – extension to children. London: Department of Health; 26 Jul 2013. Available from: https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/225360/Children_s_flu_letter_2013.pdf
2. Public Health England (PHE). Flu plan. Winter 2015/16. London: PHE; May 2016. Available from: https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/526143/Flu_Plan_Winter_2015_to_2016superseded.pdf
3. Pebody R, Warburton F, Andrews N, Ellis J, von Wissmann B, Robertson C, et al. Effectiveness of seasonal influenza vaccine in preventing laboratory-confirmed influenza in primary care in the United Kingdom: 2014/15 end of season results. *Euro Surveill.* 2015;20(36):30013. DOI: 10.2807/1560-7917.ES.2015.20.36.30013 PMID: 26535911
4. Pebody R, Warburton F, Ellis J, Andrews N, Potts A, Cottrell S, et al. Effectiveness of seasonal influenza vaccine for adults and children in preventing laboratory-confirmed influenza in primary care in the United Kingdom: 2015/16 end-of-season results. *Euro Surveill.* 2016;21(38):30348. DOI: 10.2807/1560-7917.ES.2016.21.38.30348 PMID: 27684603
5. Bolotin S, Pebody R, White PJ, McMenamin J, Perera L, Nguyen-Van-Tam JS, et al. UK Severe Influenza Surveillance System (USISS) Steering Group. A new sentinel surveillance system for severe influenza in England shows a shift in age distribution of hospitalised cases in the post-pandemic period. *PLoS One.* 2012;7(1):e30279. DOI: 10.1371/journal.pone.0030279 PMID: 22291929
6. Farrington CP. Estimation of vaccine effectiveness using the screening method. *Int J Epidemiol.* 1993;22(4):742-6. DOI: 10.1093/ije/22.4.742 PMID: 8225751
7. Thomas HL, Andrews N, Green HK, Boddington NL, Zhao H, Reynolds A, et al. Estimating vaccine effectiveness against severe influenza in England and Scotland 2011/2012: applying the screening method to data from intensive care surveillance systems. *Epidemiol Infect.* 2014;142(1):126-33. PMID: 23591102
8. Public Health England (PHE). Influenza immunisation programme for England, GP patient groups, Season 2015 to 2016. London: PHE; May 2016. Available from: https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/544552/Seasonal_flu_GP_patient_groups_annual_report_2015_2016.pdf
9. Public Health England (PHE). National Childhood Influenza Vaccination Programme 2015 to 2016, Seasonal influenza vaccine uptake for children of primary school age. London: PHE; May 2016. Available from: https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/544542/Childhood_Influenza_Vaccination_Programme_Report_2015_2016.pdf
10. Health Protection Scotland (HPS). Flu vaccine effectiveness in Scottish primary school age children from the 2015/16 season. Glasgow: HPS. [Accessed 25 Jan 2017]. Available from: <http://www.hps.scot.nhs.uk/resourcedocument.aspx?id=5529>
11. Nohynek H, Baum U, Syrjänen R, Ikonen N, Sundman J, Jokinen J. Effectiveness of the live attenuated and the inactivated influenza vaccine in two-year-olds - a nationwide cohort study Finland, influenza season 2015/16. *Euro Surveill.* 2016;21(38):30346. DOI: 10.2807/1560-7917.ES.2016.21.38.30346 PMID: 27684447
12. Grohskopf LA, Sokolow LZ, Broder KR, Olsen SJ, Karron RA, Jernigan DB, et al. Prevention and Control of Seasonal Influenza with Vaccines. *MMWR Recomm Rep.* 2016;65(5):1-54. DOI: 10.15585/mmwr.rr6505a1 PMID: 27560619
13. Rondy M, Castilla J, Launay O, Costanzo S, Ezpeleta C, Galtier F, et al. Moderate influenza vaccine effectiveness against hospitalisation with A(H3N2) and A(H1N1) influenza in 2013-14: Results from the InNHOVE network. *Hum Vaccin Immunother.* 2016;12(5):1217-24. DOI: 10.1080/21645515.2015.1126013 PMID: 27065000
14. Penttinen PM, Friede MH. Decreased effectiveness of the influenza A(H1N1)pdm09 strain in live attenuated influenza vaccines: an observational bias or a technical challenge? *Euro Surveill.* 2016;21(38):30350. DOI: 10.2807/1560-7917.ES.2016.21.38.30350 PMID: 27684999
15. Ambrose CS, Bright H, Mallory R. Letter to the editor: Potential causes of the decreased effectiveness of the influenza A(H1N1)pdm09 strain in live attenuated influenza vaccines. *Euro Surveill.* 2016;21(45):30394. DOI: 10.2807/1560-7917.ES.2016.21.45.30394 PMID: 27918259
16. Public Health England (PHE). Surveillance of influenza and other respiratory viruses in the United Kingdom: Winter 2015 to 2016. London: PHE; May 2016. Available from: https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/526405/Flu_Annual_Report_2015_2016.pdf
17. Joint Committee on Vaccination and Immunisation (JCVI). JCVI minutes of the meeting, 23 August 2016. JCVI. [Accessed 25 Jan 2017]. Available from: <https://app.box.com/s/iddfb4ppwkmjtjusr2tc/1/2199012147/98304591342/1>

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Febrile illness in healthcare workers caring for Ebola virus disease patients in a high-resource setting

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Citation style for this article:

Fink D, Cropley I, Jacobs M, Mepham S. Febrile illness in healthcare workers caring for Ebola virus disease patients in a high-resource setting. *Euro Surveill.* 2017;22(4):pii=30449. DOI: <http://dx.doi.org/10.2807/1560-7917.ES.2017.22.4.30449>

Article submitted on 08 February 2016 / accepted on 21 July 2016 / published on 26 January 2017

Ebola virus disease (EVD) patients treated in high-resource facilities are cared for by large numbers of healthcare staff. Monitoring these healthcare workers (HCWs) for any illness that may represent transmission of Ebola virus is important both for the individuals and to minimise the community risk. International policies for monitoring HCWs vary considerably and their effectiveness is unknown. Here we describe the United Kingdom (UK) experience of illness in HCWs who cared for three patients who acquired EVD in West Africa. Five of these 93 high-level isolation unit (HLIU) HCWs presented with fever within 21 days of working on the unit; one of these five presented outside of the UK. This article discusses different approaches to monitoring of HCW symptom reporting. The potential impact of these approaches on HLIU staff recruitment, including travel restrictions, is also considered. An international surveillance system enhancing collaboration between national public health authorities may assist HLIU HCW monitoring in case they travel.

Introduction

In the recent West Africa Ebola outbreak (2013–2016), healthcare workers (HCWs) in affected countries were at particular risk of Ebola virus (EBOV) transmission and many hundreds died from EVD [1]. Only 27 medically evacuated or imported EVD cases were treated in Europe and the United States (US) during the outbreak [2], and yet despite high-resource facilities three transmissions of EBOV to HCWs occurred: two in the US and one in Spain. The exact exposures responsible for these secondary cases are not known, although in addition to providing personal care during life, the Spanish nursing assistant was involved in burial of the index case [3,4]. In 2009 the European Network of Infectious Diseases published a consensus framework for the design and operation of high-level isolation units (HLIUs) for the management of highly infectious diseases [5]. Although occupational health and safety is explicitly recognised as a high priority there is no

strong evidence base for guiding monitoring of HCWs post HLIU exposure. Since early 2014, as part of an international effort, hundreds of HCWs across nine high-resource countries have cared for EVD patients and there have been numerous iterations of national guidelines concerning all aspects of EVD management including HLIU HCW monitoring [6,7]. This report reviews the recent United Kingdom (UK) experience of monitoring HLIU HCWs and managing symptomatic individuals. We consider the impact of biocontainment strategy on HCW monitoring policy and the relative strengths and weaknesses of different approaches. We reflect how best HLIU policy might protect individual and public safety without imposing exacting sanctions on a limited and often voluntary HCW population.

Biocontainment strategy and implications for healthcare worker monitoring

In the US and most European countries, isolation units for managing EVD patients consist in negative pressure rooms where HCWs wear full-body personal protective equipment (PPE). HCWs are considered to have ‘direct contact’ with EVD patients in this setting irrespective of PPE adherence. In contrast in the UK, two HLIUs for managing cases of confirmed Hazard Group 4 viral haemorrhagic fevers (VHFs) employ a primary method of biocontainment that is quite distinct from methods used elsewhere in the world. The patient is managed within a single-bed flexible-film negative pressure isolator (Trexler isolator), which in turn is located within a negative pressure room. Care is delivered by staff wearing surgical scrubs through half suits built into the wall of the isolator itself. Early experimental pressure and virus viability studies support the clinical safety of the isolator over nearly four decades of use in the UK for management of viral haemorrhagic fevers [8].

Public Health England (PHE) defines any HCWs providing patient care in the HLIU as Category 1 contacts. Category 1 describes individuals with ‘no direct

TABLE

Healthcare workers presenting with febrile illness and admitted for assessment after caring for patients with Ebola virus disease in high-level isolation units in the United Kingdom, 2014–2015

Role on HLIU	Assessment location	EBOV real-time RT-PCR testing	Diagnosis
Doctor	RFH, London, UK	No	Appendicitis
Nurse	RFH, London, UK	Yes	Influenza A
Doctor	RFH, London, UK	Yes	Enterovirus
Doctor	RFH, London, UK	Yes	Unspecified febrile illness
Doctor	Shanghai Public Health Clinical Centre, Shanghai, China	Yes	Tonsillitis

EBOV: Ebola virus; RT-PCR: reverse transcription-PCR; HLIU: high-level isolation unit; RFH: Royal Free Hospital; UK: United Kingdom.

contact' with a person with EVD. Contact by HCWs with patients while appropriately wearing the half suits of the Trexler isolator is not considered direct contact. However should Category 1 contacts record a temperature greater than 37.5°C or develop symptoms consistent with EVD within 21 days of caring for a confirmed EVD patient they are advised to contact the physician in charge of the HLIU. This is considered passive reporting. There are no restrictions on any activities, including work and travel, of HCWs who provide care in the HLIU and remain asymptomatic.

The recent United Kingdom experience of high-level isolation unit healthcare worker monitoring

During the recent West Africa Ebola outbreak, three cases of confirmed EVD were managed in the HLIU at the Royal Free Hospital between August 2014 and March 2015. Cumulatively this amounted to 45 patient days inside the bed isolator. Simultaneously two doctors and four nurses work 12-hour shifts in HLIU, equating to 180 doctor shifts and 360 nursing shifts during this period. In total, 46 individual doctors and 47 individual nurses undertook shifts and so 93 individuals provided direct patient care within HLIU.

Five of 93 (5.4%) HCWs who had provided direct patient care on the HLIU presented with a febrile illness within 21 days of last possible exposure to EBOV. Four of the cases were managed in the UK and one in China, where the individual was on vacation. One individual assessed in the UK had clinical features of appendicitis, which was confirmed by computed tomography scan, and did not undergo testing for EBOV infection. The other four cases had non-specific febrile illnesses and all were managed in an isolation facility with appropriate infection control precautions and were tested for EBOV infection by PCR. EBOV PCR was negative in each case, and an alternative diagnosis was subsequently confirmed in three cases (Table). One individual had an undefined febrile illness that resolved spontaneously within 48 hours.

Discussion

Incidence of febrile illness in high-level isolation unit healthcare workers

The prompt diagnosis of EVD is fundamental to both individual and public health. Assessment of symptomatic HCWs who have cared for EVD patients is complex requiring prompt, coordinated, direct admission to an appropriate isolation facility, adherence to robust infection control protocols, highly trained personnel, and the expeditious sending of blood samples to a reference laboratory for EBOV testing. Our experience of this assessment pathway in the UK demonstrates that febrile illness in HCW within 21 days of last possible exposure to EBOV is not rare, occurring in 5/93 (5.4%) of HCW directly involved in patient care on HLIU. Although the reporting behaviour of HLIU HCWs might be expected to be more exacting than HCWs without exposure to Risk Group 4 viruses, this is comparable to the background rate of sick leave in National Health Service (NHS) staff of ca 4% at any time [9]. Although fever is a common symptom, reasons for NHS sick leave will include other symptoms and conditions that do not cause fever (e.g. mechanical injury). The specific incidence of febrile illness in all UK HCWs in general is not known. During winter months, encompassed by our data, a significant proportion of illness is likely to be caused by self-limiting febrile illnesses.

Confirming alternative diagnoses

A fundamental principle of any monitoring policy in the HLIU setting remains that self-limiting febrile illnesses cannot reliably be differentiated from EVD on clinical grounds alone [10]. Apart from the case of appendicitis, which was clinically identified and did not undergo EBOV testing, the spectrum of illnesses diagnosed in the UK experience was minor and these did not require inpatient care in their own right. Besides excluding EVD, it is important to confirm an alternative diagnosis in the context of persistent fever. For a HCW population this may have separate infection control implications such as nosocomial transmission of other communicable diseases. An alternative diagnosis may also mitigate the need for repeat EBOV testing that might be indicated for high-risk exposures [11]. Deferring

routine HCW influenza and other immunisation that may cause fever until more than 21 days have elapsed since last possible exposure to EBOV would seem prudent to avoid unnecessary testing. Prior to working on HLIU, routine vaccination on recruitment to the HLIU staff might be considered as a preventative measure against non-EVD febrile illness and potentially reduce the burden of EBOV testing. In time routine prophylaxis may encompass vaccination against EBOV. However this may not be possible in the context of acute clinical need and an unpredictable burden of care.

Limitations of self-reported healthcare worker monitoring data

Divergent HCW monitoring policies exist between high-resource countries [6,7,12,13]. This variance is the probable consequence of different biocontainment strategies. In December 2015 the Centers for Disease Control and Prevention (CDC) published an update of *Interim U.S. Guidance for Monitoring and Movement of Persons with Potential Ebola Virus Exposure* [13]. This classified as 'low (but not zero) risk' all US-based HCWs wearing appropriate PPE caring for symptomatic EVD patients while in the 'patient-care area' or having any contact with a patient's body fluids in any area. This cohort of HCWs, the vast majority of clinical and laboratory staff involved in patient care, were to be subject to 'direct active monitoring' for 21 days post exposure, requiring direct observation of symptoms and temperature by a public health authority at least once daily. This is opposed to 'active monitoring' where individuals would themselves report daily temperature. In reference to high-resource setting HCWs within the CDC guidance, 'no identifiable risk' described those HCWs with no exposure to the immediate patient-care area or to body fluids; 'some risk' described HCWs after close contact (defined as being within one metre) with a person with EVD without appropriate PPE; 'high risk' described HCWs after direct contact with a person with EVD or their body fluids without appropriate PPE. There were no formal restrictions on travel or work, including in healthcare settings, in the 'low (but not zero) risk' group. However, CDC advised discussion of plans relating to work or travel within 21 days after care of an EVD patient with local public health authorities before undertaking these activities. Further, should international travel be undertaken during this time, guidelines recommended notification of CDC and the ministry of health in the destination country with transfer of monitoring oversight [14].

In the UK reporting by HCWs is passive compared with this active and direct active reporting in the US. It is difficult to compare the risk stratification nomenclature for CDC and PHE guidelines given that they advise on different risk exposures from different containment strategies. In the sense that they both apply to HCWs undergoing routine and safe care of persons with EVD in non-endemic settings, category 1 in PHE guidelines is equivalent to 'low (but not zero) risk identifiable risk' in the CDC policy. However, due to the perceived added

protection of the bed isolator, unlike CDC guidelines, PHE does not mandate direct active or active monitoring of symptoms or temperature for any HCWs [15].

Given that no secondary transmission of EVD occurred in the UK it is difficult to compare directly publicly available data on monitoring of HCWs in the US and our own experience. In the US, 147 contacts of two nurses diagnosed with EVD were actively monitored and 12 (8%) tested [16]. The monitored population in that case had a range of risk exposures, unlike the relatively homogeneous exposures of the UK HCW cohort, and other symptoms without fever may have triggered EVD testing in this setting. Despite these significant limitations of comparing monitoring strategies, our study suggests that passive monitoring of HLIU HCWs results in similar presentation rates with 5.4% reporting febrile illness in our study. Febrile illness is likely a common phenomenon in HCWs but there are very little data regarding its diagnosis and monitoring especially in the context of emerging infectious disease outbreaks.

It is known that presenteeism, work attendance despite illness, is common in HCWs. Questionnaire-based studies of NHS staff report rates of presenteeism as high as 70% [17]. In the HLIU setting symptomatic staff may be reluctant to impose further operational demands on an already limited cohort of specialist colleagues by declaring a fever at its onset. The perceived very low risk of transmission and the high likelihood of alternative diagnoses may also influence reporting [18,19]. Therefore, despite the seriousness of EVD acquisition and clear instruction on the importance of reporting all relevant symptoms as an integral part of HLIU training, it is difficult to exclude response bias from any analysis of self-reported illness in HCWs. There is no evidence that unsupervised active monitoring reduces this compared with passive monitoring. For example, despite active monitoring and direct communication with public health authorities, it is possible that the second nurse diagnosed with EVD in the US travelled on an internal flight with febrile symptoms [20].

In the absence of clinical evidence, a more detailed understanding of HCW illness reporting behaviour and expected rates of febrile illness in these cohorts might better inform monitoring strategies.

International collaboration

We describe one symptomatic UK HCW who was managed in conjunction with Chinese health authorities. We believe this is the first instance of EBOV PCR performed in a HCW respectively exposed and tested in two separate non-endemic countries. The individual worked in the UK HLIU, and subsequently developed febrile symptoms after arrival in China where a test for EVD was performed. Although one might argue that this represents irresponsible behaviour on the part of the HCW, there was no formal or informal requirement for the individual to alert UK health authorities before international travel within 21 days of working in HLIU.

Guidelines at the European level could be helpful for a harmonised approach in the European Union in such a circumstance.

The latest CDC guidelines in early 2016 advised discussion with local authorities and notification of CDC before such travel with a view to establishing collaborative monitoring across international borders [13]. There are no published reports of any such bilateral or unilateral arrangements. Although this travel guidance may discourage HCW international travel altogether, avoiding the complexities of international public health collaboration, it does have inherent problems. Formal travel negotiations after HLIU exposure may negatively impact recruitment of HCWs. The relatively unpredictable HLIU workload means that this may be particularly relevant for those HCWs whose travel requires VISA application or other costly advanced planning. Further, the guidelines are difficult to enforce and travel restrictions or penalties for unreported travel would likely be negatively received by the healthcare community.

Our case emphasises the cross-border cooperation that highly infectious diseases may require. During the SARS outbreak, international travel was restricted for potential SARS contacts and screening strategies demanded trans-continental communication between health authorities [21]. Despite informal and formal international collaboration addressing emergency response to infectious disease threats, no systematic framework exists for monitoring and reporting contacts of persons with infectious diseases or, more particularly, HCWs across borders [22,23]. Given finite HLIU bed space and resources consideration to sharing such care between countries may be a necessary extension of such relationships. The German government has offered the services of its medical evacuation aircraft to other members of the European Union (EU) under the EU Civil Protection Mechanism [24]. Outbreaks with the potential for global spread remind us that we should continue to develop public health communication not simply across European borders but across continental borders too.

Pragmatic monitoring

Direct active monitoring may improve the sensitivity of HCW monitoring but at significant costs. More invasive monitoring strategies, which might preclude routine work after any HLIU exposure, may negatively impact on HCW recruitment for this essential work [25].

There may be a role for pragmatic active surveillance such as monitoring via text message that has been trialled successfully in Australia representing a balance between active and passive monitoring [26]. In the event of fever, testing and home self-isolation rather than hospital admission may be appropriate to improve symptom reporting given the low risk category 1 exposure of HCW in HLIU. A formal international public health network with policy and capacity that transcends borders would empower this surveillance strategy. The

principle of collective, as well as personal, responsibility would complement the remarkable contribution that diverse HCWs make to protecting global health.

Conflict of interest

None declared.

Authors' contributions

DF co-conceived and wrote the manuscript. IC collected data and contributed with comments. MJ revised the manuscript. SM co-conceived and revised the manuscript. All authors have seen and approved the final manuscript.

References

1. Kilmarx PH, Clarke KR, Dietz PM, Hamel MJ, Husain F, McFadden JD, et al. , Centers for Disease Control and Prevention (CDC). Ebola virus disease in health care workers--Sierra Leone, 2014. *MMWR Morb Mortal Wkly Rep.* 2014;63(49):1168-71. PMID: 25503921
2. Uyeki TM, Mehta AK, Davey RT, Liddell AM, Wolf T, Vetter P, et al. , Working Group of the U.S.–European Clinical Network on Clinical Management of Ebola Virus Disease Patients in the U.S. and Europe. Clinical Management of Ebola Virus Disease in the United States and Europe. *N Engl J Med.* 2016;374(7):636-46. DOI: 10.1056/NEJMoa1504874 PMID: 26886522
3. Parra JM, Salmerón OJ, Velasco M. The first case of Ebola virus disease acquired outside Africa. *N Engl J Med.* 2014;371(25):2439-40. DOI: 10.1056/NEJMc1412662 PMID: 25409262
4. Liddell AM, Davey RT, Mehta AK, Varkey JB, Kraft CS, Tsegay GK, et al. Characteristics and Clinical Management of a Cluster of 3 Patients With Ebola Virus Disease, Including the First Domestically Acquired Cases in the United States. *Ann Intern Med.* 2015;163(2):81-90. DOI: 10.7326/M15-0530 PMID: 25961438
5. Bannister B, Puro V, Fusco FM, Heptonstall J, Ippolito G, EUNID Working Group. Framework for the design and operation of high-level isolation units: consensus of the European Network of Infectious Diseases. *Lancet Infect Dis.* 2009;9(1):45-56. DOI: 10.1016/S1473-3099(08)70304-9 PMID: 19095195
6. Public Health England (PHE) and Department of Health. Viral haemorrhagic fever: ACDP algorithm and guidance on management of patients. Last updated 19 Nov 2015. Available from: <https://www.gov.uk/government/publications/viral-haemorrhagic-fever-algorithm-and-guidance-on-management-of-patients>
7. European Centre for Disease Prevention and Control (ECDC). Algorithm for Ebola virus disease contact management. Stockholm: ECDC. Last updated: 12 Nov 2014. Available from: http://ecdc.europa.eu/en/healthtopics/ebola_marburg_fevers/algorithm-evd-contact-management/Pages/default.aspx
8. Hutchinson JG, Gray J, Flewett TH, Emond RT, Evans B, Trexler PC. The safety of the Trexler isolator as judged by some physical and biological criteria: a report of experimental work at two centres. *J Hyg (Lond).* 1978;81(2):311-9. DOI: 10.1017/S0022172400025158 PMID: 701792
9. Health and Social Care Information Centre. NHS staff: average worker takes an estimated 9.5 working days off sick a year. 2013. [Accessed 14 Aug 2015]. Available from: <http://www.hscic.gov.uk/staffsicknessspr>
10. Lado M, Walker NF, Baker P, Haroon S, Brown CS, Youkee D, et al. Clinical features of patients isolated for suspected Ebola virus disease at Connaught Hospital, Freetown, Sierra Leone: a retrospective cohort study. *Lancet Infect Dis.* 2015;15(9):1024-33. DOI: 10.1016/S1473-3099(15)00137-1 PMID: 26213248
11. O'Shea MK, Clay KA, Craig DG, Matthews SW, Kao RLC, Fletcher TE, et al. Diagnosis of Febrile Illnesses Other Than Ebola Virus Disease at an Ebola Treatment Unit in Sierra Leone. *Clin Infect Dis.* 2015;61(5):795-8. DOI: 10.1093/cid/civ399 PMID: 25991466
12. Robert Koch Institut (RKI). Recommended measures for medical and other staff who were involved in patient care or outbreak response during the Ebola outbreak in West Africa. Berlin: RKI. Available from: http://www.rki.de/EN/Content/infections/epidemiology/outbreaks/Ebola_virus_disease/Ebola_returning_HCW.html

13. Centers for Disease Control and Prevention (CDC). Interim US. Guidance for Monitoring and Movement of Persons with Potential Ebola Virus Exposure. Atlanta: CDC. [Accessed 23 Nov 2015]. Available from: <http://www.cdc.gov/vhf/ebola/exposure/monitoring-and-movement-of-persons-with-exposure.html#mm-table>
14. Interim US. Guidance for Monitoring and Movement of Persons with Potential Ebola Virus Exposure Atlanta: CDC. [Accessed 27 Jul 2015]. Available from: <http://www.cdc.gov/vhf/ebola/exposure/monitoring-and-movement-of-persons-with-exposure.html#table-monitoring-movement>
15. Public Health England (PHE). Public health recommendations for asymptomatic contacts of an Ebola case in the UK. London: PHE. [Accessed 14 Aug 2015]. Available from: https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/402483/Public_health_recommendations_for_asymptomatic_contacts_of_an_Ebola_case.pdf
16. Chevalier MS, Chung W, Smith J, Weil LM, Hughes SM, Joyner SN, et al. , Centers for Disease Control and Prevention (CDC). Ebola virus disease cluster in the United States--Dallas County, Texas, 2014. *MMWR Morb Mortal Wkly Rep.* 2014;63(46):1087-8. PMID: 25412069
17. National Health Service (NHS). NHS staff survey national briefing; 2013. [Accessed 14 Aug 2015]. Available from: http://www.nhsstaffsurveys.com/Caches/Files/NHS%20staff%20survey_nationalbriefing_2013_FINAL.pdf
18. Nichol K, Bigelow P, O'Brien-Pallas L, McGeer A, Manno M, Holness DL. The individual, environmental, and organizational factors that influence nurses' use of facial protection to prevent occupational transmission of communicable respiratory illness in acute care hospitals. *Am J Infect Control.* 2008;36(7):481-7. DOI: 10.1016/j.ajic.2007.12.004 PMID: 18786451
19. Stein AD, Makarawo TP, Ahmad MFR. A survey of doctors' and nurses' knowledge, attitudes and compliance with infection control guidelines in Birmingham teaching hospitals. *J Hosp Infect.* 2003;54(1):68-73. DOI: 10.1016/S0195-6701(03)00074-4 PMID: 12767850
20. McCarty CL, Basler C, Karwowski M, Erme M, Nixon G, Kippes C, et al. , Centers for Disease Control and Prevention (CDC). Response to importation of a case of Ebola virus disease--Ohio, October 2014. *MMWR Morb Mortal Wkly Rep.* 2014;63(46):1089-91. PMID: 25412070
21. Heymann DL. SARS and emerging infectious diseases: a challenge to place global solidarity above national sovereignty. *Ann Acad Med Singapore.* 2006;35(5):350-3. PMID: 16830003
22. Aghaizu A, Elam G, Ncube F, Thomson G, Szilágyi E, Eckmanns T, et al. Preventing the next 'SARS' - European healthcare workers' attitudes towards monitoring their health for the surveillance of newly emerging infections: qualitative study. *BMC Public Health.* 2011;11(1):541. DOI: 10.1186/1471-2458-11-541 PMID: 21740552
23. McDougall CW, Upshur REG, Wilson K. Emerging norms for the control of emerging epidemics. *Bull World Health Organ.* 2008;86(8):643-5. [Accessed 25 May 2016]. Available from: <http://www.who.int/bulletin/volumes/86/8/08-051771/en/>
24. European Commission. Ebola: Germany provides additional medical evacuation capacity. European Commission. [Accessed 10 May 2016]. Available from: http://ec.europa.eu/echo/news/ebola-germany-provides-additional-medical-evacuation-capacity_en
25. Drazen JM, Kanapathipillai R, Campion EW, Rubin EJ, Hammer SM, Morrissey S, et al. Ebola and quarantine. *N Engl J Med.* 2014;371(21):2029-30. DOI: 10.1056/NEJMe1413139 PMID: 25347231
26. Tracey LE, Regan AK, Armstrong PK, Dowse GK, Effler PV. EbolaTracks: an automated SMS system for monitoring persons potentially exposed to Ebola virus disease. *Euro Surveill.* 2015;20(1):20999. DOI: 10.2807/1560-7917.ES2015.20.1.20999 PMID: 25613652

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