Impact factor 5·7

Eurosurveillance

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

Vol. 21 | Weekly issue 19 | 12 May 2016

ecdc EUROPEAN CENTRE FOR DISEASE PREVENTION AND CONTROL

www.eurosurveillance.org

A note from the editors: enterovirus D68 epidemiology in France and Germany–food for thought

Eurosurveillance editorial team [1]
1. European Centre for Disease Prevention and Control (ECDC), Stockholm, Sweden

Correspondence: Eurosurveillance editorial team (eurosurveillance@ecdc.europa.eu)

Citation style for this article:
Eurosurveillance editorial team. A note from the editors: enterovirus D68 epidemiology in France and Germany–food for thought. Euro Surveill. 2016;21(19):pii=30229. DOI: http://dx.doi.org/10.2807/1560-7917.ES.2016.21.19.30229

Article published on 12 May 2016

Worldwide emergence of enterovirus (EV) D68 causing severe respiratory illness particularly in children, between 2008 and 2014, has been described in numerous articles. In 2014, EV-D68 gained particular attention when a large outbreak in children, associated with severe respiratory illness and possible neurological illness, occurred in the United States [1-3]. This event triggered a number of surveillance activities in various countries, some of them published in *Eurosurveillance* [4-8]. In our current issue we present investigations into the occurrence of EV-D68 in two European countries namely France and Germany.

The articles present data from patients hospitalised or visiting hospital emergency departments with respiratory symptoms. Schuffenecker et al. report on samples collected by eleven laboratories of the French EV surveillance network from eight of 22 Regions over six months in 2014 [9]. These eleven laboratories represent about one-third of the laboratories participating in the French EV network. Böttcher et al. analysed samples during two entire years, 2013 and 2014, at three large tertiary hospital laboratories in Germany [10]. These laboratories, situated mainly in the western part of the country, contribute ca 25% of the EV-positive samples in the nationwide RespVir surveillance [11].

Both reports are based on a considerable number of screened respiratory samples: 6,229 samples with 212 EV-D68 detections corresponding to 200 cases in France; 14,838 samples with 39 EV-D68-positive cases in Germany.

In line with the literature, the German analysis suggests seasonality of EV-D68 infections, with most cases occurring between September and November (weeks 36–48), and even though covering only six months, also the French analysis shows peaks in October (week 43) and November (week 48). Moreover, cases in both countries occurred mainly in children younger than five years, although French authors caution that a bias towards preferential sampling of children cannot be ruled out.

Clinical manifestation in children was characterised by asthma and bronchiolitis in France where ca 11% of the hospitalised paediatric cases and 14% of the hospitalised adult cases needed treatment in intensive care units, mostly due to severe respiratory symptoms. In Germany, clinical details were only available for a limited number of cases and in these pneumonia or obstructive bronchitis were the most common causes for hospitalisation. It should be noted that no neurological involvement was described over the two years for any of the EV-D68 cases diagnosed in the three German university hospital laboratories. In France however, four patients presented with neurological signs including one child who developed acute flaccid paralysis following EV-D68-associated pneumonia [6].

Sequence data show that the EV-D68 strains from all three German hospitals detected in 2013 and 2014 cluster together with worldwide circulating strains. The majority of sequences belonged to the B2 lineage; however, in both countries, clade A EV-D68 viruses were more frequent in adults than children. All German strains assigned to subclade A2 were identified in adult patients and the authors demonstrated insertion of two amino acids at the C-terminus of VP1 of subclade A2 strains. However, there are at present no clear clinical implications associated with this change.

The two reports add to the body of evidence on EV-D68 epidemiology and circulating strains in Europe. They also illustrate the importance and usefulness of continuous molecular surveillance of EV in respiratory samples in combination with clinical information to detect changing trends and increasing severity of infections early. Last but not least they show that existing

surveillance systems for respiratory infections can be adapted for such purposes.

References

1. Midgley CM, Jackson MA, Selvarangan R, Turabelidze G, Obringer E, Johnson D, et al. Severe respiratory illness associated with enterovirus D68 - Missouri and Illinois, 2014. MMWR Morb Mortal Wkly Rep. 2014;63(36):798-9.PMID: 25211545
2. Pastula DM, Aliabadi N, Haynes AK, Messacar K, Schreiner T, Maloney J, et al. , Centers for Disease Control and Prevention (CDC). Acute neurologic illness of unknown etiology in children - Colorado, August-September 2014.MMWR Morb Mortal Wkly Rep. 2014;63(40):901-2.PMID: 25299607
3. European Centre for Disease Prevention and Control (ECDC). Enterovirus 68 rapid risk assessment updated. Stockholm: ECDC 24; Nov. 2014. Available from: http://www.ecdc.europa.eu/en/press/news/_layouts/forms/New
4. Skowronski DM, Chambers C, Sabaiduc S, Murti M, Gustafson R, Pollock S, et al. Systematic community- and hospital-based surveillance for enterovirus-D68 in three Canadian provinces, August to December 2014. Euro Surveill. 2015;20(43):30047. DOI: 10.2807/1560-7917.ES.2015.20.43.30047 PMID: 26804195
5. Midgley SE, Christiansen CB, Poulsen MW, Hansen CH, Fischer TK. Emergence of enterovirus D68 in Denmark, June 2014 to February 2015.Euro Surveill. 2015;20(17):21105. DOI: 10.2807/1560-7917.ES2015.20.17.21105 PMID: 25955773
6. Lang M, Mirand A, Savy N, Henquell C, Maridet S, Perignon R, et al. Acute flaccid paralysis following enterovirus D68 associated pneumonia, France, 2014. Euro Surveill. 2014;19(44):20952. DOI: 10.2807/1560-7917.ES2014.19.44.20952 PMID: 25394254
7. Pfeiffer HC, Bragstad K, Skram MK, Dahl H, Knudsen PK, Chawla MS, et al. Two cases of acute severe flaccid myelitis associated with enterovirus D68 infection in children, Norway, autumn 2014. Euro Surveill. 2015;20(10):21062. DOI: 10.2807/1560-7917.ES2015.20.10.21062 PMID: 25788251
8. Meijer A, Benschop KS, Donker GA, van der Avoort HG. Continued seasonal circulation of enterovirus D68 in the Netherlands, 2011-2014.Euro Surveill. 2014;19(42):20935. DOI: 10.2807/1560-7917.ES2014.19.42.20935 PMID: 25358039
9. Schuffenecker I, Mirand A, Josset L, Henquell C, Hecquet D, Pilorgé L, et al. Epidemiological and clinical characteristics of patients infected with enterovirus D68, France, July to December 2014. Euro Surveill. 2016;21(19):30226. DOI: 10.2807/1560-7917.ES.2016.21.19.30226
10. Böttcher S, Prifert C, Weißbrich B, Adams O, Aldabbagh S, Eis-Hübinger AM, et al. Detection of enterovirus D68 in patients hospitalised in three tertiary university hospitals in Germany, 2013 to 2014. Euro Surveill. 2016;21(19):30227. DOI: 10.2807/1560-7917.ES.2016.21.19.30227
11. Respiratorisches Viren – Netzwerk (RespVir). German. [Accessed 20 May 2015]. Available from: http://rvdev.medical-dpc.com/

License and copyright

Epidemiological and clinical characteristics of patients infected with enterovirus D68, France, July to December 2014

I Schuffenecker [1 2 3], A Mirand [3 4 5], L Josset [1 2], C Henquell [4 5], D Hecquet [6], L Pilorgé [7], J Petitjean-Lecherbonnier [8], C Manoha [9], J Legoff [10], C Deback [11], S Pillet [12], Q Lepiller [13], JM Mansuy [14], S Marque-Juillet [15], D Antona [16], H Peigue-Lafeuille [4 5], B Lina [1 2]

1. Centre National de Référence des Enterovirus et Parechovirus, Laboratoire de Virologie, Hospices Civils de Lyon, Lyon, France
2. Laboratoire Virpath, EA4610, Faculté de médecine Lyon Est, Université Claude Bernard Lyon 1, Lyon, France
3. These authors contributed equally to this work
4. CHU Clermont-Ferrand, Laboratoire de Virologie, Centre National de Référence des Enterovirus et Parechovirus – laboratoire associé, Clermont-Ferrand, France
5. Université d'Auvergne, EA4843 "Epidémiologie et pathogénie des infections à entérovirus", Clermont-Ferrand, France
6. Laboratoire de Virologie, CHU Amiens, Amiens, France
7. Laboratoire de Virologie, CHRU de la Cavale Blanche, Brest, France
8. Laboratoire de Virologie, CHU Caen, Caen, France
9. Laboratoire de Virologie, CHU Dijon, Dijon, France
10. Laboratoire de Microbiologie, Hôpital Saint-Louis, APHP, Paris, France
11. Laboratoire de Virologie, Hôpital Paul Brousse, APHP, Villejuif, France
12. Laboratoire des agents infectieux et hygiène, CHU de Saint-Etienne, Saint-Etienne, France
13. Laboratoire de Virologie, Hôpitaux Universitaires de Strasbourg, Strasbourg, France
14. Laboratoire de Virologie, CHU Toulouse, Toulouse, France
15. Laboratoire de Microbiologie, CH Versailles, Versailles, France
16. Institut de Veille sanitaire, Département des Maladies Infectieuses, Saint-Maurice, France

Correspondence: Isabelle Schuffenecker (isabelle.schuffenecker@chu-lyon.fr)

Citation style for this article:
Schuffenecker I, Mirand A, Josset L, Henquell C, Hecquet D, Pilorgé L, Petitjean-Lecherbonnier J, Manoha C, Legoff J, Deback C, Pillet S, Lepiller Q, Mansuy JM, Marque-Juillet S, Antona D, Peigue-Lafeuille H, Lina B. Epidemiological and clinical characteristics of patients infected with enterovirus D68, France, July to December 2014. Euro Surveill. 2016;21(19):pii=30226. DOI: http://dx.doi.org/10.2807/1560-7917.ES.2016.21.19.30226

Article submitted on 04 August 2015 / accepted on 05 February 2016 / published on12 May 2016

In 2014, the United States (US) experienced a nationwide outbreak of enterovirus D68 (EV-D68) infection with 1,152 cases reported mainly in hospitalised children with severe asthma or bronchiolitis. Following the US alert, 11 laboratories of the French enterovirus (EV) surveillance network participated in an EV-D68 survey. A total of 6,229 respiratory samples, collected from 1 July to 31 December 2014, were screened for EV-D68 resulting in 212 EV-D68-positive samples. These 212 samples corresponded to 200 EV-D68 cases. The overall EV-D68 positivity rates among respiratory samples were of 5% (184/3,645) and 1.1% (28/2,584) in hospitalised children and adults respectively. The maximum weekly EV-D68 positivity rates were of 16.1% for children (n = 24/149; week 43) and 2.6% for adults (n = 3/115; week 42). Of 173 children with EV-D68 infection alone, the main symptoms were asthma (n = 83; 48.0%) and bronchiolitis (n = 37; 21.4%). One child developed acute flaccid paralysis (AFP) following EV-D68-associated pneumonia. Although there was no significant increase in severe respiratory tract infections reported to the French public health authorities, 10.7% (19/177) of the EV-D68 infected children and 14.3% (3/21) of the EV-D68 infected adults were hospitalised in intensive care units. Phylogenetic analysis of the viral protein 1 (VP1) sequences of 179 EV-D68 cases, revealed that 117 sequences (65.4%), including that of the case of AFP, belonged to the B2 variant of clade B viruses. Continuous surveillance of EV-D68 infections is warranted and could benefit from existing influenza-like illness and EV surveillance networks.

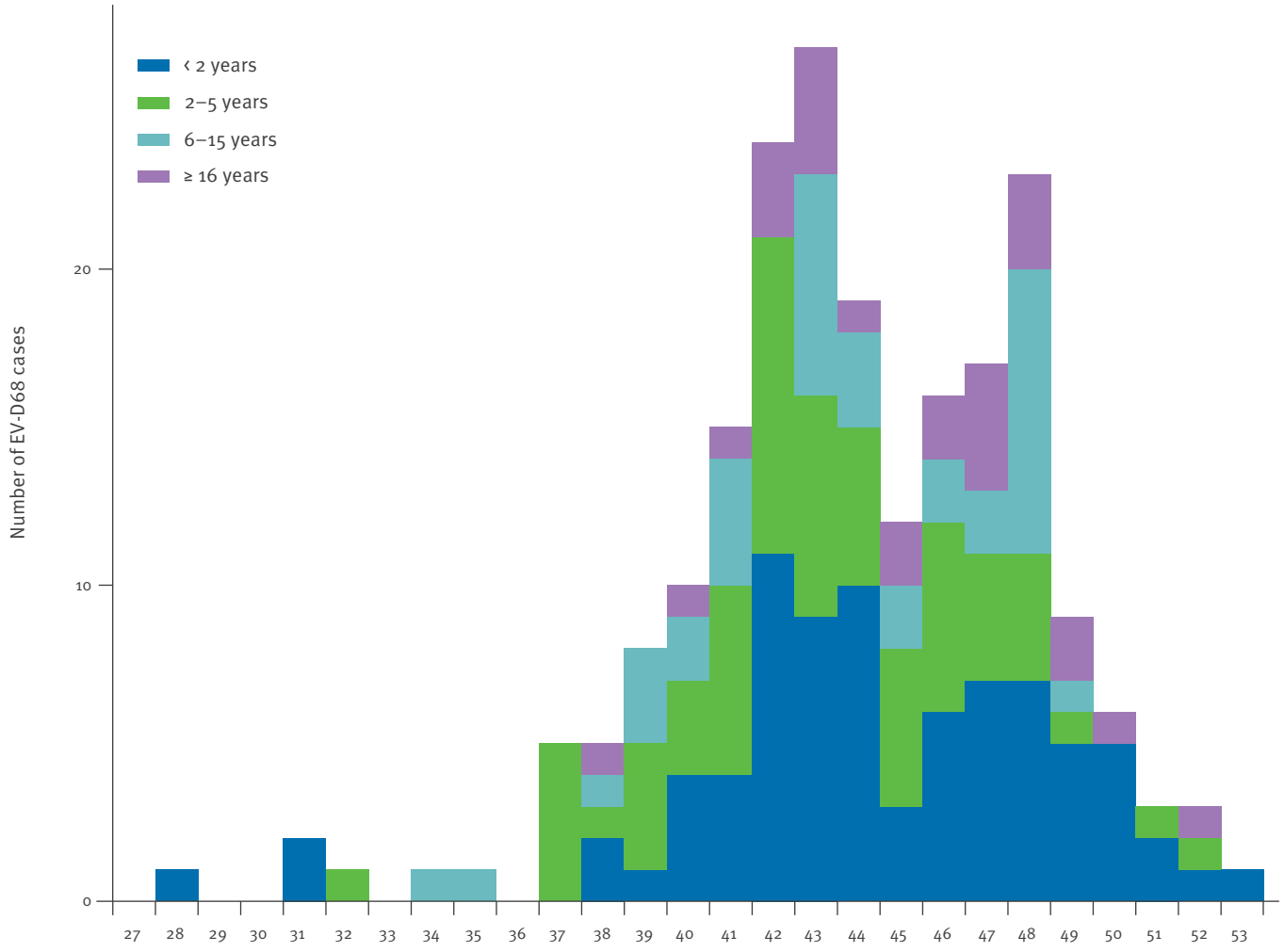
Introduction

Enterovirus D68 (EV-D68) was first identified in the United States (US) in 1962 in four paediatric patients with acute respiratory infections (ARI) [1-11]. Until 2014, only sporadic cases of infection with this virus as well as small outbreaks (10 publications during 2006–2011) were reported in Asia, Europe and the US [1-11], with disease manifestations mainly ranging from mild respiratory symptoms to severe ARI requiring intensive care and mechanical ventilation.

In 2014, the US experienced a nationwide outbreak of EV-D68 infection associated with an upsurge of severe respiratory cases admitted to emergency departments. Between mid-August and mid-December, 1,152 EV-D68 cases were reported by the Centers for Disease Control and Prevention (CDC) in 49 states, mainly in hospitalised children with severe asthma or bronchiolitis and occasionally in children with acute flaccid myelitis [12]. The overall disease burden was however, probably

Figure 1

Distribution of enterovirus D68 cases by week and by age, France, July–December 2014 (n=209)



much higher [13,14]. During the autumn, European countries did not report a global increase in hospital admissions for severe respiratory infections or a significant upsurge of ARI [15]. However, reports from Norway and the Netherlands suggested that EV-D68 circulation might have increased [16,17].

In France, enterovirus (EV) surveillance and molecular typing involve a network of hospital virology laboratories and focus mainly on EV neurological infections in hospitalised patients [18]. In hospitalised patients with respiratory infections, human rhinoviruses and enteroviruses (HRV/EV) infections have been more systematically investigated since early 2010, due to the recent development of HRV/EV and commercial multiplex reverse-transcription polymerase chain reaction (RT-PCR) assays, but they remain underdiagnosed. In addition, no routine typing of EV and HRV is performed, even in severe respiratory cases. In late September 2014, a French child developed severe acute flaccid paralysis (AFP) following EV-D68 pneumonia [19]. Taking all these factors into account, the National Institute of Public Health encouraged the French EV surveillance network to conduct a systematic analysis of respiratory samples collected from hospitalised patients to evaluate both the level of EV-D68 circulation and its clinical impact.

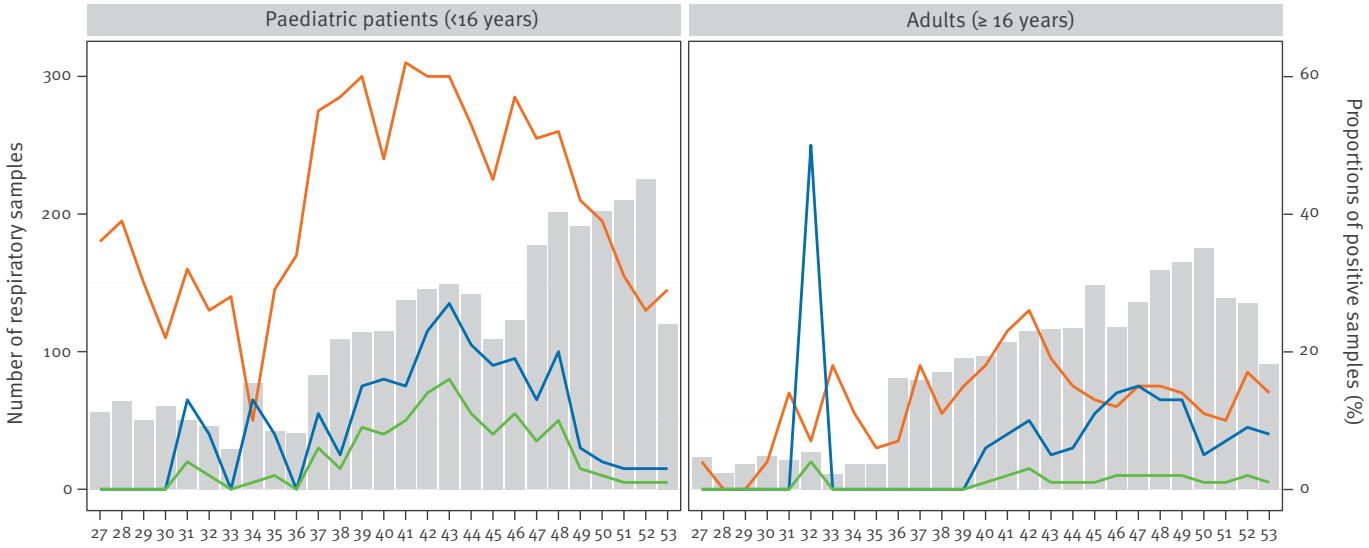
Methods

French enterovirus surveillance network

EV surveillance in France involves 34 virology/microbiology laboratories in university and general hospitals, including the two EV National Reference Laboratories (NRLs) (based in Lyon and Clermont-Ferrand). Each laboratory reports monthly on a specific website (http://cnr.chu-clermontferrand.fr/CNR) the number and type of samples analysed for EV, the relevant clinical data and EV serotype (when available). Throughout the year, EV-positive samples including mainly cerebrospinal fluid (CSF) specimens are genotyped in nine laboratories of the EV surveillance network (including the two NRLs) [18]. On 9 October 2014, the French EV surveillance laboratories were contacted by the Lyon NRL to take part in a national EV-D68 surveillance study. Participation in the French EV-D68 project was

Figure 2

Distribution of human rhinovirus/enterovirus-and enterovirus D68-positive samples per week, France, July–December 2014 (n=6,229 respiratory samples)



EV: enterovirus; HRV/EV: human rhinovirus/enterovirus.

Orange line: proportion of HRV/EV-positive samples among respiratory samples; green line: proportion of EV-D68-positive samples among respiratory samples; blue line: proportion of EV-D68 among HRV/EV-positive samples; grey histogram: number of respiratory samples tested.

voluntary. Some of the virological data (available as of 1 December, 2014) were also included in a European-wide EV-D68 surveillance study [20].

Screening of respiratory samples for enterovirus D68

Each participating laboratory was requested to test all the respiratory tract specimens collected from 1 July to 31 December 2014 from children (<16 years of age) and adults (≥16 years of age) admitted to or visiting the emergency unit of hospitals or university hospitals. Respiratory tract samples were systematically tested for HRV/EV by the RT-PCR assays routinely used at each participating laboratory. EV or HRV/EV-positive samples were thereafter tested for EV-D68 either by a specific EV-D68 real-time RT-PCR assay [17] or by sequencing of the partial viral protein (VP)4–VP2 sequences [21]. The sensitivity of the HRV/EV and the EV-D68 assays was initially evaluated in each laboratory with a titrated aliquot of the Fermon strain provided by the Lyon NRL. Detection of HRV/EV and EV-D68 in samples was performed either in the participating laboratories, or at the NRLs. Besides HRV/EV screening, all other viral and bacteriological tests were performed according to the physicians' requests.

Molecular typing of enterovirus D68-positive samples and phylogenetic analyses

Complete VP1 sequences of EV-D68 strains were amplified using EV-D68-specific in-house primers and sequenced using the Sanger method. When a complete VP1 sequence could not be obtained, a partial VP1 or VP4–VP2 sequence was determined [21-23]. All the sequences were generated by the EV NRLs and deposited into the GenBank database under accession numbers KP196362–78, KP307989–92, KP406467–96, KT220441–6, KT220448–505, LN681318–38, and LN874222–53.

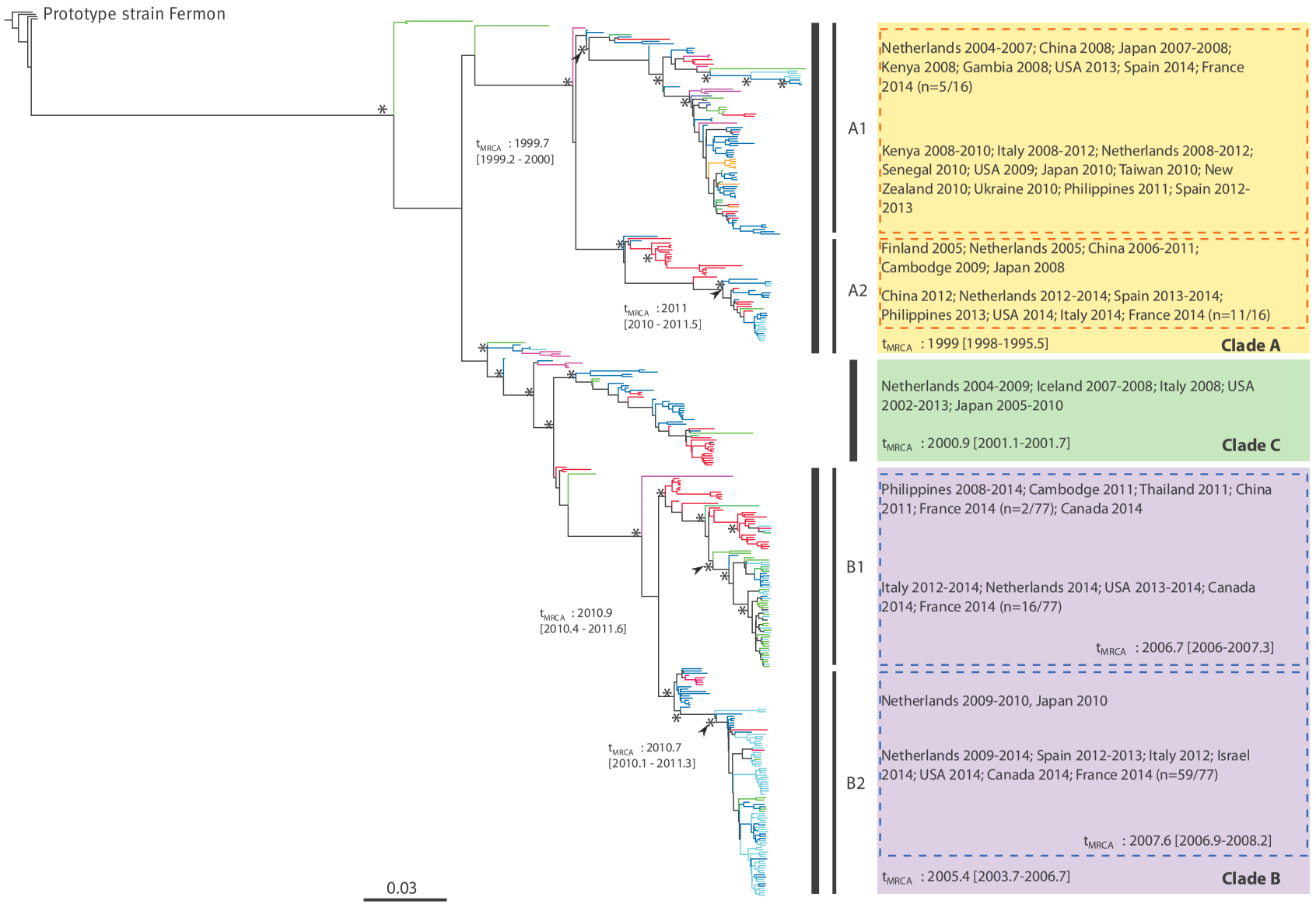
A nucleotide (nt) alignment (340 nt, n=391) including all the EV-D68 VP1 sequences available from GenBank (as of 4 June, 2015) and those determined in this study was compiled. Redundant sequences (sharing 100% nt homology) were discarded. Phylogenetic relationships between sequences were inferred using a Bayesian method implemented in the Bayesian Evolutionary Analysis Sampling Trees (BEAST) package (v1.7) (http://beast.bio.ed.ac.uk) [24]. The uncorrelated lognormal molecular clock was employed with a flexible Bayesian skyline plot coalescent prior (15 piece-wise constant groups) and the generalised time reversible (GTR) model of nt substitution. The Markov chains Monte Carlo (MCMC) were run for 200 million generations, with subsampling every 10,000 iterations. Maximum Clade Credibility trees were calculated with the TreeAnnotator programme (v1.5.4). Topological support was assessed by estimating the values of the posterior probability (pp) density of each node.

Patients and clinical characteristics

For each EV-D68-infected patient, a review of the medical chart was carried out retrospectively to document the following data: age and sex; symptoms including fever (≥38.5 °C), cough, rhinitis, pharyngitis, bronchitis

Figure 3

Phylogeny of partial viral protein 1 (VP1) coding sequences of enterovirus D68 (n=391 sequences)



The maximum credibility tree is inferred with the partial VP1 sequence (340nt, position 2,521–2,859 relative to the Fermon EV-D68 strain). The phylogenetic relationships were inferred with a Bayesian method using a relaxed molecular clock model. The tree was reconstructed using Figtree (v1.4.2). For clarity, the sequence names are not included in the tree (but are shown on Suppl. Figure 2). Asterisks indicate key nodes with posterior probability density values›0.90. Each tip branch represents a sampled virus sequence. Times of the most recent common ancestor (tMRCA) with the 95 % highest probability density (HPD) are indicated. The continents where the virus strains were sampled are indicated by different colours on the tree branches: Europe, blue; France, light blue; North America, green; Asia, red; Africa, Pink; Oceania, orange.

or bronchiolitis, acute respiratory distress, pneumonia, meningitis, polyradiculoneuritis; severity criteria [25,26] at admission such as need for intensive care and/or need for oxygen; length of hospitalisation including in intensive care unit (ICU); final diagnosis; presence or absence of underlying asthma or wheezing, prematurity, atopy, and chronic respiratory disease. Informed consent was not required for this surveillance study. A standardised Excel sheet including all the items was specifically designed for the present study and completed by each participating laboratory. The Lyon NRL compiled and analysed all the anonymised data.

Statistical analysis

Categorical variables with two or more than two levels (e.g. main diagnosis) were analysed using Fisher's exact test and G-test, respectively. The association between explanatory variables and severity was analysed using univariate logistic regression. Continuous variables (e.g. hospitalisation duration) were treated as binary variables and classified according to their median value. Statistical analysis was conducted using R software.

Results

Detection and distribution of enterovirus D68 cases

Eleven laboratories of the French EV network (including the Lyon and Clermont-Ferrand NRLs) participated in the EV-D68 enhanced surveillance. These laboratories were located in eight administrative regions (Table 1). Two of the laboratories analysed only specimens collected from patients under 16 years of age. Performances of the HRV/EV assays and the EV-D68 real-time RT-PCR were comparable among the participating laboratories, as tested on dilutions of a titrated EV-D68 Fermon strain (data not shown).

A total of 6,229 respiratory samples were systematically screened, including 3,645 from children and 2,584 from adults (Table 1). Among the respiratory samples collected from children, 1,501 (41.2%) were HRV/EV positive, of which 184 (12.3%) were positive for EV-D68. Among the respiratory samples collected from adults, 368 (14.2%) were HRV/EV positive, of which 28 (7.6%) were positive for EV-D68. The overall EV-D68 positivity rates among the respiratory samples tested were of 5.0% and of 1.1% in children and adults, respectively (Table 1). Overall the EV-D68-positive respiratory samples (n=212) corresponded to 200 EV-D68 cases including 178 children and 22 adults (Table 1).

While routinely genotyping EV-positive clinical samples that had been detected in laboratories not involved in the EV-D68 study, the NRLs identified nine additional cases (5 children and 4 adults) during the study period. Seven of these were hospitalised patients and two lived in an elderly nursing home. The nine cases were considered in the overall epidemiological analysis, which therefore comprised a total of 209 cases.

Overall, the first EV-D68 case was detected on 11 July 2014 (Figure 1; week 28). The majority (179/209; 85.6%) of the EV-D68 cases were detected from weeks 39 to 49 and two peaks could be observed, one in October (week 43) and one in November (week 48).

The samples of the nine cases, which were detected through routine analysis, were not taken into account to calculate positivity rates, which were based on the total of 212 systematically screened respiratory samples. At week 43, in children, the EV-D68-positive samples represented up to 16.1% (n=24/149) of the respiratory samples tested in that week and 26.7% (n=24/90) of the HRV/EV-positive-samples (Figure 2). At week 42, in adults, the EV-D68-positive samples represented up to 2.6% (n=3/115) of the respiratory samples tested and 10% (n=3/30) of the HRV/EV-positive samples (Figure 2). Circulation of the virus persisted until at least the end of December 2014.

EV-D68 infections were detected in all the regions covered by the participating laboratories, i.e. eight of the 22 French administrative regions (Suppl. Figure 1, available from: http://cnr-chu-clermontferrand.fr/CNR/Pages/Accueil/Publis.aspx).

Clinical characteristics of patients infected by enterovirus D68

EV-D68 infections were detected in both children and adults (Figure 1). Based on medical chart review and final diagnosis, a bacterium or a parasite was likely to be responsible for the symptoms of six children and five adults. The six paediatric patients presented with arthritis due to *Kingella kingae* (1 case); pyelonephritis due to *Escherichia coli* (1 case); gastroenteritis due to norovirus and conjunctivitis due to *Haemophilus influenzae* (1 case); sepsis due to *Streptococcus parasanguis* (1 case); febrile syndrome due to *Plasmodium falciparum* (1 case); meningitis-like syndrome due to *Haemophilus influenzae* (1 case). The five adult patients had either severe sepsis and acute respiratory distress syndrome (ARDS) due to *Pneumocystis carinii* or pneumopathy due to *Pneumocystis jirovecii* (2 cases), *Streptococcus pneumoniae* (1 case) or *Escherichia coli* (1 case). Detailed clinical characteristics of the 11 patients are available upon request. These patients were excluded from the 209 previously described patients, when considering the overall description of clinical characteristics, which thus comprised 198 patients, including 177 children and 21 adults (Table 2). The 11 EV-D68 co-infected patients were also not considered in the univariate analyses that were performed to determine if certain characteristics were associated with disease severity.

Paediatric patients

In the 177 children taken into account to investigate the clinical characteristics, EV-D68 was detected in all age

Table 1

Detection of human rhinovirus/enterovirus and enterovirus D68 through systematic screening of respiratory samples, France, July–December 2014 (n=6,229 respiratory samples)

Town of the laboratory, administrative region	Screening period	RT–PCR assay used for HRV/EV detection	Samples tested for HRV/EV n	HRV/ EV-positive samples n (%)	EV-D68-positive samples among HRV/ EV positive samples n (%)	EV-D68-positive samples among samples tested for HRV/EV n (%)	EV-D68-positive patients n
Paediatric patients (<16 years)							
Amiens, Picardie	1 Jul–31 Dec	Luminex xTAG RVP FAST	397	125 (31.5)	18 (14.4)	18 (4.5)	18
Brest, Bretagne	1 Jul–14 Dec	RespiFinder SMART 22 FAST v2	142	75 (52.8)	7 (9.3)	7 (4.9)	7
Caen, Normandie	1 Sep–31 Dec	RespiFinder SMART 22 FAST v2	614	353 (57.5)	50 (14.2)	50 (8.1)	48
Clermont-Ferrand, Auvergne	1 Jul–31 Dec	Rhino and EV/Cc r–gene	289	121 (41.9)	24 (19.8)	24 (8.3)	23
Dijon, Bourgogne	1 Jul–31 Dec	Rhino and EV/Cc r–gene	115	36 (31.3)	6 (16.7)	6 (5.2)	5
Lyon, Rhône-Alpes	1 Jul–31 Dec	Rhino and EV/Cc r–gene	1,060	349 (32.9)	35 (10.0)	35 (3.3)	33
Paris, Ile de France (Saint Louis)	1 Jul–7 Dec	RespiFinder SMART 22 FAST v2	77	35 (45.5)	0 (0.0)	0 (0.0)	0
Paris, Ile de France (Paul Brousse)	1 Jul–31 Dec	Rhino and EV/Cc r–gene	321	122 (38.0)	6 (4.9)	6 (1.9)	6
Saint–Etienne, Rhône-Alpes	10 Oct–31 Dec	Rhino and EV/Cc r–gene	204	80 (39.2)	14 (17.5)	14 (6.9)	14
Strasbourg, Alsace	19 Sep–31 Dec	Luminex xTAG RVP FAST	304	147 (48.4)	11 (7.5)	11 (3.6)	11
Versailles, Ile de France	1 Jul–31 Dec	Rhino and EV/Cc r–gene	122	58 (47.5)	13 (22.4)	13 (10.7)	13
Total	–	–	**3,645**	**1,501 (41.2)**	**184 (12.3)**	**184 (5.0)**	**178**
Adult patients (≥16 years)							
Amiens, Picardie	1 Jul–31 Dec	Luminex xTAG RVP FAST	216	36 (16.7)	7 (19.4)	7 (3.2)	4
Brest, Bretagne	1 Jul–14 Dec	RespiFinder SMART 22 FAST v2	130	29 (22.3)	4 (13.8)	4 (3.1)	4
Caen, Normandie	1 Sep–31 Dec	RespiFinder SMART 22 FAST v2	416	78 (18.8)	1 (1.3)	1 (0.2)	1
Clermont–Ferrand, Auvergne	1 Jul–31 Dec	Rhino and EV/Cc r–gene	367	54 (14.7)	4 (7.4)	4 (1.1)	3
Dijon, Bourgogne	1 Jul–31 Dec	Rhino and EV/Cc r–gene	214	25 (11.7)	1 (4.0)	1 (0.5)	1
Lyon, Rhône–Alpes	1 Sep–31 Dec	Rhino and EV/Cc r–gene	1,036	123 (11.9)	11 (8.9)	11 (1.1)	9
Paris, Ile de France (Paul Brousse)	1 Jul–31 Dec	Rhino and EV/Cc r–gene	40	7 (17.5)	0 (0.0)	0 (0.0)	0
Saint–Etienne, Rhône Alpes	10 Oct–31 Dec	Rhino and EV/Cc r–gene	41	1 (2.4)	0 (0.0)	0 (0.0)	0
Versailles, Ile de France	1 Jul–31 Dec	Rhino and EV/Cc r–gene	124	15 (12.1)	0 (0.0)	0 (0.0)	0
Total	–	–	**2,584**	**368 (14.2)**	**28 (7.6)**	**28 (1.1)**	**22**

EV: enterovirus; HRV/EV: human rhinovirus/enterovirus; RT-PCR: reverse-transcription polymerase chain reaction.

The study involved 11 voluntary laboratories of the 34 in the EV surveillance network (including two different virology laboratories from the Paris area). A total of 212 EV-D68-positive samples corresponding to 200 EV-D68 cases were detected by the systematic screening of respiratory tract samples collected from children (<16 years-old) and adults (≥16 years-old) admitted to or visiting emergency units of hospitals or university hospitals.

groups and the most affected age group was < 2 years (< 2 years: 76 patients, including ≤ 28 days: 6 patients; 2–5 years: 73 patients; 6–15 years: 28 patients). The median age of the patients was 2.33 years (range: 3 days–13.5 years). Information on hospitalisation was available for 174 patients. A total of 160/174 (92.0%) patients were hospitalised and 14/174 (8.0%) were outpatients (short stay at the emergency unit but no overnight hospitalisation). A final diagnosis was available for 173 (97.7%) patients and a total of 166/173 (96.0%) presented with acute respiratory infections. The main diagnoses were asthma (n = 83; 48.0%) and bronchiolitis (n = 37; 21.4%). Other diagnoses are summarised in Table 2. Among the children hospitalised for asthma (82/83; Table 2), 64 (78.0%) had a previous history of asthma or wheezing. In univariate analysis however, the history of asthma or wheezing as a determinant of severity or hospitalisation in ICU was not statistically significant (Table 3).

Four patients (2.3%) presented with neurological signs (Table 2). One four-year-old patient developed AFP following EV-D68 associated pneumonia; CSF showed pleocytosis with normal protein and glucose levels and spinal magnetic resonance imagery showed gadolinium enhancement of the ventral nerve roots of the cauda equine [19]. One patient aged 20 months developed meningitis-like symptoms. Two infants with underlying epilepsy developed severe seizures in a context of bronchiolitis or pneumonia. Three children presented with isolated neonatal fever, one with a severe sepsis syndrome and one with hypotonia. One EV-D68 infection was diagnosed in the context of a sudden infant death syndrome (SIDS) in a two-month-old girl; detection of EV-D68 in blood was negative and no other pathogen was detected.

Nineteen children (10.7%) were hospitalised in ICUs (median duration: 3 days; range: 1–137 days) (Table 2). Of these, two ex-premature babies with bronchopulmonary dysplasia were infected by EV-D68 while already in neonatal ICU and developed severe respiratory decompensation. Among the 17 remaining patients (see clinical presentation in Table 2), 15 had pre-existing chronic conditions (prematurity: 4; asthma/wheezing: 9; pulmonary vein atresia: 1, ventricular septal defect: 1, drepanocytosis: 1; epilepsy: 2) and two patients, who presented with pneumothorax (without asthma) or AFP, had no underlying disease. All but one patient hospitalised in ICU had favourable outcomes. The patient who developed AFP was extubated after 4.5 months in ICU, but still showed severe sequelae of right upper limb after 12 months. No death could be directly imputed to EV-D68.

Adult patients

The median age of the 21 adult patients was 36.7 years (range: 17.2–98.9 years). Fourteen were hospitalised and five were outpatients (2 patients not documented) (Table 2). A diagnosis and clinical signs were available for 17 patients. The diagnoses were as follows: asthma (n = 4; all with underlying history of asthma); pneumonia (n = 4), chronic obstructive pulmonary disease (COPD) exacerbation (n = 3; all with stage III COPD), upper respiratory tract infection (n = 2), bronchitis (n = 1), influenza-like illness (n = 1) and pneumothorax (n = 1). One patient was asymptomatic (allograft follow-up).

Three patients were hospitalised in ICU for two, three and six days, respectively; two of them presented with pneumonia: a 25 year-old patient who developed a severe respiratory distress without underlying risk factors during the week 29 of gestation and a 23 year-old patient with underlying Duchenne muscular dystrophy; the third patient presented with exacerbation of COPD. All the adult cases had favourable outcomes.

Enterovirus D68 sequencing and phylogenetic analysis

EV-D68 was tentatively sequenced in 207 of 209 patients. Among these 207, EV-D68 infection was confirmed in 201 patients either by VP1 sequencing (n = 179) or by VP4–VP2 sequencing (n = 22). In six patients, the virus could not be sequenced, probably because of the low viral load (cycle thresholds of EV-D68 real-time RT-PCRs were between 39.3 and 40.7). A total of 178/201 (88.6%) EV-D68 viruses belonged to clade B and 23/201 (11.4%) belonged to clade A [27]. Of the 159 clade B viruses for which the VP1 sequence was obtained, 42 (26.4%) and 117 (73.6%) were assigned to the sublineage B1 and B2, respectively (data not shown). Clade A and B viruses were identified throughout the screening period and the proportion of A and B viruses per week did not vary significantly (data not shown). Clade A viruses were detected more frequently in adults (10/23, 43.5%) than in children (13/178, 7.3%) ($p < 0.001$). Proportions of A and B viruses did not differ significantly between patients hospitalised in ICU and patients not hospitalised in ICU.

To investigate a large sample drawn from different geographical origins, a Bayesian analysis was performed on partial VP1 sequences, including those of 93 viruses from France and 298 viruses from other geographical regions (Figure 3 and Suppl. Figure 2, available from: http://cnr-chu-clermontferrand.fr/CNR/Pages/Accueil/Publis.aspx). The results suggested that all the recent EV-D68 strains formed one genogroup which could be further divided in two major lineages: the first corresponded to clade A lineage while the second included clades B and C [27]. This phylogenetic topology was confirmed by a Bayesian analysis on complete VP1 sequences (data not shown) and was concordant with the topology described by Lauinger et al. [11]. Sixteen French strains fell within the clade A and clustered in two highly supported lineages (posterior probability, pp > 0.97) designated A1 (n=5 strains) and A2 (n=11 strains). The French A1 viruses clustered with strains collected in 2013–2014 in the US, Spain and the Netherlands. The French A2 viruses clustered with viruses recovered between 2012 and 2014 from three

Table 2

Clinical characteristics of enterovirus D68 cases, France, July–December 2014 (n = 198 patients)[a]

Characteristic	Paediatric patients (<16 years)			Adult patients (≥16 years)		
	Patients N	Hospitalised patients N	Patients in ICU N	Patients N	Hospitalised patients N	Patients in ICU N
Sex-ratio (M/F)	1.39 (103/74)			0.62 (8/13)		
Median hospitalisation duration	4 days (range: 1–172 days) (n = 147)[b]			3 days (2–18) (n = 12)[b]		
Median hospitalisation duration in ICU	3 days (range: 1–132 days) (n = 17)[b]			3 days (2–6) (n = 3)		
Number of patients with oxygen therapy	78 (n = 171)[b,c]			6 (n = 17)[b,d]		
Number of patients with history of asthma/wheezing	85 (n = 168)[b]			4 (n = 10)[b]		
Respiratory presentation						
Asthma	83[e]	82[e]	8	4	4	0
Severe asthma	*24*[e]	*24*[e]	*8*	*1*	*1*	*0*
Bronchiolitis	37[e]	34[e]	4[f]	0	0	0
Severe bronchiolitis	*4*[e]	*4*[e]	*4*[f]	*0*	*0*	*0*
COPD exacerbation	0	0	0	3	3	1
Respiratory distress only	4	4	3	0	0	0
Pneumonia	11[e]	11[e]	1[f]	4	4	2
Upper respiratory tract infection	26	18	0	2	0	0
Other	7[g]	7	2	3[h]	2	0
Neurological presentation						
Acute flaccid paralysis	1	1	1	0	0	0
Seizures	2[e]	2[e]	2[f]	0	0	0
Other	1 (meningitis-like)	1	0	0	0	0
Other presentation						
Hypotonia	1	1	0	0	0	0
Neonate fever (≥38.5 °C)	3	2	0	0	0	0
Other	2[i]	1	0	0	0	0
Asymptomatic	0	0	0	1[j]	0	0
Not documented	4	1	0	4	1	0
Total	177	160	19	21	14	3

COPD: chronic obstructive pulmonary disease; EV: enterovirus; F: female; ICU: intensive care unit; M: male.

A given patient could have more than one clinical characteristic.

[a] We excluded six paediatric and five adult patients for whom the clinical signs were likely to be due to a bacterium or a parasite, from the total of 209 cases.

[b] The number of patients for whom the information was available is indicated in parentheses.

[c] Two ex-premature babies with bronchodysplasia were already under continuous oxygen therapy.

[d] Four patients with underlying COPD (n = 3) or Duchenne muscular dystrophy (n = 1) were already under continuous oxygen therapy.

[e] Three patients presented with asthma and pneumonia; one patient with bronchiolitis and seizures; one with pneumonia and seizures.

[f] One patient presented with bronchiolitis and seizures; one with pneumonia and seizures.

[g] Pneumothorax (n=1); acute thoracic syndrome (n=1); bronchitis (n=5).

[h] Influenza-like illness (n=1); pneumothorax (n=1); bronchitis (n=1).

[i] Infant sepsis (n=1); sudden infant death syndrome (n=1).

[j] Allograft follow-up.

different continents. The remaining 77 French strains belonged to two lineages designated B1 (pp = 0.94; n=18 strains) and B2 (pp = 1; n=59) within the clade B. The B1 lineage included most of the strains sampled in 2014 in the US and 18 French strains, while the B2 lineage was almost exclusively composed of strains recovered in Europe and comprised the majority of strains detected in France in 2014 (59/93, 63.4%). The AFP case was associated with a B2 strain [19]. The EV-D68 sequences detected in Europe between 2012 and 2014 were closely related to those from viruses detected in 2014 in Israel (n = 2), US (n = 4) and Canada (n = 1).

Discussion

From mid-August 2014 until the end of December, EV-D68 caused a geographically widespread outbreak of respiratory disease of unprecedented magnitude in the US, leading to substantial hospitalisation for

Table 3

Univariate analysis of potential factors for severe disease in children infected with enterovirus D68, France, July–December 2014 (n=177)

Characteristic	Severity[a]				ICU admission				Oxygen therapy				Hospitalisation duration[b]			
	No	Yes	OR (95% CI)	P	No	Yes	OR (95% CI)	P	No	Yes	OR (95% CI)	p	≤4 days	>4 days	OR (95% CI)	P
Sex																
Male	75[a]	23[a]	0.90 (0.44–1.85)	0.7781	90	12	1.22 (0.46–3.43)	0.6937	54	45	0.98 (0.54–1.82)	0.9608	55	30	1.06 (0.54–2.14)	0.8579
Female	53[a]	18[a]			64	7			39	33			41	21		
Prematurity																
Yes	18	6	0.99 (0.34–2.57)	0.9850	20	4	1.72 (0.46–5.32)	0.3755	15	9	0.65 (0.26–1.57)	0.3476	8	8	2.07 (0.72–6.02)	0.1729
No	104	35			129	15			74	68			85	41		
History of asthma or wheezing																
Yes	59	22	1.30 (0.64–2.65)	0.4733	76	8	0.72 (0.26–1.87)	0.5005	32	51	3.48 (1.86–6.65)	**0.0001**	50	26	0.98 (0.49–1.94)	0.9423
No	66	19			75	11			59	27			45	24		
History of atopy																
Yes	18	8	1.49 (0.57–3.67)	0.3970	27	2	0.60 (0.09–2.31)	0.5171	13	16	1.59 (0.71–3.6)	0.2630	21	8	0.65 (0.25–1.55)	0.3494
No	104	31			122	15			76	59			70	41		
History of chronic respiratory insufficiency																
Yes	3	4	4.29 (0.91–22.6)	0.0642	5	2	3.39 (0.46–17.12)	0.1632	4	3	0.86 (0.17–4.03)	0.8484	0	3	NA (NA)	NA
No	119	37			144	17			85	74			92	47		
EV-D68 clade																
A	9	1	3.03 (0.54–56.72)	0.3009	10	1	1.32 (0.23–25)	0.7949	5	6	0.71 (0.2–2.46)	0.5870	8	1	4.38 (0.77–82.55)	0.1699
B	113	38			136	18			82	70			84	46		
Age																
<2 years	59	15	Ref	Ref	64	10	Ref	Ref	40	33	Ref	Ref	35	28	Ref	Ref
2–5 years	44	15	1.34 (0.59–3.05)	0.4806	56	5	0.57 (0.17–1.71)	0.3325	30	31	1.25 (0.63–2.48)	0.5173	41	13	0.4 (0.17–0.87)	**0.0230**
>5 years	25	11	1.73 (0.69–4.29)	0.2363	34	4	0.75 (0.19–2.44)	0.6516	23	14	0.74 (0.32–1.64)	0.4611	20	10	0.63 (0.25–1.53)	0.3100

CI: confidence interval; EV: enterovirus; ICU: intensive care unit; NA: not applicable because of the small number of reports; OR: odds ratio; P: p-value; Ref: reference.

[a] Severity criteria were defined as elsewhere [25,26] and included the need for intensive care and need for oxygen. Severity criteria were only known for 169 cases.

[b] Dichotomised according to median value. Median hospitalisation time (for inpatients) was four days.

severe respiratory disease. In the context of the US alert, a systematic screening of EV-D68 was performed by 11 voluntary hospital laboratories of the French EV surveillance network on 6,229 respiratory samples collected between 1 July and 31 December 2014.

This report concerns the largest number of EV-D68 cases ever documented for France. Due to the implementation of systematic screening of EV-D68, a total of 200 EV-D68 infections were diagnosed and EV-D68 was detected in all the administrative regions from where the participating laboratories were involved (i.e. 8 of the 22 administrative regions), suggesting that EV-D68 might have circulated even more widely throughout the country. Previously, two small clusters of cases had been reported in 2008 (19 cases; Oct–Nov; Basse-Normandie region) and 2009 (10 cases; Sep–Nov; Champagne-Ardennes region), respectively [8,9] and only 66 EV-D68 cases were reported to the National Institute for Public Health between 2006 and 2013. However, during the 2007 to 2013 period, EV-D68 infections were probably underestimated, because HRV/EV screening in ARI was restricted to a limited number of laboratories (particularly before 2010), genotyping of HRV/EV-positive samples was rarely performed and the specific detection of EV-D68 by real-time RT-PCR was unavailable. On the other hand, no EV-D68 case was detected by systematic screening of respiratory samples collected in Lyon from September until December 2013 (data not shown), whereas 42 cases were identified between July and December 2014. This suggests that the circulation level of EV-D68 was higher in 2014 than in 2013, at least in the Lyon area and possibly elsewhere in France. In this respect, surveillance studies in

the Philippines [28], Italy [10] and the Netherlands [7] showed that EV-D68 may follow a cyclic pattern of circulation with a two-year interval.

The overall EV-D68 detection rate that we observed in a hospital-based setting between July and December 2014 in France (3.4%; maximum 8.4% on week 43) was similar to that observed in a European-wide survey (2.1% [20]) conducted on 17,384 respiratory samples from 17 countries collected mainly from hospitalised patients between July and November 2014 – and in which the virological results for 117 French patients, available as of 1 December, 2014, were included. It was much lower than that reported by the CDC during the August to December period (36% of 2,600 respiratory samples) (http://www.cdc.gov/non-polio-enterovirus/about/EV-D68.html). However, the proportion reported by the CDC was calculated mainly from severe cases, which may hamper comparisons. Comparison between findings in France and the US may also be hampered by increased public/physician awareness and more active case finding in the US.

At the time of the US alert, and despite existing surveillance systems for respiratory tract illness (RTI) or influenza-like illness [29,30], no upsurge of the number of hospitalisations for RTI, or of the number of HRV/EV positive respiratory samples, was reported in France. This suggests that the impact of the circulation of EV-D68 on public health was more limited in France and Europe than in the US and may explain why only rare alerts were reported in Europe [15-17].

Our longitudinal study provided a comprehensive description of the epidemiological and clinical characteristics of EV-D68 infections in hospitalised patients during the entire study period. Most cases (87.5%) were detected in children, as observed in the US [14]. The EV-68 detection rate in respiratory samples from children was of 9.7% (n = 100/1,035) in the September to October period and was similar to that observed at the same period in hospitalised children from the Oslo area [16]. Most children (93%) with an EV-D68 infection presented with respiratory symptoms, mainly asthma and bronchiolitis, as described in hospitalised patients in the 2014 US outbreak, an outbreak in Canada in the same year, and in previous reports [6,8,13,14,31]. EV-D68 could also be associated with respiratory distress without underlying asthma or bronchiolitis, especially in ex-premature babies with bronchopulmonary dysplasia. Among the children who were hospitalised for asthma, 78% had a history of asthma or wheezing, consistent with US reports. In our study, underlying asthma or wheezing was not identified as a risk factor for developing more severe asthma or being hospitalised in ICU, however statistical power may have been limited by the sample size.

Viral factors may also contribute to the disease. Even though identical VP1 sequences were detected in both mild and severe RTI cases, full length analysis of viral genomes is warranted to determine whether specific mutations in coding or non-coding regions influence severity, as observed for poliovirus or EV-A71 [32,33].

Neurological signs were observed in four patients. Only one AFP case was reported during this survey [19] and no increase in AFP cases was reported to the public health authorities during the EV-D68 circulation period. For the three remaining cases of patients with meningitis-like symptoms or with seizures, although such disease manifestations have not been previously described with EV-D68, they are frequently associated with EV infections particularly in young children. However, we cannot rule out the possibility that other viral or bacterial infection could have contributed to these neurological signs. Of note, in 2014, no EV-D68 was detected in 1,197 CSF specimens genotyped throughout the EV national surveillance. So, apart from the AFP-associated case, the spectrum of illnesses associated with EV-D68 was similar to that of rhinoviruses, as previously reported [1,3-10,13,14,16,17,19,31]. Although no significant increase in severe respiratory disease was reported to the French national public health authorities in autumn 2014, the present study showed that EV-D68 did have a clear clinical impact, with 10.7% of the paediatric cases and 14.3% of the adult cases being hospitalised in ICUs. Moreover, its implication in nosocomial infections should be considered [17,34]. This highlights the need for clinical laboratories to take EV-D68 in account in the differential diagnosis of patients with severe respiratory symptoms, including in adult patients.

EV-D68 infections in France in 2014 were mainly associated with the B2 variant, as in other European countries [20]. However, it was not possible to determine whether the B2 variant was circulating in France before 2014 because the molecular characterisation of EV/HRV-positive respiratory samples is not routinely performed, as exemplified by our finding of only one French EV-D68 VP1 sequence in GenBank from prior to 2014 (sequence from genogroup C; 1999). In the Netherlands, virus surveillance between 2004 and 2014 provided evidence of the successive replacement of the major lineage by another lineage in each period of increased virus reporting. While clade C predominated until 2008, an outbreak in 2010 was mainly associated with the circulation of clade A strains [7]. The B2 viruses also circulated in 2010 but to a lesser extent, [6,7] and became predominant in 2014 [17]. This type of circulation pattern – the replacement of an earlier variant during periods of low virus incidence – is reminiscent of that observed for EV-A71 [35,36]. The succession of predominant lineages could be driven by the immunity of the general population. In this respect, Imamura et al. [37] showed that there were antigenic differences between the recent lineages of EV-D68 circulating strains. Finally, the different lineages were present simultaneously over several countries and continents. The close genetic relatedness between EV-D68 strains sampled from distant countries suggests that

this virus is subject to frequent geographical turnover. Further studies based on larger samples of complete VP1 sequences are needed to investigate the dynamics of EV-D68 geographical transportation between countries and over continents.

This study comprised some limitations. The screening was not population-based as it depended on the voluntary participation of only about one-third of EV network laboratories in France. We also lacked historical EV-D68 screening data at a national level for comparison, and the sample size was limited in terms of the statistical power support in univariate analyses. Moreover, we cannot exclude that respiratory samples may have been collected for viral screening more frequently from children than from adults and that EV-D68 positivity rate may have been underestimated in adults. Our data were however likely not biased towards more severe infections as they were based on testing results of respiratory samples collected for routine viral screening of respiratory infections.

The autumn of 2014 was marked by increased EV-D68 detection in many parts of the world [12-17,31], associated, at least in parts of the US and Canada, with a significant upsurge of severe respiratory infections, sometimes followed by neurological signs. A similar outbreak may possibly also occur in Europe in the future, and the results of our study show that in France, a number of EV-D68 infections had a clinical impact. This justifies the need for continuous surveillance of EV-D68 infections in Europe. The surveillance could rely on existing and effective surveillance programmes such as the influenza and influenza-like illness surveillance systems, the EV surveillance networks and the surveillance of AFP cases. The increasing awareness of HRV/EV as major respiratory pathogens and the development of commercial molecular assays for these viruses has allowed the implementation of HRV/EV diagnosis in an increasing number of virology laboratories [33,38]. Moreover, virus characterisation should be encouraged, at least in the event of severe respiratory signs.

Acknowledgements

We would like to thank Delphine Falcon, Katy Pinet, Chantal Gousse for EV-D68 screening and typing. We are grateful to Nathalie Rodde, Gwendoline Jugie, Emilie Leroy for excellent technical assistance in HRV/EV genotyping. We acknowledge Dr Mélanie Ribault, Dr Matthieu Verdan et Pr André Labbé, Dr Jean-Sébastien Casalegno, Dr Christine Raybaud, Dr Claire Gay, Dr Emmanuelle Laurent, Dr Lucie Molet for collecting clinical data for the EV-D68 cases diagnosed through a systematic approach. We acknowledge Pantxica Bellecave, Marianne Coste-Burel, Gisèle Lagathu and Pierrette Dhont for transmitting clinical data for the EV-D68 cases diagnosed through a non-screening strategy. We are grateful to Lynn Richardson for revision of the English.

Conflict of interest

None declared.

Authors' contributions

IS designed the study and coordinated the laboratory network involved in this study, together with AM. IS, AM, DH, LP, JPL, CM, JL, CD, SP, QL, JMM and SMJ provided respiratory samples and collected epidemiological and clinical data. IS compiled and analysed the clinical data. LJ performed the statistical analyses. AM performed the phylogenetic analyses. IS, AM and LJ wrote the first draft of the paper. All the authors, including BL, CH, DA and HPL, reviewed the manuscript critically.

References

1. Imamura T, Oshitani H. Global reemergence of enterovirus D68 as an important pathogen for acute respiratory infections.Rev Med Virol. 2015;25(2):102-14. DOI: 10.1002/rmv.1820 PMID: 25471236
2. Khetsuriani N, Lamonte-Fowlkes A, Oberst S, Pallansch MA, Centers for Disease Control and Prevention. Enterovirus surveillance--United States, 1970-2005.MMWR Surveill Summ. 2006;55(8):1-20.PMID: 16971890
3. Ikeda T, Mizuta K, Abiko C, Aoki Y, Itagaki T, Katsushima F, et al. Acute respiratory infections due to enterovirus 68 in Yamagata, Japan between 2005 and 2010. Microbiol Immunol. 2012;56(2):139-43. DOI: 10.1111/j.1348-0421.2012.00411.x PMID: 22309616
4. Imamura T, Fuji N, Suzuki A, Tamaki R, Saito M, Aniceto R, et al. Enterovirus 68 among children with severe acute respiratory infection, the Philippines. Emerg Infect Dis. 2011;17(8):1430-5. DOI: 10.3201/eid1708.101328 PMID: 21801620
5. Tokarz R, Kapoor V, Wu W, Lurio J, Jain K, Mostashari F, et al. Longitudinal molecular microbial analysis of influenza-like illness in New York City, May 2009 through May 2010. Virol J. 2011;8(1):288. DOI: 10.1186/1743-422X-8-288 PMID: 21658237
6. Rahamat-Langendoen J, Riezebos-Brilman A, Borger R, van der Heide R, Brandenburg A, Schölvinck E, et al. Upsurge of human enterovirus 68 infections in patients with severe respiratory tract infections. J Clin Virol. 2011;52(2):103-6. DOI: 10.1016/j.jcv.2011.06.019 PMID: 21802981
7. Meijer A, van der Sanden S, Snijders BE, Jaramillo-Gutierrez G, Bont L, van der Ent CK, et al. Emergence and epidemic occurrence of enterovirus 68 respiratory infections in The Netherlands in 2010. Virology. 2012;423(1):49-57. DOI: 10.1016/j.virol.2011.11.021 PMID: 22177700
8. Petitjean-Lecherbonnier J, Dina J, Nguyen E, Gouarin S, Lebigot E, Vabret A. [Molecular diagnosis of respiratory enterovirus infections: Use of PCR and molecular identification for a best approach of the main circulating strains during 2008]. Pathol Biol (Paris). 2011;59(2):113-21. DOI: 10.1016/j.patbio.2010.07.010 PMID: 20828940
9. Renois F, Bouin A, Andreoletti L. Enterovirus 68 in pediatric patients hospitalized for acute airway diseases.J Clin Microbiol. 2013;51(2):640-3. DOI: 10.1128/JCM.02640-12 PMID: 23224095
10. Piralla A, Girello A, Grignani M, Gozalo-Margüello M, Marchi A, Marseglia G, et al. Phylogenetic characterization of enterovirus 68 strains in patients with respiratory syndromes in Italy. J Med Virol. 2014;86(9):1590-3. DOI: 10.1002/jmv.23821 PMID: 24155220
11. Lauinger IL, Bible JM, Halligan EP, Aarons EJ, MacMahon E, Tong CYW. Lineages, sub-lineages and variants of enterovirus 68 in recent outbreaks.PLoS One. 2012;7(4):e36005. DOI: 10.1371/journal.pone.0036005 PMID: 22536453
12. Greninger AL, Naccache SN, Messacar K, Clayton A, Yu G, Somasekar S, et al. A novel outbreak enterovirus D68 strain associated with acute flaccid myelitis cases in the USA (2012-14): a retrospective cohort study. Lancet Infect Dis. 2015;15(6):671-82. DOI: 10.1016/S1473-3099(15)70093-9 PMID: 25837569
13. Midgley CM, Jackson MA, Selvarangan R, Turabelidze G, Obringer E, Johnson D, et al. Severe respiratory illness associated with enterovirus D68 - Missouri and Illinois, 2014. MMWR Morb Mortal Wkly Rep. 2014;63(36):798-9.PMID: 25211545
14. Oermann CM, Schuster JE, Conners GP, Newland JG, Selvarangan R, Jackson MA. Enterovirus d68. A focused review and clinical highlights from the US outbreak.Ann Am Thorac Soc. 2015;12(5):775-81. DOI: 10.1513/AnnalsATS.201412-592FR PMID: 25714788

15. European Centre for Disease Prevention and Control (ECDC). Enterovirus 68 detected in the USA, Canada and Europe – Second update 24 November 2014. Stockholm: ECDC; 2014
16. Bragstad K, Jakobsen K, Rojahn AE, Skram MK, Vainio K, Holberg-Petersen M, et al. High frequency of enterovirus D68 in children hospitalised with respiratory illness in Norway, autumn 2014. Influenza Other Respi Viruses. 2015;9(2):59-63. DOI: 10.1111/irv.12300 PMID: 25534826
17. Poelman R, Schölvinck EH, Borger R, Niesters HGM, van Leer-Buter C. The emergence of enterovirus D68 in a Dutch University Medical Center and the necessity for routinely screening for respiratory viruses.J Clin Virol. 2015;62:1-5. DOI: 10.1016/j.jcv.2014.11.011 PMID: 25542461
18. Antona D, Lévêque N, Chomel JJ, Dubrou S, Lévy-Bruhl D, Lina B. Surveillance of enteroviruses in France, 2000-2004.Eur J Clin Microbiol Infect Dis. 2007;26(6):403-12. DOI: 10.1007/s10096-007-0306-4 PMID: 17534678
19. Lang M, Mirand A, Savy N, Henquell C, Maridet S, Perignon R, et al. Acute flaccid paralysis following enterovirus D68 associated pneumonia, France, 2014. Euro Surveill. 2014;19(44):20952. DOI: 10.2807/1560-7917.ES2014.19.44.20952 PMID: 25394254
20. Poelman R, Schuffenecker I, Van Leer-Buter C, Josset L, Niesters HGM, Lina B, ESCV-ECDC EV-D68 study group. European surveillance for enterovirus D68 during the emerging North-American outbreak in 2014.J Clin Virol. 2015;71:1-9. DOI: 10.1016/j.jcv.2015.07.296 PMID: 26364237
21. Savolainen C, Mulders MN, Hovi T. Phylogenetic analysis of rhinovirus isolates collected during successive epidemic seasons.Virus Res. 2002;85(1):41-6. DOI: 10.1016/S0168-1702(02)00016-3 PMID: 11955637
22. Nix WA, Oberste MS, Pallansch MA. Sensitive, seminested PCR amplification of VP1 sequences for direct identification of all enterovirus serotypes from original clinical specimens.J Clin Microbiol. 2006;44(8):2698-704. DOI: 10.1128/JCM.00542-06 PMID: 16891480
23. Linsuwanon P, Payungporn S, Samransamruajkit R, Posuwan N, Makkoch J, Theanboonlers A, et al. High prevalence of human rhinovirus C infection in Thai children with acute lower respiratory tract disease. J Infect. 2009;59(2):115-21. DOI: 10.1016/j.jinf.2009.05.009 PMID: 19556008
24. Drummond AJ, Rambaut A. BEAST: Bayesian evolutionary analysis by sampling trees.BMC Evol Biol. 2007;7(1):214. DOI: 10.1186/1471-2148-7-214 PMID: 17996036
25. Refabert L. Management of bronchiolitis. Proceedings of the French consensus conference; 2000 Sep 21; Paris, France. French. Available from http://www.has-sante.fr/portail/upload/docs/application/pdf/bronchio.pdf
26. Marguet C, Groupe de Recherche Sur Les Avancées En PneumoPédiatrie. [Management of exacerbated asthma in children].Rev Mal Respir. 2007;24:427-39. DOI: 10.1016/S0761-8425(07)91567-3 PMID: 17468701
27. Tokarz R, Firth C, Madhi SA, Howie SRC, Wu W, Sall AA, et al. Worldwide emergence of multiple clades of enterovirus 68. J Gen Virol. 2012;93(Pt 9):1952-8. DOI: 10.1099/vir.0.043935-0 PMID: 22694903
28. Imamura T, Suzuki A, Lupisan S, Okamoto M, Aniceto R, Egos RJ, et al. Molecular evolution of enterovirus 68 detected in the Philippines. PLoS One. 2013;8(9):e74221. DOI: 10.1371/journal.pone.0074221 PMID: 24073203
29. Mosnier A, Caini S, Daviaud I, Bensoussan JL, Stoll-Keller F, Bui TT, et al. , GROG network. Ten influenza seasons in France: distribution and timing of influenza A and B circulation, 2003-2013.BMC Infect Dis. 2015;15(1):357. DOI: 10.1186/s12879-015-1056-z PMID: 26289794
30. Casalegno JS, Ottmann M, Bouscambert-Duchamp M, Valette M, Morfin F, Lina B. Impact of the 2009 influenza A(H1N1) pandemic wave on the pattern of hibernal respiratory virus epidemics, France, 2009.Euro Surveill. 2010;15(6):19485.PMID: 20158981
31. Drews SJ, Simmonds K, Usman HR, Yee K, Fathima S, Tipples G, et al. Characterization of enterovirus activity, including that of enterovirus D68, in pediatric patients in Alberta, Canada, in 2014. J Clin Microbiol. 2015;53(3):1042-5. DOI: 10.1128/JCM.02982-14 PMID: 25588657
32. Cordey S, Petty TJ, Schibler M, Martinez Y, Gerlach D, van Belle S, et al. Identification of site-specific adaptations conferring increased neural cell tropism during human enterovirus 71 infection. PLoS Pathog. 2012;8(7):e1002826. DOI: 10.1371/journal.ppat.1002826 PMID: 22910880
33. Vignuzzi M, Stone JK, Arnold JJ, Cameron CE, Andino R. Quasispecies diversity determines pathogenesis through cooperative interactions in a viral population.Nature. 2006;439(7074):344-8. DOI: 10.1038/nature04388 PMID: 16327776
34. Bal A, Schuffenecker I, Casalegno JS, Josset L, Valette M, Armand N, et al. Enterovirus D68 nosocomial outbreak in elderly people, France, 2014. Clin Microbiol Infect. 2015;21(8):e61-2. DOI: 10.1016/j.cmi.2015.05.008 PMID: 25986875
35. Huang S-W, Hsu Y-W, Smith DJ, Kiang D, Tsai H-P, Lin K-H, et al. Reemergence of enterovirus 71 in 2008 in taiwan: dynamics of genetic and antigenic evolution from 1998 to 2008. J Clin Microbiol. 2009;47(11):3653-62. DOI: 10.1128/JCM.00630-09 PMID: 19776232
36. Hassel C, Mirand A, Lukashev A, TerletskaiaLadwig E, Farkas A, Schuffenecker I, et al. Transmission patterns of human enterovirus 71 to, from and among European countries, 2003 to 2013. Euro Surveill. 2015;20(34):30005. DOI: 10.2807/1560-7917.ES.2015.20.34.30005 PMID: 26530407
37. Imamura T, Okamoto M, Nakakita S, Suzuki A, Saito M, Tamaki R, et al. Antigenic and receptor binding properties of enterovirus 68. J Virol. 2014;88(5):2374-84. DOI: 10.1128/JVI.03070-13 PMID: 24371050
38. Jacobs SE, Lamson DM, St George K, Walsh TJ. Human rhinoviruses.Clin Microbiol Rev. 2013;26(1):135-62. DOI: 10.1128/CMR.00077-12 PMID: 23297263

License and copyright

Detection of enterovirus D68 in patients hospitalised in three tertiary university hospitals in Germany, 2013 to 2014

S Böttcher [1], C Prifert [2], B Weißbrich [2], O Adams [3], S Aldabbagh [4], AM Eis-Hübinger [4], S Diedrich [1]
1. National Reference Centre for Poliomyelitis and Enteroviruses, Robert Koch-Institute, Germany
2. Institute of Virology and Immunobiology, University of Würzburg, Germany
3. Institute of Virology, University Hospital of Düsseldorf, Germany
4. Institute of Virology, University of Bonn Medical Centre, Germany

Correspondence: Sabine Diedrich (diedrichs@rki.de)

Citation style for this article:
Böttcher S, Prifert C, Weißbrich B, Adams O, Aldabbagh S, Eis-Hübinger AM, Diedrich S. Detection of enterovirus D68 in patients hospitalised in three tertiary university hospitals in Germany, 2013 to 2014. Euro Surveill. 2016;21(19):pii=30227. DOI: http://dx.doi.org/10.2807/1560-7917.ES.2016.21.19.30227

Article submitted on 22 May 2015 / accepted on 09 March 2016 / published on 12 May 2016

Enterovirus D68 (EV-D68) has been recognised as a worldwide emerging pathogen associated with severe respiratory symptoms since 2009. We here report EV-D68 detection in hospitalised patients with acute respiratory infection admitted to three tertiary hospitals in Germany between January 2013 and December 2014. From a total of 14,838 respiratory samples obtained during the study period, 246 (1.7%) tested enterovirus-positive and, among these, 39 (15.9%) were identified as EV-D68. Infection was observed in children and teenagers (0–19 years; n=31), the majority (n=22) being under five years-old, as well as in adults › 50 years of age (n=8). No significant difference in prevalence was observed between the 2013 and 2014 seasons. Phylogenetic analyses based on viral protein 1 (VP1) sequences showed co-circulation of different EV-D68 lineages in Germany. Sequence data encompassing the entire capsid region of the genome were analysed to gain information on amino acid changes possibly relevant for immunogenicity and revealed mutations in two recently described pleconaril binding sites.

Introduction

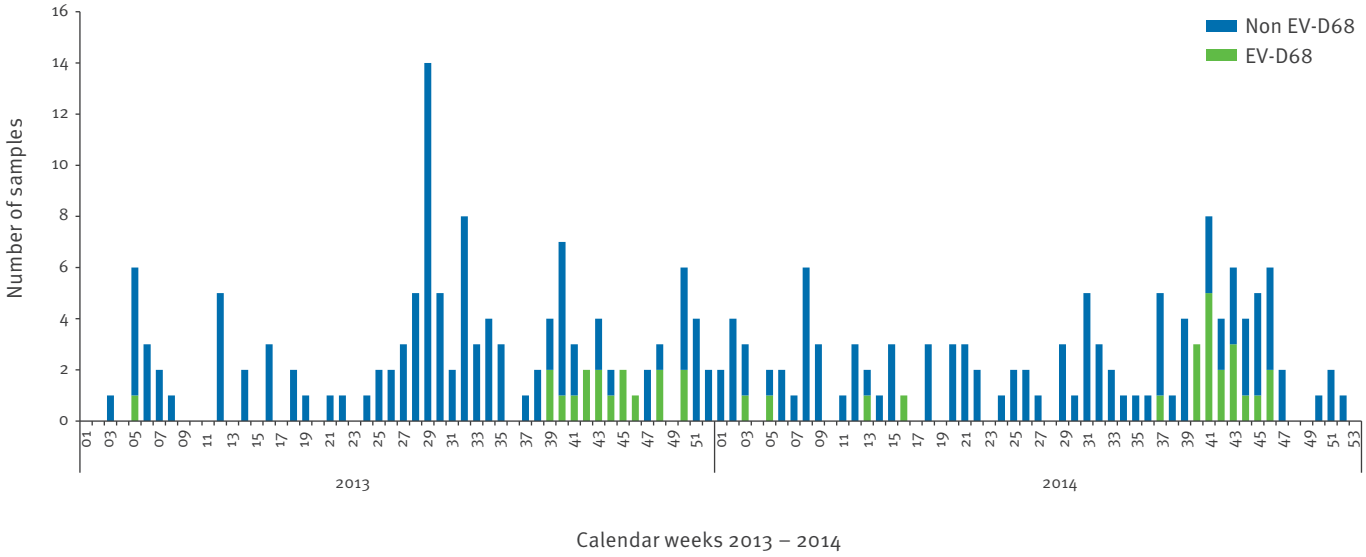
Within the *picornaviridae* family the genus Enterovirus is known to include more than 120 human enterovirus (EV) serotypes, causing a broad range of symptoms mainly in children below the age of five years. The major clinically relevant manifestations of non-polio enteroviruses (NPEV) include meningitis/encephalitis or acute flaccid paralyses (AFP), atypical hand, foot and mouth-disease or myocarditis. Some serotypes have been identified to be predominantly associated with respiratory diseases. Of those, EV-D68 has, since its first description in 1962, been detected sporadically worldwide until 2009 [1]. Subsequently, several epidemic clusters of EV-D68 associated with increases of respiratory illnesses have been reported, [1,2]. The largest outbreak so far was reported in autumn 2014 from the United States (US) with more than 1,100 EV-D68 detections in children hospitalised with acute severe respiratory infections [1,3].

In Germany, surveillance of respiratory virus infections is conducted mainly with regards to influenza representing a vaccine preventable disease, and is based on sentinel surveillance systems including outpatients with influenza like illness (ILI) and/or acute respiratory infection (ARI) (AGI Influenza RKI [4]; ARE NLGA [5]). Furthermore, a laboratory network reporting detection of respiratory viruses in hospitalised patients was established in 2009 (RespVir [6]). Besides influenza, pathogens recorded within these systems include respiratory syncytial virus (RSV), human metapneumovirus (HMPV), parainfluenza viruses (HPIV), coronaviruses (HCoV), adenoviruses (HAdV), rhinoviruses (HRV), and EV. Since the latter viruses are not routinely differentiated, no valid data on EV circulation including EV-D68 in Germany are available.

The aim of the study was to investigate the prevalence of EV-D68 in Germany by analysing EV-positive respiratory tract samples collected from patients admitted to three German university hospitals in two consecutive years. Furthermore, nucleotide (nt) sequence analysis of the complete viral protein 1 (VP1) region was performed for comparison of EV-D68 strains circulating in Germany with recent published strains from other countries. Complete capsid sequences from selected strains based on phylogenetic analysis were obtained to provide more data for better understanding of any changes in antigenicity.

Figure 1

Number of enterovirus (EV)-positive samples obtained by three university laboratoriesa stratified as EV-D68 and non-EV-D68, by week, Germany, 2013–2014 (n=246)



[a] The laboratories were the Institute of Virology and Immunobiology, University of Würzburg, the Institute of Virology, University Hospital of Düsseldorf and the Institute of Virology, University of Bonn Medical Centre.

Methods

Setting

Three German university laboratories provided data and samples collected from January 2013 through December 2014 to this study: the Institute of Virology and Immunobiology, University of Würzburg (laboratory 1), the Institute of Virology, University Hospital of Düsseldorf (laboratory 2) and the Institute of Virology, University of Bonn Medical Centre (laboratory 3).

Sample collection

Respiratory samples (e.g. nasopharyngeal swabs, bronchial lavages) were collected from patients with respiratory diseases admitted to the affiliated tertiary hospitals. The samples were routinely screened for a broad panel of respiratory pathogens including EV/HRV and other respiratory viruses (influenza A and B, RSV, HMPV, HPIV 1–4, HCoV 229, NL63, HKU1, OC43, HAdV, parechoviruses, bocavirus) according to the individual laboratory protocols. All samples positive for EV or EV/HRV were included in this study. These EV samples represent about one fourth of the overall number of EV positive samples detected in the nationwide RespVir surveillance [6].

The diagnostic procedures for the detection of respiratory viruses of the three university laboratories are as follows: laboratory 1: FTD 'Respiratory Pathogens 21' (Fast track Diagnostics, Luxembourg), laboratory 2: Bonzel et al., 2008 [7], laboratory 3: Dierssen et al., 2008 [8] and Poelman et al., 2014 [9]. All methods have been proven to detect EV-D68 in national and international proficiency tests.

Polymerase chain reaction amplification of enterovirus D68 viral protein 1 region

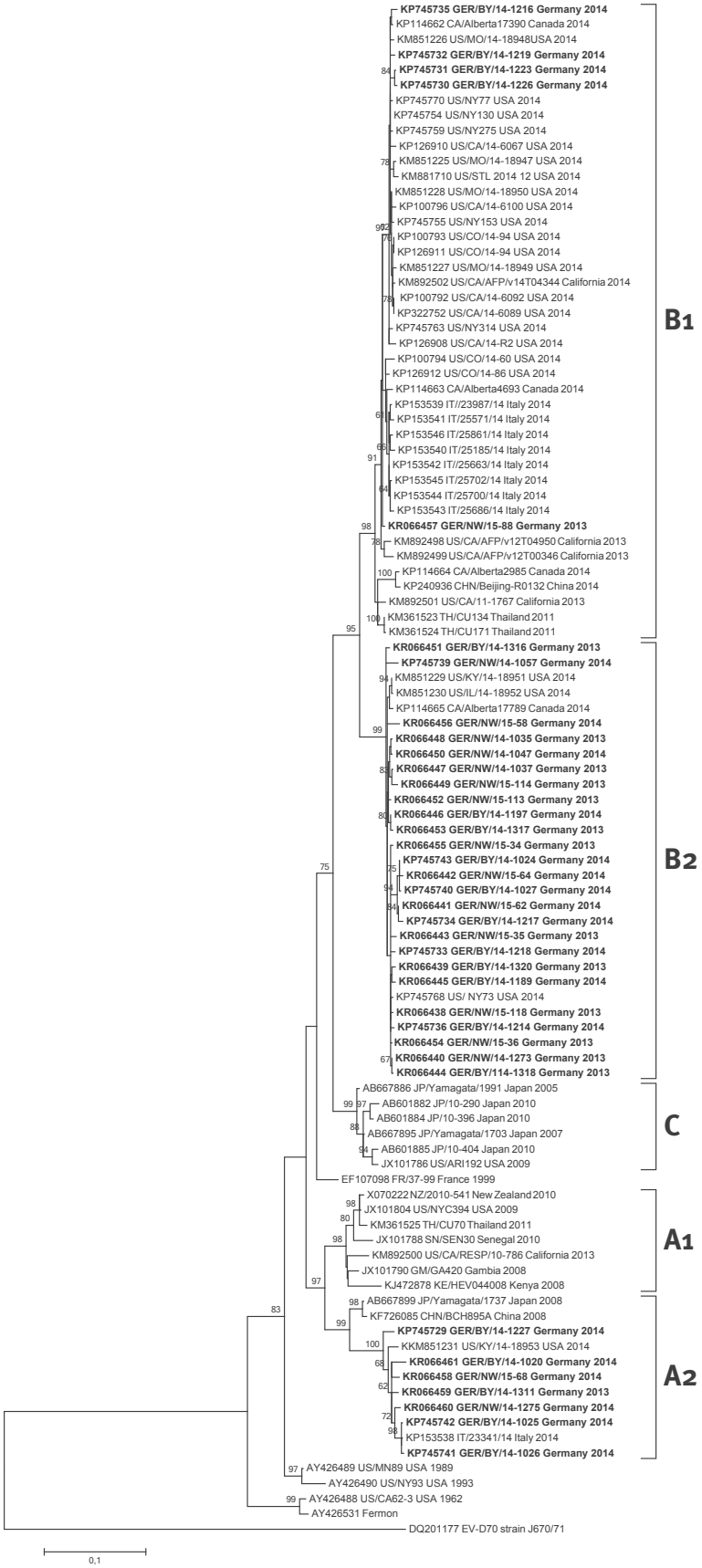
For highly sensitive amplification of the complete VP1 region of EV-D68 strains directly from clinical material a specific one-step reverse-transcription polymerase chain reaction (RT-PCR) assay was established at the German National Reference Centre for Poliomyelitis and Enteroviruses (NRZ PE). Amplification was performed using One-Step-RT-PCR Kit (Qiagen, Hilden, Germany) followed by a nested PCR using HotStarTaq-Mastermix (Qiagen, Hilden Germany) according to the manufacturer's protocol. RT-PCR and nested PCR were done with 600 nM of primers (Table 1). The RT-PCR was conducted with primers NRZ 267/268 and with the following temperature profile: 10 min 22 °C, 45 min 50 °C, 15 min 95 °C for RT followed by 40 cycles (30 s 94 °C; 30 s 55 °C; 90 s 72 °C) and final elongation for 10 min at 72 °C. The nested PCR was carried out with primers 269/270 by using a touchdown protocol with 10 cycles (30 s 94 °C; 30 s 60 °C; 90 s 72 °C) with a decrease of 1 °C per cycle of the initial 60 °C annealing temperature, followed by 30 cycles (30 s 94 °C; 30 s 50 °C; 90 s 72 °C) and final elongation for 10 min at 72 °C. The resulting product of 1,129 bp was treated with ExoSAP-IT (Affymetrix) before cycle sequencing with primers NRZ 269, NRZ 270 and NRZ 271 using the BigDye 3.1 kit (Applied Biosystems, Weiterstadt, Germany).

Phylogenetic analysis

Sequences were assembled using Sequencher software version 5.2.4. Alignments were performed using MAFFT [10] and the phylogenetic relationships among the strains circulating in Germany and representative strains taken from GenBank were estimated using

Figure 2

Phylogenetic analysis[a] of enterovirus D68 sequences (n=37) obtained by three university laboratories[b], Germany, 2013–2014



The phylogenetic analysis is based on the complete viral protein 1 region. Bootstrap values are shown at the nodes. The scale bar indicates the number of nucleotide (nt) substitutions per site.

[a] The complete VP1 region nt sequence corresponded to bases 2,389 –3,315 of EV-D68 prototype strain Fermon (GenBank accession number: AY426531).

[b] The laboratories were the Institute of Virology and Immunobiology, University of Würzburg, the Institute of Virology, University Hospital of Düsseldorf and the Institute of Virology, University of Bonn Medical Centre.

the maximum likelihood (ML) method based on the Tamura–Nei model conducted with molecular evolutionary genetics analysis (MEGA6) using a bootstrap procedure with 1,000 replicates [11].

Molecular typing of non-enterovirus D68 enteroviruses

Molecular typing of non-EV-D68 enteroviruses was carried out by sequencing of the VP1 region using published PCR systems with slightly modified conditions due to use of the Qiagen One Step RT-PCR kit instead of Invitrogen Superscript II and III as described in references [12,13]. Details of methodology are available upon request. For those samples remaining VP1 PCR negative, sequencing of partial 5'non-coding region (5'NCR) [14] allowed assignment to enterovirus group A–D. Samples with no clear basic local alignment search tool (BLAST) result were classified as NPEV.

Analysis of immunogenic sites in the capsid proteins of enterovirus D68

To provide sequence data for further understanding of possible changes in the immunogenic sites of the capsid, 23 strains representing members of all three current subclades (A2, B1, B2) were selected for sequencing of the entire capsid (P1) genomic region encoding all four capsid proteins as well as adjacent 5'NCR region. Amplification of the VP4/VP2/VP3 region of the genome from clinical material was performed with primers listed in Table 1 using the following cycling protocol: 45 min 50 °C, 15 min 95 °C followed by 25 cycles (30 s 94 °C; 30 s 55 °C; 90 s 72 °C) and final elongation for 10 min at 72 °C. Nested PCR was carried out 15 min at 95 °C followed by 25 cycles (30 s 94 °C; 30 s 55 °C; 30 s 72 °C) and final elongation for 10 min at 72 °C. PCR products were directly sequenced after EXOSAP-IT treatment with primers used for nested PCR. Amplification and sequencing of partial 5'NCR was done as described recently [14].

Results

Enterovirus D68 detection

From January 2013 to the end of December 2014, 14,838 respiratory samples from patients admitted to three tertiary university hospitals were analysed, with 246 (1.7%) being EV- positive. EV-positive samples were retrospectively typed with molecular methods resulting in a total of 39 EV-D68 detections with 17 (0.2%) detections in 2013 and 22 (0.3%) detections in 2014 (Table 2).

When analysed in more detail, variations in EV-D68 prevalence among patients admitted to each of the three hospitals were noticed. While in one hospital a moderate raise in EV-D68 infections among total samples analysed was observed in 2014 compared with 2013 (0.2% in 2013 vs 0.6% in 2014), another hospital showed a higher EV-D68 rate in 2013 (0.6% in 2013 vs 0.1% in 2014).

Weekly distribution of EV-D68 positive samples, as shown in Figure 1, peaked in late summer and autumn months (September–November). This was also reflected by the EV-D68-positivity rates among EV-positive samples, which in calendar weeks 36 to 48 corresponding to September to November (last column, Table 2) ranged from 23.8 to 54.5%. Regarding the individual hospitals, EV-D68 was detected nearly consistently among EVs during weeks 36 to 48 in hospital 1 and 2 (54.5% in 2013 vs 50.0% in 2014; and 27.3% in 2013 vs 23.8% in 2014). Hospital 3 showed higher EV-D68 rate in 2013 (45.5%) than in 2014 (25.0%), however, some caution is needed concerning this hospital because of the relatively small number of EV-D68- positive samples which might be biased by the overall low number of EV detections. On average, no substantial differences in EV-D68 rates could be found between 2013 and 2014 suggesting two regular seasons.

The 39 EV-D68 samples detected over the whole study period were from young children aged 0–9 years (n = 28), teenagers aged 10–19 years (n = 3) and adults aged >50 years (n = 8). Within the group of young children, the majority of EV-D68 patients was under the age of five years (n = 22). The male/female ratio for EV-D68-positive patients was 1.4:1 (m = 23, f = 16). Co-infection with other viruses was observed in two EV-D68-positive samples (Cox A10, n=1; HCoV OC43, n=1). Specified clinical details were not accessible for all EV-D68 patients. For patients where data were available (n = 15), pneumonia (n = 6) or obstructive bronchitis (n = 6) were most commonly reported.

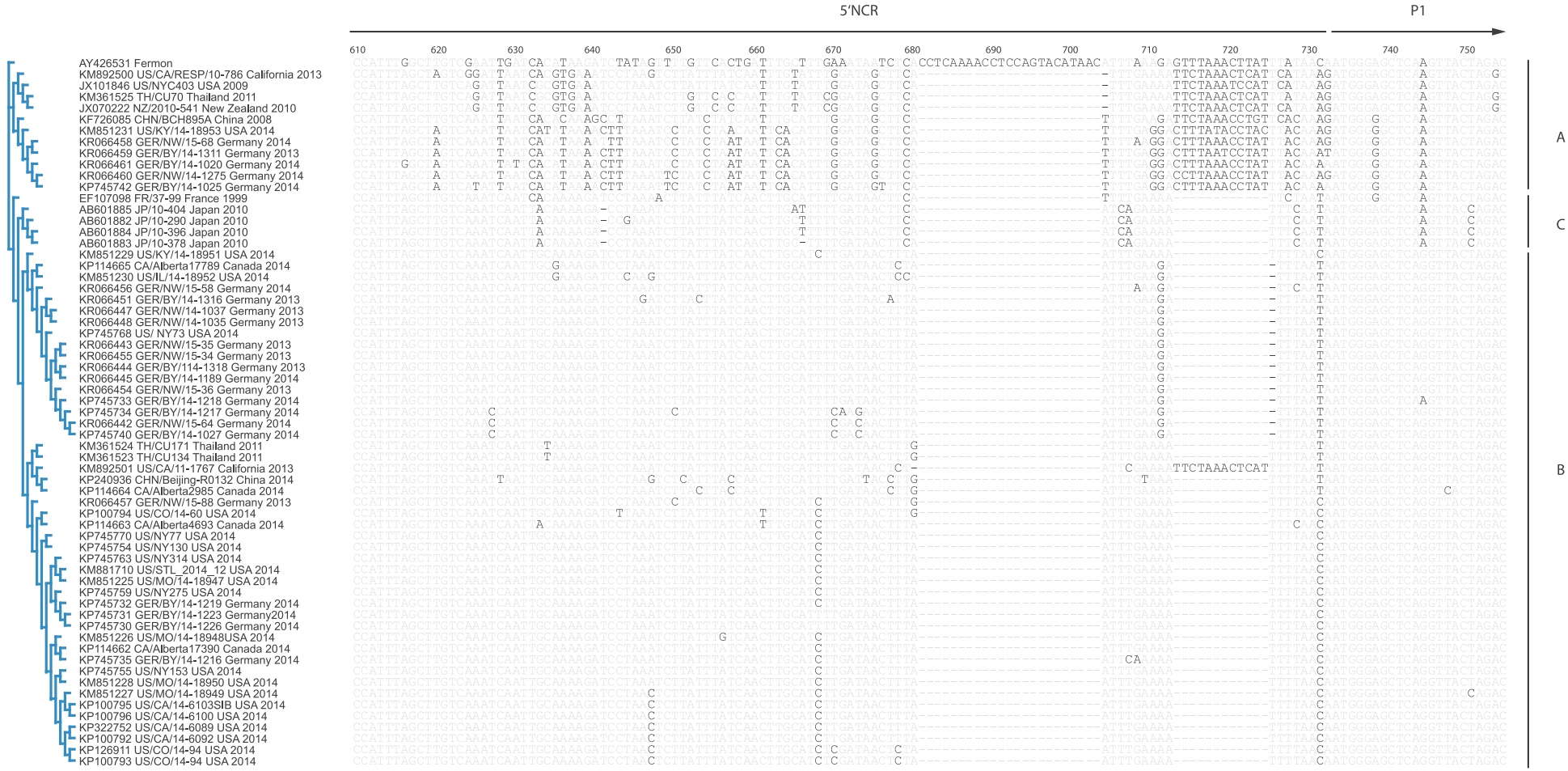
Amplification and phylogenetic analysis based on viral protein 1 region

Amplification of the complete VP1 sequence was achieved for 37 of 39 EV-D68 samples. For phylogenetic analysis complete VP1 sequences of EV-D68 strains available in GenBank were used. The ML tree confirmed the previously observed divergence of EV-D68 strains circulating since 2005 into three major subgroups A, B, and C (Figure 2) [15]. Furthermore, as recently described, subgroups A and B segregated in two subclades [16]. Among the 37 EV-D68 strains, seven belonged to clade A2 and 30 belonged to clade B (B1: n = 5, B2: n = 25). All sequences were deposited in GenBank under accession numbers KP745729–43 and KR066438–61.

Amplification and sequencing of partial VP1 region from non-EV-D68 viruses revealed serotypes from enterovirus species EV-A (n = 87) and EV-B (n = 68). Typing results are shown in more detail in Table 3. Samples remaining negative in VP1 amplification were categorised as EV-A (n = 3), EV-B (n = 6), and EV-C (n = 1) by 5´NCR sequencing. Fifteen samples were identified as rhinovirus (HRV-A: n = 8, HRV-C: n = 7). Twenty-nine samples gave no clear BLAST result and were classified as NPEV (Table 3).

Figure 3

Analysis of the 3' end of the 5'non-coding region of enterovirus D68 identified in Germany[a], 2013–2014 (n=23)



[a] Sequencesfrom this study are compared with clade A, B, and C EV-D68 reference sequences obtained from GenBank.

Table 1

Primers used for amplification and sequencing of the complete capsid (P1) region of enterovirus D68

Target region and primer	Sequence 5'–3'	Orientation	Location[a]
VP1			
NRZ 267	ATG YTA GST ACW CAT RTB GTB TGG GAY TT	Sense	2,125–2,153
NRZ 268	ATC CAY TGR ATM CCW GGG CCY TCR AAR C	Antisense	3,557–3,530
NRZ 269	AAT GCY AAY GTT GGY TAY GTY ACH TGT T	Sense	2,239–2,266
NRZ 270	AAG AYC CYA CAA ARA CYC CHC CRW ARC CKG G	Antisense	3,358–3,327
NRZ 271	CAA GCA ATG TTY GTA CCH ACT GG	Sense	2,854–2,876
VP2/4			
NRZ 272	GTG GTC CAG GCT GCG TTG GCG	Sense	350–370
NRZ 273	TTR AAC TCA CAA CAC ATT GGA GCR ATT G	Antisense	1,658–1,631
NRZ 274	ATG AAC AAG GTG TGA AGA GTC TAT TGA GC	Sense	405–433
NRZ 275	ACT GGT ATT ATT GCT AGY GTC CAC TG	Antisense	1,580–1,555
VP3			
NRZ 276	TGA CAT CAT GAA AGG TGA AGA AGG AGG	Sense	1,371–1,397
NRZ 277	GTG CGA GTT TGT ATG GCT TCY TCT GG	Antisense	2,564–2,539
NRZ 278	GTT CTT CCC TGG ATG AAT GCY GCT CC	Sense	1,504–1,529
NRZ 279	CTC TCR ATY TGR TAG GCT GCC TCT G	Antisense	2,432–2,408

VP: viral protein.

[a] Nucleotide locations are relative to the genome of EV-D68 prototype strain Fermon (GenBank accession number: AY426531).

Analysis of complete capsid region (P1) amino acid- and partial 5'non-coding region sequences

Twenty-three EV-D68 sequences of strains belonging to subclades A2, B1, and B2 from this study were compared with 40 sequences available through GenBank. There were only few amino acids exclusively defining a single clade, however clade A was characterised by E143 and V291 in VP2, N525 and V533 in VP3 and a deletion of N692 in VP1. Strains assigned to subclade A2 carried an arginine and lysine insertion at position 859 of VP1. No differences between strains circulating in 2014 and strains circulating before 2014 were observed with regard to the defined loop structures of the capsid proteins VP1, VP2, and VP3 representing neutralising immunogenic sites (VP2 EF loop, VP3 BC loop, VP1 BC loop and DE loop; alignment available upon request). Notably, two amino acid positions that have been reported to interact with the antiviral pleconaril differed in strains assigned to subclade B1 compared with the other EV-D68 strains: M341A(VP3) and V746I(VP1) [17]. Whether or not these changes influence pleconaril efficacy requires experimental confirmation.

Within the 3' end of the 5'NCR, all strains included in the comparison showed a 23 or 24 nt deletion (681–703/704 compared with prototype strain Fermon). In addition, all B and C strains carried a 12 or 13 nt deletion (713–724/725 compared with Fermon), except strain KM892501 (Figure 3).

Discussion

In this study we provide epidemiological and phylogenetic information on EV-D68 in hospitalised patients admitted with respiratory diseases to three tertiary hospitals in Germany from January 2013 through December 2014. During this period, EV-D68 circulation appeared to have a seasonal pattern, with an increase in numbers of patient samples testing positive for this virus from the beginning of the autumn until the early winter months. The apparent seasonality was also reflected in the EV-D68 positivity rates among enterovirus-positive samples from September to November (calendar week 36–48), which ranged between 23.8% and 54.5%, compared to between 8.3% and 23.4% annually.

Overall EV-D68 infections could be detected in children and teenagers, with most detections in those under five years-old. Adults over 50 years of age were also affected. The male/female ratio of 1.4:1 among all respiratory isolates indicated a male predominance, which has also been previously described for enterovirus-infected patients [18].

Among the total annual numbers of analysed respiratory samples, EV-D68 was detected at a rate of 0.2% (2013) and 0.3% (2014). The similar rates between the two years suggest that each year was characterised by a regular season. In support of this, similar prevalences have been reported for hospitalised patients [19-21] as well as outpatients [18,22] from several studies worldwide in non-epidemic years. In contrast, for years with described increased EV-D68 activity, an overall annual EV-D68 detection rate of›1% has been observed in hospitalised patients [20,23,24] as well as outpatients [22,25].

Table 2

Overview of respiratory samples analysed and enterovirus (EV) and EV-D68 detection rates by three university laboratories[a], Germany, 2013–2014 (n=14,838 respiratory samples)

Laboratory/hospital	Year	Respiratory samples N	EV positive N (% of respiratory samples)	EV-D68 positive N (% of respiratory samples)	EV-D68/EV positives (%) annually	Number of EV-D68/EV positives (%) in calendar week 36 - 48
1	2013	3,526	46 (1.3)	6 (0.2)	6/46 (13.0)	6/11 (54.5)
	2014	2,696	64 (2.4)	15 (0.6)	15/64 (23.4)	12/24 (50.0)
2	2013	3,351	55 (1.6)	6 (0.2)	6/55 (10.9)	3/11 (27.3)
	2014	3,753	44 (1.2)	6 (0.2)	6/44 (13.6)	5/21 (23.8)
3	2013	813	25 (3.1)	5 (0.6)	5/25 (20)	5/11 (45.5)
	2014	699	12 (1.7)	1 (0.1)	1/12 (8.3)	1/4 (25.0)
Total 2013		**7,690**	**126 (1.6)**	**17 (0.2)**	**17/126 (13.5)**	**14/33 (42.4)**
Total 2014		**7,148**	**120 (1.7)**	**22 (0.3)**	**22/120 (18.3)**	**18/49 (36.7)**
Total 2013–2014		**14,838**	**246 (1.7)**	**39 (0.3)**	**39/246 (15.8)**	**32/82 (39.0)**

[a] The laboratories were the Institute of Virology and Immunobiology, University of Würzburg, the Institute of Virology, University Hospital of Düsseldorf and the Institute of Virology, University of Bonn Medical Centre.

A more detailed data analysis revealed variation in EV-D68 prevalence among patients admitted to each of the three hospitals in our study between the years, suggesting a broad range in EV-D68 positivity rates from one season to another. The annual rates of EV-D68 positivity among all respiratory samples for each hospital during the study period remained however<1%.

Worldwide reports on the detection of EV-D68 in patients with respiratory diseases increased rapidly during the last few years especially during the 2008 to 2010 period [2]. The observation of an upsurge in hospitalised patients due to EV-D68 infection in the US and Canada in 2014 [1,3,26] resulted in the recognition of EV-D68 as an (re)emerging pathogen. In response to this, the European Society for Clinical Virology (ESCV) launched a study in collaboration with the European Centre for Disease Prevention and Control (ECDC) to collect information on EV-D68 infections in paediatric patients in September/October 2014 in Europe [16]. We contributed to this study and therefore samples collected between September 2014 and November 2014 reported here were also included in that study. However, no epidemiological data on EV-D68 in Germany covering a period as extended as this current study have been described so far. The only data available to date came from an EV-D68-specific screening of samples collected within the German ILI/ARI outpatient study during the August to October 2014 period, resulting in identification of 25 EV-D68-positive samples among 325 samples (7.7%) screened [27].

Coinciding with the upsurge of severe acute respiratory diseases in the US and Canada in 2014, a cluster of 12 paediatric patients with AFP following respiratory illness was reported from Colorado [28,29]. Among 11 of these children, five (45%) tested EV-D68 positive in respiratory specimens [28,29]. A further investigation of AFP cases reported nationwide in the US during the same period (August through October) found 88 cases of AFP in 32 States, revealing a similar EV-D68 positive rate [30]. In contrast, no development of central nervous system (CNS) complications was reported in the patients from this study. Furthermore, in the context of the Global Polio Eradication Initiative programme, the German enterovirus surveillance [31] reported no significant increase of AFP cases in 2013 and 2014 compared to the 2006 to 2014 average in Germany (Katrin Neubauer, personal communication 15 May 2015).

As part of the EVSurv, laboratory diagnostics focus mainly on stool samples from patients with symptoms of aseptic meningitis/encephalitis and/or AFP to exclude polioviruses. Stool samples are nevertheless not suitable for EV-D68 detection, due to the biological properties of this virus [32,33]. In spite of this, among 24,246 specimens tested for enteroviruses between 2006 and 2014 within the EVSurv, three stool samples from paediatric patients with signs of aseptic meningitis were reported as EV-D68-positive (2 in 2010, 1 in 2013). These most probably resulted from spill-over from the respiratory tract, but nevertheless suggest circulation of EV-D68 in Germany before 2013 and presumably possible association with CNS disorders.

As different EV-D68 clades are evolving over time [15], the increased detection of EV-D68 in the recent decade may have been due to changes in antigenicity [34]. Comparison of the complete capsid sequences of 23 strains isolated in 2013 and 2014 in Germany with reference strains obtained from GenBank did not reveal any amino acid residues in antigenic sites that were unique to the 2013 or 2014 strains.

Furthermore, all German strains described here showed clade specific deletions within the 3' end of 5'NCR as reviewed in Imamura and Oshitani in 2014 [2]. All clade A strains identified in this study displayed

Table 3

Enterovirus typing results from respiratory samples, Germany, 2013–2014 (n= 207 samples)[a]

Species	Serotype	Number[a]
EV-A	Cox A10	12
	Cox A2	8
	Cox A4	21
	Cox A5	3
	Cox A6	23
	Cox A8	1
	Cox A16	10
	EV-A71	9
	NA	3
EV-B	Cox B2	6
	Cox B3	8
	Cox B4	7
	Cox B5	5
	Echo 11	2
	Echo 18	5
	Echo 25	2
	Echo 27	1
	Echo 3	5
	Echo 6	1
	Echo 30	19
	Cox A9	7
	NA	6
EV-C	NA	1
EV-D	EV-D68	39
HRV A	NA	8
HRV C	NA	7
NPEV	NA	29

NA: not applicable.

[a] Due to two samples carrying two different serotypes, the total number of serotypes in the table differ from the total number of samples analysed.

the previously described deletion within the DE loop in VP1 [21]. Interestingly, all German strains assigned to subclade A2 were identified in seven of eight adult patients. Those strains carried a subclade A2 specific insertion of two amino acids (arginine and lysine) at the very end of the C-terminus of VP1. So far, no function has been assigned to this region. Comparison with prototype sequences of all other known enterovirus serotypes revealed a highly diverse C-terminus of VP1 downstream from a conserved proline-rich region (data not shown, available upon request). Furthermore, we recognised two amino acid exchanges in subclade B1 strains (M341A in VP3 and V746I in VP1) at positions that have been described to interact with pleconaril [17] (data not shown, available upon request).

In summary, no significant changes in the EV-D68 prevalence in patients admitted to three German tertiary hospitals could be observed in 2014 compared with 2013. On the basis of amino acid sequences of the capsid proteins no unique changes in 2014 strains compared with 2013 strains or clade specific differences were found. However, the insertion of two amino acids at the C-terminus of VP1 of subclade A2 strains, combined with their occurrence in adults, warrants further experimental investigation regarding neutralisation properties of antibodies directed against (i) the strains not containing this insertion versus (ii) the strains containing the insertion. Furthermore, continuous molecular surveillance of enteroviruses in respiratory samples using defined criteria is a necessity to be able to interpret potential epidemiological and clinical situations like those recently reported from North America and Canada.

Acknowledgements

We thank the technical staff in all four labs for excellent performance.

Conflict of interest

None declared.

Authors' contributions

Sindy Böttcher has drafted the manuscript, initiated the study and developed and performed EV-D68 specific PCR-assays. Christiane Prifert performed EV/HRV screening and conducted phylogenetic analyses. Benedikt Weißbrich provided respiratory samples for enterovirus characterisation. Ortwin Adams provided respiratory samples for enterovirus characterisation. Souhaib Aldabbagh performed EV/HRV screening. Anna-Maria Eis-Hübinger provided respiratory samples for enterovirus characterization. Sabine Diedrich conceptualised the study and has participated in writing of the manuscript together with Sindy Böttcher.

References

1. European Centre for Disease Prevention and Control (ECDC). Enterovirus 68 rapid risk assessment updated. Stockholm: ECDC 24; Nov. 2014. Available from: http://www.ecdc.europa.eu/en/press/news/_layouts/forms/News_DispForm.aspx?List=8db7286c-fe2d-476c-9133-18ff4cb1b568&ID=1125
2. Imamura T, Oshitani H. Global reemergence of enterovirus D68 as an important pathogen for acute respiratory infections.Rev Med Virol. 2015;25(2):102-14. DOI: 10.1002/rmv.1820 PMID: 25471236
3. Midgley CM, Jackson MA, Selvarangan R, Turabelidze G, Obringer E, Johnson D, et al. Severe respiratory illness associated with enterovirus D68 - Missouri and Illinois, 2014. MMWR Morb Mortal Wkly Rep. 2014;63(36):798-9.PMID: 25211545
4. der Heiden MA, Köpke K, Buda S, Buchholz U, Haas W. Estimates of excess medically attended acute respiratory infections in periods of seasonal and pandemic influenza in Germany from 2001/02 to 2010/11.PLoS One. 2013;8(7):e64593. DOI: 10.1371/journal.pone.0064593 PMID: 23874380
5. Niedersächsische Landesgesundheitsamt (NLGA). Akute respiratorische Erkrankungen (ARE). NLGA. German. [Accessed 20 May 2015]. Available from: www.nlga.niedersachsen.de
6. Respiratorisches Viren – Netzwerk (RespVir). German. [Accessed 20 May 2015]. Available from: http://rvdev.medical-dpc.com/
7. Bonzel L, Tenenbaum T, Schroten H, Schildgen O, Schweitzer-Krantz S, Adams O. Frequent detection of viral coinfection in children hospitalized with acute respiratory tract infection using a real-time polymerase chain reaction.Pediatr Infect Dis J. 2008;27(7):589-94. DOI: 10.1097/INF.0b013e3181694fb9 PMID: 18520973

8. Dierssen U, Rehren F, Henke-Gendo C, Harste G, Heim A. Rapid routine detection of enterovirus RNA in cerebrospinal fluid by a one-step real-time RT-PCR assay.J Clin Virol. 2008;42(1):58-64. DOI: 10.1016/j.jcv.2007.11.016 PMID: 18164234
9. Poelman R, Schölvinck EH, Borger R, Niesters HG, van Leer-Buter C. The emergence of enterovirus D68 in a Dutch University Medical Center and the necessity for routinely screening for respiratory viruses.J Clin Virol. 2015;62:1-5. DOI: 10.1016/j.jcv.2014.11.011 PMID: 25542461
10. Katoh K, Standley DM. MAFFT multiple sequence alignment software version 7: improvements in performance and usability.Mol Biol Evol. 2013;30(4):772-80. DOI: 10.1093/molbev/mst010 PMID: 23329690
11. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0.Mol Biol Evol. 2013;30(12):2725-9. DOI: 10.1093/molbev/mst197 PMID: 24132122
12. Nix WA, Oberste MS, Pallansch MA. Sensitive, seminested PCR amplification of VP1 sequences for direct identification of all enterovirus serotypes from original clinical specimens.J Clin Microbiol. 2006;44(8):2698-704. DOI: 10.1128/JCM.00542-06 PMID: 16891480
13. Leitch EC, Harvala H, Robertson I, Ubillos I, Templeton K, Simmonds P. Direct identification of human enterovirus serotypes in cerebrospinal fluid by amplification and sequencing of the VP1 region.J Clin Virol. 2009;44(2):119-24. DOI: 10.1016/j.jcv.2008.11.015 PMID: 19135410
14. Kuryk Ł, Wieczorek M, Diedrich S, Böttcher S, Witek A, Litwińska B. Genetic analysis of poliovirus strains isolated from sewage in Poland.J Med Virol. 2014;86(7):1243-8. DOI: 10.1002/jmv.23803 PMID: 24123142
15. Tokarz R, Firth C, Madhi SA, Howie SR, Wu W, Sall AA, et al. Worldwide emergence of multiple clades of enterovirus 68. J Gen Virol. 2012;93(Pt 9):1952-8. DOI: 10.1099/vir.0.043935-0 PMID: 22694903
16. Poelman R, Schuffenecker I, Van Leer-Buter C, Josset L, Niesters HG, Lina B, ESCV-ECDC EV-D68 study group. European surveillance for enterovirus D68 during the emerging North-American outbreak in 2014.J Clin Virol. 2015;71:1-9. DOI: 10.1016/j.jcv.2015.07.296 PMID: 26364237
17. Liu Y, Sheng J, Fokine A, Meng G, Shin WH, Long F, et al. Structure and inhibition of EV-D68, a virus that causes respiratory illness in children. Science. 2015;347(6217):71-4. DOI: 10.1126/science.1261962 PMID: 25554786
18. Meijer A, Benschop KS, Donker GA, van der Avoort HG. Continued seasonal circulation of enterovirus D68 in the Netherlands, 2011-2014.Euro Surveill. 2014;19(42):20935. DOI: 10.2807/1560-7917.ES2014.19.42.20935 PMID: 25358039
19. Piralla A, Girello A, Grignani M, Gozalo-Margüello M, Marchi A, Marseglia G, et al. Phylogenetic characterization of enterovirus 68 strains in patients with respiratory syndromes in Italy. J Med Virol. 2014;86(9):1590-3. DOI: 10.1002/jmv.23821 PMID: 24155220
20. Ikeda T, Mizuta K, Abiko C, Aoki Y, Itagaki T, Katsushima F, et al. Acute respiratory infections due to enterovirus 68 in Yamagata, Japan between 2005 and 2010. Microbiol Immunol. 2012;56(2):139-43. DOI: 10.1111/j.1348-0421.2012.00411.x PMID: 22309616
21. Imamura T, Suzuki A, Lupisan S, Okamoto M, Aniceto R, Egos RJ, et al. Molecular evolution of enterovirus 68 detected in the Philippines. PLoS One. 2013;8(9):e74221. DOI: 10.1371/journal.pone.0074221 PMID: 24073203
22. Meijer A, van der Sanden S, Snijders BE, Jaramillo-Gutierrez G, Bont L, van der Ent CK, et al. Emergence and epidemic occurrence of enterovirus 68 respiratory infections in The Netherlands in 2010. Virology. 2012;423(1):49-57. DOI: 10.1016/j.virol.2011.11.021 PMID: 22177700
23. Piralla A, Lilleri D, Sarasini A, Marchi A, Zecca M, Stronati M, et al. Human rhinovirus and human respiratory enterovirus (EV68 and EV104) infections in hospitalized patients in Italy, 2008-2009. Diagn Microbiol Infect Dis. 2012;73(2):162-7. DOI: 10.1016/j.diagmicrobio.2012.02.019 PMID: 22494556
24. Tokarz R, Kapoor V, Wu W, Lurio J, Jain K, Mostashari F, et al. Longitudinal molecular microbial analysis of influenza-like illness in New York City, May 2009 through May 2010. Virol J. 2011;8(1):288. DOI: 10.1186/1743-422X-8-288 PMID: 21658237
25. Renois F, Bouin A, Andreoletti L. Enterovirus 68 in pediatric patients hospitalized for acute airway diseases.J Clin Microbiol. 2013;51(2):640-3. DOI: 10.1128/JCM.02640-12 PMID: 23224095
26. Drews SJ, Simmonds K, Usman HR, Yee K, Fathima S, Tipples G, et al. Characterization of enterovirus activity, including that of enterovirus D68, in pediatric patients in Alberta, Canada, in 2014. J Clin Microbiol. 2015;53(3):1042-5. DOI: 10.1128/JCM.02982-14 PMID: 25588657
27. Reiche J, Böttcher S, Diedrich S, Buchholz U, Buda S, Haas W, et al. Low-level Circulation of Enterovirus D68-Associated Acute Respiratory Infections, Germany, 2014. Emerg Infect Dis. 2015;21(5):837-41. DOI: 10.3201/eid2105.141900 PMID: 25898320
28. Messacar K, Schreiner TL, Maloney JA, Wallace A, Ludke J, Oberste MS, et al. A cluster of acute flaccid paralysis and cranial nerve dysfunction temporally associated with an outbreak of enterovirus D68 in children in Colorado, USA. Lancet. 2015;385(9978):1662-71. DOI: 10.1016/S0140-6736(14)62457-0 PMID: 25638662
29. Greninger AL, Naccache SN, Messacar K, Clayton A, Yu G, Somasekar S, et al. A novel outbreak enterovirus D68 strain associated with acute flaccid myelitis cases in the USA (2012-14): a retrospective cohort study. Lancet Infect Dis. 2015;15(6):671-82. DOI: 10.1016/S1473-3099(15)70093-9 PMID: 25837569
30. Division of Viral Diseases, National Centers for Immunization and Respiratory Diseases, CDC; Division of Vector-Borne Diseases, Division of High-Consequence Pathogens and Pathology, National Center for Emerging and Zoonotic Infectious Diseases, CDC; Children's Hospital Colorado; Council of State and Territorial Epidemiologists. Notes from the field: acute flaccid myelitis among persons aged ≤21 years - United States, August 1-November 13, 2014.MMWR Morb Mortal Wkly Rep. 2015;63(53):1243-4.PMID: 25577990
31. Respiratorisches Viren – Netzwerk (RespVir). German [Accessed 20 May 2015]. Available from: http://EVsurv.rki.de
32. Blomqvist S, Savolainen C, Råman L, Roivainen M, Hovi T. Human rhinovirus 87 and enterovirus 68 represent a unique serotype with rhinovirus and enterovirus features.J Clin Microbiol. 2002;40(11):4218-23. DOI: 10.1128/JCM.40.11.4218-4223.2002 PMID: 12409401
33. Oberste MS, Maher K, Schnurr D, Flemister MR, Lovchik JC, Peters H, et al. Enterovirus 68 is associated with respiratory illness and shares biological features with both the enteroviruses and the rhinoviruses. J Gen Virol. 2004;85(Pt 9):2577-84. DOI: 10.1099/vir.0.79925-0 PMID: 15302951
34. Imamura T, Okamoto M, Nakakita S, Suzuki A, Saito M, Tamaki R, et al. Antigenic and receptor binding properties of enterovirus 68. J Virol. 2014;88(5):2374-84. DOI: 10.1128/JVI.03070-13 PMID: 24371050

License and copyright

Research article

Is the recent emergence of mephedrone injecting in the United Kingdom associated with elevated risk behaviours and blood borne virus infection?

VD Hope [1,2], KJ Cullen [1], J Smith [4], L Jessop [5], J Parry [3,2], F Ncube [1]

1. Centre for Infectious Disease Surveillance and Control, Public Health England, London, United Kingdom
2. Centre for Research on Drugs and Health Behaviour, London School of Hygiene and Tropical Medicine, London, United Kingdom
3. Microbiology Services, Public Health England, London, United Kingdom
4. Health Protection, Public Health Wales, Cardiff, United Kingdom
5. Public Health Agency, Belfast, United Kingdom

Correspondence: Vivian Hope (Vivian.Hope@phe.gov.uk)

Citation style for this article:
Hope VD, Cullen KJ, Smith J, Jessop L, Parry J, Ncube F. Is the recent emergence of mephedrone injecting in the United Kingdom associated with elevated risk behaviours and blood borne virus infection?. Euro Surveill. 2016;21(19):pii=30225. DOI: http://dx.doi.org/10.2807/1560-7917.ES.2016.21.19.30225

Article submitted on 10 August 2015 / accepted on 08 January 2016 / published on 12 May 2016

The recent, and rapid, emergence of injection of the short-acting stimulant mephedrone (4-methylmethcathione) has resulted in concerns about increased infection risks among people who inject drugs (PWID). Data from the bio-behavioural surveillance of PWID in the United Kingdom were analysed to examine the impact of mephedrone injection on infections among PWID. During the year preceding the survey, 8.0% of PWID (163/2,047) had injected mephedrone. In multivariable analyses, those injecting mephedrone were younger, less likely to have injected opiates, and more likely to have injected cocaine or amphetamines, used needle/syringe programmes or sexual health clinics, been recruited in Wales and Northern Ireland or shared needles/syringes. There were no differences in sexual risks. Those injecting mephedrone more often had hepatitis C antibodies (adjusted odds ratio (AOR) = 1.51; 95% confidence interval (CI): 1.08–2.12), human immunodeficiency virus (AOR = 5.43; 95% CI: 1.90–15.5) and overdosed (AOR = 1.70; 95% CI: 1.12–2.57). There were no differences in the frequency of injecting site infections or prevalence of hepatitis B. The elevated levels of risk and infections are a concern considering its recent emergence. Mephedrone injection may currently be focused among higher-risk or more vulnerable groups. Targeted responses are needed to prevent an increase in harm.

Introduction

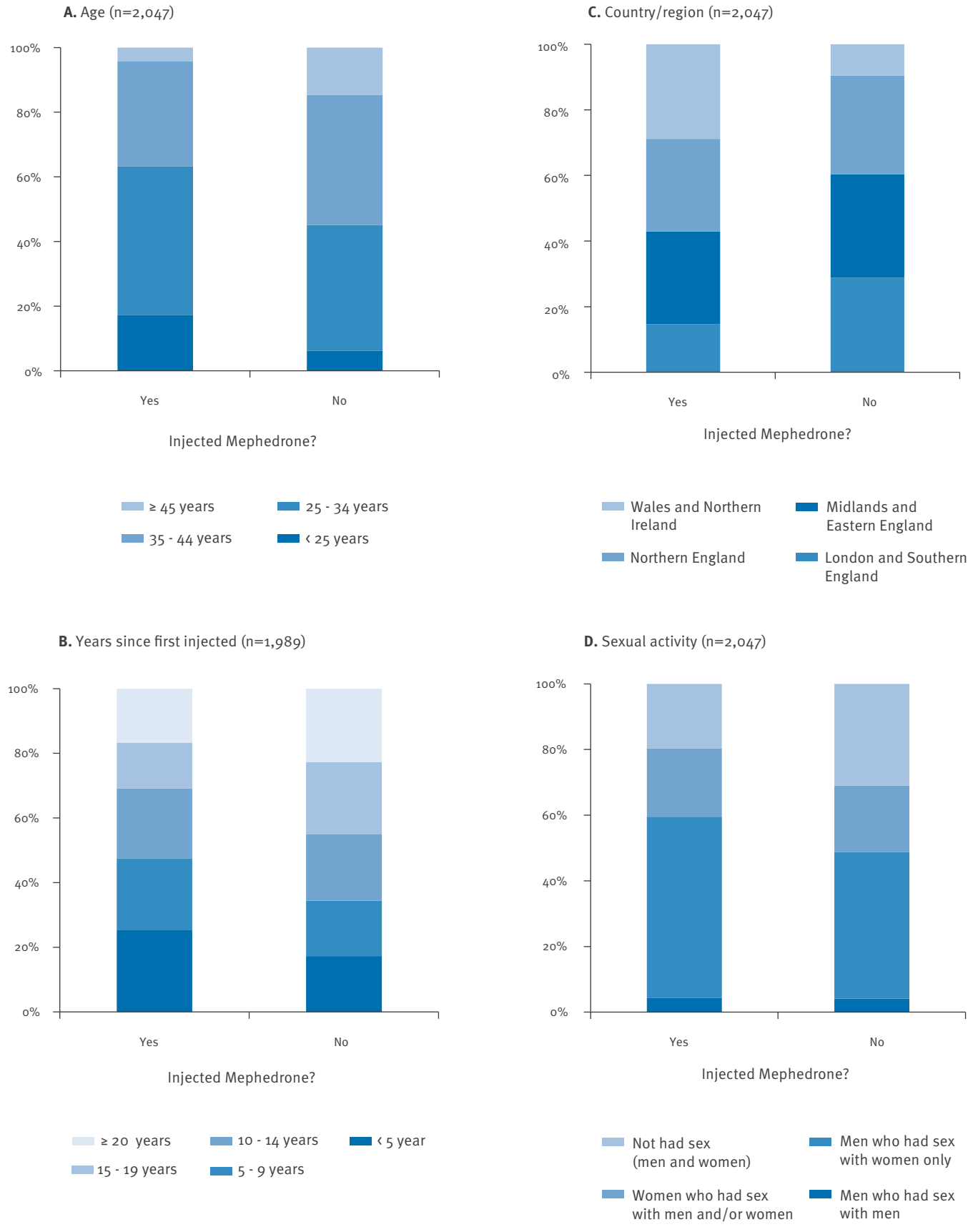
Over the past decade, the emergence of the use of 'new psychoactive substances' has caused major concerns in many countries [1,2]. New psychoactive substances encompass a range of synthetic substances, including synthetic cannabinoids, cathinones, piperazines, tryptamines and phenethylamines, that are not controlled by two United Nations Conventions (the 1961 Convention on Narcotic Drugs and the 1971 Convention on Psychotropic Substances), but which may pose a public health threat that is comparable to that of the substances listed in these conventions [3]. The use of synthetic cathinones, and especially drugs marketed as mephedrone have caused particular concern in a number of countries including the United Kingdom (UK) [3-6].

Mephedrone is the common name for 4-methylmethcathione, it is a relatively short-acting stimulant with reported effects similar to amphetamine and MDMA [5,7]. It can be administered in a variety of ways, including snorting, ingestion and injection, and compulsive re-dosing over a period of many hours has been reported, due to rapid comedown when snorted or induced tolerance when injected repeatedly [5,8]. The use of drugs marketed as 'mephedrone' under street names such as 'drone', 'm-cat' and 'meow meow', have increased since its use was first reported around 2007. The subsequent emergence of the injection of synthetic cathinones, including mephedrone, has caused particular concerns in Europe [9], with the injection of these drugs having been associated with increases in human immunodeficiency virus (HIV) infections and risk behaviours among people who inject drugs (PWID) in several central European countries [10,11]. The use, and in particular the injection, of mephedrone by some populations of men who have sex with men (MSM), particularly during sex, has also recently been reported in Europe and elsewhere [12-14], often in settings where unsafe sex and sharing of injecting equipment occur [12,13].

In the UK, the use of mephedrone was first noted in 2008 [15,16], leading to 4-methylmethcathione being

Figure

Variations in the extent of mephedrone injecting, by age, time in years since first injection, region of recruitment, and sexual activity, United Kingdom, 2013 (n = 2,047)



controlled under the Misuse of Drugs Act in 2010 [17]. The injection of this drug is a more recent practice that was first reported in the UK in 2012; it occurred among people who had switched from snorting as well as among people who had previously injected other drugs including opioids and stimulants [18]. Of particular concern is the compulsion to re-dose when using mephedrone, increasing the frequency of injecting from two or three times daily to 15–20 times, raising the risk of injecting site damage and of infection through poor injection hygiene and the reuse and sharing of injecting equipment [19].

In response to the emergence of mephedrone injecting and the associated concerns about the risks, mephedrone was added to the list of drugs specifically asked about in the UK's national bio-behavioural surveillance system of infections and risks among PWID in 2013. In this paper, we used data from this large national survey to (i) assess the current extent of mephedrone injecting in the UK, (ii) examine the factors associated with mephedrone injecting and (iii) describe the frequency of a range of health harms among those injecting mephedrone.

Methods

PWID have been recruited into a voluntary unlinked anonymous monitoring system in the UK since 1990; methodological details of this series of annual cross-sectional surveys have been published previously [20,21]. Briefly, agencies providing services to PWID (e.g. needle and syringe programmes (NSPs) and providers of addiction services such as opiate substitution therapy (OST)) at sentinel locations (n = 67 in 2013) throughout the UK except Scotland, invite clients who have ever injected psychoactive drugs to participate in the survey each year. The sentinel sites are selected so as to reflect both the geographical distribution and range of services offered to PWID. Those who consent to participate provide a biological sample, currently a dried blood spot (DBS), and self-complete a brief questionnaire focused on the injection of psychoactive drugs. In 2013, the answer categories to the question asking about the drugs injected during the preceding year was revised to include a new response category: 'mephedrone (m-cat)'. The survey has multi-site ethics approval.

The DBS specimens were tested for antibodies to HIV (anti-HIV), the hepatitis B core antigen (anti-HBc) and hepatitis C virus (anti-HCV). The anti-HIV test was an in-house IgG capture enzyme-linked immunosorbent assay (GACELISA) with similar performance to the HIV 1+2 GACELISA (Abbott Murex Diagnostics Ltd, Dartford, UK). Reactive specimens underwent further testing according to an algorithm that included a second ELISA and Western blot [22]. Anti-HCV testing employed a previously validated commercial enzyme-immunoassay (Ortho HCV 3.0 SAVe, Ortho Diagnostics, New Jersey) [23]. For hepatitis C, a previously described algorithm using antibody avidity testing was applied to the survey samples to identify probable recent hepatitis C infections, i.e. samples with weak antibody avidity $<40\%$ in the presence of HCV RNA [24]. For anti-HBc, an in-house IgG class-specific antibody capture enzyme immunoassay (EIA) was used.

For those who had injected during the preceding year, bivariate associations ($p<0.05$) between the outcome variable, i.e. having injected mephedrone, and covariates (demographics, injecting practices, drugs injected, sexual practice and use of health services) were examined using Pearson's chi-squared test. Where possible associations were found ($p<0.10$), these were further examined via logistic regression using the forward stepwise procedure to select variables for inclusion in the model, with selection based on the likelihood ratio test ($p<0.05$). All analyses were undertaken using SPSS 19.

Associations between mephedrone injecting and a range of health harms were explored by examining the frequency of mephedrone injecting among those with and without harms (anti-HIV, anti-HBc, anti-HCV, reported recent symptoms of injection site infections or injuries, and reported recent overdose). Data were adjusted for age, sex and region as these factors are known to be associated with these harms [20,21,25,26].

Results

Sample characteristics

During 2013, the survey recruited 2,047 individuals who had injected psychoactive drugs during the preceding year. Almost half (47%; n = 953) were aged 35 years or older (mean age: 36 years, median: 35 years), 26% (n = 522) were women and 5% (n = 107) had been born outside of the UK. Almost one fifth, 18% (n = 369), reported that they had been homeless during the preceding year and almost three quarters, 72% (n = 1,471), reported that they had ever been imprisoned.

The majority, 85% (n = 1,733), reported using an NSP service during the preceding year and 69% (n = 1,418) were currently in receipt of a maintenance drug regime such as OST or on detoxification. During the year preceding the survey, 10% (n = 204) had visited a sexual health (genito-urinary medicine) clinic, 20% (n = 411) a walk-in (minor injury/primary care) clinic, 30% (n = 617) an Emergency Department and 65% (n = 1,331) a general practitioner. Overall, 75% (n = 1,531) had ever had a voluntary confidential test for HIV, 81% (n = 1,667) for hepatitis C, and 72% (n = 1,471) had received at least one dose of hepatitis B vaccine.

Drugs injected and injecting risks

The most commonly injected drug during the year preceding the survey was heroin (92%; n = 1,879). Two-fifths reported that they had injected crack cocaine (43%; n = 885), almost three-tenths had injected amphetamines (29%; n = 591) and just over one-tenth had injected powder cocaine (12%; n = 245). Injecting

Table 1

Factors associated with injecting mephedrone during the preceding year among people who inject drugs, United Kingdom, 2013 (n = 2,047)

Characteristic[a]		Injected mephedrone in the preceding year?							
	Yes		Total	p value	Odds ratio	95% CI	Adjusted odds ratio	95% CI	
	%	n							
All		8.0	163	2,047	NA				
Demographic characteristics									
Age	‹25 years	19	28	144	‹0.001	NA			
	25–34 years	9.3	75	809					
	35–44 years	6.5	53	811					
	≥45 years	2.5	7	283					
	Per year increase in age					0.94	0.92–0.96	0.95	0.92–0.97
Number of years since first injected	‹5 years	12	41	356	0.003	1.00	Ref	b	
	5–9 years	10	36	349		0.88	0.55–1.42		
	10–14 years	8.5	35	413		0.71	0.44–1.14		
	15–19 years	5.4	23	429		0.44	0.26–0.74		
	≥20 years	6.1	27	442		0.50	0.30–0.83		
	Not known	1.7	1	58		0.13	0.02–1.00		
Region/Country	Midlands and Eastern England	7.2	46	640	‹0.001	1.00	Ref	1.00	Ref
	London and Southern England	4.2	24	567		0.57	0.34–0.95	0.60	0.35–1.02
	Northern England	7.5	46	613		1.05	0.69–1.60	0.91	0.58–1.43
	Wales and Northern Ireland	21	47	227		3.37	2.17–5.23	3.06	1.91–4.89
Homeless preceding year	Not last year/never	7.4	125	1,678	0.067	1.00	Ref	b	
	Yes last year	10	38	369		1.43	0.97–2.09		
Anal or vaginal sex during preceding year	Men who had sex with men	8.2	7	85	0.017	1.64	0.70–3.84	b	
	Men who had sex with women only	9.7	90	931		1.95	1.29–2.96		
	Women who had sex with men and/or women c	8.2	34	415		1.63	0.99–2.68		
	Not had sex (men and women)	5.2	32	616		1.00	Ref		
Injecting practice during the preceding year									
Injected heroin	No	21	35	168	‹0.001	1.00	Ref	1.00	Ref
	Yes	6.8	128	1,879		0.28	0.18–0.42	0.35	0.22–0.56
Injected amphetamine (speed)	No	4.7	68	1,456	‹0.001	1.00	Ref	1.00	Ref
	Yes	16	95	591		3.91	2.82–5.43	2.42	1.68–3.50
Injected cocaine	No	6.8	123	1,802	‹0.001	1.00	Ref	1.00	Ref
	Yes	16	40	245		2.66	1.81–3.91	2.36	1.53–3.63
Used needles or syringes previously used by someone else	No	6.8	117	1,729	‹0.001	1.00	Ref	1.00	Ref
	Yes	14	46	318		2.33	1.62–3.35	1.95	1.31–2.92
Health services usage									
Used needle and syringe programme preceding year	Not last year /Never	4.8	15	314	0.023	1.00	Ref	1.00	Ref
	Last year	8.5	148	1,733		1.86	1.08–3.21	1.89	1.04–3.42
Prescribed opiate substitution therapy	Previously/Never	10	63	629	0.016	1.00	Ref	b	
	Currently	7.0	99	1,418		0.67	0.48–0.93		
Used sexual health (genito-urinary medicine) clinic preceding year	No	6.9	114	1,649	‹0.001	1.00	Ref	1.00	Ref
	Yes	17	35	204		2.79	1.85–4.20	2.10	1.32–3.35
	Not known	7.2	14	194		1.05	0.59–1.86	0.99	0.54–1.82

CI: confidence interval; NA: not applicable; Ref: reference value.

[a] No associations with: sex; being born in the United Kingdom, ever being imprisoned, injecting crack during the preceding 12 months, using a walk-in (minor injury/primary care) clinic during the preceding 12 months, using an emergency department during the preceding 12 months, visiting a general practitioner during the preceding 12 months, ever having had a voluntary confidential test for human immunodeficiency virus, ever having had a voluntary confidential test for hepatitis C, and uptake of vaccine against hepatitis B.

[b] Entered in multivariate analyses but not in the final model.

[c] The number of women reporting sex with women was small (‹ 50) and they are thus not reported separately.

mephedrone during the preceding year was reported by 8% (n = 163) of participants. Overall, 41% (n = 847) of the participants reported injecting only one of these five drugs during the preceding year and 19% (n = 391) reported injecting three or more of them. Those reporting that they had injected mephedrone were more likely to report injecting three or more of the other four drugs (63% (n = 102) vs 15% (n = 289); p<0.001). Of those who reported injecting mephedrone, 13% (n = 21) had also injected all of the other four drugs (i.e. heroin, crack cocaine, amphetamines and powder cocaine); 8% (n = 13) had not injected any of these four other drugs.

Those injecting mephedrone were younger (mean age: 32 years, median: 31 years vs mean age: 36 years, median: 35 years; p<0.001), had been injecting for fewer years (mean duration: 11 years, median: 10.5 years vs mean duration: 14 years, median: 13 years; p=0.001), and were more likely to be living in Wales or Northern Ireland (p<0.001, the level of use was very similar in both of these areas) (Figure). Overall, 16% (n = 318) of all of the participants reported that they had knowingly receptively shared needles or syringes (i.e. injected with needles or syringes that had previously been used by someone else) during the preceding year. Reporting sharing was more common among those injecting mephedrone than those not (28%; n = 46 vs 14%; n = 272; p<0.001). Similarly, those injecting mephedrone were more likely to report having *ever* receptively shared a needle or syringe (59%; n = 96 vs 46%; n = 864; p=0.001).

Sexual risk and condom use

The majority of all survey participants were sexually active, with just over two-thirds (70%; n = 1,431) reporting that they had had anal or vaginal sex in the preceding year; 5.6% (n = 85) of the men reported sex with other men. Heterosexual men were more likely to report injecting mephedrone than MSM or women (p=0.017, Figure). Of those sexually active, 35% (n = 503) reported having two or more sexual partners during the preceding year overall; 53% (n = 45) of the MSM had two or more partners, 37% (n = 348) of heterosexual men, and 27% (n = 110) of the women (who either had sex with men and/or women, there were<50 women reporting female partners). Of those with two or more partners, 17% (n = 84) reported always using condoms; 13% (n = 6) of the MSM, 17% (n = 59) of heterosexual men, and 17% (n = 19) of the women. Mephedrone injection was not associated with the extent of condom use among those with two or more sexual partners.

Factors associated with mephedrone injecting

The bivariate and multivariable associations are shown in Table 1. In the multivariable analysis, mephedrone injecting during the preceding year was associated with younger age. It was more common in Wales and Northern Ireland, among those who had injected amphetamine or powder cocaine, those who had shared needles or syringes, those using NSPs or sexual health (genito-urinary medicine) clinics. It was less common among those who had injected heroin.

Health harms and mephedrone injecting

Testing of the DBS samples collected in the survey found that overall, 1.1% (n = 23) of the participants had anti-HIV, 15% (n = 311) anti-HBc, and 50% (n = 1,027) anti-HCV. Having had an abscess, sore or open wound at an injection site during the preceding year was reported by 25% (n = 502) of the participants, and an overdose during the preceding year was reported by 14% (n = 277). After adjustment, injecting mephedrone was found to be more common among those with anti-HIV or anti-HCV, and among those reporting an overdose during the preceding year (Table 2).

Those who reported that they had ever had a voluntary confidential test for HIV or hepatitis C were also asked about the result of their last test. These data were used to assess the proportion of those with anti-HIV and anti-HCV who were aware of their infections. Of those anti-HCV-positive, it was possible to assess awareness for 87% (n = 898); of these, 46% (n = 417) were aware of their infection and awareness was similar among those injecting mephedrone and those not (43% vs 47%, p=0.393). Among those with HIV, it was possible to assess awareness for 87% (n = 20); of these, 95% (n = 19) were aware of their infection and again there was no difference in awareness between those injecting and those not injecting mephedrone (100% vs 94% respectively, p=1.000, Fisher's exact test).

A laboratory testing algorithm was applied to the samples collected in the survey to identify probable recent infections with HCV [24], i.e. those with weak anti-HCV avidity in the presence of HCV RNA. This algorithm identified 28 probable recent infections among those participants who had been at risk of hepatitis C infection (n=1,048); thus overall, 2.7% of those who had been at risk had recently become infected with HCV. There was no difference in the extent of these probable recent infections between those who reported injecting mephedrone and those who did not (2.5% vs 2.7% respectively, p=0.936).

Discussion

Considering the recent, and rapid, emergence of the injection of mephedrone, the elevated levels of risk and harm found in our study among those who had injected mephedrone are a concern. Within two years of mephedrone injection first being reported in the UK it was being injected by one in 12 PWID. Worryingly, those with HIV were more than five times as likely to report mephedrone injecting, and mephedrone injecting was also more common among those with antibodies to HCV and those who had recently overdosed. Although there were no differences in sexual risk, injecting risks were significantly higher among those injecting mephedrone.

Table 2

Health harms and extent of mephedrone injecting among people who inject drugs, United Kingdom, 2013 (n = 2,047)

Injected mephedrone during the preceding year?	Had harm		n	p value	Odds ratio	(95% CI)	Adjusted odds ratio	(95% CI)[a]
Had an abscess, sore or open wound during preceding year								
Not injected mephedrone	24%	461	1,884	0.846	1.00	Ref	1.00	Ref
Injected mephedrone	25%	41	163		1.04	0.72–1.50	1.10	0.75–1.62
Had antibodies to hepatitis C								
Not injected mephedrone	50%	941	1,884	0.491	1.00	Ref	1.00	Ref
Injected mephedrone	53%	86	163		1.12	0.81–1.54	1.51	1.08–2.12
Had antibodies to hepatitis B core antigen								
Not injected mephedrone	16%	298	1,884	0.007	1.00	Ref	1.00	Ref
Injected mephedrone	8.0%	13	163		0.46	0.26–0.82	0.73	0.40–1.33
Had antibodies to HIV								
Not injected mephedrone	1.0%	18	1,884	0.014	1.00	Ref	1.00	Ref
Injected mephedrone	3.1%	5	163		3.28	1.20–8.95	5.43	1.90–15.5
Had an overdose during preceding year								
Not injected mephedrone	13%	243	1,884	0.004	1.00	Ref	1.00	Ref
Injected mephedrone	21%	34	163		1.78	1.19–2.66	1.70	1.12–2.57

CI: confidence interval; HIV: human immunodeficiency virus; Ref: reference value.

[a] Adjusted for age, sex and region/country as these factors are known to be associated with the outcomes.

Our findings suggest the spread of mephedrone injecting within the UK has been fairly rapid since this was first reported in 2012. The rapid emergence of the injection of synthetic cathinones, often as substitute for or in addition to other drugs, has also been reported in several central European countries [9-11]. However, the extent of mephedrone injecting varied markedly across the UK, from around one in 25 in London and the south of England, through around one in 14 elsewhere in England, to one in five in both Wales and Northern Ireland. This indicates that the emergence of mephedrone injecting in both Wales and Northern Ireland has been more extensive and rapid than in England. The reasons for these geographical differences are unknown and further research is required to explore this.

Mephedrone injecting was more common among those who reported injecting other stimulants (amphetamine and powder cocaine) and was less common among those injecting heroin. This is perhaps to be expected given that mephedrone is also a stimulant. This finding suggests that the emergence of mephedrone injecting might, in part at least, be driven by issues such as drug availability, price and/or drug purity, leading to drug substitution among existing populations of people who inject stimulants [27]. As opiate injecting has most probably declined in the UK [28], particularly in England [29], and is now focused in an ageing cohort [30], the emergence of mephedrone injecting may also be part of a generational shift towards the injection of stimulants [31]. Those who had injected mephedrone were overall younger and had been injecting for a shorter time than those who had only injected other drugs. Considering this, and that a small number of those sampled reported injecting only mephedrone, it is possible that a new group of PWID who inject mephedrone, either alone or in conjunction with other drugs, might be emerging [18]. These findings thus indicate that currently mephedrone injecting is mostly occurring among existing populations of PWID, but they also suggest the emergence of a new group of younger PWID with potentially higher risks.

Those reporting mephedrone injection were twice as likely to report sharing injecting equipment, indicating that they are a high risk group. This is supported by the higher HIV and hepatitis C prevalence and overdoses being more common among those injecting mephedrone. The data on the proportions aware of their infection with HIV or hepatitis C indicate that awareness does not vary between those injecting and those not injecting mephedrone, which suggests that there might be no difference in the recency of these infections (recent infections are probably less likely to have been diagnosed than longer standing ones). This is corroborated by our data on probable recent HCV infections, which indicate that there is no difference in the incidence of HCV infection between those injecting mephedrone and those not injecting mephedrone. Our findings thus suggest that mephedrone injection in the UK is currently mainly concentrated among groups of PWID that already have elevated levels of risk, infection and harm.

Considering the higher levels of injecting risk behaviours and infections among those injecting mephedrone, our findings indicate that the emergence of mephedrone injection in the UK has the potential to increase the transmission of infections among PWID, particularly if its use is sustained or becomes more widespread. The rapid emergence of the injection of synthetic cathinones has already been implicated in increases in viral hepatitis and HIV transmission in a number of other European countries [10,11,32].

Mephedrone injection was not associated with increased sexual risk in our study, although it was more commonly reported among those who were sexually active and younger. However, overall levels of unsafe sexual practice were high. Mephedrone use and injecting has been associated with sexual risk in some populations, specifically subgroups of MSM where it has been linked to high risk behaviours and infections [12,13,33,34], and mephedrone use has also been reported to have positive effects on libido [35]. Those injecting mephedrone in our study were more likely to have used a sexual health service, suggesting that mephedrone injection may be related to increased sexual health needs that may not have been detected by the limited data on sexual behaviour collected in our study, and further investigation is required.

The findings presented here suggest that interventions are needed to address mephedrone injection. Responses should first look at ways to improve injection practice and hygiene, as well as promoting awareness among PWID of the risks and harms that are associated with injecting mephedrone [9,18,19]. However, to date, the UKs response to the injection of psychoactive drugs has had a strong focus on the traditional predominant drug, heroin, with an emphasis on a combination of high coverage NSPs and easy to access OST, which have been shown to be effective for reducing infections [36]. Although stimulant injection is not a new phenomenon in the UK, this has predominantly been in the form of crack cocaine injection alongside heroin (both need to be dissolved in acidic solutions), whereas the injection of amphetamines has been comparatively rare but may have increased in recent years [31]. With the emergence of mephedrone injection, responses will need to adapt to the increased use of water-soluble drugs and make greater use of treatments that are appropriate for users of stimulant drugs [37]. There may also be a need to explore the provision of these services in non-traditional settings, such as community-based outreach services and sexual health clinics.

This study has a number of potential limitations. Firstly, the illicit and marginalised nature of injecting drug use makes the recruitment of a representative sample problematic. To maximise representativeness, this survey used an accepted approach for surveillance surveys involving recruitment at multiple sites through targeted services for PWID as a sampling frame [38,39]. In the UK, there is extensive provision of such targeted services, and the uptake and use of these is high, with very few of the PWID recruited through community-based studies found not to be in contact with these services [40]. For emerging drugs such as mephedrone, there may be new groups of users or populations where new patterns of injecting have emerged, such as some sub-groups of MSM. These groups may be less likely to be in contact with services or have different patterns of service use. This may possibly lead to such users being under-represented. Secondly, the behavioural data used here are based on self-reports, the accuracy of which may be subject to recall bias; however, the reliability of self-reported risk behaviours among PWID has been shown in other studies [41,42]. Considering these issues, the findings presented here should be generalised with caution.

Conclusion

Although the associations found here need further investigation, they suggest that the injection of mephedrone may be focused among younger and higher-risk groups of PWID, who may be particularly vulnerable to harm. Most of those injecting mephedrone were also using other drugs; however, a number were not. These findings, together with the younger age of those injecting mephedrone, suggest that new groups of PWID may also be emerging. Services in contact with PWID, including NSPs and sexual health clinics, will need to be alert to these elevated infection risks and the harm reduction needs of those injecting mephedrone. In the UK, the level of reported needle and syringe sharing among PWID is currently stable and lower than it was a decade ago, while the overall prevalences of HIV and hepatitis C among PWID have changed little in recent years; targeted responses, such as risk reduction interventions for those injecting mephedrone, are therefore needed to prevent an increase in the transmission of infections among PWID. Considering the increasing range of new psychoactive substances [43], vigilance should also be maintained for possible emergence other injected substances.

Acknowledgements

We are grateful to all of the people who took part in the surveys and to the various services across the England and Wales who assisted with their recruitment. We would like to thank the support staff who worked on the surveys and those who undertook the laboratory work. The views expressed here are those of the authors and do not necessarily reflect the views of Public Health England.

Conflict of interest

None declared.

Authors' contributions

All authors contributed to preparing the manuscript, with VH coordinating. VH and FN manage the implementation of the

survey, with KC, JS and LJ assisting. JP oversees the laboratory testing. Analyses were undertaken by VH.

References

1. Europol–European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) joint report on a new psychoactive substance: 4-methylmethcathinone (mephedrone). Lisbon: EMCDDA; March 2010. Available from: www.emcdda.europa.eu/html.cfm/index132196EN.html
2. Winstock AR, Ramsey JD. Legal highs and the challenges for policy makers.Addiction. 2010;105(10):1685-7. Available from: DOI: 10.1111/j.1360-0443.2010.02934.x PMID: 20840205
3. European Monitoring Centre for Drugs and Drug Addiction (EMCDDA). Responding to new psychoactive substances. Drugs in focus (ISSN 1681-5157). Lisbon: EMCDDA; 2011.
4. Musshoff F, Hottmann L, Hess C, Madea B. ["Legal highs" from the German internet--"bath salt drugs" on the rise]. Arch Kriminol. 2013;232(3-4):91-103. German.PMID: 24358620
5. German CL, Fleckenstein AE, Hanson GR. Bath salts and synthetic cathinones: an emerging designer drug phenomenon. Life Sci. 2014;97(1):2-8. Available from: DOI: 10.1016/j.lfs.2013.07.023 PMID: 23911668
6. Schifano F, Corkery J, Ghodse AH. Suspected and confirmed fatalities associated with mephedrone (4-methylmethcathinone, "meow meow") in the United Kingdom.J Clin Psychopharmacol. 2012;32(5):710-4. Available from: DOI: 10.1097/JCP.0b013e318266c70c PMID: 22926609
7. Winstock A, Mitcheson L, Ramsey J, Davies S, Puchnarewicz M, Marsden J. Mephedrone: use, subjective effects and health risks.Addiction. 2011;106(11):1991-6. Available from: DOI: 10.1111/j.1360-0443.2011.03502.x PMID: 21592252
8. Schifano F, Albanese A, Fergus S, Stair JL, Deluca P, Corazza O, et al. Mephedrone (4-methylmethcathinone; 'meow meow'): chemical, pharmacological and clinical issues. Psychopharmacology (Berl). 2011;214(3):593-602. Available from: DOI: 10.1007/s00213-010-2070-x PMID: 21072502
9. European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) Injection of synthetic cathinones in Europe. Perspectives on Drugs series. Lisbon: EMCCDA; May 2014. Available from; www.emcdda.europa.eu/attachements.cfm/att_228233_EN_POD2014_Injection%20of%20synthetic%20cathinones.pdf
10. Péterfi A, Tarján A, Horváth GC, Csesztregi T, Nyírády A. Changes in patterns of injecting drug use in Hungary: a shift to synthetic cathinones.Drug Test Anal. 2014;6(7-8):825-31. Available from: DOI: 10.1002/dta.1625 PMID: 24692417
11. Oprea C, Ceausu E, Ruta S. Ongoing outbreak of multiple blood-borne infections in injecting drug users in Romania. Public Health. 2013;127(11):1048-50. Available from: DOI: 10.1016/j.puhe.2013.08.018 PMID: 24239282
12. Kirby T, Thornber-Dunwell M. High-risk drug practices tighten grip on London gay scene.Lancet. 2013;381(9861):101-2. Available from: DOI: 10.1016/S0140-6736(13)60032-X PMID: 23320280
13. Peyrière H, Jacquet JM, Eiden C, Tuaillon E, Psomas C, Reynes J. Viral and bacterial risks associated with mephedrone abuse in HIV-infected men who have sex with men. AIDS. 2013;27(18):2971-2. Available from: DOI: 10.1097/QAD.0000000000000029 PMID: 25119693
14. Kelly BC, Wells BE, Pawson M, Leclair A, Parsons JT, Golub SA. Novel psychoactive drug use among younger adults involved in US nightlife scenes.Drug Alcohol Rev. 2013;32(6):588-93. Available from: DOI: 10.1111/dar.12058 PMID: 23795887
15. European Monitoring Centre for Drugs and Drug Addiction (EMCDDA)–Europol 2008 annual report on the implementation of Council Decision 2005/387/JHA. Lisbon: EMCDDA; May 2009. Available from: www.emcdda.europa.eu/html.cfm/index132901EN.html
16. Advisory Council on the Misuse of Drugs (ACMD). Consideration of cathinones. London: ACMD; 2010.
17. A change to the misuse of drugs act 1971: control of mephedrone and other cathinone derivatives. London: Home Office; April 2010. Available from: http://webarchive.nationalarchives.gov.uk/20130125102358/http://www.homeoffice.gov.uk/about-us/corporate-publications-strategy/home-office-circulars/circulars-2010/010-2010/
18. Daly M. 'Drone strikes. Druglink. 2012;27(6):8-11. Available from: http://www.drugwise.org.uk/wp-content/uploads/Druglink-Nov-Dec-2012.pdf
19. Van Hout MC, Bingham T. "A costly turn on": patterns of use and perceived consequences of mephedrone based head shop products amongst Irish injectors.Int J Drug Policy. 2012;23(3):188-97. Available from: DOI: 10.1016/j.drugpo.2012.01.008 PMID: 22342322
20. Hope VD, Judd A, Hickman M, Sutton A, Stimson GV, Parry JV, et al. HIV prevalence among injecting drug users in England and Wales 1990 to 2003: evidence for increased transmission in recent years. AIDS. 2005;19(11):1207-14. Available from: DOI: 10.1097/01.aids.0000176222.71355.a1 PMID: 15990575
21. Sweeting MJ, Hope VD, Hickman M, Parry JV, Ncube F, Ramsay ME, et al. Hepatitis C infection among injecting drug users in England and Wales (1992-2006): there and back again? Am J Epidemiol. 2009;170(3):352-60. Available from: DOI: 10.1093/aje/kwp141 PMID: 19546152
22. Connell JA, Parry JV, Mortimer PP, Duncan J. Novel assay for the detection of immunoglobulin G antihuman immunodeficiency virus in untreated saliva and urine.J Med Virol. 1993;41(2):159-64. Available from: DOI: 10.1002/jmv.1890410212 PMID: 8283178
23. Judd A, Parry J, Hickman M, McDonald T, Jordan L, Lewis K, et al. Evaluation of a modified commercial assay in detecting antibody to hepatitis C virus in oral fluids and dried blood spots. J Med Virol. 2003;71(1):49-55. Available from: DOI: 10.1002/jmv.10463 PMID: 12858408
24. Cullen KJ, Hope VD, Croxford S, Shute J, Ncube F, Parry JV. Factors associated with recently acquired hepatitis C virus infection in people who inject drugs in England, Wales and Northern Ireland: new findings from an unlinked anonymous monitoring survey.Epidemiol Infect. 2014;143(7):1398-407. PMID: 25119383
25. Judd A, Hickman M, Hope VD, Sutton AJ, Stimson GV, Ramsay ME, et al. Twenty years of selective hepatitis B vaccination: is hepatitis B declining among injecting drug users in England and Wales? J Viral Hepat. 2007;14(8):584-91. Available from: DOI: 10.1111/j.1365-2893.2007.00844.x PMID: 17650293
26. Powis B, Strang J, Griffiths P, Taylor C, Williamson S, Fountain J, et al. Self-reported overdose among injecting drug users in London: extent and nature of the problem. Addiction. 1999;94(4):471-8. Available from: DOI: 10.1046/j.1360-0443.1999.9444712.x PMID: 10605843
27. Business as usual? A status report on new psychoactive substsances (NPS) and 'club drugs' in the UK. London: Drugscope; 2014. Available from: http://www.drugwise.org.uk/wp-content/uploads/businessasusual.pdf
28. UK Focal Pont at Public Health England. United Kingdom drug situation: Annual report to the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) 2013. Davies C, Murray R, eds. London: Public Health England; October 2013. Available from: www.nta.nhs.uk/uploads/2478ofocalpointreport2013.pdf
29. Hay G, Rael dos Santos A, Worsley J. Estimates of the prevalence of opiate use and/or crack cocaine use, 2011/12: Sweep 8 report. Liverpool: John Moores University; April 2014. Available from: www.nta.nhs.uk/facts-prevalence.aspx
30. UK Focal Pont at Public Health England. United Kingdom drug situation: Annual Report to the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) 2009. Davies C, English L, Lodwick A, McVeigh J, Bellis, M, eds. London: Public Health England; October 2009. Available from: http://www.nta.nhs.uk/uploads/2009.pdf
31. Public Health England, Health Protection Scotland, Public Health Wales, and Public Health Agency Northern Ireland. Shooting Up: Infections among people who inject drugs in the United Kingdom 2013. London: Public Health England, November 2014. Available from: https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/370707/Shooting_Up_2014.pdf
32. Giese C, Igoe D, Gibbons Z, Hurley C, Stokes S, McNamara S, et al. outbreak control team. Injection of new psychoactive substance snow blow associated with recently acquired HIV infections among homeless people who inject drugs in Dublin, Ireland, 2015. Euro Surveill. 2015;20(40):30036. Available from: DOI: 10.2807/1560-7917.ES.2015.20.40.30036 PMID: 26537764
33. Gilbart VL, Simms I, Gobin M, Oliver I, Hughes G. High-risk drug practices in men who have sex with men.Lancet. 2013;381(9875):1358-9. Available from: DOI: 10.1016/S0140-6736(13)60882-X PMID: 23601946
34. Simms I, Gilbart VL, Byrne L, Jenkins C, Adak GK, Hughes G, et al. Identification of verocytotoxin-producing Escherichia coli O117:H7 in men who have sex with men, England, November 2013 to August 2014. Euro Surveill. 2014;19(43):20946. Available from: DOI: 10.2807/1560-7917.ES2014.19.43.20946 PMID: 25375900
35. Bourne A, Reid D, Hickson F, Torres Rueda S, Weatherburn P. The Chemsex study: drug use in sexual settings among gay and bisexual men in Lambeth, Southwark & Lewisham. London: Sigma Research, London School of Hygiene and Tropical Medicine; 2014.

36. MacArthur GJ, van Velzen E, Palmateer N, Kimber J, Pharris A, Hope V, et al. Interventions to prevent HIV and Hepatitis C in people who inject drugs: a review of reviews to assess evidence of effectiveness. Int J Drug Policy. 2014;25(1):34-52. Available from: DOI: 10.1016/j.drugpo.2013.07.001 PMID: 23973009

37. Drug misuse and dependence: UK guidelines on clinical management. London: Department of Health and devolved administrations, 2007. Available from: www.nta.nhs.uk/uploads/clinical_guidelines_2007.pdf

38. European Centre for Disease Prevention and Control (ECDC). Mapping of HIV/STI behavioural surveillance in Europe. ECDC Technical Report. Stockholm: ECDC; September 2009. Available from: http://www.ecdc.europa.eu/en/publications/Publications/0909_TER_Mapping_of_HIV_STI_Behavioural_Surveillance_in_Europe.pdf

39. Topp L, Iversen J, Wand H, Day C, Kaldor J, Maher L, Collaboration of Australian Needle Syringe Programs. Representativeness of injecting drug users who participate in HIV surveillance: results from Australia's Needle and Syringe Program Survey.J Acquir Immune Defic Syndr. 2008;47(5):632-8. Available from: DOI: 10.1097/QAI.0b013e31816a1d68 PMID: 18491422

40. Hickman M, Hope V, Brady T, Madden P, Jones S, Honor S, et al. Hepatitis C virus (HCV) prevalence, and injecting risk behaviour in multiple sites in England in 2004. J Viral Hepat. 2007;14(9):645-52. Available from: DOI: 10.1111/j.1365-2893.2007.00855.x PMID: 17697017

41. Latkin CA, Vlahov D, Anthony JC. Socially desirable responding and self-reported HIV infection risk behaviors among intravenous drug users.Addiction. 1993;88(4):517-26. Available from: DOI: 10.1111/j.1360-0443.1993.tb02058.x PMID: 8485429

42. De Irala J, Bigelow C, McCusker J, Hindin R, Zheng L. Reliability of self-reported human immunodeficiency virus risk behaviors in a residential drug treatment population.Am J Epidemiol. 1996;143(7):725-32. Available from: DOI: 10.1093/oxfordjournals.aje.a008806 PMID: 8651235

43. European Monitoring Centre for Drugs and Drug Addiction (EMCDDA). European drug report: trends and developments. Lisbon: EMCDDA; June 2015. Available from: www.emcdda.europa.eu/publications/edr/trends-developments/2015

License and copyright

ECDC publishes risk assessment ahead of the 2016 Olympic and Paralympic Games in Rio de Janeiro

Eurosurveillance editorial team [1]
1. European Centre for Disease Prevention and Control (ECDC), Stockholm, Sweden

Correspondence: Eurosurveillance editorial team (eurosurveillance@ecdc.europa.eu)

Citation style for this article:
Eurosurveillance editorial team. ECDC publishes risk assessment ahead of the 2016 Olympic and Paralympic Games in Rio de Janeiro. Euro Surveill. 2016;21(19):pii=30228. DOI: http://dx.doi.org/10.2807/1560-7917.ES.2016.21.19.30228

Article submitted on 12 May 2016 / accepted on 12 May 2016 / published on 12 May 2016

On 10 May 2016, the European Centre for Disease Prevention and Control (ECDC) published a risk assessment [1] on the 'Potential risks to public health related to communicable diseases at the Olympic and Paralympic Games in Rio de Janeiro, Brazil 2016'. The Games will start in early August 2016.

The risk assessment states that visitors to the Games will be mostly at risk to acquire a vector-borne infection or gastrointestinal illness. Therefore, visitors are advised to adopt protective measures against mosquito bites such as wearing long-sleeved shirts and trousers, and to apply mosquito repellent. Standard hygiene measures, for example drink factory produced beverages, consume thoroughly cooked meals and wash fruits and vegetables before eating these, to prevent gastrointestinal illnesses, are also recommended.

Since the Games will be held during the winter season in Rio de Janeiro the weather conditions are less favourable for mosquitoes leading to significant reduction for risk for mosquito-borne infections such as chikungunya, dengue and Zika virus disease, except for the area of Manaus where some of the football matches will take place. Still it cannot be excluded that travellers can become infected.

In respect to the outbreak of Zika virus disease in Brazil, the risk assessment points out that ECDC information to travellers, in particular pregnant women and women who are planning to become pregnant as well as travellers with immune disorders or severe chronic illnesses remains valid and should be recalled [2].

Ahead of travel, visitors should inform themselves of the advice issued by the Pan American Health Organisation as well as by Brazilian and other national health authorities regarding vaccine-preventable diseases which they could contract during the visit.

The detailed risk assessment considers also other risks such as colonisation (digestive tract carriage) with multidrug-resistant Enterobacteriaceae, sexually transmitted diseases.

References

1. European Centre for Disease Prevention and Control (ECDC). Potential risks to public health related to communicable diseases at the Olympics and Paralympics Games in Rio de Janeiro, Brazil 2016. Stockholm, 2016. Available from: http://ecdc.europa.eu/en/publications/_layouts/forms/Publication_DispForm.aspx?List=4f55ad51-4aed-4d32-b960-af70113dbb90&ID=1486
2. European Centre for Disease Prevention and Control (ECDC). Rapid Risk Assessment. Zika virus disease epidemic: potential association with microcephaly and Guillain–Barré syndrome. Fifth update, 11 April 2016. Stockholm: ECDC; 2016. Available from: http://ecdc.europa.eu/en/healthtopics/zika_virus_infection/zika-outbreak/Pages/risk-assessment.aspx#sthash.D6VMM46M.dpuf

License and copyright